

# Genetic variation among pines and spruces in assimilation efficiencies and photosynthetic regulation under elevated CO<sub>2</sub>

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## Abstract

**Key message** After 3 years of elevated CO<sub>2</sub> treatments, spruce  $P_n$  regulation from aCO<sub>2</sub> to eCO<sub>2</sub> was strongly related to total dry mass change (%), whereas pines displayed the same trend, but the relationship was not statistically significant.

**Abstract** Assimilation to internal CO<sub>2</sub> (AC<sub>i</sub>) response curve parameters were grown and quantified under ambient (aCO<sub>2</sub>) and elevated (eCO<sub>2</sub>) CO<sub>2</sub> treatments for two commercially important tree genera, *Pinus* and *Picea* spp. The species include *Pinus strobus* (WP), *P. resinosa* (RP), *P. banksiana* (JP), *P. rigida* (PP), *Picea glauca* (WS), *P. rubens* (RS), *P. mariana* (BS), and *P. abies* (NS). Seedlings were 4 years old and dosed for 3 years at the time of measurements. Overall, pines had greater maximum rates of carboxylation ( $V_{\text{cmax}}$ ) and maximum assimilation ( $A_{\text{max}}$ ) values than spruces, and there was a significant downregulation in  $V_{\text{cmax}}$  and  $A_{\text{max}}$  for both genera in eCO<sub>2</sub>, but more so for the spruces. For the maximum rate of electron transport ( $J_{\text{max}}$ ) and the rate of triose phosphate utilization (TPU), there was no significant genus effect, but there was a significant downregulation in eCO<sub>2</sub>. For these four traits, all spruces downregulated, whereas each pine species responded quite differently. White pine downregulated the most, followed by RP; no change for JP, and PP traits increased under eCO<sub>2</sub>. At an intermediate CO<sub>2</sub> level, net photosynthesis @570 ppm CO<sub>2</sub> ( $P_{n570}$ ) was 13.0% greater for pines and 9.0% lower

for spruces under eCO<sub>2</sub> compared with aCO<sub>2</sub>. Comparing responses under eCO<sub>2</sub> to aCO<sub>2</sub>,  $P_{n570}$  was equal for WS but lower for the other spruces; however, WP declined, RP showed no difference, JP had greater  $P_{n570}$ , and PP had substantially greater  $P_{n570}$ . For pines, there appears to be a consistent enhanced sink effect on  $P_n$  across all species. Corresponding  $P_{n570}$  change from aCO<sub>2</sub> to eCO<sub>2</sub> across spruce species showed a strong positive and statistically significant correlation to biomass stimulation that supports the theory of sink regulation of  $P_n$ .

**Keywords** Biochemical efficiency · Elevated CO<sub>2</sub> · Fitness · Net photosynthesis · Pines · Spruces

## Introduction

Ecological adaptations related to carbon assimilation are important to plant fitness and have significant growth and ecological implications for both intra- and interspecific competition (Bazzaz 1979). Assimilation traits vary widely among species, environments, and plant age (Wullschlegel 1993; Hoddinott and Scott 1996; Bauer et al. 2001; Zhou et al. 2011). Downregulation of the photosynthetic apparatus (e.g., chlorophyll and biochemical efficiency traits), also known as biochemical downregulation, can often occur in response to elevated CO<sub>2</sub> (eCO<sub>2</sub>) and results in the reduction in carboxylation efficiency and electron transport among other related traits (Greenup et al. 2003; Ellsworth et al. 2004; Crous et al. 2008). However, many questions remain on the magnitude of the change, what limits these effects, and their impact on gas exchange. We know that the short-term response to an increase in CO<sub>2</sub> stimulates net photosynthesis ( $P_n$ ) (Teskey 1997; Werten et al. 2010). However, there can also be the corresponding net photosynthesis

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downregulation ( $P_{\text{ndr}}$ ), which is a decrease in the photosynthetic response to  $e\text{CO}_2$  with time. Studies have shown that  $P_{\text{ndr}}$  generally ranges from no downregulation to complete downregulation, depending on species and study (Tjoelker et al. 1998a; Centritto and Jarvis 1999; Greenep et al. 2003; Luomala et al. 2003; Zhou et al. 2011).

Pines and spruces belong to the Pinaceae, which is the second largest conifer family in geographical distribution across the Northern Hemisphere. This family contains most of the commercially important conifer species. The spruces we examined include white spruce [WS; *Picea glauca* (Moench) Voss], a transcontinental species that grows under highly variable conditions, including extreme climates and soils and is considered to be mid to late successional depending on the ecosystem (Nienstaedt and Zasada 1990). Black spruce [BS; *Picea mariana* (Mill.) B.S.P.] is another transcontinental species often found on wetter organic sites, but which can also be found on a variety of soils, and is considered early to mid successional depending on the ecosystem (Viereck and Johnston 1990). Red spruce [RS; *Picea rubens* (Sarg.)] has a much more restricted geographical range but is an important and characteristic component of the late successional Acadian Forest Region in eastern Canada and the northeastern United States, extending along the Appalachian Mountains into North Carolina and Tennessee, primarily as isolated montane remnants (Blum 1990). Norway spruce [NS; *Picea abies* (L.) H. Karst] is a fast-growing conifer native to central and northern Europe, used for reforestation in eastern Canada.

The pines examined include: white pine (WP: *Pinus strobus* L.), largely a temperate zone species, usually associated with well-drained sites across eastern Canada and the United States, extending into northern Georgia (Wendel and Smith 1990). Red pine (RP: *P. resinosa* Ait.) is native to the Great Lakes–St. Lawrence forest region, where it is largely confined to dry, sandy soils (Rudolf 1990). Jack pine (JP: *P. banksiana* Lamb.) is largely a boreal tree species, ranging from the Rocky Mountains to the Maritimes, and is also usually found on dry, sandy soils (Rudolph and Laidly 1990). Pitch pine (PP: *P. rigida* Mill.) has a more restricted geographical range occurring from southeastern Ontario and Maine to northern Georgia and grows primarily on shallow, less fertile soils, with a sandy or gravelly texture (Little and Garrett 1990).

Paleoecological reconstructions (Peters 1990) and pollen coring (Schauffler and Jacobson 2002; Lindbladh et al. 2003) support the conclusion that species migrate singly rather than as intact plant communities. Thus, the predicted doubling of  $\text{CO}_2$  this century could affect comparative fitness among these tree species. As northern conifers are long lived, it would be prudent to investigate atmospheric  $\text{CO}_2$  effects on the comparative fitness among genera and species. How species gas exchange and biochemical efficiency

parameters interact with  $\text{CO}_2$  levels may determine relative competitive fitness in an  $e\text{CO}_2$  environment. Our goal was to examine and compare biochemical efficiency traits and gas exchange at an intermediate  $\text{CO}_2$  level for pine and spruce species under ambient  $\text{CO}_2$  ( $a\text{CO}_2$ ) and  $e\text{CO}_2$  environments. We also tested the hypothesis of sink regulation of photosynthesis (Paul and Foyer 2001; Ainsworth et al. 2004) by comparing changes in  $P_n$  in  $e\text{CO}_2$  to changes in biomass data from Major et al. (2018). Also, do faster growing trees respond better in an  $e\text{CO}_2$  environment? We hypothesized that elevated  $\text{CO}_2$  responses in pines and spruces would differ both among genera and among species. Our specific objectives were to (1) quantify variation in gas exchange and biochemical efficiency traits among pines and spruces, (2) examine responses and interactions under  $e\text{CO}_2$ , and (3) test the theory of sink regulation of photosynthesis across species and genera.

## Materials and methods

### Material and growth conditions

The seed sources used for each of the pine and spruce species are listed in Table 1. Seeds were sown in June 2003 in hydrated 36-mm Jiffy™ peat pellets (Jiffy Products Ltd, Lincoln, NB, Canada) (90 ml) full volume. 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 well-watered  $a\text{CO}_2$  conditions. Seedlings were stored on site overwinter in cold storage at  $-5.0\text{ }^\circ\text{C}$ . Eight (four per  $\text{CO}_2$  treatment) specially constructed growth chambers measuring  $2.15 \times 4.30 \times 2.5\text{ m}$  and covered with 4-mm polyethylene greenhouse film, located at the CFS–AFC greenhouses with air cooling and outside air exchangers,

**Table 1** Geographic coordinates of populations for pine and spruce seed sources used

Species	Location	Latitude (N)	Longitude (W)
<b>Pines</b>			
<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'
<b>Spruces</b>			
<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' E

were used to grow seedlings planted in 25 cm diameter  $\times$  30 cm deep felt root control bags (Root Control Inc., Oklahoma City, OK, USA). The growth chambers were monitored and maintained to outside ambient temperatures and relative humidity conditions and to either 370 or 740 ppm of CO<sub>2</sub>, representing aCO<sub>2</sub> and eCO<sub>2</sub> levels, respectively. A greenhouse monitoring system, calibrated monthly, was used to dose the chambers with CO<sub>2</sub> 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.

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, and rates were 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, 8:20:30 at 35 ppm N was used. Note that half of the four chambers randomly assigned to aCO<sub>2</sub> or eCO<sub>2</sub> were assigned to be regularly irrigated and the other half moisture stressed. Our experimental measurements described below were performed only on the irrigated chambers. Soil moisture under irrigated conditions was set to be maintained at  $> -0.1$  MPa, calculated from a soil retention curve, and measured using a soil moisture probe (CS615, Campbell Scientific, Logan, UT) placed in each chamber, and recorded with a data logger (CR10X, Campbell Scientific).

### Sampling, measurements, and parameter estimation

Assimilation to internal CO<sub>2</sub> (AC<sub>i</sub>) response curves was measured between and including 20 September and 13 October 2006 measuring 4–6 curves per day between 10:00 and 15:00 h, but only on irrigated, non-moisture-stressed blocks. The maximum temperatures that occurred during this time ranged from 25.2 to 11.8 °C, with an average of 18 °C. Samples were always allowed to acclimate to set conditions before measurement. On each sampling date, a species, block, replicate number within a block, and CO<sub>2</sub> treatment were randomly selected for gas exchange measurements. There were four replicates (two from each block) for each species  $\times$  CO<sub>2</sub> treatment for a total of 64 AC<sub>i</sub> response curves. The AC<sub>i</sub> response curves were measured in situ using a Li-Cor 6400 portable gas exchange system with a CO<sub>2</sub> mixer (LI-COR, Lincoln, Nebraska, USA) on the exposed top part of the canopy. Sample branches were not cut until gas exchange measurements had been completed. Instrument settings were set for 25 °C at an air flow of 250  $\mu\text{mol s}^{-1}$

and at a light level of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Relative humidity (RH) was adjusted using a moisture desiccant to provide a RH of approximately 50–60% in the sample chamber, resulting in a vapor pressure deficit (VPD) of approximately 1.0 kPa. Gas exchange was measured three times at 11 external CO<sub>2</sub> measurement concentrations in this sequence: 350, 290, 230, 150, 100, 350, 350, 570, 890, 1500, and 1900 ppm CO<sub>2</sub>. Needle samples were removed for needle area and dry mass determination. Projected needle area was determined using a Li-COR 3100 (LI-COR, Lincoln, Nebraska, USA). Dry mass was determined after drying for 48 h at 65 °C. We used mass as a denominator in  $P_n$  and AC<sub>i</sub> determination to more equitably compare across species and genera; however, specific needle areas (SNA) were also determined. The mass unit used was hectogram (hg) or 100 g to achieve units all above 1 for ease of AC<sub>i</sub> response calculations.

The AC<sub>i</sub> response curves were fitted using commercially available software (Photosyn Assistant; Dundee Scientific, Dundee, Scotland, UK). The program uses algorithms based on the biochemical model of leaf photosynthesis developed by Farquhar et al. (1980), and modified by von Caemmerer and Farquhar (1981), Harley and Sharkey (1991), and Harley et al. (1992). Using this model, CO<sub>2</sub> assimilation can be mathematically described by saturation kinetics, which account for the change in assimilation rate at increasing  $C_i$ . Through an iteration procedure, the Photosyn Assistant software provides estimates for the maximum rate of carboxylation ( $V_{\text{cmax}}$ ), maximum rate of electron transport ( $J_{\text{max}}$ ), and phosphorus (P) limited rate of triose phosphate utilization ( $TPU$ ). The model calculations are based on the assumption that one of these parameters is at a maximum and limits  $A$ . In the program, a least-squares fit is used to estimate respiration ( $R$ ) and CO<sub>2</sub> compensation point. The maximum assimilation ( $A_{\text{max}}$ ) as measured at saturated CO<sub>2</sub> (1900 ppm) and saturating light was abbreviated  $A_{\text{max}}$ , which was the maximum  $A$  rate recorded in situ. In addition, gas exchange parameters were examined at a common 570 ppm CO<sub>2</sub>, the near mid-point of the two CO<sub>2</sub> treatments and thus  $P_n$  @ 570 ppm CO<sub>2</sub> is abbreviated  $P_{n570}$ . Other gas exchange parameters examined were stomatal conductance at 570 ppm CO<sub>2</sub> ( $C_{s570}$ ) and water-use efficiency at 570 ppm CO<sub>2</sub> ( $WUE_{570}$ ), determined by dividing  $P_{n570}$  by  $C_{s570}$ .

### Statistical analyses

The data were subjected to analyses of variance (ANOVAs) with greenhouse chamber (block), genus, species, and CO<sub>2</sub> level considered as fixed effects. Species were nested within genus. The following ANOVA model was used:

$$Y_{ijklm} = \mu + B_i + G_j + S_{k(j)} + C_l + GC_{jl} + SC_{k(j)l} + e_{ijklm}, \quad (1)$$

where  $Y_{ijklm}$  is the dependent seedling trait of the  $i$ th greenhouse chamber,  $j$ th genus,  $k$ th species,  $l$ th CO<sub>2</sub> treatment,  $m$ th seedling, and  $\mu$  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 ( $j = 1, 2$ ),  $S_{k(j)}$  is the effect of the  $k$ th species nested within the  $j$ th genus ( $k = 1, 2, 3, 4$ ),  $C_l$  is the effect of the  $l$ th CO<sub>2</sub> treatment ( $l = 1, 2$ ),  $GC_{jl}$  is the interaction effect of the  $j$ th genus and  $l$ th CO<sub>2</sub> treatment,  $SC_{k(j)l}$  is the interaction effect of the  $k$ th species and the  $l$ th CO<sub>2</sub> treatment, and  $e_{ijklm}$  is the random error component. Effects were considered statistically significant at the  $P = 0.10$  level, although individual  $P$  values are provided so that readers can make their own interpretations, and Tukey's mean separation test was used. The data satisfied normality and equality of variance assumptions. The general linear model from Systat (Chicago, IL, USA) was used for analysis.

Covariate analysis was used to evaluate the relationship of mean  $V_{\text{cmax}}$  and  $A_{\text{max}}$  with SNA and to test genus effect. In these analyses, three sources of variation were studied: (1) covariate (i.e., SNA), (2) independent effect (i.e., genus), and (3) independent effect  $\times$  covariate. The analyses were done based on the following model:

$$Y_{ij} = B_0 + B_{0i} + B_1 X_{ij} + B_{1i} X_{ij} + e_{ij}, \quad (2)$$

where  $Y_{ij}$  is the dependent trait of the  $i$ th species of the  $j$ th genus treatment.  $B_0$  and  $B_1$  are the average regression coefficients,  $B_{0i}$  and  $B_{1i}$  are the genus specific coefficients,  $X_{ij}$  is the independent variable, and  $e_{ij}$  is the error term. Results were considered statistically significant at  $P = 0.10$ . The general linear model from Systat was used for analysis. The biomass data used for examining the relationship to net photosynthesis change are from Major et al. (2018). Using the mean numbers from above, we were able to determine  $P_{n570}$  percentage increase and net total mass percentage increase from ambient to elevated CO<sub>2</sub> and examine this relationship.

## Results

### Biochemical efficiency parameters

Genus and CO<sub>2</sub> effects and the species  $\times$  CO<sub>2</sub> interaction were significant for  $V_{\text{cmax}}$  (Table 2). Pines and spruces had a mean  $V_{\text{cmax}}$  of 32.1 and 28.1  $\mu\text{mol hg}^{-1} \text{s}^{-1}$ , respectively (Fig. 1a). Under aCO<sub>2</sub> and eCO<sub>2</sub> conditions, mean  $V_{\text{cmax}}$  values were 32.7 and 27.6  $\mu\text{mol hg}^{-1} \text{s}^{-1}$ , respectively. Overall, both genera responded similarly, with  $V_{\text{cmax}}$  decreasing in eCO<sub>2</sub> environment, but with pines decreasing by 12.6% and spruces by 25.2%. The significant species  $\times$  CO<sub>2</sub> interaction would appear to be both a rank change and magnitude effect (Fig. 1b, c). Although the spruces all responded similarly by showing a 5–7  $\mu\text{mol hg}^{-1} \text{s}^{-1}$  decline from aCO<sub>2</sub> to eCO<sub>2</sub>, three of the pines responded with different magnitudes of  $V_{\text{cmax}}$  decline, whereas PP showed a  $V_{\text{cmax}}$  increase from aCO<sub>2</sub> to eCO<sub>2</sub>. The 60% decrease in WP was substantial, whereas decreases in RP and JP were modest and similar to the spruces; however, PP increased from 22.4 to 34.1  $\mu\text{mol hg}^{-1} \text{s}^{-1}$ .

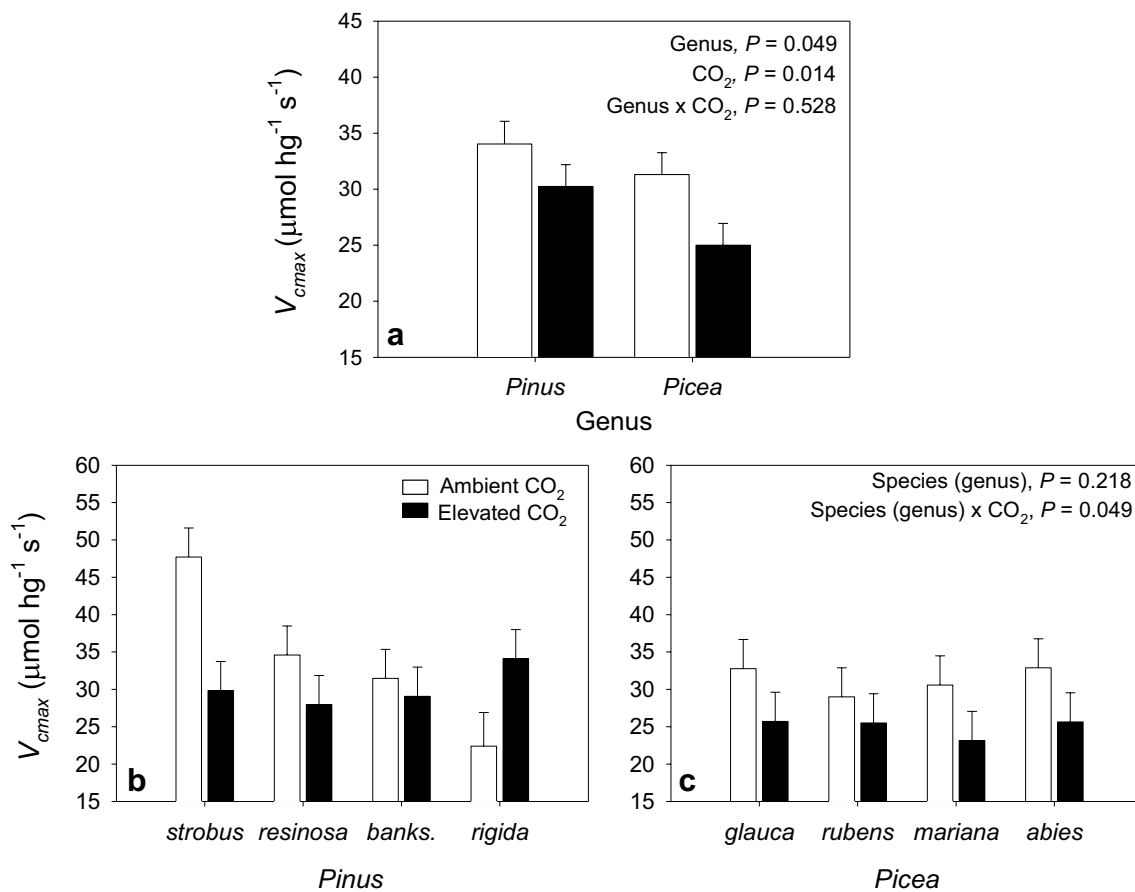
Genus differences were not significant for  $J_{\text{max}}$ , however, CO<sub>2</sub> and species  $\times$  CO<sub>2</sub> interactions were significant (Table 2). Overall,  $J_{\text{max}}$  for aCO<sub>2</sub> and eCO<sub>2</sub> were 124 and 103  $\mu\text{mol hg}^{-1} \text{s}^{-1}$  for both pines and spruces, respectively (Fig. 2a). The species  $\times$  CO<sub>2</sub> interaction results had a similar pattern as  $V_{\text{cmax}}$ , with all spruces except RS showing a strong decline: WP and RP showed very strong declines, JP showed no difference between treatments, and PP increased from aCO<sub>2</sub> to eCO<sub>2</sub> conditions (Fig. 2b, c). The  $TPU$  was also significant for CO<sub>2</sub> and species  $\times$  CO<sub>2</sub> interaction but not for genus (Table 2); across species  $TPU$  was 8.4 and 7.5  $\mu\text{mol hg}^{-1} \text{s}^{-1}$  for aCO<sub>2</sub> and eCO<sub>2</sub>, respectively (Tables 3, 4). All spruces downregulated  $TPU$  under eCO<sub>2</sub>; for the pines, WP declined substantially, RP

**Table 2** Assimilation trait ANOVAs, including source of variation, degrees of freedom ( $df$ ), mean square values (MS), and  $P$  values

Source of variation	$df$	Maximum rate of carboxylation ( $V_{\text{cmax}}$ ) ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )		Maximum rate of electron transport ( $J_{\text{max}}$ ) ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )		Triose-phosphate utilization ( $TPU$ ) ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )		Maximum assimilation ( $A_{\text{max}}$ ) ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )		CO <sub>2</sub> compensation point (ppm)	
		MS	$P$ value	MS	$P$ value	MS	$P$ value	MS	$P$ value	MS	$P$ value
Block	1	70.64	0.287	4974.81	<b>0.039</b>	12.37	<b>0.049</b>	33.84	0.273	230.6	0.325
Genus	1	248.93	<b>0.049</b>	682.76	0.434	4.36	0.237	117.46	<b>0.045</b>	4798.8	<b>&lt;0.001</b>
CO <sub>2</sub>	1	399.68	<b>0.014</b>	6979.70	<b>0.015</b>	14.17	<b>0.036</b>	144.94	<b>0.026</b>	184.5	0.378
CO <sub>2</sub> *Genus	1	24.59	0.528	1582.47	0.236	5.48	0.186	27.20	0.325	17.8	0.783
Spp(Genus)	6	88.01	0.218	709.14	0.693	2.84	0.480	50.77	0.111	507.9	<b>0.062</b>
CO <sub>2</sub> *Spp(Genus)	6	141.09	<b>0.049</b>	2147.65	<b>0.092</b>	7.56	<b>0.036</b>	61.27	<b>0.057</b>	19.3	0.998
Error	46	60.84		1097.45		3.04		27.53		232.9	
$R^2$			0.438		0.390		0.419		0.445		0.443

$P$  values < 0.10 are in bold print

Spp species,  $df$  degrees of freedom



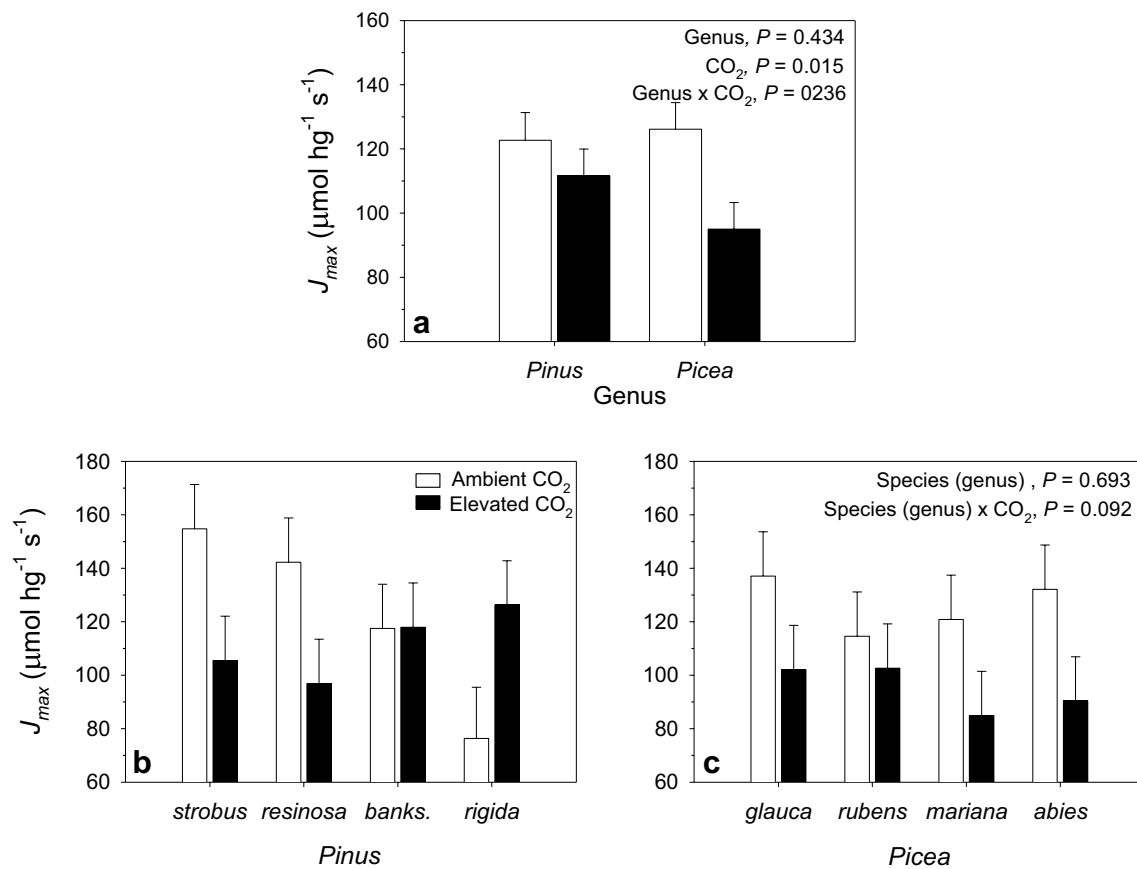
**Fig. 1** a Maximum rate of carboxylation ( $V_{cmax}$ ) (mean  $\pm$  SE) by genus and  $\text{CO}_2$  treatment, b by pine species and  $\text{CO}_2$  treatment, and c by spruce species and  $\text{CO}_2$  treatment

declined modestly; and both JP and PP increased under  $\text{eCO}_2$ .

Maximum assimilation rate was significant for genus,  $\text{CO}_2$ , and the species  $\times$   $\text{CO}_2$  interaction, at 23.3 and 20.5  $\mu\text{mol hg}^{-1} \text{s}^{-1}$  for pine and spruce, respectively (Tables 2, 3, 4). Across species mean  $A_{max}$  was 23.4 and 20.4  $\mu\text{mol hg}^{-1} \text{s}^{-1}$  for  $\text{aCO}_2$  and  $\text{eCO}_2$ , respectively. The species  $\times$   $\text{CO}_2$  interaction was due to pine rank changes. Spruces were consistent in that  $A_{max}$  was always greater under  $\text{aCO}_2$  than  $\text{eCO}_2$ . However, for pines, responses were species specific: WP displayed a substantial decline, RP a modest decline, JP was equal, and PP had a substantial increase in  $A_{max}$  under  $\text{eCO}_2$ . The  $\text{CO}_2$  compensation point was significantly different among the genera and species. The overall mean was 47.8 and 65.3 ppm  $\text{CO}_2$  for pines and spruces, respectively (Tables 2, 3, 4). The species effect was driven by JP and PP having greater values than WP and RP. Respiration was significant only at the genus level, and was 1.3 and 2.5  $\mu\text{mol hg}^{-1} \text{s}^{-1}$  for pines and spruces, respectively (Tables 3, 4, 5).

### Gas exchange parameters

At an intermediate  $\text{CO}_2$  level, 570 ppm,  $P_{n570}$  was significant for genus and both the genus  $\times$   $\text{CO}_2$  and species  $\times$   $\text{CO}_2$  interactions (Table 5). The genus  $\times$   $\text{CO}_2$  interaction was a rank change; for pines,  $P_{n570}$  under  $\text{eCO}_2$  was greater than  $\text{aCO}_2$ , with 14.6 and 12.7  $\mu\text{mol hg}^{-1} \text{s}^{-1}$ , respectively; whereas for spruce, there was a decline in  $P_{n570}$  from  $\text{aCO}_2$  to  $\text{eCO}_2$  with 12.1 and 11.1  $\mu\text{mol hg}^{-1} \text{s}^{-1}$ , respectively (Fig. 3a). Overall, pine and spruce had 13.7 and 11.6  $\mu\text{mol hg}^{-1} \text{s}^{-1}$ , respectively. The species  $\times$   $\text{CO}_2$  interaction was due to both magnitude differences and rank change (Fig. 3b, c). For spruces,  $P_{n570}$  under  $\text{eCO}_2$  was either equal (WS) or less than  $P_{n570}$  under  $\text{aCO}_2$ . For pines, the results were more variable, with WP  $P_{n570}$  showing a decline from  $\text{aCO}_2$  to  $\text{eCO}_2$ , but now for RP, they were equal, and for JP,  $P_{n570}$  was greater in  $\text{eCO}_2$  than  $\text{aCO}_2$  and doubled for PP when comparing  $\text{aCO}_2$  with  $\text{eCO}_2$ . Stomatal conductance at 570 ppm  $\text{CO}_2$  was only significant for species  $\times$   $\text{CO}_2$  interaction as there were rank changes for both pines and spruces (Tables 3, 4,



**Fig. 2** **a** Maximum rate of electron transport ( $J_{\max}$ ) (mean  $\pm$  SE) by genera and  $\text{CO}_2$  treatment, **b** by pine species and  $\text{CO}_2$  treatment, and **c** by spruce species and  $\text{CO}_2$  treatment

**Table 3** Physiological traits (mean  $\pm$  SE) under ambient (Amb.) and elevated (Elev.)  $\text{CO}_2$  by pine species

Physiological trait	$\text{CO}_2$	White pine	Red pine	Jack pine	Pitch pine	Pines
$TPU$ ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )	Amb.	$10.79 \pm 0.87$	$9.52 \pm 0.87$	$7.62 \pm 0.87$	$5.72 \pm 0.87$	$8.41 \pm 0.44$
	Elev.	$7.58 \pm 0.87$	$7.45 \pm 0.87$	$8.44 \pm 0.87$	$8.44 \pm 0.87$	$8.05 \pm 0.44$
	Mean					$8.23 \pm 0.31$
$A_{\max}$ ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )	Amb.	$33.0 \pm 2.6$	$25.9 \pm 2.6$	$21.9 \pm 2.6$	$15.8 \pm 2.6$	$24.3 \pm 1.4$
	Elev.	$25.9 \pm 2.6$	$20.1 \pm 2.6$	$22.2 \pm 2.6$	$24.2 \pm 2.6$	$22.4 \pm 1.4$
	Mean					$23.3 \pm 0.9$
$\text{CO}_2$ compensation point (ppm)	Amb.	$38.4 \pm 7.6$	$46.7 \pm 7.6$	$58.8 \pm 7.6$	$52.1 \pm 7.6$	$49.0 \pm 3.8$
	Elev.	$36.7 \pm 7.6$	$41.4 \pm 7.6$	$56.5 \pm 7.6$	$51.9 \pm 7.6$	$46.6 \pm 3.8$
	Mean					$47.8 \pm 2.7$
$C_{s570}$ ( $\text{mmol hg}^{-1} \text{s}^{-1}$ )	Amb.	$12.80 \pm 2.37$	$11.78 \pm 2.37$	$8.22 \pm 2.37$	$5.23 \pm 2.37$	$9.51 \pm 1.18$
	Elev.	$9.62 \pm 2.37$	$10.08 \pm 2.37$	$11.40 \pm 2.37$	$17.9 \pm 2.37$	$12.25 \pm 1.18$
	Mean					$10.88 \pm 0.85$
$WUE_{570}$	Amb.	$163.8 \pm 21.9$	$134.6 \pm 21.9$	$163.0 \pm 21.9$	$180.6 \pm 21.9$	$160.5 \pm 10.9$
	Elev.	$167.0 \pm 21.9$	$167.0 \pm 21.9$	$127.4 \pm 21.9$	$100.9 \pm 21.9$	$140.9 \pm 10.9$
	Mean					$150.7 \pm 7.8$
Respiration ( $\mu\text{mol hg}^{-1} \text{s}^{-1}$ )	Amb.	$0.56 \pm 0.76$	$1.51 \pm 0.76$	$2.72 \pm 0.76$	$1.47 \pm 0.76$	$1.56 \pm 0.38$
	Elev.	$0.10 \pm 0.76$	$0.50 \pm 0.76$	$1.83 \pm 0.76$	$1.60 \pm 0.76$	$1.01 \pm 0.38$
	Mean					$1.29 \pm 0.27$

$TPU$  triose-phosphate utilization,  $A_{\max}$  maximum assimilation,  $C_{s570}$  stomatal conductance @ 570 ppm,  $WUE_{570}$  water-use efficiency @ 570 ppm



**Table 4** Physiological traits (mean  $\pm$  SE) under ambient (Amb.) and elevated (Elev.) CO<sub>2</sub> by spruce species

Physiological trait	CO <sub>2</sub>	White spruce	Red spruce	Black spruce	Norway spruce	Spruces
TPU ( $\mu\text{mol hg}^{-1} \text{ s}^{-1}$ )	Amb.	8.72 $\pm$ 0.87	8.01 $\pm$ 0.87	8.20 $\pm$ 0.87	8.97 $\pm$ 0.87	8.48 $\pm$ 0.44
	Elev.	7.17 $\pm$ 0.87	7.04 $\pm$ 0.87	6.59 $\pm$ 0.87	6.94 $\pm$ 0.87	6.94 $\pm$ 0.44
	Mean					7.71 $\pm$ 0.31
$A_{\text{max}}$ ( $\mu\text{mol hg}^{-1} \text{ s}^{-1}$ )	Amb.	23.4 $\pm$ 2.6	21.0 $\pm$ 2.6	21.6 $\pm$ 2.6	24.0 $\pm$ 2.6	22.7 $\pm$ 1.4
	Elev.	20.0 $\pm$ 2.6	18.7 $\pm$ 2.6	16.0 $\pm$ 2.6	18.7 $\pm$ 2.6	18.4 $\pm$ 1.4
	Mean					20.5 $\pm$ 0.9
CO <sub>2</sub> compensation point (ppm)	Amb.	77.5 $\pm$ 7.6	65.1 $\pm$ 7.6	70.8 $\pm$ 7.6	56.7 $\pm$ 7.6	67.5 $\pm$ 3.8
	Elev.	67.7 $\pm$ 7.6	61.9 $\pm$ 7.6	66.7 $\pm$ 7.6	56.0 $\pm$ 7.6	63.1 $\pm$ 3.8
	Mean					65.3 $\pm$ 2.7
$C_{s570}$ ( $\text{mmol hg}^{-1} \text{ s}^{-1}$ )	Amb.	6.52 $\pm$ 2.37	8.62 $\pm$ 2.37	7.08 $\pm$ 2.37	13.02 $\pm$ 2.37	8.81 $\pm$ 1.18
	Elev.	8.30 $\pm$ 2.37	9.28 $\pm$ 2.37	7.98 $\pm$ 2.37	10.75 $\pm$ 2.37	9.08 $\pm$ 1.18
	Mean					8.94 $\pm$ 0.85
WUE <sub>570</sub>	Amb.	159.4 $\pm$ 21.9	140.8 $\pm$ 21.9	182.1 $\pm$ 21.9	121.0 $\pm$ 21.9	150.8 $\pm$ 10.9
	Elev.	127.6 $\pm$ 21.9	124.3 $\pm$ 21.9	131.5 $\pm$ 21.9	125.8 $\pm$ 21.9	127.3 $\pm$ 10.9
	Mean					139.1 $\pm$ 7.8
Respiration ( $\mu\text{mol hg}^{-1} \text{ s}^{-1}$ )	Amb.	3.33 $\pm$ 0.76	2.72 $\pm$ 0.76	2.88 $\pm$ 0.76	2.36 $\pm$ 0.766	2.82 $\pm$ 0.38
	Elev.	2.64 $\pm$ 0.76	2.30 $\pm$ 0.76	2.33 $\pm$ 0.76	1.68 $\pm$ 0.766	2.24 $\pm$ 0.38
	Mean					2.53 $\pm$ 0.27

TPU triose-phosphate utilization,  $A_{\text{max}}$  maximum assimilation,  $C_{s570}$  stomatal conductance @ 570 ppm, WUE<sub>570</sub> water-use efficiency @ 570 ppm

**Table 5** Gas exchange @ 570 ppm, specific needle area, and respiration ANOVAs, including source of variation, degrees of freedom (*df*), mean square values (MS), and *P* values

Source of variation	<i>df</i>	Net photosynthesis @ 570 ppm ( $P_{n570}$ ) ( $\mu\text{mol hg}^{-1} \text{ s}^{-1}$ )		Stomatal conductance @ 570 ppm ( $C_{s570}$ ) ( $\text{mmol hg}^{-1} \text{ s}^{-1}$ )		Water-use efficiency @ 570 ppm (WUE <sub>570</sub> )		Specific needle area		Respiration ( $\mu\text{mol hg}^{-1} \text{ s}^{-1}$ )	
		MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value	MS	<i>P</i> value
Block	1	1.28	0.735	22.62	0.321	3.20 $\times 10^3$	0.203	13.8	0.629	13.30	<b>0.020</b>
Genus	1	66.18	<b>0.018</b>	58.65	0.113	2.11 $\times 10^3$	0.300	2102.3	< <b>0.001</b>	24.27	<b>0.002</b>
CO <sub>2</sub>	1	3.68	0.565	35.38	0.216	7.31 $\times 10^3$	<b>0.057</b>	609.6	<b>0.002</b>	5.11	0.143
CO <sub>2</sub> * Genus	1	32.49	<b>0.092</b>	24.10	0.306	0.06 $\times 10^3$	0.862	390.0	<b>0.013</b>	< 0.01	0.972
Spp (Genus)	6	14.08	0.285	19.56	0.523	1.29 $\times 10^3$	0.671	1554.3	< <b>0.001</b>	3.33	0.218
CO <sub>2</sub> *Spp (Genus)	6	21.01	<b>0.099</b>	49.26	<b>0.060</b>	2.75 $\times 10^3$	0.223	79.2	0.250	0.26	0.994
Error	46	10.99		22.44		1.92 $\times 10^3$		58.2		2.30	
<i>R</i> <sup>2</sup>			0.384		0.351		0.296		0.827		0.382

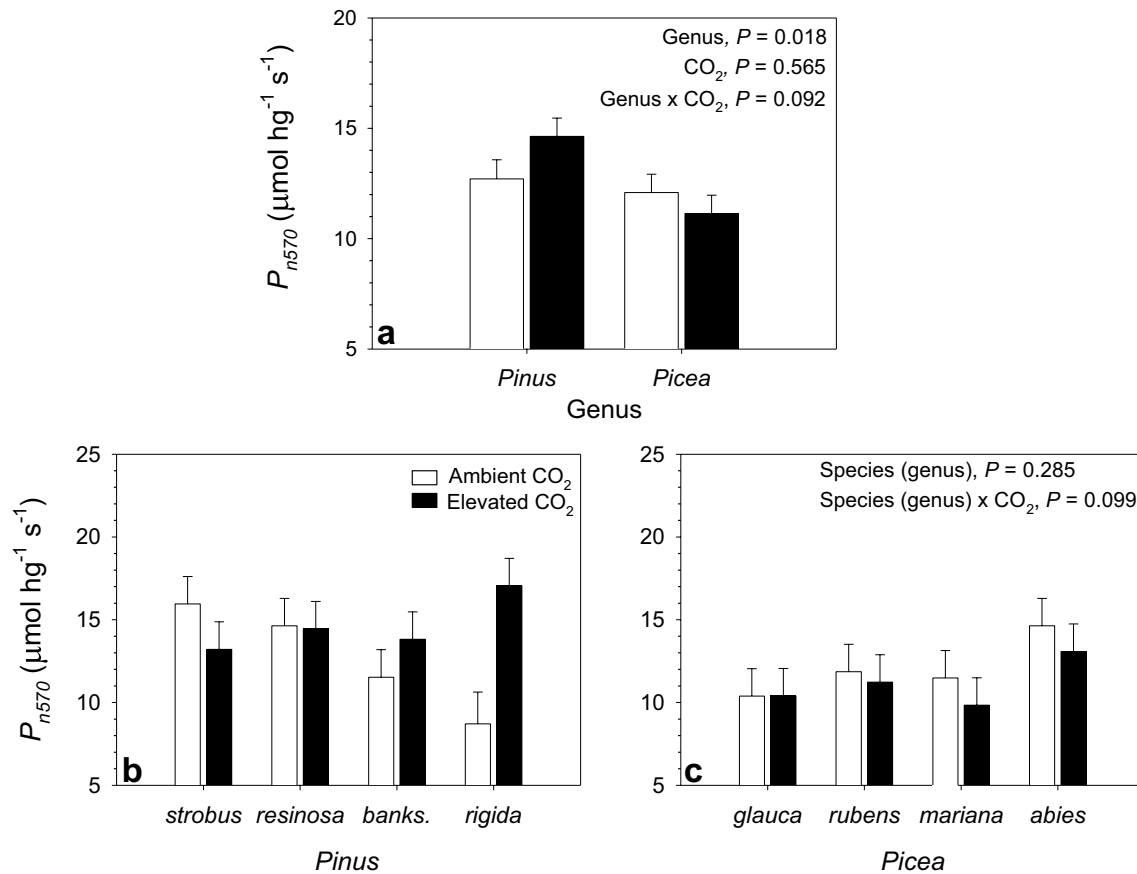
Spp species, *df* degrees of freedom

*P* values < 0.10 are in bold print

5). Differences in WUE<sub>570</sub> were significant for CO<sub>2</sub>. Under aCO<sub>2</sub> and eCO<sub>2</sub>, overall the average WUE was 155.7 and 134.1 across genera, respectively (Tables 3, 4, 5). Pines had a greater but not significant response for WUE than spruces at 150.7 and 139.1, respectively.

Specific needle area was significantly different among genera, CO<sub>2</sub> levels, the genus  $\times$  CO<sub>2</sub> interaction, and among species (Table 5). The genus  $\times$  CO<sub>2</sub> interaction effect was due to a large magnitude effect (no rank change) (Fig. 4a).

For pines, SNA was 55.6 and 54.3 cm<sup>2</sup> g<sup>-1</sup> for aCO<sub>2</sub> and eCO<sub>2</sub>, respectively; for spruces, SNA was 72.2 and 60.9 cm<sup>2</sup> g<sup>-1</sup>, respectively. Overall, genus SNA was 55.0 and 66.6 cm<sup>2</sup> g<sup>-1</sup> for pines and spruces, respectively. For aCO<sub>2</sub> and eCO<sub>2</sub>, it was 63.9 and 57.7 cm<sup>2</sup> g<sup>-1</sup>, respectively. Specific needle area of WP—the soft pine—was greater than that of all the spruces, which in turn was greater than that of the hard pines (RP, JP, and PP). It should be noted that even though there was a statistically nonsignificant species  $\times$  CO<sub>2</sub> interaction,



**Fig. 3** **a** Net assimilation measured at  $\text{CO}_2$  concentration of 570 ppm ( $A_{570}$ ) by genera and  $\text{CO}_2$  treatment, **b** by pine species and  $\text{CO}_2$  treatment, and **c** by spruce species and  $\text{CO}_2$  treatment

WP and all the spruces showed a decrease in  $SNA$  from  $a\text{CO}_2$  to  $e\text{CO}_2$ , whereas RP and JP showed no effect between  $\text{CO}_2$  treatments, and PP had greater  $SNA$  under  $e\text{CO}_2$  than  $a\text{CO}_2$  of 55.5 and 46.1  $\text{cm}^2 \text{g}^{-1}$ , respectively, which was greater than the standard error of 3.8  $\text{cm}^2 \text{g}^{-1}$ .

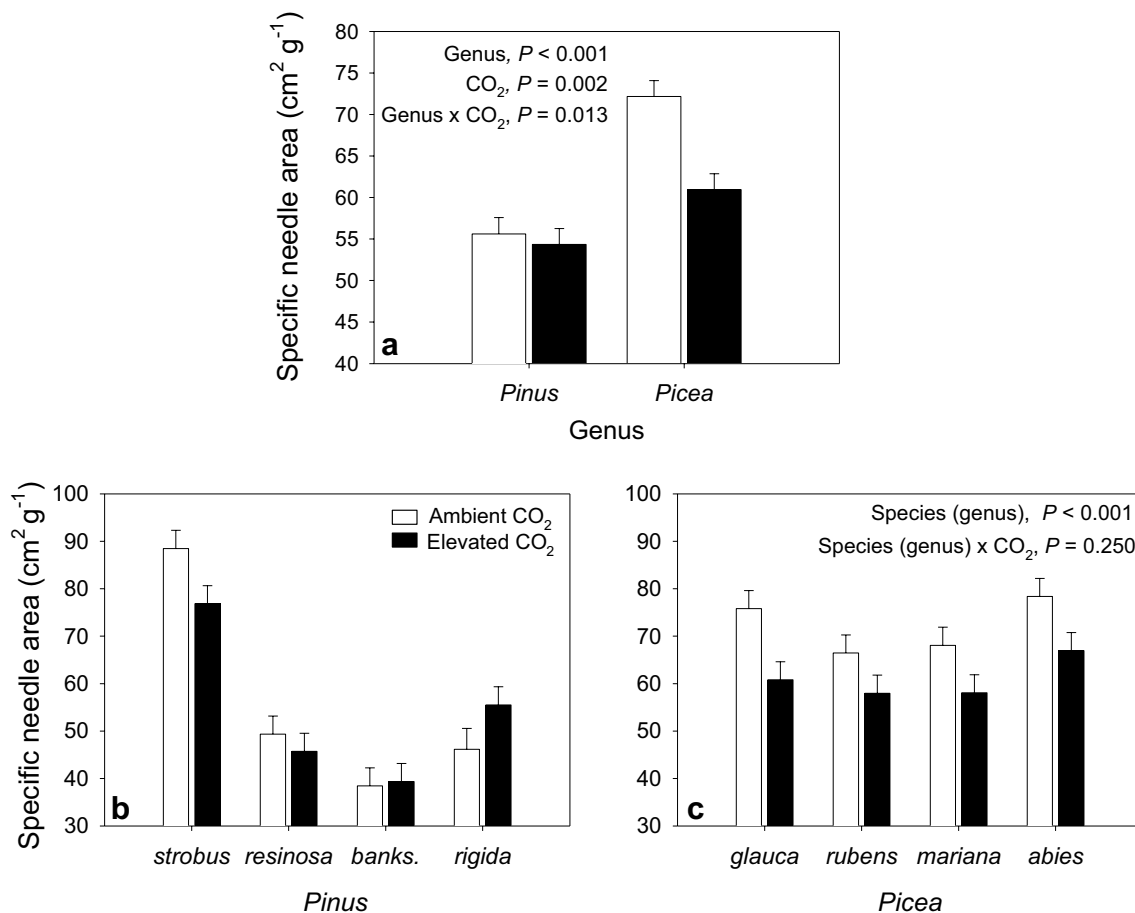
### Trait relationships

Covariate analysis of  $V_{\text{cmax}}$  using  $SNA$  as covariate and testing for genus showed no significant genus x  $SNA$  interaction ( $P = 0.490$ ), but the genus ( $P = 0.007$ ) and  $SNA$  ( $P = 0.002$ ) effects were significant. The result was a two-genus response with similar positive  $V_{\text{cmax}}$  slopes in relation to  $SNA$  (Fig. 5a). Covariate analysis of  $A_{\text{max}}$  using  $SNA$  as covariate and testing for genus showed no significant genus x  $SNA$  interaction ( $P = 0.486$ ). Further analysis showed significant genus ( $P = 0.006$ ) and  $SNA$  ( $P = 0.002$ ) effects. The result was a positive and similar  $A_{\text{max}}$  slope for pine and spruce in relation to  $SNA$  (Fig. 5b). Thus, for both species, for every unit increase in  $SNA$ , there was a corresponding 0.3 increase in  $V_{\text{cmax}}$  and 0.2  $\mu\text{mol hg}^{-1} \text{s}^{-1}$

increase in  $A_{\text{max}}$ . There was also a similar covariate result for  $J_{\text{max}}$  (not shown). Covariate analysis of  $J_{\text{max}}$  using  $V_{\text{cmax}}$  as a covariate and testing for genus showed a significant genus x  $V_{\text{cmax}}$  interaction ( $P = 0.083$ ). The genus effect was significant ( $P < 0.001$ ). Thus, the result was a different slope for spruces and pines (Fig. 6).

Using the mean numbers from both treatments, we were able to determine the net  $P_{n570}$  percentage change and total mass percentage increases under elevated  $\text{CO}_2$ . There was a very strong relationship among spruces in the biomass stimulation (%) and net  $P_{n570}$  change (%) in  $a\text{CO}_2$  in relation to  $e\text{CO}_2$  ( $P = 0.007$ ,  $r = 0.993$ ) (Fig. 7a). For pines, the biomass stimulation (%) in relation to  $P_{n570}$  change (%) in  $a\text{CO}_2$  in relation to  $e\text{CO}_2$  displayed the same trend as spruces but was not statistically significant ( $P = 0.214$ ,  $r = 0.786$ ) (Fig. 7b). A conceptual  $AC_i$  model is presented showing the relative differences of pine and spruce under  $a\text{CO}_2$  and  $e\text{CO}_2$  (Fig. 8). Maximum rate of carboxylation ( $V_{\text{cmax}}$ ),  $J_{\text{max}}$ ,  $TPU$ ,  $A_{\text{max}}$ , and respiration are listed in areas of their determination.





**Fig. 4** Specific needle area (mean  $\pm$  SE) by genera and  $\text{CO}_2$  treatment, **b** by pine species and  $\text{CO}_2$  treatment, and **c** by spruce species and  $\text{CO}_2$  treatment

## Discussion

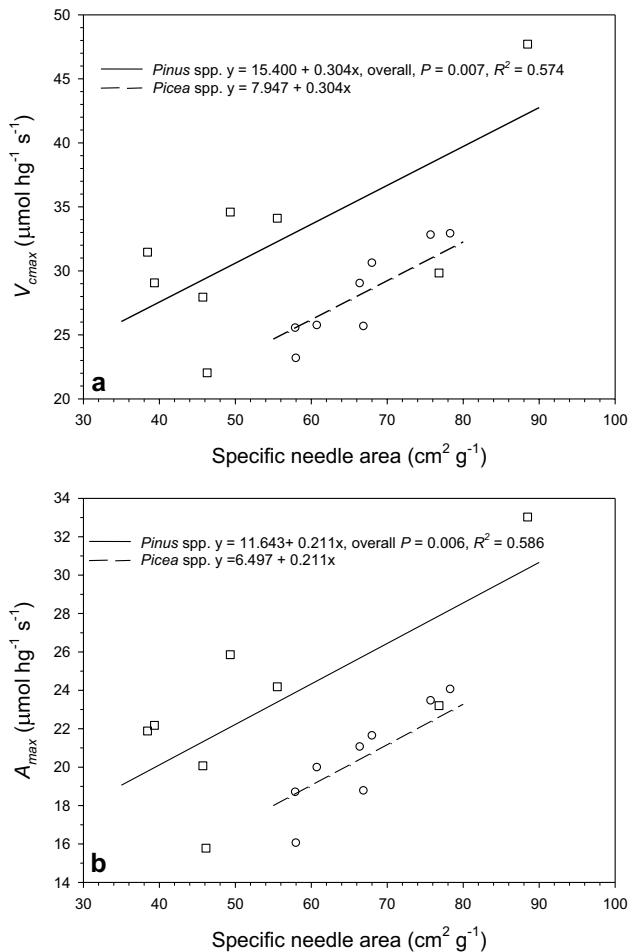
### Biochemical efficiency parameters: genus and $\text{CO}_2$ effects

Biochemical downregulation is generally considered a decrease in the photosynthetic capacity with time in response to, in this case,  $\text{eCO}_2$  (Tissue et al. 1999; Jach and Ceulemans 2000; Crous and Ellsworth 2004; Ainsworth and Long 2005). Overall, pines and spruces  $V_{\text{cmax}}$  downregulated by 12.6% and 25.2%, and  $J_{\text{max}}$  downregulated by 9.9% and 32.8%, respectively, from  $\text{aCO}_2$  to  $\text{eCO}_2$  treatments. Bauer et al. (2001) examined biochemical traits of RS and WP among three other species (*Betula alleghaniensis*, *Quercus rubra*, and *Acer rubrum*), and found  $V_{\text{cmax}}$  and  $J_{\text{max}}$  downregulated for both RS and WP. However, RS downregulated the least, which is also what we found among the four spruces we tested, and WP downregulated the most among the eight species we tested. Among RS, BS, and their hybrids, spruces  $V_{\text{cmax}}$  and  $J_{\text{max}}$  downregulated by 40% and 36%, respectively (Major et al.

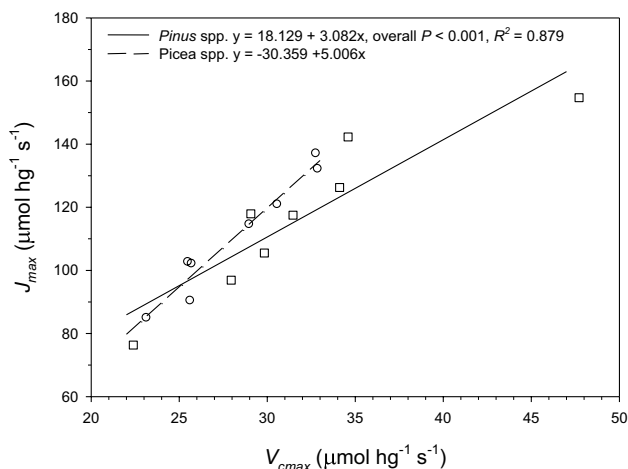
2014), similar to the numbers we found for spruces. Centritto and Jarvis (1999) also found  $V_{\text{cmax}}$  declined in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) by 36% in  $\text{eCO}_2$ .

Overall, *TPU* downregulated by 4.5 and 22.2% in pines and spruces, respectively, under  $\text{eCO}_2$  treatments. Bauer et al. (2001) found that *TPU* downregulated 23.4% from approximately  $7.9\text{--}6.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Among RS, BS, and their hybrids, *TPU* downregulated by 20% from 7.8 to  $6.5 \mu\text{mol hg}^{-1} \text{s}^{-1}$ , similar to the four spruces tested here (Major et al. 2014). There was also a significant  $\text{CO}_2$  effect for  $A_{\text{max}}$ , with pines and spruces downregulating 7.6% and 23.4%, respectively, similar to other studies (Centritto and Jarvis 1999; Tissue et al. 1999; Major et al. 2014). From  $\text{aCO}_2$  to  $\text{eCO}_2$ , Sitka spruce (Centritto and Jarvis 1999) and ponderosa pine (*Pinus ponderosa* P. Laws. ex C. Laws; Tissue et al. 1999)  $A_{\text{max}}$  declined 20.0% and 24.9%, respectively.

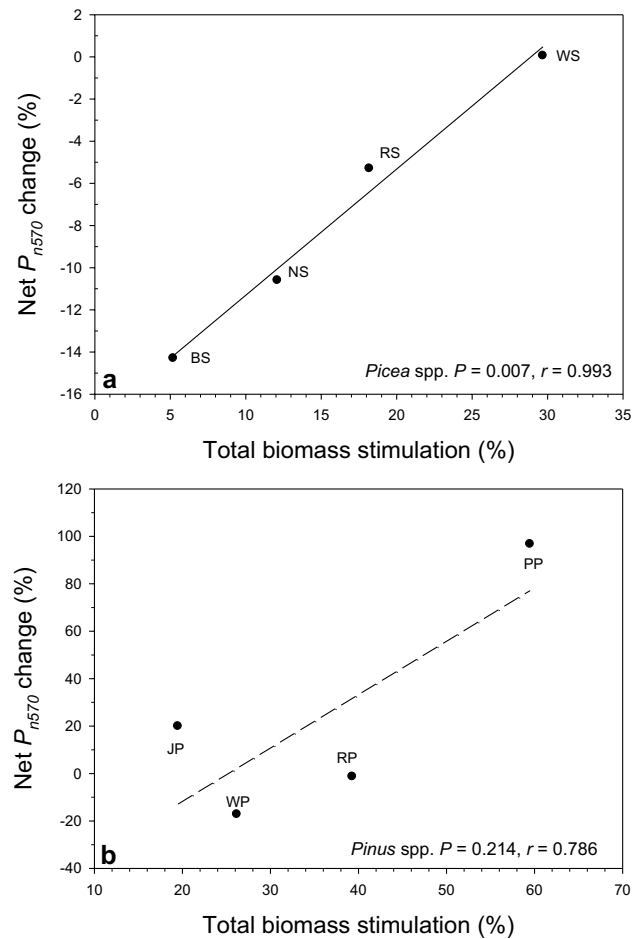
Needle respiration did not change with  $\text{CO}_2$  treatment, consistent with findings for *Pinus radiata* (Griffin et al. 2000), *P. sylvestris* (Jach and Ceulemans 2000), and *Picea abies* (Hattenschwiler and Korner 1996). Spruce had greater



**Fig. 5** Relationship between **a** maximum rate of carboxylation ( $V_{cmax}$ ) and **b** maximum net assimilation ( $A_{max}$ ) versus specific needle area for pine and spruce species grown under ambient and elevated  $\text{CO}_2$  environments



**Fig. 6** Relationship between maximum rate of electron transport ( $J_{max}$ ) and maximum rate of carboxylation ( $V_{cmax}$ ) by genus

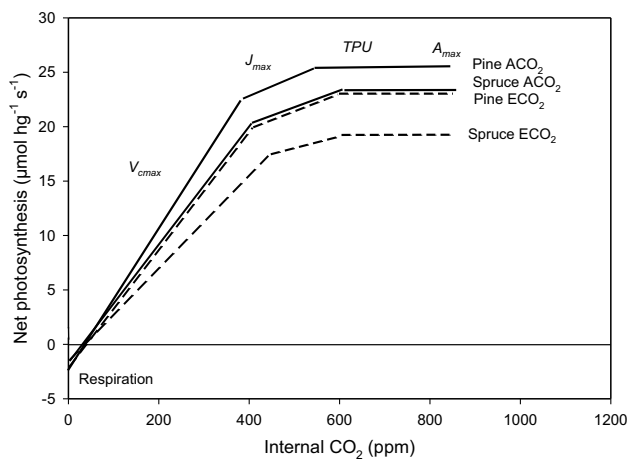


**Fig. 7** Relationship between  $P_{n570}$  change (%) from  $\text{aCO}_2$  to  $\text{eCO}_2$  for **a** pine species, and **b** spruce species to total biomass stimulation (%) under  $\text{eCO}_2$  compared with  $\text{aCO}_2$ . Symbols for species are as follows: WP white pine, RP red pine, JP jack pine, PP pitch pine, WS white spruce, RS red spruce, BS black spruce, NS Norway spruce

needle respiration and  $\text{CO}_2$  compensation point than pines, which may be a reflection of the greater spruce SNA.

### Genus and $\text{CO}_2$ effects for gas exchange parameters

Photosynthetic regulation ( $P_{nr}$ ) due to  $\text{eCO}_2$  has also been observed in a number of other tree species (Tjoelker et al. 1998a; Centritto and Jarvis 1999; Zhou et al. 2011). However,  $P_{nr}$  is different than biochemical downregulation in that it is more temporal and can often reflect both biochemical downregulation and species C sink demands (Jach and Ceulemans 2000; Rogers and Ellsworth 2002; Ellsworth et al. 2004; Ainsworth et al. 2004; Ainsworth and Long 2005). When comparing  $P_{n570}$  under  $\text{aCO}_2$  to  $\text{eCO}_2$ , pines showed a 13.0% increase, whereas the response in spruces decreased by 9.0%, which did not directly reflect the biochemical downregulation of these species.



**Fig. 8** Conceptual model of assimilation to internal  $\text{CO}_2$  ( $\text{AC}_i$ ) curves for pine and spruces under  $\text{aCO}_2$  and  $\text{eCO}_2$ . Maximum rate of carboxylation ( $V_{\text{cmax}}$ ), maximum rate of electron transport ( $J_{\text{max}}$ ), maximum rate of triose phosphate utilization (TPU), maximum assimilation ( $A_{\text{max}}$ ) and respiration are listed in areas of their determination

There are generally four plausible causes recognized for  $P_{\text{ndr}}$ . First, exposure to elevated  $\text{CO}_2$  often produces a decline in underlying photosynthetic capacities, carboxylation efficiencies, electron transport, and chlorophyll content (Tissue et al. 1999; Urban and Marek 1999; Ellsworth et al. 2004). As observed, we found that  $V_{\text{cmax}}$ ,  $J_{\text{max}}$ ,  $A_{\text{max}}$ , and TPU declined for both genera, but differentially. Second, the accumulation of nonstructural carbohydrates is usually associated with  $P_{\text{ndr}}$  and is commonly interpreted as evidence of a lack of sink activity (Tjoelker et al. 1998a; Centritto and Jarvis 1999; Griffin et al. 2000; Ainsworth and Long 2005). The increase in nonstructural carbohydrates is also most likely the driver of the decline in underlying biochemical capacity. Third, it has been observed in experiments with mature trees on relatively infertile sites that limiting N can cause  $P_{\text{ndr}}$  by reducing growth (Oren et al. 2001; Crous et al. 2008). Oren et al. (2001) found that fertilization of the  $\text{eCO}_2$  plots restored growth, and Crous et al. (2008) found fertilization reversed  $P_{\text{ndr}}$ . Fourth, severe root constriction appears to have induced  $P_{\text{ndr}}$  in some potted plant studies, particularly when using fast-growing species, which eventually reduces the effect of roots as a C sink (Will and Teskey 1997). In the context of  $P_{\text{ndr}}$ , our experiment was grown under stress-free conditions (fertilized and watered on a weekly basis), and we used large felt root bags with generally slow-growing conifers.

Overall, we did not find significant differences for  $\text{CO}_2$  or genus effects for  $C_{\text{s}570}$ , although the trend was toward increased  $C_{\text{s}}$  in  $\text{eCO}_2$ . In a study involving *P. koraiensis*, the  $\text{eCO}_2$  plants had greater  $C_{\text{s}}$  than under  $\text{aCO}_2$  (Zhou and Han 2005). In a study of three conifer species, *Picea mariana*

and *Larix laricina* displayed a modest reduction in  $C_{\text{s}}$  in response to  $\text{eCO}_2$ , whereas there was no response seen in *Pinus banksiana* (Tjoelker et al. 1998a) or *P. radiata* (Greenup et al. 2003). Our overall WUE values had a significant  $\text{CO}_2$  effect; however, due to the greater  $C_{\text{s}}$  trend in  $\text{eCO}_2$ , WUE was greater in  $\text{aCO}_2$  than in  $\text{eCO}_2$  for both genera. This was also found in *P. radiata* (Greenup et al. 2003). In contrast, Le Thiec and Dixon (1996) found WUE was greater for *Picea abies* and *Quercus rubra* grown at  $\text{eCO}_2$  than  $\text{aCO}_2$ . This was also the case for loblolly pine (*Pinus taeda* L.; Werten et al. 2010).

For pines, SNA showed no difference between  $\text{CO}_2$  treatments. In the literature, there were no differences found for *Pinus sylvestris* (Jach and Ceulemans 2000) or *Pinus taeda* and *Liquidambar styraciflua*; (Springer et al. 2005) for SNA under different  $\text{CO}_2$  treatments. The SNA decline from  $\text{aCO}_2$  to  $\text{eCO}_2$  for BS was also observed by (Tjoelker et al. 1998b). The decline in SNA in  $\text{eCO}_2$  for spruces would result in a greater difference between  $\text{CO}_2$  treatments if measured by area-based gas exchange as observed by Tjoelker et al. (1998a).

### Biochemical and gas exchange parameters: species and $\text{CO}_2$ effect

Species can show large variation in gas exchange, biochemical efficiency traits, and growth response to elevated  $\text{CO}_2$  (Tjoelker et al. 1998a, b; Bauer et al. 2001; Liu et al. 2011). In a similar study, Zhou et al. (2011) found that  $V_{\text{cmax}}$  and  $J_{\text{max}}$  were reduced in one pine species, *Pinus koraiensis*, but not in another, *P. sylvestrisformis*, under  $\text{eCO}_2$ . What is remarkable in comparing the genera is that, although there were species differences within the genera overall, spruces were fairly consistent in their biochemical trait response: all traits decreased under  $\text{eCO}_2$ . Moreover, for the pines, there were changes in  $\text{CO}_2$  treatment rank among species. In pines, biochemical trait results for WP and RP consistently displayed greater values under  $\text{aCO}_2$  than in  $\text{eCO}_2$ , with WP showing the largest biochemical downregulation. In JP, biochemical traits were largely unchanged between  $\text{CO}_2$  treatments. In PP, biochemical traits were greater in  $\text{eCO}_2$  than in  $\text{aCO}_2$ . This phenomenon was also found in *Populus tremuloides* when measured on a mass basis (Tjoelker et al. 1998a) and for two subtropical species, *Ormosia pinnata* and *Castanopsis hystrix* (Liu et al. 2011). This biochemical upregulation probably reflects the far greater biomass stimulation among all eight species and will be discussed further later. Interestingly, our SNA,  $P_{\text{n}570}$ , and quantum efficiency were greater for PP for light response curves in  $\text{eCO}_2$  than  $\text{aCO}_2$  (Major, unpublished).

For spruces,  $P_{\text{ndr}}$  reflected the underlying biochemical parameters, with some notable differences. White spruce was the only spruce that did not have  $P_{\text{ndr}}$  whereas RS and

NS both had modest  $P_{\text{ndr}}$ . Among the spruces, BS had the greatest  $P_{\text{ndr}}$ . However, for pines,  $P_{\text{ndr}}$  not only reflected the underlying biochemical traits, but also showed an overall greater  $P_{\text{n570}}$  under  $\text{eCO}_2$  (Fig. 3a). White pine was the only pine species to have  $P_{\text{ndr}}$  in  $\text{eCO}_2$ , but not as large as the biochemical traits. Red pine went from a downregulation in biochemical traits to equal  $P_{\text{n570}}$  in both  $\text{CO}_2$  treatments. Jack pine went from equal biochemical trait values to showing greater values of  $P_{\text{n570}}$  in  $\text{eCO}_2$ . Pitch pine went from greater biochemical values in  $\text{eCO}_2$  to even greater  $P_{\text{n570}}$  under  $\text{eCO}_2$ . Thus, there appears to be an enhanced sink effect for pines that was consistent across all pine species.

### Relationships to other traits

Why does BS have the greatest  $P_{\text{ndr}}$  among spruce, and WS the least  $P_{\text{ndr}}$ ? Why does WP have the greatest  $P_{\text{ndr}}$  among pines whereas  $P_{\text{n}}$  is upregulated in PP? The sink regulation of  $P_{\text{n}}$  theory suggests examining the change in total dry mass in response to  $\text{eCO}_2$  (Paul and Foyer 2001; Ainsworth et al. 2004). For the spruce species reported here, we found an average 15% increase in total dry mass accumulation in  $\text{eCO}_2$  ( $P < 0.001$ ) compared with  $\text{aCO}_2$  (Major et al. 2018). Major et al. (2015a) also found an average 15.5% increase in total dry mass across RS, BS, and three hybrid types in  $\text{eCO}_2$ , which is also near the average seen in a meta-analysis of tree species prepared by Ainsworth and Long (2005). In this study, biomass yield stimulation was lowest for BS at 5.2% and was as high as 18.2% in RS. This is very similar to a previous study that found BS had the lowest biomass stimulation with 6.5%, whereas RS had a stimulation of 17.5% (Major et al. 2014, 2015a). In this study, WS had the greatest biomass stimulation at 29.7% among spruces, and NS had intermediate (12.1%) biomass stimulation. The corresponding  $P_{\text{ndr}}$  for spruces showed a strong positive correlation to biomass stimulation ( $P = 0.007$ ,  $r = 0.993$ ), supporting the theory of sink regulation of  $P_{\text{n}}$ . In a previous study, there was a very strong positive relationship among RS, BS, and three hybrid indices between assimilation regulation (%) and percentage change in total dry mass  $\text{aCO}_2$  to  $\text{eCO}_2$  ( $R^2 = 0.931$ ,  $P = 0.008$ ) (Major et al. 2014). Photosynthetic traits have been correlated with productivity (Major and Johnsen 1996; Reich et al. 1998; Tjoelker et al. 1998a; Johnsen et al. 1999), but it is clear that the correlations among photosynthesis, growth rate, and chlorophyll content in forest trees can be complicated and depend on many factors, such as time scale, environmental conditions, species, and age (Pereira 1994; Reich et al. 1998).

Why does PP have such a remarkable response to  $\text{eCO}_2$  compared with the other seven species? In Canada, PP is a rare species found near the northern limit of its geographical range along the border between the provinces of Ontario and Quebec, and New York State. Further east, the northernmost

populations of PP are found in northern Maine (e.g., the Acadia National Forest), just south of the New Brunswick border. It is not a commercial species in North America, and thus, is one of the least studied conifers. Pitch pine is similar to the pines of the southeast USA in that it does have the ability to produce a strong second, late-season bud flush and shoot growth. We have examined some 8-year-old provenance trials of PP planted together with RP and JP located in south-central ON and south-central NB and NS and have documented this second late-season bud flush (Major unpub.). Also, 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. Among the four eastern pines, PP is currently more restricted in its northern geographical range, and may begin to express a competitive advantage under increasing  $\text{CO}_2$ , and could expand its range, aided by the predicted warming and drying, and associated changes in fire regimes.

We found positive relationships between  $V_{\text{cmax}}$ ,  $A_{\text{max}}$ , and  $SNA$  for both pines and spruces: the more needle area per unit needle weight, the greater the biochemical efficiency. Exposure to  $\text{eCO}_2$  generally results in both less  $SNA$  and lower  $V_{\text{cmax}}$  or  $A_{\text{max}}$ . The thicker needles may be contributing to the decline; however, the declines are largely biochemical as the light intensity was  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which is beyond the saturation point for these genera (Tjoelker et al. 1998a; Jach and Ceulemans 2000). Spruces consistently showed lower  $V_{\text{cmax}}$ ,  $A_{\text{max}}$ , and  $SNA$  in  $\text{eCO}_2$ ; however, the pines were not consistent in the response to  $\text{eCO}_2$  and the pine results showed more scattered data around the regression line. Interestingly, pines had greater biochemical efficiencies than spruces for a given  $SNA$ , and were 7.5 and  $5.1 \mu\text{mol hg}^{-1} \text{s}^{-1}$  greater for  $V_{\text{cmax}}$  and  $A_{\text{max}}$ , respectively.

The differential genus response of the  $J_{\text{max}}-V_{\text{cmax}}$  relationship probably indicates a differential N allocation between the thylakoid and soluble proteins (Centritto and Jarvis 1999) or an N reallocation between photosynthetic components (Crous et al. 2008). Centritto and Jarvis (1999) and Crous et al. (2008), examining *Picea sitchensis* and *Pinus taeda*, respectively, under N x  $\text{CO}_2$  treatments, did not observe a change in the  $J_{\text{max}}:V_{\text{cmax}}$  ratio, but each of these studies only dealt with one species. However, a review by Wullschlegel (1993) showed that these two parameters are largely species specific. Based on our findings, pines under  $\text{aCO}_2$  had the greatest biochemical efficiencies across the  $\text{CO}_2$  response curve. Significantly below that would be a similar response by spruce under  $\text{aCO}_2$  and pines under  $\text{eCO}_2$  treatments. The lowest biochemical efficiencies would be for spruce under  $\text{eCO}_2$  treatment. Why are the efficiencies greater for pines? Perhaps for two reasons: first, pines grow faster, under  $\text{aCO}_2$ , with a mean of 38% greater total biomass than spruces (Major et al. 2018), and thus they require greater efficiency

to meet this sink demand. Second, hard pines typically have much lower leaf area index (LAI) values or needle dry mass than spruces. In a study examining BS and JP in Saskatchewan and Manitoba on northern and southern sites, the LAI was 4.2 and 5.6 for BS, respectively, and 2.4 and 2.2 for JP, respectively (Gower et al. 1997). Thus, pines may need to be more efficient to meet the greater growth demand. This may have considerable implications as post-European settlement forestry practices such as the use of clearcut harvesting have resulted in increased competition among pines and spruces (Harrison et al. 1999). In addition, artificial regeneration practices include both genera mixed even in the same planting trays in an effort to hedge against the direction of climate change and markets (i.e., J.D. Irving Limited, NB). Thus, whether in direct individual or stand competition, it would appear that under increasing CO<sub>2</sub>, pines would have a competitive advantage in early growth in response to increasing CO<sub>2</sub>.

Will faster growing trees respond better in an eCO<sub>2</sub> world? Our study showed mixed results, as does the literature on this question. The theory that inherently faster growing species would have a competitive advantage compared with inherently slower growing plants in eCO<sub>2</sub> is supported by some studies (Atkin et al. 1999; Poorter and Navas 2003) but not by others (Tjoelker et al. 1998a; Ghannoum et al. 2010; Major et al. 2014, 2015a). The general theory is that faster growing, early successional species are adapted to a wider range of environmental conditions and have a comparative advantage to any overabundant resource, whereas specialist species take advantage of a particular environmental condition or resource. Comparing genera, faster growing genera such as pines do respond better than spruces to eCO<sub>2</sub>. For spruces, in most experiments involving comparisons of RS, RS is among the slowest-growing species (Johnsen et al. 1998; Bauer et al. 2001; Major et al. 2003, 2015a, b). As BS is an early successional species, it grows faster than RS, but RS responded better to eCO<sub>2</sub> than BS in this study and in those of Major et al. (2014, 2015a, b). Others have hypothesized that shade-tolerant species respond better than shade-intolerant species to eCO<sub>2</sub>, which would include RS and WS, which had the greatest response of all spruces to eCO<sub>2</sub>. In a meta-analysis of 74 species, total biomass response ratios (eCO<sub>2</sub> to aCO<sub>2</sub>) among woody seedlings and saplings were significantly greater for shade-tolerant than shade-intolerant species (Kerstiens 2001). This phenomenon has subsequently been observed in physiological comparisons in long-term free-air CO<sub>2</sub> enrichment experiments (Mohan et al. 2007; Ellsworth et al. 2012). The exact physiological or structural cause of our observed species differences in growth stimulation by eCO<sub>2</sub> remain uncertain, but may be related to an ability to increase photosynthetic carbon capture even under different light conditions (Kerstiens 2001; Ellsworth et al. 2012).

## Conclusion

Overall pines have greater biochemical efficiencies and maintain even greater efficiencies in eCO<sub>2</sub> than spruces. Although there were substantial response differences among spruces, they were consistent in that their biochemical trait responses all decreased under eCO<sub>2</sub>. Whereas, for pines, there were CO<sub>2</sub> treatment rank changes for a number of traits among species. When comparing  $P_{n570}$  under aCO<sub>2</sub> to eCO<sub>2</sub>, pines had a 13.0% increase, whereas spruces had a 9.0% decrease in their responses, respectively. The PP upregulation is probably a reflection of by far the largest biomass stimulation amongst all species. The corresponding  $P_{ndr}$  for spruces showed a remarkable correlation to biomass growth stimulation ( $P=0.007$ ,  $r=0.993$ ), giving strong support to the sink regulation of  $P_n$  theory, whereas for pines, the same trend was found but was not statistically significant. Will faster growing trees respond better in an eCO<sub>2</sub> world? Our study shows mixed results, as does the literature on this question. Some have hypothesized that shade-tolerant trees respond better than shade-intolerant trees to eCO<sub>2</sub>, and this was supported by our results from the spruce responses. Whether in direct individual or stand competition it would appear that under increasing CO<sub>2</sub>, pines would have a competitive advantage in early growth in response to increasing CO<sub>2</sub>. Among the four eastern pines, PP is currently more restricted in its northern geographical range, and may begin to express a competitive advantage under increasing CO<sub>2</sub>, and could expand its range, aided by the predicted associated climate warming.

**Author contribution statement** JEM designed the experiment and was lead author, AM contributed to the analyses and writing of the manuscript; JWM undertook the processing, organized the experiment, and contributed to the writing of the manuscript.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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