



Interactive effects of CO₂ and soil water treatments on growth and biomass allocation in pines and spruces



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ARTICLE INFO

Keywords:

Biomass allocation
Elevated CO₂
Fitness
Growth
Pine
Shade tolerance
Soil moisture stress
Spruce

ABSTRACT

Growth, components of growth, and biomass allocation were quantified for eight species in two commercially important genera, *Pinus* and *Picea*, grown in a 2 × 2 factorial of atmospheric CO₂ and soil moisture stress. Four of the pines and three of the spruces are native to eastern North America; a fourth spruce, Norway spruce (NS: *P. abies*), is native to Europe but is used for reforestation in northeastern North America. Height, basal diameter (BD), and total biomass response of pines were often more than two times greater than that of spruces under elevated CO₂ (eCO₂). A significant species × CO₂ interaction for total biomass was a result of species' differential response to eCO₂: *Pinus rigida* had the greatest biomass stimulation (59%), followed by *P. resinosa* (39%), *P. strobus* (26%), and *P. banksiana* (19%). Among spruces, *Picea glauca* showed the greatest response (30%), and *P. mariana* the least response under eCO₂ (5%). Overall, soil moisture stress reduced total productivity by 12%. Most pines did have greater growth under moisture stress, and NS and BS grew well. Percent needle mass was lower under eCO₂, but this was not due directly to eCO₂ but to ontological changes. Controlling for size, pines had 20% greater needle biomass than spruces, while having a negative relationship to total biomass. A comparison of total biomass under eCO₂ in relation to aCO₂ by species showed that overall, the greater the species mass, the greater the mass gain under eCO₂, and the greater the mass loss under drought conditions. In addition, our results for spruces lend strong support to the theory that late-successional species have greater growth response under eCO₂ than early to mid-successional species. A diverse portfolio of tree species for artificial reforestation would help forest management adapt to the many uncertainties over future environments and markets, but our results on responses in spruces and pines to eCO₂ and soil moisture stress support a shift toward increased use of pines in forest management and artificial reforestation.

1. Introduction

A predicted doubling in atmospheric CO₂ concentration during this century and potential changes in soil moisture conditions resulting from global climate changes cause great uncertainty regarding future comparative fitness among forest tree species (Parker et al., 2000; Feng and Fu, 2013; Sherwood and Fu, 2014). Paleoecological reconstructions support the contention that species migrate singly rather than as intact plant communities (Peters, 1990; Schaffler and Jacobson, 2002). Pollen cores at a number of locations in Maine, USA show that red spruce (RS; *Picea rubens* (Sarg.)) has been abundant for the last 1000 years, a period of relatively cool and wet conditions compared with the previous 1000–7000 years when it was warmer and drier, favoring pines (*Pinus* spp.) over spruces (Schaffler and Jacobson, 2002; Lindbladh et al., 2003). White pine (WP: *Pinus strobus* L.) was the predominant pine during that warm, dry period, whereas pitch pine

(PP: *P. rigida* Mill.), jack pine (JP: *P. banksiana* Lamb.), and red pine (RP: *P. resinosa* Ait.) were less common. Examining the postglacial history of the three native *Picea* species in New England during the two key periods since deglaciation when *Picea* were abundant—the late glacial/early Holocene (14,000 to 8000 cal yr B.P.) and the late Holocene (1400 cal yr B.P. to present)—Lindbladh and Schaffler (2003) demonstrated a shift from a forest dominated by white spruce (WS; *Picea glauca* (Moench) Voss) and black spruce (BS; *Picea mariana* (Mill.) B.S.P.) during the late glacial/early Holocene to a forest dominated by RS and BS during the late Holocene.

Elevated CO₂ (eCO₂) typically increases tree growth and has been shown to modify component physiological processes, including net assimilation (A), water-use efficiency, cold tolerance, and dry-matter partitioning (Greenwood and Volkaert, 1992; Johnsen, 1993; Samuelson and Seiler, 1994; Major et al., 2014, 2018). Mid-Holocene and recent dry, warm periods over the past 2000 years have been

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<https://doi.org/10.1016/j.foreco.2019.03.056>

Received 15 February 2019; Received in revised form 26 March 2019; Accepted 27 March 2019
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detected from Maine to Minnesota based on the record of vegetation changes from well-dated pollen profiles (Davis et al., 1980; Gajewski, 1988). Thus, forest management, including the choice of species for a given region or locale, may need to be altered as climate (moisture and temperature) changes and atmospheric CO₂ increases. Pines and spruces are largely sympatric at the landscape level, but changes in climate conditions can change composition in as little as two generations in the case of spruce and pines (Schauffler and Jacobson, 2002). It is uncertain if and how these two genera and their respective species will respond to eCO₂ and changing soil moisture conditions, and thus deployment decisions for reforestation must be formulated to adapt to these anticipated environmental changes.

The Pinaceae, to which pines and spruces belong, is the largest conifer family, with 11 genera spread across most of the Northern Hemisphere. Pines and spruces are the most commercially important conifer species. The spruces we examined are: WS, a transcontinental, mid- to late-successional species that grows under highly variable conditions, including extreme climates (Nienstaedt and Zasada, 1990); black spruce, a transcontinental, early to mid-successional species, usually found on wet organic sites, but productive stands can be found on a wide variety of soil types (Viereck and Johnston, 1990); red spruce a commercially important and characteristic component of the late-successional forest of the Acadian Forest Region in eastern Canada and in the northeastern United States, extending along the Appalachians to North Carolina and Tennessee, primarily as isolated montane remnants (Blum, 1990); and Norway spruce (NS; *Picea abies* (L.) H. Karst), a large and fast-growing conifer native to central and northern Europe, used for reforestation in eastern Canada.

The pines we examined are: WP, a dominant species found on well-drained sites across eastern Canada and the United States, extending into northern Georgia (Wendel and Smith, 1990); red pine, which is associated with the Great Lakes–St. Lawrence Forest Region, where it is largely confined to sandy soils on well-drained sites (Rudolf, 1990); jack pine, a boreal temperate pine ranging from the eastern slopes of the Rocky Mountains to Maritime Provinces in the east and usually found on drier, sandy soils (Rudolph and Laidly, 1990); and pitch pine, which occurs from southeastern Ontario and Quebec and across Maine extending southward to northern Georgia, where it grows on shallow, less fertile soils, with a sandy or gravelly texture (Little and Garrett, 1990).

A number of studies have reported that faster-growing species have greater absolute and relative growth responses to eCO₂ than inherently slower-growing species (Atkin et al., 1999; Poorter and Navas, 2003), although this trend was not reported by others (Tjoelker et al., 1998; Ghannoum et al., 2010). A meta-analysis of 74 total biomass response ratios across a range of ambient CO₂ (aCO₂) to eCO₂ showed that response ratios differed widely and significantly, but that shade-tolerant tree species generally had greater response ratios than shade-intolerant species (Bazzaz et al., 1990; Kerstiens, 2001). We hypothesized that species' biomass traits will respond to and interact with eCO₂ and soil moisture stress, contributing to altered relative fitness under eCO₂. Our goal was to examine and compare growth traits, components of growth, and biomass allocation responses among four spruce and four pine species under eCO₂ and soil moisture stress. Our specific objectives were to (1) determine variation in biomass sequestration, components of growth, and biomass allocation among species and genera, (2) determine how these biomass traits respond to and interact with eCO₂ and soil moisture stress, and (3) assess if relative size or shade tolerance affects response to eCO₂ and soil moisture stress.

2. Materials and methods

2.1. Material and growth conditions

We examined four pines and four spruces; seed sources are listed in Table 1. Seeds were sown in hydrated 36-mm Jiffy™ peat pellets (Jiffy

Table 1

Geographic coordinates of populations for pine and spruce seed sources used.

Species	Location	Latitude (N)	Longitude (W)
Pines			
<i>Pinus strobus</i>	Doaktown, NB	46°33'	66°07'
<i>Pinus resinosa</i>	Debert, NS	45°26'	63°27'
<i>Pinus banksiana</i>	Debert, NS	45°26'	63°27'
<i>Pinus rigida</i>	Brockville, ON	44°35'	75°41'
Spruces			
<i>Picea glauca</i>	Richard Brook, NB	47°31'	68°13'
<i>Picea rubens</i>	Lawrencetown, NS	44°52'	65°09'
<i>Picea mariana</i>	Glenco, NB	47°57'	66°48'
<i>Picea abies</i>	Central France	45°46'	3°04' East

Products Ltd, Lincoln, NB, Canada) (90 mL full volume) in June 2003. Trays were randomly located across greenhouse benches at the Canadian Forest Service - Atlantic Forestry Centre (CFS-AFC) in Fredericton, NB, Canada (45°52'N, 66°31'W). First-year growth was under aCO₂ and well-watered conditions. Seedlings were stored overwinter in cold storage on site at −5.0 °C. Eight (four per CO₂ treatment) 2.15 × 4.30 × 2.5 m specially constructed chambers, located at the CFS-AFC greenhouses and covered with 4-mm polyethylene greenhouse film and with air cooling and outside air exchanger, were used to grow seedlings planted in 25 cm diameter × 30 cm deep felt root-control bags (Root Control Inc., Oklahoma City, OK, USA). The chambers were monitored and maintained to outside ambient temperatures and relative humidity conditions and to either 370 or 740 ppm of CO₂, representing aCO₂ and eCO₂ levels, respectively, using a greenhouse monitoring system, calibrated monthly, that dosed the chambers when they fell below the target values (Major et al., 2007). Light levels were measured outside and inside chambers using quantum sensors LI-190SA (LI-COR, Lincoln, Nebraska, USA). Using the light extinction method detailed by Parent and Messier (1996), light levels were approximately 70% of outside levels, resulting in an approximate maximum of 1300–1400 μmol m^{−2} s^{−1}.

Four groups of three seedlings, for a total of 12 seedlings for each of the eight species, were randomly established in each greenhouse chamber in May 2004. Seedlings were watered and fertilized once a week. Fertilization was applied in solution, with application rates modified over the growing season: in May, we used 11:41:8 (N:P:K) plus micronutrients at 50 ppm N (Plant Products Co. Ltd., Brampton, ON); from June to the end of August, we used 20:8:20 at 100 ppm N; and from September to the end of October, we used 8:20:30 at 35 ppm N. Two soil moisture treatments were each assigned to four tents in factorial combination with the CO₂ treatments. Soil moisture conditions were set either between −0.1 and −0.5 MPa (irrigated treatment) or −0.7 and −1.0 MPa (drought treatment), calculated from a soil retention curve, measured with a soil moisture probe (CS615, Campbell Scientific, Logan, UT) put in each chamber, and recorded with a data logger (CR10X, Campbell Scientific). In addition to electronic monitoring and because of imperfections in the irrigation system, we visually examined the beds and did some spot hand watering as required.

2.2. Harvest, measurements, and parameter estimation

At age four, after 3 years' treatment, all samples were measured for stem height, basal diameter (BD), and needle, wood (wood and bark), and root biomass. Dry biomass was measured after oven drying the seedling components at 65 °C for 48 h. From the morphometric data, the following parameters were determined: percent needle, wood, and root biomass; and shoot-to-root ratio = needle + wood biomass/root biomass.

Table 2

Height and basal diameter variance components (Var. Comp.) and ANOVAs including source of variation, degrees of freedom (df), mean square values (MS), P values, and coefficient of determination (R^2). P values < 0.05 are in bold print.

Source of variation	df	Total height (cm)			Basal diameter (mm)		
		MS	Var. Comp. (%)	P value	MS	Var. Comp. (%)	P value
Block	1	6.9	< 0.1	0.732	5.9	0.2	0.175
Genus	1	9609.0	14.4	< 0.001	726.7	21.3	< 0.001
CO ₂	1	1616.7	2.4	< 0.001	121.9	3.6	< 0.001
Water	1	306.3	0.5	0.024	20.8	0.6	0.011
Genus × CO ₂	1	249.5	0.4	0.041	27.5	0.8	0.004
Genus × water	1	74.3	0.1	0.263	16.8	0.5	0.022
CO ₂ × water	1	63.8	0.1	0.300	42.8	1.3	< 0.001
Genus × CO ₂ × water	1	168.1	0.3	0.093	0.7	< 0.1	0.642
Spp ¹ (genus)	6	6760.0	60.6	< 0.001	275.9	48.6	< 0.001
Spp(genus) × CO ₂	6	68.7	0.6	0.327	4.8	0.8	0.173
Spp(genus) × water	6	37.3	0.3	0.705	6.2	1.1	0.074
Spp(genus) × CO ₂ × water	6	91.1	0.8	0.165	4.4	0.8	0.215
Error	223	59.0	19.5		3.2	20.5	
R^2				0.805			0.796

¹ Spp = species.

2.3. Statistical analyses

The study was established as a randomized block experimental design. Water, CO₂ level, genus, and species nested within genus [(species (genus))] were all considered as fixed effects. The three row tree plots were averaged as these were not independent of each other in the experiment. The data were subjected to analyses of variance (ANOVA) using the following ANOVA model:

$$Y_{ijklmn} = \mu + B_i + G_j + C_k + W_l + GC_{jk} + GW_{jl} + CW_{kl} + GCW_{jkl} + S_{m(j)}SC_{m(j)k} + SW_{m(j)l} + SCW_{m(j)kl} + e_{ijklmn} \quad (1)$$

where Y_{ijklmn} is the dependent seedling trait of the i^{th} greenhouse chamber, of the j^{th} genus, of the k^{th} CO₂ treatment, of the l^{th} water treatment, of the m^{th} species, of the n^{th} seedling, and μ is the overall mean. B_i is the effect of the i^{th} greenhouse chamber ($i = 1, 2$), G_j is the effect of the j^{th} genus ($j = 1, 2$), C_k is the effect of the k^{th} CO₂ treatment ($k = 1, 2$), W_l is the effect of the l^{th} water treatment ($l = 1, 2$), GC_{jk} is the interaction effect of the j^{th} genus and the k^{th} CO₂ treatment, GW_{jl} is the interaction effect of the j^{th} genus and l^{th} water treatment, CW_{kl} is the interaction effect of the k^{th} CO₂ treatment and the l^{th} water treatment, GCW_{jkl} is the interaction effect of the j^{th} genus, k^{th} CO₂ treatment and l^{th} water treatment. $S_{m(j)}$ is the effect of the m^{th} species nested within the j^{th} genus ($m = 1, 2$), $SC_{m(j)k}$ is the interaction effect of the m^{th} species nested within j^{th} genus and k^{th} CO₂ treatment, $SW_{m(j)l}$ is the interaction effect of the m^{th} species nested within j^{th} genus and l^{th} water treatment, and the m^{th} species nested within j^{th} genus, $SCW_{m(j)kl}$ is the interaction effect of the m^{th} species nested within j^{th} genus, k^{th} CO₂ treatment, and l^{th} water treatment, and e_{ijklmn} is the random error component. Effects were considered statistically significant at the $P = 0.05$ level, but individual P values are provided so that readers can make their own interpretations. The data satisfied normality and equality of variance assumptions. The general linear model from Systat (Chicago, IL) was used for analysis. Species within genus were tested using Tukey's mean separation test ($P = 0.05$).

In order to separate allocation changes from size-induced changes (ontogeny), we analyzed biomass allocation changes while correcting for size changes using analysis of covariance (ANCOVA). In these analyses, three sources of variation were studied: (1) covariate (i.e., total biomass, correcting for size effect), (2) independent effect (i.e., CO₂ or genus), and (3) independent effect × covariate. This analysis helps differentiate changes in biomass allocation determined by ANOVA (Eq. (1)) from changes in allocation due to changes in tree size. The ANCOVA CO₂ results will thus have been corrected for tree size.

The analyses were done based on the following model:

$$Y_{ij} = B_0 + B_{0i} + B_1X_{ij} + B_{1i}X_{ij} + e_{ij} \quad (2)$$

where Y_{ij} is the dependent trait of the i^{th} plant of the j^{th} genus or CO₂ treatment, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are the species or CO₂ treatment specific coefficients, X_{ij} is the independent variable, and e_{ij} is the error term. The covariate analysis used has far fewer numbers (16 in total) than the ANOVA. In order to identify any trends, we increased the P level to 0.10. This only impacted the shoot-to-root ratio, which identified a genus × root biomass interaction that can clearly be seen in Fig. 7b. Individual P values are provided for all traits so that readers can make their own interpretations. When referring to the species effect, it should be assumed to be the species nested within the respective genus. The general linear model from Systat was used for analysis. The variance component analysis uses the sum of squares for its calculation as outlined by Hicks (1982: 55–57).

3. Results

3.1. Total height and basal diameter

Genus, species, CO₂, water, and the genus × CO₂ interaction were significant for total height and accounted for 14.4, 60.6, 2.4, 0.5, and 0.4% of total variation, respectively (Table 2). The CO₂ × genus interaction was a magnitude effect (no rank change); the relative increase in total height was 2x greater for pine (10.5%) than for spruce (5.4%) (Fig. 1a). The significant negative drought effect was similar for both genera (Fig. 1b). Overall, pine and spruce had total heights of 70.9 and 58.6 cm, respectively, or a difference of 21%. Total heights after four growing seasons for pines were 94.1a, 81.1b, 58.5c, and 50.2d cm for PP, JP, WP, and RP, respectively (Fig. 1c). Total heights for spruces were 63.2a, 60.1ab, 57.5bc, and 53.7c cm for BS, WS, NS, and RS, respectively (Fig. 1d).

Genus, species, CO₂, water and genus × CO₂, genus × water, and CO₂ × water interactions were significant effects for BD and accounted for 21.3, 48.6, 3.6, 0.6, 0.8, 0.5, and 1.3% of total variation, respectively (Table 2). Average BD under aCO₂ was 14.7 and 12.0 mm for pines and spruces, respectively (not shown). The genus × CO₂ interaction was a magnitude effect resulting from a more muted effect on growth for spruces (Table 2). Pines showed two times the increase in BD than spruce, with 14 and 6% response to eCO₂, respectively. The genus × water interaction showed a BD decrease of 7.2% for pines and negligible decrease for spruces under drought treatment. The CO₂ × water interaction was due to a greater negative drought effect

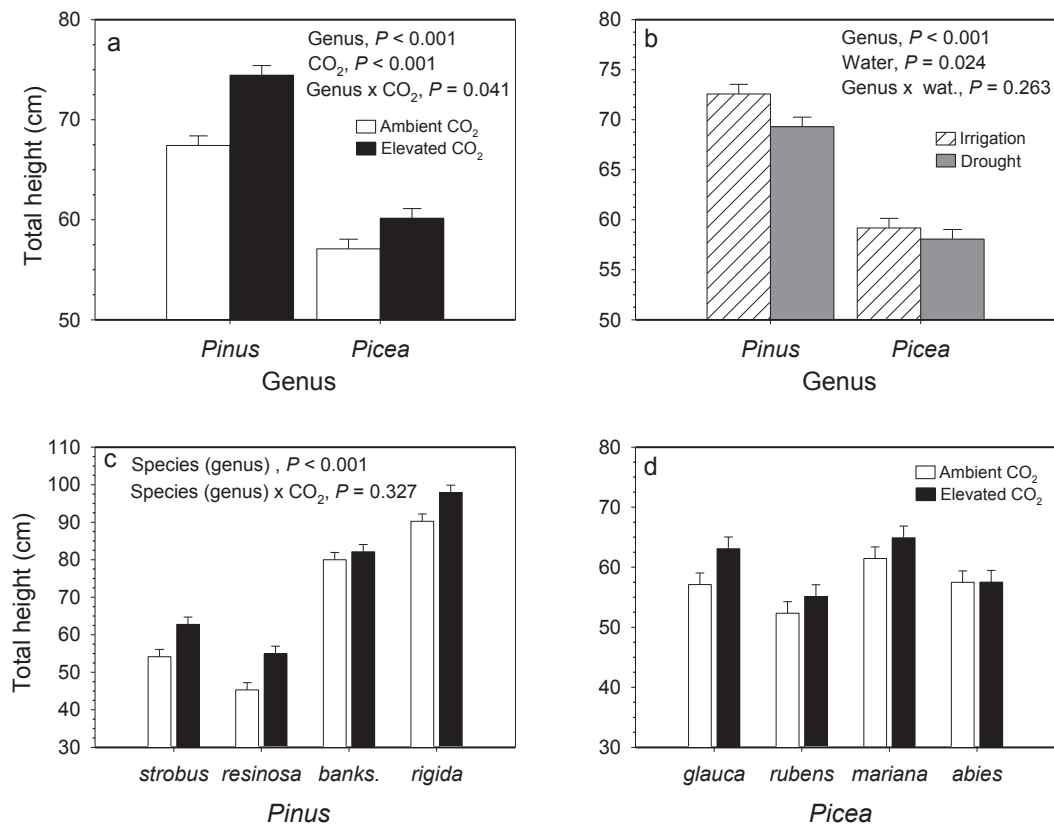


Fig. 1. Total height (mean \pm SE) (a) by genus and CO_2 treatments, (b) by genus and soil moisture stress treatments, (c) by pine species and CO_2 treatments, and (d) by spruce species and CO_2 treatments. Note the y-axis scale difference between pines (c) and spruces (d).

under eCO_2 than aCO_2 . Basal diameters for pines were 20.1a, 17.3b, 13.8c, and 11.5d mm for PP, JP, WP, and RP, respectively. Basal diameters for spruces were 13.6a, 13.3a, 12.7a, and 9.8b mm for WS, NS, BS, and RS, respectively.

3.2. Biomass

Needle biomass was significantly affected by genus, species, CO_2 , water, and genus $\times \text{CO}_2$, species $\times \text{CO}_2$, and species \times water interactions, which accounted for 42.2, 18.1, 3.8, 1.3, 1.6, 3.5, and 1.8% of the total variation, respectively (Table 3). The significant $\text{CO}_2 \times$ genus

interaction for needle biomass was a magnitude effect, with pines showing a greater response at 36.5 and 47.1 g (30%) compared with spruce at 19.3 and 21.5 g (13%) under aCO_2 and eCO_2 conditions, respectively (Fig. 2a and b). Average needle biomass for all species under irrigated and drought treatments was 33.0 and 29.2 g, and under aCO_2 and eCO_2 , was 27.9 and 34.3 g, respectively. Overall, pines and spruces had 41.8 and 20.4 g of needle biomass, respectively. Needle biomass for pines was 56.2a, 43.4b, 35.3c, and 32.3c g for PP, JP, WP, and RP, respectively. Needle biomass for spruces was 24.6a, 22.3ab, 19.7b, and 15.1c g for BS, NS, WS, and RS, respectively. The species $\times \text{CO}_2$ interaction was due to magnitude effects, with some pine

Table 3

Needle, wood, and root dry mass variance components (Var. Comp.) and ANOVAs including source of variation, degrees of freedom (df), mean square values (MS), P values, and coefficient of determination (R^2). P values < 0.05 are in bold print.

Source of Variation	df	Needle biomass (g)			Wood biomass (g)			Root biomass (g)		
		MS	Var. Comp. (%)	P value	MS	Var. Comp. (%)	P value	MS	Var. Comp. (%)	P value
Block	1	295.0	0.4	0.055	247.4	0.3	0.108	0.01	< 0.1	0.980
Genus	1	28982.9	42.2	< 0.001	6473.8	6.7	< 0.001	797.31	5.8	< 0.001
CO_2	1	2618.9	3.8	< 0.001	4870.2	5.1	< 0.001	653.58	4.7	< 0.001
Water	1	890.2	1.3	0.001	582.5	0.6	0.014	580.28	4.2	< 0.001
Genus $\times \text{CO}_2$	1	1103.8	1.6	< 0.001	1364.8	1.4	< 0.001	217.77	1.6	0.001
Genus \times water	1	175.6	0.3	0.138	302.4	0.3	0.076	63.26	0.5	0.067
$\text{CO}_2 \times$ water	1	238.8	0.3	0.084	1078.5	1.1	0.001	214.30	1.6	0.001
Genus $\times \text{CO}_2 \times$ water	1	9.1	< 0.1	0.735	9.8	0.0	0.749	2.57	< 0.1	0.711
Spp ¹ (genus)	6	2075.6	18.1	< 0.001	9079.2	56.7	< 0.001	1014.65	44.1	< 0.001
Spp(genus) $\times \text{CO}_2$	6	394.9	3.5	< 0.001	540.5	3.4	< 0.001	88.76	3.9	< 0.001
Spp(genus) \times water	6	210.9	1.8	0.017	264.6	1.7	0.013	55.62	2.4	0.008
Spp(genus) $\times \text{CO}_2 \times$ water	6	117.3	1.0	0.187	133.9	0.8	0.213	33.49	1.5	0.101
Error	223	79.4	25.6		95.1	21.9		18.67	29.9	
R^2				0.746			0.782			0.702

¹ Spp = species.

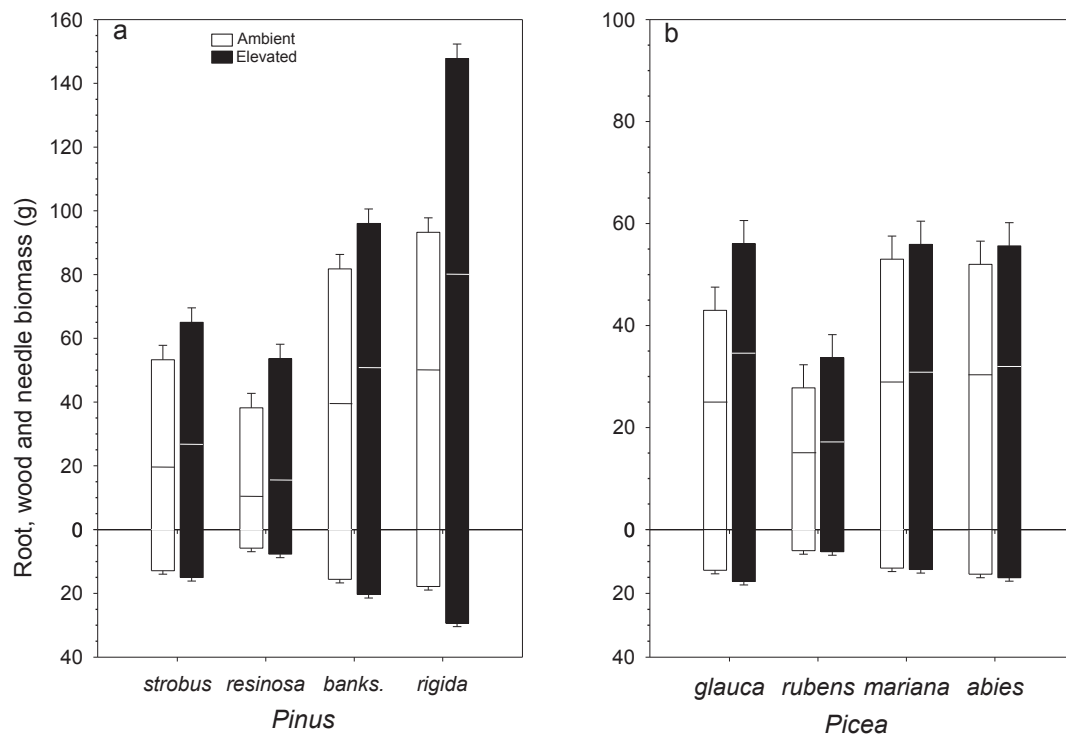


Fig. 2. Root, wood, and needle biomass (mean \pm SE) (a) by pine species and CO₂ treatments, and (b) by spruce species and CO₂ treatments. Note (1) the y-axis scale difference between pines and spruces, and (2) the lines in the vertical bars separate the needle biomass above, and wood biomass below.

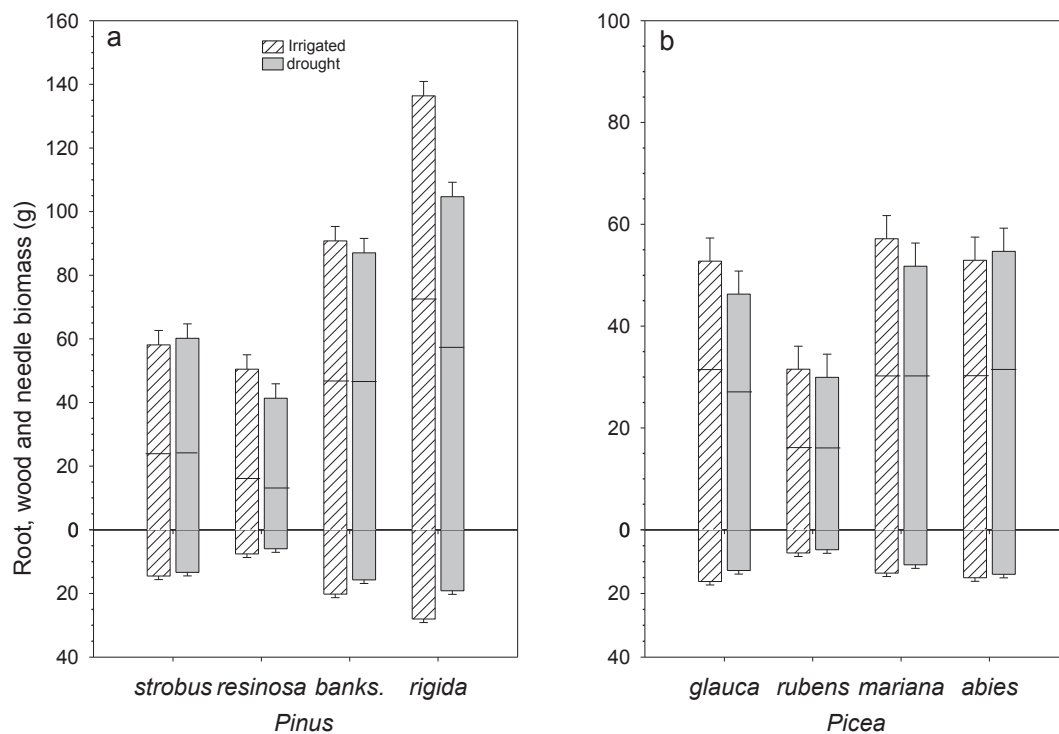


Fig. 3. Root, wood, and needle biomass (mean \pm SE) (a) by pine species and soil moisture stress treatments, and (b) by spruce species and soil moisture stress treatments. Note (1) the y-axis scale difference between pines and spruces, and (2) the lines in the vertical bars separate the needle biomass above, and wood biomass below.

species—particularly PP, RP, WP—responding better than spruces. Once again, the species \times water interaction was largely due to magnitude effects, with some species (PP and RP) responding to drought more negatively than others (WP, JP, and all spruces) (Fig. 3a and b).

Wood biomass was significant for genus, species, CO₂, water, and

genus \times CO₂, CO₂ \times water species \times CO₂ and species \times water interactions, which accounted for 6.7, 56.7, 5.1, 0.6, 1.4, 1.1, 3.4, and 1.7% of the total variation, respectively (Table 3). The significant CO₂ \times genus effect was a magnitude effect, with pines sequestering more biomass under eCO₂ than spruces. The pines sequestered wood

biomass of 30.1 and 43.5 g in aCO₂ and eCO₂, respectively; whereas spruces had a wood biomass of 24.7 and 28.8 g in aCO₂ and eCO₂, respectively (CO₂, $P < 0.001$; genus, $P < 0.001$). The significant CO₂ × water interaction was due to the muted response to eCO₂ under drought conditions. The significant water effect was a result of an average decline in wood biomass from 33.3 to 30.3 g for irrigated and drought treatments, respectively. Wood biomass for pines was 64.3a, 45.5b, 23.8c, and 13.5d g for PP, JP, WP, and RP, respectively (Fig. 2a). Wood biomass for spruces was 31.5a, 29.8a, 29.8a, and 15.6b g for BS, NS, WS, and RS, respectively (Fig. 2b). The significant species × CO₂ interaction was the result of different magnitudes of species' response. Among pines, PP showed the greatest absolute (29.9 g) and relative (60.5%) wood biomass responses to eCO₂. Jack pine had the lowest relative response (29%), and RP had the lowest absolute response to eCO₂ (5.7 g). Among spruces, WS had the greatest absolute (8.4 g) and relative (32%) wood biomass response to eCO₂; whereas BS had the lowest absolute (2.2 g) and relative (8%) wood biomass response to eCO₂. The significant species × water response for pines showed that PP had the greatest relative (29%) and absolute (16.9 g) decline, and WP and JP had among the lowest responses to drought (Fig. 3a). Among the spruces, wood biomass responses to drought were fairly weak (Fig. 3b).

Root biomass was significant for genus, species, CO₂, water, and genus × CO₂, CO₂ × water species × CO₂ and species × water interactions, which accounted for 5.8, 44.1, 4.7, 4.2, 1.6, 1.6, 3.9, and 2.4% of total variation, respectively (Table 3). The significant genus × CO₂ response was also due to magnitude response differences by genus. Pine root biomass response to eCO₂ was greater in relative (39%) and absolute terms (5.1 g) than spruce biomass response (12.3% and 1.4 g). The significant CO₂ × water interaction was due to magnitude differences in response to eCO₂, where the response to eCO₂ was lessened under the drought treatment. Root biomass for pines was 23.6a, 18.0b, 14.0c, and 6.8d g for PP, JP, WP, and RP, respectively (Figs. 2 and 3). Root biomass for spruces was 14.5a, 14.5a, 12.3a, and 6.8b g for NS, WS, BS, and RS, respectively. Pitch pine showed a strong root biomass response to eCO₂ (65% increase) compared with the other pines, which averaged 28%. There was a small increase in root biomass for BS and RS, 4 and 5%, respectively. White spruce and NS had a 28 and 8% increase, respectively, in root biomass in response to eCO₂. The species × water interaction in root biomass was most evident among pines, with PP declining the most (46%) relatively and absolutely (8.8 g), whereas WP declined the least (8.2%) relatively and absolutely (1.1 g). Among the spruces, WS declined the most (26%, 3.4 g), and NS the least (7.8% 1.1 g).

Genus, species, CO₂, water, and genus × CO₂, CO₂ × water, species × CO₂, and species × water interactions were significant for total biomass and accounted for 18.2, 40.8, 5.0, 1.4, 1.7, 0.9, 3.6, and 1.9% of total variation, respectively (Table 4). The significant total biomass genus × CO₂ interaction was due to magnitude effects, with pines showing a greater effect under eCO₂ than spruces (Table 3, Fig. 2a and b). The significant CO₂ × water interactions were magnitude effects as described above; i.e., a muted response to eCO₂ under drought conditions. Total biomass for pines was 144.1a, 106.9b, 73.1c, and 52.6d g for PP, JP, WP, and RP, respectively (Fig. 2a and b). Total biomass for spruces was 68.3a, 66.7a, 64.0a, and 37.5b g for NS, BS, WS, and RS, respectively. Among pines, PP had the greatest total biomass response to eCO₂ (59.0%, 66.0 g). The other three pines had similar absolute gains of 17 g, on average, amounting to a 39.3, 26.2, and 19.5% response to eCO₂ for RP, WP, and JP, respectively. For spruces, WS had the greatest response (30%, 16.6 g), whereas BS had the least response to eCO₂ (5.2%, 3.4 g). Red spruce and NS had an 18.2 and 12.1% response to eCO₂, respectively. As for the significant species × water interaction, the greatest decline due to drought was for PP (32.7% 40.6 g) followed by RP (22.7%, 10.7 g) (Fig. 3a); WP and JP had the least decline among pines. For spruces, the greatest decline in response to drought was for WS (16.8%, 9.9 g), with the least decline

showed by NS and RS (Fig. 3b).

3.2.1. Allocation

Percent needle biomass was significant for genus, species, CO₂, genus × CO₂, and CO₂ × water, which accounted for 37.6, 50.6, 0.3, 0.2, and 0.3% of the total variation, respectively (Table 5). The genus × CO₂ interaction was due to a decrease in percent needle biomass for pines in eCO₂ and no change for spruce between CO₂ treatments (Fig. 4a). The CO₂ × water interaction was due to a decrease in percent needle biomass from aCO₂ to eCO₂ under well-watered conditions; under drought, there was no change in percent needle biomass between CO₂ treatments (Fig. 4b). Pines and spruces had on average 47.6 and 35.7% needle biomass, respectively. Percent needle biomass for pines was 61.7a, 48.8b, 40.9c, and 38.9d%, for RP, WP, JP, and PP, respectively (Fig. 4c). Percent needle biomass for spruces was 40.7a, 37.8b, 33.1c, and 31.3c% for RS, BS, NS, and WS, respectively (Fig. 4d). Percent needle biomass declined for all pines under eCO₂, except for PP where it remained unchanged.

Percent wood biomass was significant for genus, species, CO₂, water, CO₂ × water, which accounted for 28.2, 55.4, 0.9, 1.0, and 0.5% of the total variation, respectively (Table 5). The significant CO₂ × water interaction was due to rank change, with percent wood biomass increasing from aCO₂ to eCO₂ under the well-watered treatment, whereas under drought conditions, aCO₂ and eCO₂ percent wood biomass were equal. Spruces had more percent wood biomass than pines, with 44.3 and 36.1%, respectively (Fig. 5a and b). For pines, percent wood biomass was 44.9a, 42.2b, 32.1c, and 25.5d for PP, JP, WP, and RP, respectively (Fig. 5c). For spruces, percent wood biomass was 46.1a, 45.8ab, 44.2b, and 41.1c for WS, NS, BS, and RS, respectively (Fig. 5d). Percent wood biomass increased in eCO₂ for all pines except RP. Percent wood biomass increased for all spruces except WS.

Percent root biomass was significant for genus, species, and water, which accounted for 26.6, 33.8, and 4.6% of the total variation, respectively (Table 5). Percent root biomass was greater in the well-watered than the drought treatment, with 18.9 and 17.3%, respectively. Spruces had a greater percent root biomass than pines, with 20.0 and 16.2%, respectively. For pines, percent root biomass was 19.1a, 16.9b, 16.2b, and 12.7c for WP, JP, PP, and RP, respectively. For spruces, percent root biomass was 22.6a, 21.2a 18.2b, 18.0b for WS, NS, RS, and BS, respectively.

Shoot-to-root ratio was significant for genus, species, water, and species × CO₂ × water interaction and accounted for 22.2, 37.9, 3.6, and 2.7% of the total variation, respectively (Table 4). Shoot-to-root ratio was greater for drought than well-watered treatments, at 5.1 and 4.6, respectively. Pines had greater shoot-to-root ratio than spruces, at 5.5 and 4.2, respectively. Shoot-to-root ratio for pines was 7.1a, 5.3b, 5.1b, and 4.3c for RP, PP, JP, and WP, respectively. Shoot-to-root ratio for spruces was 4.8a, 4.8a, 3.8b, and 3.5b for BS, RS, NS, and WS, respectively.

3.2.2. Covariate analysis (correcting for size differences)

Covariate analysis of percent needle biomass in relation to total biomass testing for genus × total biomass showed that there was no significant interaction ($P = 0.971$). Further analysis showed that genus was significant ($P < 0.001$), and also there was a significant relationship between percent needle biomass to total biomass ($P < 0.001$). The result was a negative relationship between percent needle biomass and total biomass, with pines having 20% greater values than spruce when controlling for total biomass (Fig. 6a). For every 10 g increase in total biomass, there was a corresponding 2% decrease in percent needle biomass. Further covariate analysis (controlling for size) within genus showed that CO₂ or water itself did not result in the reduced or changed percent needle biomass.

Covariate analysis of percent wood biomass in relation to total biomass showed no genus × total biomass interaction ($P = 0.693$). Further analysis showed a significant genus effect ($P < 0.001$) for

Table 4

Shoot, total dry mass, and shoot to root ratio variance components (Var. Comp.) and ANOVAs. including source of variation, degrees of freedom (df), mean square values (MS), *P* values, and coefficient. of determination (R^2). *P* values < 0.05 are in bold print.

Source of Variation	df	Total biomass (g)			Shoot-to-root biomass ratio		
		MS	Var. Comp. (%)	<i>P</i> value	MS	Var. Comp. (%)	<i>P</i> value
Block	1	1075	0.3	0.135	11.83	2.7	< 0.001
Genus	1	77,808	18.2	< 0.001	98.79	22.2	< 0.001
CO ₂	1	21,470	5.0	< 0.001	0.88	0.2	0.220
Water	1	6093	1.4	< 0.001	15.94	3.6	< 0.001
Genus × CO ₂	1	7212	1.7	< 0.001	0.86	0.2	0.224
Genus × water	1	1490	0.3	0.079	0.98	0.2	0.194
CO ₂ × water	1	3961	0.9	0.004	0.52	0.1	0.346
Genus × CO ₂ × water	1	60	< 0.1	0.723	0.01	< 0.1	0.879
Spp ¹ (genus)	6	29,017	40.8	< 0.001	28.15	37.9	< 0.001
Spp(genus) × CO ₂	6	2584	3.6	< 0.001	1.00	1.4	0.115
Spp(genus) × water	6	1377	1.9	0.010	0.21	0.3	0.902
Spp(genus) × CO ₂ × water	6	659	0.9	0.224	1.99	2.7	0.003
Error	223	478	24.8		0.58	28.7	
R^2				0.753			0.714

¹ Spp = species.

Table 5

Percent needle, wood, and root variance components (Var. Comp.) and ANOVAs including source of variation, degrees of freedom (df), mean square values (MS), *P* values, and coefficient of determination (R^2). *P* values < 0.05 are in bold print.

Source of Variation	df	Needle biomass (%)			Wood biomass (%)			Root biomass (%)		
		MS	Var. Comp. (%)	<i>P</i> value	MS	Var. Comp. (%)	<i>P</i> value	MS	Var. Comp. (%)	<i>P</i> value
Block	1	2.8	< 0.1	0.605	44.1	0.3	0.019	69.0	2.0	< 0.001
Genus	1	8935.9	37.6	< 0.001	4169.4	28.2	< 0.001	897.6	26.6	< 0.001
CO ₂	1	67.3	0.3	0.011	127.1	0.9	< 0.001	9.4	0.3	0.150
Water	1	0.0	0.0	0.984	152.3	1.0	< 0.001	153.9	4.6	< 0.001
Genus × CO ₂	1	49.9	0.2	0.029	20.5	0.1	0.108	6.5	0.2	0.233
Genus × water	1	28.3	0.1	0.100	27.7	0.2	0.062	0.0	< 0.1	0.977
CO ₂ × water	1	69.5	0.3	0.010	73.4	0.5	0.003	0.1	< 0.1	0.913
Genus × CO ₂ × water	1	15.1	0.1	0.229	7.4	< 0.1	0.333	1.4	< 0.1	0.584
Spp ¹ (genus)	6	2007.7	50.6	< 0.001	1366.4	55.4	< 0.001	190.3	33.8	< 0.001
Spp(genus) × CO ₂	6	12.9	0.3	0.284	13.6	0.5	0.116	5.7	1.0	0.272
Spp(genus) × water	6	15.6	0.4	0.177	16.3	0.7	0.058	2.2	0.4	0.811
Spp(genus) × CO ₂ × water	6	18.8	0.5	0.097	9.6	0.4	0.295	9.0	1.6	0.069
Error	223	10.4	9.6		7.8	11.7		4.5	29.5	
R^2				0.904			0.882			0.706

¹ Spp = species.

wood mass in relation to total mass ($P < 0.001$). The result was a positive relationship between percent wood biomass and total biomass (Fig. 6b). Thus, spruces had 14% more of their biomass allocated to wood than did pines when controlling for total biomass. For every 10 g total biomass increase, there was a 1.7% increase in percent wood biomass. Further covariate analysis within genus showed that the CO₂ effect did not directly cause the increase or change in percent wood biomass under eCO₂.

Covariate analysis of percent root biomass in relation to total biomass showed no genus × total biomass interaction ($P = 0.531$). Further analysis showed that there was no significant percent root biomass relationship to total biomass ($P = 0.314$) but there was a significant genus effect ($P = 0.006$), resulting in two horizontal lines for percent root to total biomass. Spruce had a greater percent root biomass than pine by almost 4% (Fig. 7a).

Covariate analysis of shoot biomass in relation to root biomass, showed a significant genus × root biomass interaction ($P = 0.066$) and that pines have a steeper shoot-to-root slope than spruces, with 4.54 and 2.85 slopes, respectively (Fig. 7b). Breaking it down to component parts, needle biomass to root biomass showed no genus × root biomass interaction ($P = 0.171$) but a significant genus effect ($P < 0.001$), with a slope of 1.41 ($P < 0.001$; graph not shown). With each 1 g increase in root biomass, there is a 1.4 g increase in needle biomass. In addition,

pines had a 16 g greater needle mass than spruces over the range of root mass. Wood biomass, however, did show a significant genus × root biomass interaction ($P = 0.073$), which contributed to the interaction found in the shoot-to-root biomass.

Analysis of eCO₂ total biomass in relation to aCO₂ total biomass by species showed that the greater the species mass, the greater the mass gain under eCO₂ (Fig. 8a). As seen in the ANOVA of total biomass (Table 4), there is a significant species × CO₂ interaction. Thus, the response to eCO₂ is species specific. Analysis of the irrigated total biomass in relation to drought total biomass by species also showed that the greater the species total biomass, the greater the drought effect (Fig. 8b). Again, as seen in the total biomass ANOVA, there was a significant species × water interaction. Thus, the response to drought is species specific.

4. Discussion

4.1. Growth response to eCO₂

Height, BD, and total biomass were enhanced by eCO₂ for all species, but to different degrees, consistent with other findings (Marfo and Dang, 2009; Liu et al., 2011; Granda et al., 2014). After 3 years of treatments, pines clearly assimilated more than spruces. Furthermore,

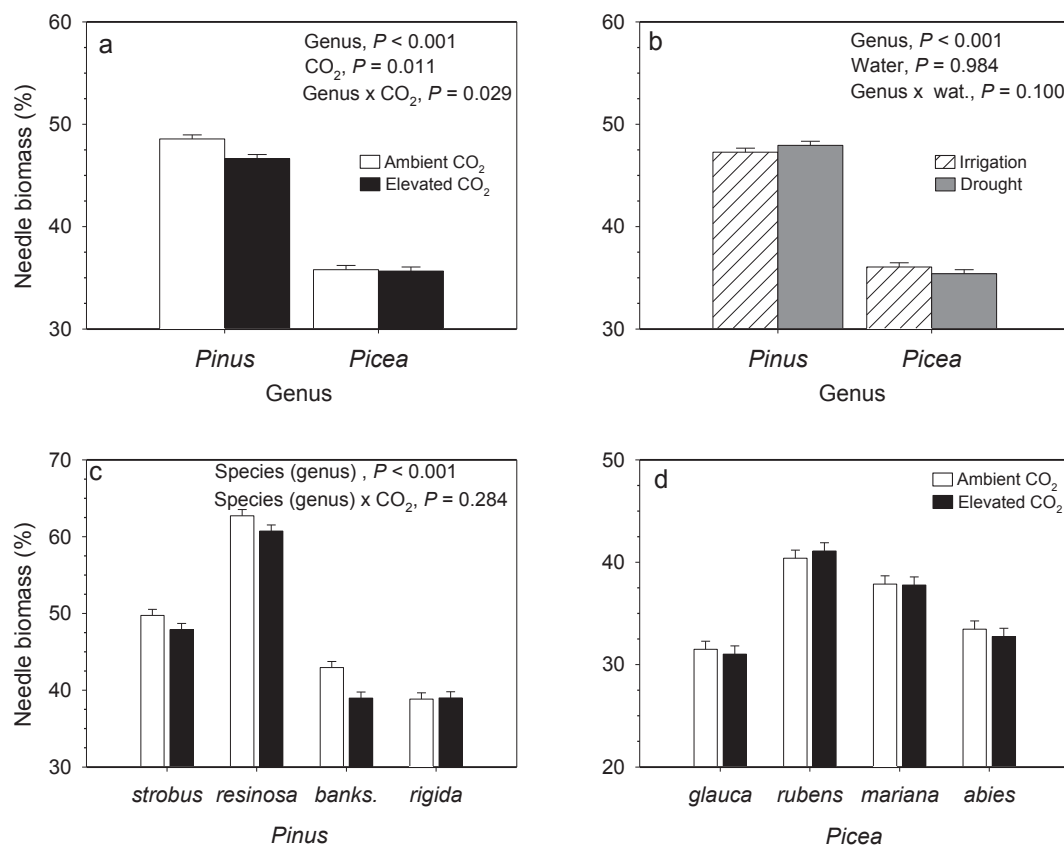


Fig. 4. Percent needle biomass (mean ± SE) (a) by genus and CO₂ treatments, (b) by genus and soil moisture stress treatments, (c) by pine species and CO₂ treatments, and (d) by spruce species and CO₂ treatments.

there was more growth variation among pines than spruces in response to eCO₂. Response of PP to eCO₂ was remarkable, as it had the greatest response compared with the other seven species and had almost twice the total biomass response to eCO₂ than the average of the other three pines. Interestingly, under aCO₂, JP and PP were the largest of the eight species and had somewhat similar total biomass with 97.4 and 111.1 g, respectively. However, JP had only a 20% biomass stimulation in response to eCO₂, whereas, PP had a 59% stimulation in total biomass. This threefold difference in biomass stimulation was partially driven by height (Fig. 1c) and BD stimulation. Besides visual observations, there are five lines of evidence that roots were not root bound. (1) PP had by far the greatest root biomass in aCO₂ (17.8 g) and in eCO₂ (29.3 g) of the eight species. (2) PP showed the greatest increase in root mass (65%). (3) This was greater than the aboveground biomass increase (58%) for PP. (4) Root bound seedlings reduce carbohydrate sink demand for root growth; however, net photosynthesis (P_n) did not decline in PP, but increased the most in eCO₂ (Major et al. 2018). (5) The root control bags were 25 cm across and 30 cm deep, giving them a volume of 14,725 cm³. PP root dry mass per unit soil volume was quite low, with an average of 0.002 g.cm³ most of which would be in the seedling “stump.”

In Canada, PP is a rare species, found along the international border between New York State and the provinces of Ontario and Quebec, near the northern limit of its geographical range (Mosseler et al., 2004). Further east, the northernmost populations of PP are found in northern Maine (e.g., Acadia National Park). Pitch pine has the ability to produce a second, late-season shoot growth or lammas growth. We have examined some 15-year-old provenance trials of PP planted together with RP and JP that have been established at 11 locations across ON, NB, and NS and have documented comparative growth results, including this second late-season bud flush (Major et al., 2020a, unpublished results). In addition, the early growth of PP is superior to either JP or RP, with

some selections growing to 10 cm in basal diameter within 8 years after establishment. Among the four pines, PP is currently more restricted in its northern geographical range, and may begin to express a competitive advantage under increasing CO₂. Thus, PP could expand its range, aided by the predicted warming and drying, and associated increase in fire frequencies.

Red pine under aCO₂ had the lowest productivity compared with the other pines and the second lowest among all species. Observations from a pine provenance experiment comparing RP, JP, and PP showed that RP had the lowest productivity at age 8 (Major et al., 2020a, unpublished results). However, RP did demonstrate the second best overall response to eCO₂, with a 39% biomass stimulation. White pine also had among the greatest biomass response to eCO₂, with a 26% growth stimulation. It has been suggested that early successional species, such as pines, are ecological generalists, adapted to a wide range of environmental conditions, and are perhaps able to rapidly utilize overabundant resources; whereas late-successional species are specialists, requiring more specific environmental conditions (Atkin et al., 1999). Faster-growing generalist species may have a competitive advantage over inherently slower-growing specialist species in eCO₂ (Atkin et al., 1999; Poorter and Navas, 2003). Among the eight species examined, the faster-growing species generally experienced greater stimulation of total biomass under eCO₂, which supports this supposition (Fig. 8a). The observation that larger trees responded better to eCO₂ has not been supported by some studies (Tjoelker et al., 1998; Ghannoum et al., 2010).

Another hypothesis supported by our spruce results is that shade-tolerant trees will respond better to eCO₂ than shade-intolerant trees. Overall, the four spruces examined had a 16.3% stimulation response under eCO₂. The late-successional WS and RS had the greatest total biomass stimulation to eCO₂, with 29.7% and 18.2%, respectively. Norway spruce is a mid-successional species, depending on the

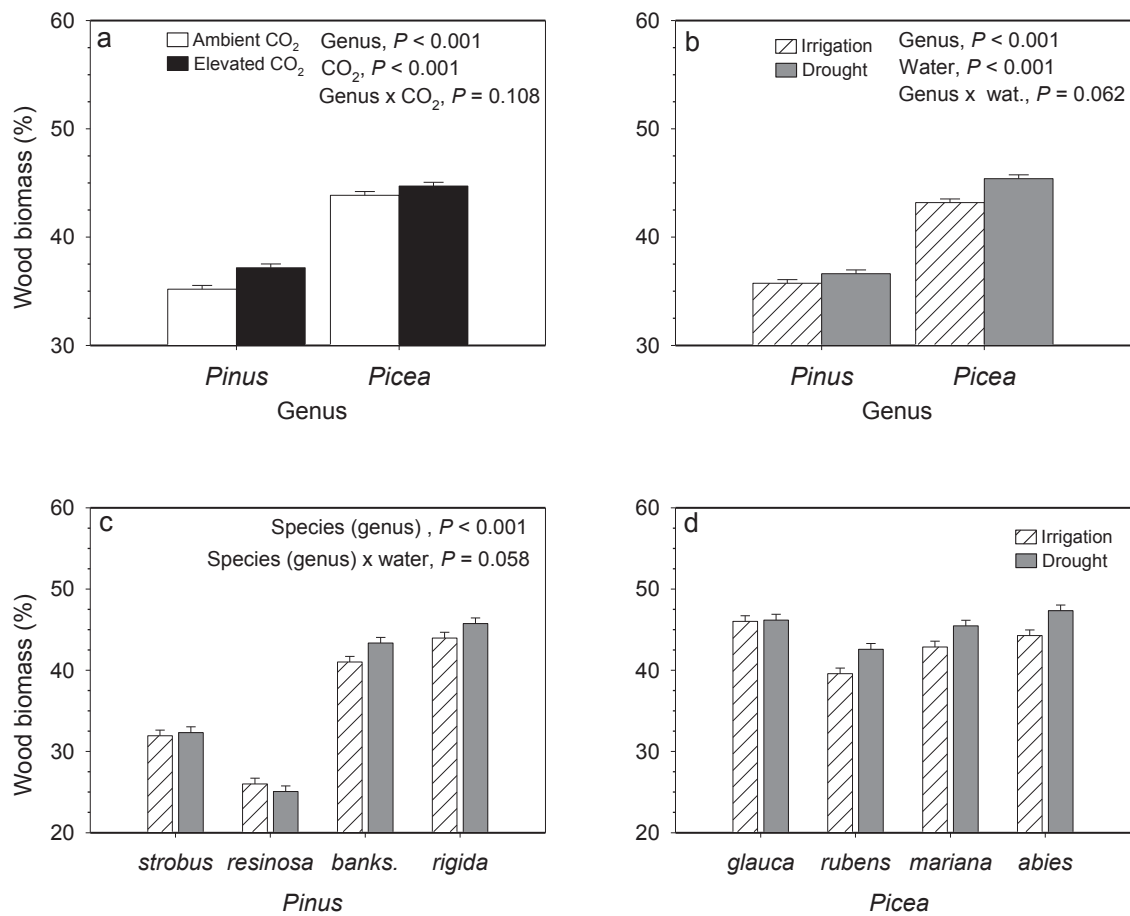


Fig. 5. Percent wood biomass (mean \pm SE) (a) by genus and CO₂ treatments, (b) by genus and soil moisture stress treatments, (c) by pine species and soil moisture stress treatments, and (d) by spruce species and soil moisture stress treatments.

ecosystem. Gaps in Norway spruce forests often regenerate naturally to tolerant hardwoods, or *Abies* (Drobyshev, 2001). Norway spruce had a lower than average biomass stimulation to eCO₂ of 12.1%. Black spruce, an early to mid-successional species, showed the lowest biomass stimulation to eCO₂, with an increase of only 5.2%. In a meta-analysis of 74 eCO₂ to aCO₂ total biomass response ratios among woody seedlings and saplings, shade-tolerant species were significantly greater than shade-intolerant species (Kerstiens, 2001). This was subsequently observed in physiological comparisons in long-term free-air CO₂ enrichment experiments (Mohan et al., 2007; Ellsworth et al., 2012). Shade-intolerant species are generally faster growing than shade-tolerant species. However, Kerstiens (2001) found that in five of 17 experiments, shade-tolerant species had greater response ratios and grew as large as, or larger than, their less shade-tolerant counterparts under eCO₂. The exact physiological or structural causes of shade-tolerance-related differences in growth stimulation by eCO₂ remain uncertain, but may be related to an ability to increase photosynthetic carbon capture even under lower light conditions and short periods of high light sun flecks (Kerstiens, 2001; Ellsworth et al., 2012).

4.2. Black and red spruce

Our current results confirmed earlier findings on growth responses in BS and RS provenances and their hybrids (Major et al. 2015a,b). First, total biomass was twice as great for BS as for RS under aCO₂, highlighting the early competitive growth advantage of BS compared with RS, and reflecting their respective roles as early to mid-successional and late-successional species, respectively. Second, in our current study, eCO₂ resulted in an overall average relative total mass

enhancement of 5.2% and 18.2% for BS and RS, respectively. In an earlier study of BS, RS, and their interspecific hybrids, BS and RS showed a 6.5 and 17.5% total biomass increase in response to eCO₂, respectively (Major et al., 2015a). In provenances originating from three Canadian provinces, eCO₂ resulted in an overall average relative total mass enhancement of 10.8 and 18.0%, for BS and RS, respectively (Major et al., 2015b). Therefore, three different experiments have demonstrated that BS, despite being larger than RS, consistently had lower eCO₂ biomass stimulation than RS.

If we extend our combined findings from the three studies described above to that of an older common-garden field experiment, BS trees were 32% taller than RS at age 22 years (Johnsen et al., 1998). We hypothesized that this ranking might change in the future with canopy closure because RS is more shade tolerant than BS, and thus, we expect that RS may grow relatively better as stand closure occurs. Results from a recent remeasurement of this provenance study at age 41, indicate that this has indeed occurred, and BS is currently only 8% taller than RS (Major et al., 2020b, unpublished results). The greater relative response of RS compared with BS in eCO₂ in our seedling experiment might project into an absolute advantage if CO₂ levels were greater at the beginning of that experiment or if such a study were conducted at low light levels. Changes in ranking or magnitude can occur with age within a species (Morgenstern, 2006), but the changes described here in the common-garden field experiment are dramatic and are probably related to varying responses to canopy closure, which would also happen sooner in an eCO₂ environment. Seedling growth response does not necessarily predict mature tree response, but as seen above with young to intermediate-aged species in common-garden experiments, the results are comparable to our findings. Within many conifer tree species,

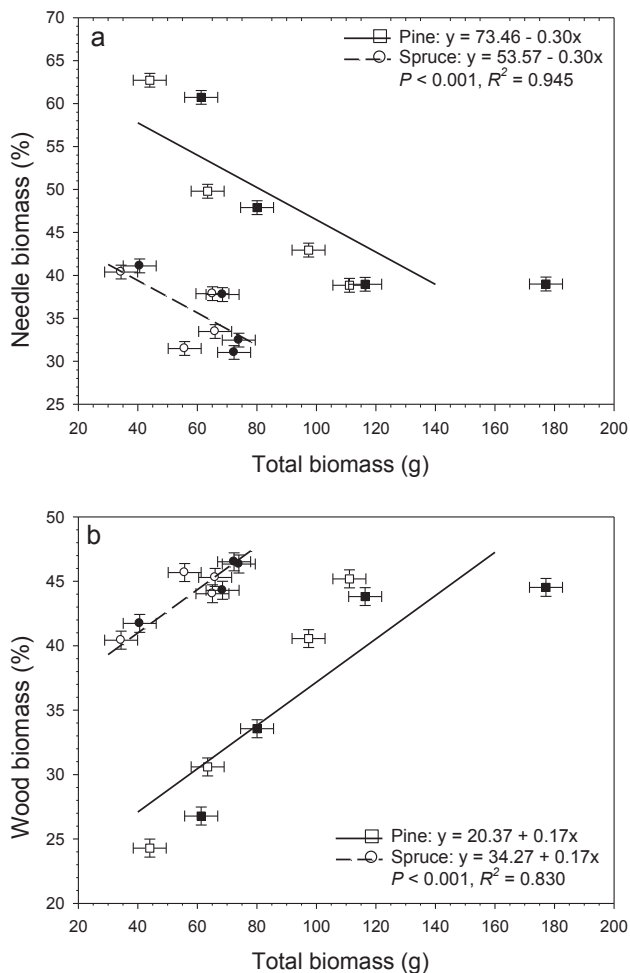


Fig. 6. (a) Percent needle biomass in relationship to total biomass, and (b) percent wood biomass in relationship to total biomass. Empty and filled symbols are ambient and elevated CO₂ treatments, respectively.

breeding for improvement, has been based on early selection because of robust age-to-age correlations supporting early selection (McKeand 1988; Xie and Ying 1996). On the subject of shading, there was some mutual shading in our experiment, but the statistics show that RP, by far the smallest pine (and second smallest species) and very shade intolerant, was not impacted (Fig. 2a) as it showed the second greatest growth response (among the eight species) to eCO₂ both for above-ground (40%) and belowground (32%) growth.

This study has many sources of variation, including two genera, four species within each genus, and two environmental treatments, so it is informative to examine how the components of biomass variation are partitioned. For the most part, the species effect accounts for most of the total variation: on average 40% and ranging from 18 (needle biomass) to 60% (total height). The genus effect accounts for the next greatest amount of total variation with, on average, 20% and ranging from six (wood biomass) to 40% (needle biomass). By comparison, CO₂ had a relatively weak effect, averaging only 4% of total variation; whereas in the allocation traits, CO₂ accounted for less than 1%. The water effect averaged around 1% of the total variation; whereas for root biomass and root-related allocation, the water effect was 4% of the total variation. Moisture stress had a relatively strong negative effect on root biomass and root-related allocation. The interaction effects were quite variable among traits. However, genus \times CO₂ and species \times CO₂ interactions were relatively consistent in accounting for 1.5 and 3.5% of total variation, respectively, with some minor exceptions. The genus and species \times CO₂ interaction reflect the varied genus and species

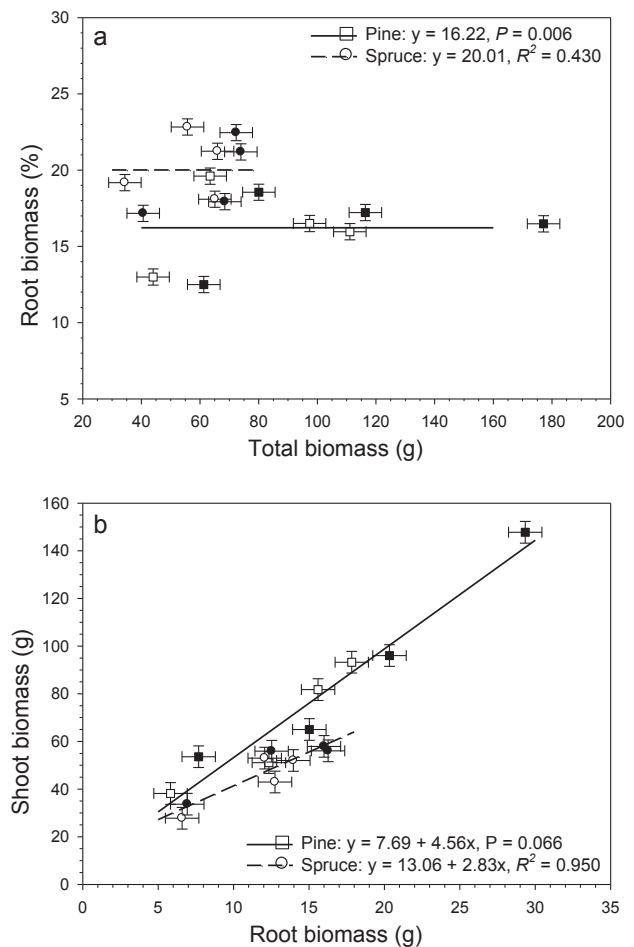


Fig. 7. (a) Percent root biomass in relationship to total biomass, and (b) Shoot biomass in relationship to root biomass. Empty and filled symbols are ambient and elevated CO₂ treatments, respectively.

responses to CO₂, particularly at the species level.

4.3. Growth response to drought

As expected, and as observed by others (Duan et al., 2015; Liu et al., 2015), species responded negatively to drought, but to different degrees. The overall negative biomass response to drought of 12% was not as great as the positive effect under eCO₂ of 27%. Thus, it would appear that the soil moisture stress itself was not strong, aside from its effect on PP and overall root growth. A contributing factor was that the greater the tree size, the greater the negative response to soil moisture stress. To help alleviate the possibility of larger trees experiencing locally lower soil moisture conditions due to their greater transpiration, we used adjacent water-porous felt root bags with sand in between to facilitate water movement among bags. As expected, pines did have greater growth under drought compared with spruces. In particular, PP, JP, and WP had the greatest overall growth under drought stress. Aside from WP, the hard pines (RP, JP, and PP) are generally considered more drought tolerant and outcompete most other tree species on dry, sandy soils (Little and Garrett, 1990; Rudolf, 1990; Rudolph and Laidly, 1990). Despite the generally negative effects of water stress on growth, the pines had larger root biomass than the spruces, despite the spruce having a larger proportion of roots than the pines. Norway spruce and BS were next in ranking of total biomass in drought, after PP, JP, and WP and had the greatest growth for spruces under soil moisture stress, consistent with their early to mid-successional ecological position within forest stand development (Viereck and Johnston, 1990;

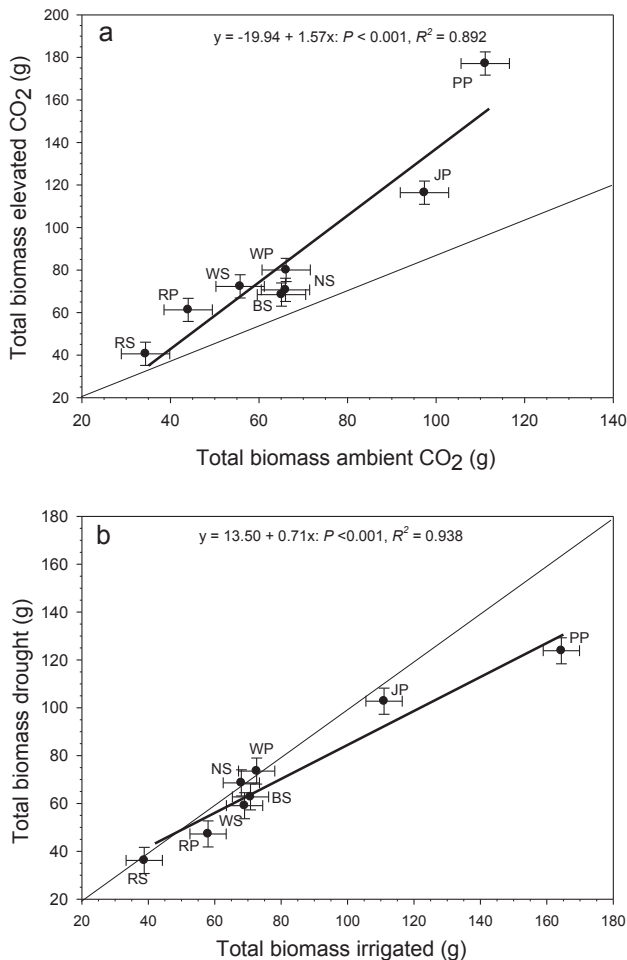


Fig. 8. (a) Total biomass under eCO₂ in relation to total biomass under aCO₂, and (b) total biomass under soil moisture stress in relation to total biomass under well-watered treatment. Thin line is the 1:1 line.

Drobyshev, 2001).

In this study, under soil moisture stress conditions, eCO₂ increased total biomass by 15% and thus helped mitigate the soil moisture stress effects as observed in other studies (Manderscheid and Weigel, 2007; Oliver et al., 2009). Elevated CO₂ often increases water-use efficiency (WUE) through either an increase in assimilation or a decrease in stomatal conductance, or both. In Major et al. (2018), assimilation in trees grown under aCO₂ and eCO₂, but measured at equal CO₂ concentration, had a significant genus × CO₂ rank change interaction. Pines had greater assimilation in eCO₂ than aCO₂. This was reversed for spruces. Stomatal conductance was not significant for CO₂ or genus effects, although the trend was toward increased stomatal conductance in eCO₂. Le Thiec and Dixon (1996) found WUE was greater for *Picea abies* and *Quercus rubra* grown at eCO₂ than at aCO₂. This was also the case for loblolly pine (*Pinus taeda* L.; Werten et al., 2010). Overall, our WUE values had a significant CO₂ effect. However, due to the greater stomatal conductance trend in eCO₂, WUE was greater in aCO₂ than in eCO₂ for both genera. Similar observations were made in *P. radiata* (Greenep et al., 2003).

4.4. Biomass allocation relationships

Overall, biomass allocation to needles was greater for pines than spruces. The greater percent needle biomass for pines was partially driven by the smaller size of RP; however, when corrected for tree size, pines still had a greater proportion of needles than spruces. This appears to be an early developmental phenomenon. Generally, hard pines

are sparsely foliated trees compared with densely foliated spruce, which can retain foliage for up to 10 years (Greenway et al., 1992). Mature pines generally have lower leaf area index (LAI) than spruces. In a study of mature JP and BS in Saskatchewan and Manitoba, the LAI in southern stands were 2.4 and 5.6 and 2.2 and 4.2 in the northern stands, respectively (Gower et al., 1997).

For both genera and seven of the eight species, percent needle biomass declined under eCO₂; whereas tree size increased under eCO₂. Covariate analysis showed that, across species within a genus, there is a negative percent needle relationship with increasing tree size. Was the decline in percent needle biomass simply a tree size effect (e.g., an ontogenetic effect)? Further covariate analysis within genus showed that eCO₂ itself did not result in the reduced percent needle biomass when controlling for tree size. Among species within a genus, there were large species differences, but no detectable direct eCO₂ effect on percent needle biomass. However, when examining a number of populations within a species, or interspecific hybrids of RS and BS, the direct eCO₂ effect is not only detectable but is larger than the ontogenetic effect when controlling for tree size (Major et al. 2015a,b). After controlling for tree size, the provenance results showed that the percent needle mass decreased with increased tree size, but this only accounted for 20% of differences in needle mass for both BS and RS; the balance of the difference was directly attributable to eCO₂.

Conversely, spruces have a greater percent wood biomass than pines, and all eight species increased percent wood biomass under eCO₂. Covariate analysis showed that among species within a genus, there was a positive relationship between percent wood and tree size. Was this due to tree size changes or due directly to eCO₂ effect, or both? Again, in this study, the CO₂ effect did not directly cause the increase in percent wood biomass under eCO₂, but this increase was related to increases in tree size caused by eCO₂. Again, when examining a number of different provenances (populations) within BS, RS, and among closely related hybrids while controlling for tree size, the percent wood biomass increased with increased tree size, but this effect only accounted for 25% of the increase, with the balance directly attributable to eCO₂. Thus, it would appear that, within species or closely related genetic entries such as interspecific hybrids, there is a clear signal, greater than the ontogenetic effect, that eCO₂ modifies allocation. Why was there less needle biomass and more wood biomass in eCO₂? It could be that fewer needles are needed under eCO₂ to satisfy the sink demand for sugars used in growth.

Soil moisture did not affect pine or spruce percent needle biomass, nor was there a significant species × soil water interaction (Table 5). While controlling for tree size, percent needle biomass did not change as a result of changes in soil moisture. However, percent wood biomass for both pines and spruces increased under soil moisture stress (Fig. 5). As we observed above, percent wood increases with tree size. Controlling for tree size, we found that soil moisture stress further increased percent wood, but only for spruces.

Spruces had greater percent root mass than pines, and there was no CO₂ or soil moisture effect on percent root mass even when controlling for tree size. Across tree sizes, percent root biomass was a steady 16.2 and 20.0% for pines and spruces, respectively. Thus, pines had greater shoot-to-root ratios than spruces, and there was no significant eCO₂ or drought effect. There was no change in shoot-to-root ratio with increasing size, with pines and spruces having 5.5 and 4.2 shoot-to-root ratios, respectively. This is somewhat paradoxical as pines generally do better than spruces on drier, sandier sites, but it should be noted again that pines overall had more root biomass than spruces.

Pines clearly assimilated more than spruces in response to eCO₂, but why might that be? In examining assimilation efficiencies and photosynthetic regulation of the same species under eCO₂, we found that pines had greater maximum rates of carboxylation and maximum assimilation rates than spruces (Major et al., 2018). We also found that there was significant downregulation of these values under eCO₂, which is commonly observed, but that assimilation downregulation was

greater for spruces than pines. In addition, we found strong support for the theory of sink regulation of assimilation across species within a genus, particularly among the spruces. Under this theory, it is sink (growth) demand that drives assimilation, and that is why there is some assimilation downregulation under increased supply of CO₂ (increased source). For pines, there appears to be a consistent enhanced sink effect on assimilation across all species (Major et al., 2018).

5. Conclusion

In order to optimize carbon sequestration and wood production while adapting to increasing levels of atmospheric CO₂, it appears that across a range of soil moisture conditions it would be more effective to shift artificial reforestation from spruces to pines. According to pollen records from the Holocene, in an environment of comparatively low CO₂ concentrations (approx. 280 ppm), the landscape of northeastern North America was dominated by pines, particularly white pine, when temperatures were warmer than at present. It was not until the maritime northeast became cooler and wetter that spruces and firs began to dominate again. With increasing CO₂, the atmosphere is predicted to become warmer, and hence potentially drier (as in the past), with an associated increase in fire frequency. Such a climate would suggest an ecological shift favoring pines, rather than spruces. Among pines, pitch pine may experience a relative fitness advantage under eCO₂. The implications for forest management would involve increased use of pines for wood production, management of natural stands, and for artificial regeneration of forests. A diverse portfolio of tree species for artificial reforestation would help forest management adapt to the many uncertainties for future environments and markets, but our results on responses in spruces and pines to eCO₂ and soil moisture stress suggests a shift toward increased use of pines in forest management and artificial reforestation.

Author contribution statement

JEM designed the experiment and was lead author, AM contributed to the analyses and writing of the manuscript.

Acknowledgments

We gratefully acknowledge useful comments received from Dr. Joanne MacDonald, John Malcolm and two anonymous reviewers. We gratefully acknowledge the organizational and technical skills of Debby Barsi, Moira Campbell, and Stephanie West. Financial support of the Canadian Forest Service, Natural Resources Canada is gratefully acknowledged.

Conflict of interest

The authors declare no conflict of interest.

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