Chlorophyll pigment and needle macronutrient responses and interactions to soil moisture and atmospheric CO₂ treatments of eight pine and spruce species

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Abstract

Key message Large differences were detected between genera and among species in chlorophyll pigment and macronutrient concentrations. Pines have greater variances than spruces and showed less downregulation of chlorophyll pigment concentrations than spruces in response to eCO₂ and DRT. There was strong genetic control of chlorophyll pigments and most macronutrients.

Abstract Chlorophyll pigment and needle macronutrient concentrations were quantified for eight tree species in two commercially important genera, *Pinus* and *Picea* grown in a 2 × 2 factorial of atmospheric CO₂ (370 and 740 ppm) and soil moisture stress (−0.1 to −0.5 and −0.7 to −1.0 MPa) treatments. Four of the pines and three of the spruces are native to eastern North America, while a fourth spruce, Norway spruce (NS: *Picea abies*), is from Europe but has been widely used for reforestation in northeastern North America. Overall, spruces had significantly greater chlorophyll *a*, *b*, (CHL*a*, CHL*b*), and total chlorophyll concentration (TCC) and carotenoid concentration (CAR) than pines. Ambient CO₂ (aCO₂) had significantly greater TCC than in response to elevated CO₂ (eCO₂), and TCC and CAR was significantly downregulated more in spruces than in pines in response to eCO₂. Pines had equal or greater TCC and CAR in response to drought treatment (DRT) than well-watered treatment, whereas spruces had significantly lower values in response to DRT. Needle N, P and Ca concentrations were greater for spruces than pines. Needle N concentrations declined in response to both eCO₂ and DRT. Needle P increased in response to eCO₂ but declined in response to DRT. Using total biomass as a covariate, needle N showed no response in pines; whereas spruces showed a slight positive response to increasing total biomass. Covariate analysis showed that TCC had a significant positive relationship to needle N and Mg, with greater TCC in spruces than in pines for a given needle N. Photosynthetic quantum efficiency (QE), derived from light response curves, had a significant positive relationship to TCC that was greater in pines than in spruces for a given TCC. Photosynthetic light convexity had a significant positive relationship to TCC that was also greater in pines than in spruces for a given TCC, indicating a sharper curvature compared to a more progressive curve for spruce. Pine species have greater variances than spruce species. While *Pinus strobus* (white pine, subgenus *Strobus*) stands out as having greater chlorophyll and nutrient concentrations than the other three pines (subgenus *Pinus*). Overall, pines showed less downregulation of chlorophyll pigment concentrations than spruces in response to eCO₂ and DRT. There was strong genetic control for chlorophyll pigments and most macronutrients.

Keywords Elevated CO₂ · Fitness · Chlorophyll pigments · Needle macronutrients · Pines and spruces · Soil moisture stress

Introduction

To better understand biological responses to climate change factors we need to examine the genetic, environment, and genetic × environment interactions. Multiple species in response to environmental change studies can help inform reforestation plans for future climate scenarios. Chlorophyll pigment and nutrient concentrations are important underlying plant traits that vary widely among species,

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and environmental conditions (Barsi et al. 2009; Minocha et al. 2009; Lukac et al. 2010). Depending on the species and severity, moisture stress and elevated CO₂, can reduce chlorophyll pigment content (Zhou et al. 2011; Rasheed et al. 2021), but by how much? Lower chlorophyll amounts intercept less light by the plant, thereby decreasing photosynthesis and growth potential. Short-term responses to increased CO₂ stimulates photosynthesis (Wertin et al. 2010; Ellsworth et al. 2012). However, downregulation of the photosynthetic apparatus (e.g., chlorophyll and biochemical efficiency traits) can occur in response to sustained elevated CO₂ (eCO₂), vary among species and can result in reductions in carboxylation efficiencies and maximum assimilation, along with other related traits (Crous et al. 2008; Major et al. 2018). Depending on the species, studies have shown that photosynthetic apparatus downregulation generally ranges from none to complete downregulation (when *A* is the same under eCO₂ as it is under ambient CO₂ (aCO₂)), (Luomala et al. 2003; Zhou et al. 2011) and that this is strongly influenced by carbon sink demand (Ainsworth et al. 2004; Major et al. 2018). Depending on future scenarios, species interactions with eCO₂ and soil moisture stress are important indicators of future fitness. Elevated CO₂ can mitigate some of the negative effects of water stress in mulberry (Sekhar et al. 2017) and cacao (Lahive et al. 2021), but was neutral or negative depending on the genotype for maritime pine (Sanchez-Gomez et al. 2017).

Nitrogen (N), an essential nutrient for building the components of chlorophyll, enzymes, proteins and other organic compounds required by plants, often limits growth in plants (Maathuis 2009). It has been positively associated with chlorophyll pigment content (Major et al. 2007a), photosynthesis (Crous et al. 2008), and overall productivity (Finzi et al. 2007). Other macronutrients, e.g., phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), are essential elements in various plant physiological functions, and as structural components of DNA and RNA, osmotic potential generation for growth, and cell wall and chlorophyll constituents (Maathuis 2009). Studies have noted variation in species requirements for each of these elements (Major et al. 2007b; Mellert and Gottlein 2012). In a review of tree nutrition, Lukac et al. (2010) postulated that although tree nutrition has not been the primary focus of climate change studies, there are likely to be nutritional changes depending on species and limits to future biomass production in response to increasing CO₂.

With climate warming, the frequency and severity of drought are predicted to increase (Trenberth et al. 2014) and water is a predominant factor in determining the geographic distribution of vegetation. Drought tolerance can vary with species and is an important factor affecting tree biomass yield and mortality (Adams et al. 2015; Allen et al. 2015). Under conditions of water stress and excessive

heat, photosynthesis is often reduced, photooxidative stress increases, leaves abscise, growth rate of remaining leaves and overall plant growth decreases (Attia et al. 2015; Teskey et al. 2015). Tree physiological responses to water availability vary among and within species (Attia et al. 2015; Duan et al. 2015).

These pines and spruces were selected because they all grow in northeastern North America. The pines examined include white pine (WP: *Pinus strobus* L.), red pine (RP: *P. resinosa* Ait.), jack pine (JP: *P. banksiana* Lamb.) and pitch pine (PP: *P. rigida* Mill.). The spruces examined include: white spruce (WS; *Picea glauca* (Moench) Voss), black spruce (BS; *Picea mariana* (Mill.) B.S.P.), red spruce (RS; *Picea rubens* (Sarg.)) and Norway spruce (NS; *Picea abies* (L.) H. Karst) that has been introduced for reforestation in Eastern Canada.

Genera and species have different ranges of tolerance and potential interactions to climatic and other environmental variables. Paleoecological reconstructions (Peters 1990) and pollen coring (Schauffler and Jacobson 2002; Lindbladh et al. 2003) support the conclusion that species migrate individually rather than as intact plant communities. The predicted doubling of CO₂ this century, resulting in increased global temperatures and increased drought will most likely affect comparative fitness among tree species. Thus, our goal was to examine and compare underlying needle chlorophyll pigment and macronutrient response and interactions in eight pine and spruce species to soil moisture and atmospheric CO₂ treatments. We hypothesized that the genera and species chlorophyll and nutrient content would respond and interact differently to eCO₂ and soil moisture stress. Our specific objectives were to (1) quantify variation in chlorophyll pigment and needle macronutrient traits among eight pine and spruce species, (2) examine genera and species responses and various interactions to soil moisture stress and eCO₂ treatments, and (3) examine underlying total chlorophyll content (TCC) relationships to needle macronutrients, photosynthetic light response traits and total biomass. We will discuss these and other results in terms of practical application for reforestation in northeastern North America.

Materials and methods

Material and growth conditions

The seed sources used for each pine and spruce species are shown in Table 1. Details of growing conditions and experimental layout are provided in Major and Mosseler (2020). In brief, 370 or 740 ppm of CO₂ (24 h), representing aCO₂ and eCO₂ levels, respectively. Soil moisture conditions were set to range between either −0.1 and −0.5 MPa, or −0.7 and −1.0 MPa (drought treatment, DRT). Thus, the experiment

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

Species	Location	Lat (N)	Long (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° 56'	3° 04' E

was a two $\times$ two factorial of atmospheric CO₂ and soil moisture treatments with two blocks of randomized replicates for a total of eight chambers. Within each chamber there were four randomized three-tree plots of each species for a total of 96 seedlings planted in 25-cm diameter $\times$ 30-cm felt root control bags (Root Control Inc., Oklahoma City, OK, USA). Seedlings were grown for three years in treatment chambers.

Chlorophyll pigment sampling and measurements

Four seedlings (one from each of the four three-tree plots within each block or chamber) were sampled from current-year foliage of each of the eight species from the two replicate blocks across the four treatments, for a total of 256 samples. Sampling was performed in the early morning from September 18 to October 4, 2006 on 4-year-old seedlings after three years of treatments. A lateral branch from the top whorl was removed and placed on ice. Six to eight needles were used for the spruces depending on needle size. Fewer pine needles were used depending on their size. The pigment extraction protocol followed that of Malavasi and Malavasi (2001). Needles were placed into 1.5-ml Eppendorf tubes stored on ice. Tubes were immersed in liquid N and frozen needles were crushed by hand using a pellet pestle in 1.0 ml of 80% acetone (v/v) with a small quantity of Alumina® added. Samples were homogenized using a laboratory stirrer and centrifuged at 10,000 rpm for 2 min to clarify the extract. The supernatant was diluted 1:1 with 80% acetone. Spectrophotometric measurements were taken at 470 nm, 647 nm, and 663 nm for carotenoids (CAR), chlorophyll *b*, and *a* (CHL*b*, CHL*a*), respectively, using an Ultrospec 2100 pro UV/Visible Spectrophotometer (Biochrom Ltd., Cambridge, England). Concentrations of CHL*a*, CHL*b*, and CAR were calculated using equations and absorbance coefficients of Lichtenthaler (1987). There are various chemical constituents of CAR, (i.e., beta-carotene, lutein and other xanthophyll pigments), but our study provides only an estimation of CAR.

Leaf nutrient analysis

For N analysis, we used the current-year foliage samples from the same plant used for the assimilation to CO₂ response curve (curves performed only on well-watered samples, Major et al. 2018) and on well-watered and DRT light response curves (Major and Mosseler 2020). The Total Kjeldahl Nitrogen (TKN) process (described below) was used to obtain needle N on 120 needle samples. The remaining needle mass allowed us to perform CNS analysis (LECO process described below) and macronutrient analysis (102 samples). We measured needle N both ways to compare the different extraction method results. Samples were collected in October 2006, dried at 65 °C for 48 h, then ground to a fine powder and analyzed for elemental nutrients. The grinder was washed with ethanol between samples. The Laboratory for Forest Soils and Environmental Quality (LFSEQ) at the University of New Brunswick (UNB) used standard protocols (e.g., Method numbers TP-SSMA 15.3.1, 15.3.3, and 15.4 from McKague (1978) for foliage analysis of P, K, Ca, Mg). Foliage dry-ashing is done by muffle furnace (500 °C for 4 h—progressive ramping), and extracted with 8 N HCl in a heated water bath at 90 °C for 30 min. and filtered. Dilute test solutions were prepared for K, Ca, Mg (cations), and were analysed on the Varian SpectraAA 400 (Varian Techntron Pty. Limited, Mulgrave, Victoria, Australia); whereas P was analysed on the Technicon Traaccs Autoanalyser (Technicon Instruments Corp., Tarrytown, New York, USA). Needle total Kjeldahl nitrogen (TKN) content was measured using standard sulphuric acid digestion Kjeldahl techniques (Carter 1983). Method #TP-LFIM (Total carbon by LECO induction furnace (LECO Corp. St. Joseph, Michigan, USA)) was used for C and N and C:N ratio determination.

Light response parameters

Photosynthetic light response curve (LRC) traits, quantum efficiency (QE) and photosynthetic light convexity were determined using a commercially available software (Photosyn Assistant; Dundee Scientific, Dundee, Scotland, UK) which modelled using a non-rectangular hyperbola (Prioul and Chartier 1977). The LRC methodology is provided in Major and Mosseler (2020). Briefly, the QE is the initial slope of the light response curve. The convexity (also known as curve convexity) describes the progressive rate of bending between the linear initial slope and the maximum assimilation plateau. Convexity ranges from 0 to 1, where low values have a very progressive curve, whereas, greater values have a sharper curve. These LRC traits were used in the covariate analysis in relation to TCC traits.

Statistical analyses

The study was established as a completely randomized block experimental design with CO₂, water treatment, genus, and species nested within genus [(species (genus))] all considered as fixed effects. Block and replicates were considered random effects. The same ANOVA model used in Major and Mosseler (2020) was used to analyze results. Effects were considered statistically significant at the $P=0.05$ level, although 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 the genus were tested using Tukey's mean separation test ($P=0.05$).

Covariate analysis was used to evaluate relationships among chlorophyll, leaf nutrient, total biomass and some light response traits as described in Major and Mosseler (2019, 2020). Results were considered statistically significant at $P=0.10$ due to fewer (16 or 32) data points, although individual P values are provided for all traits so that readers can make their own interpretations of significance. Note that when referring to the species effect in the presentation of results, species are nested within a genus. The general linear model from Systat was used for analysis. The variance component analysis was carried out using the sum of squares as outlined by Hicks (1982).

Results

Chlorophyll traits

The ANOVA results for CHL_a and CHL_b are presented in Table 2, followed the same trend as TCC and thus only TCC results are presented here. For TCC, genus, species and CO₂

effects, and genus $\times$ CO₂, genus $\times$ water, and CO₂ $\times$ water interactions were significant accounting for 41.7, 13.1, 5.7, 0.7, 1.2 and 1.8% of the total variation (Table 2). Overall, the genus had a very large effect with spruces showing 57.7% more TCC than pines (Fig. 1a). The significant genus $\times$ CO₂ interaction was not a result of a rank change but rather a magnitude effect with TCC decreasing by 15.0 and 20.0% in eCO₂, for pines and spruces, respectively. The genus $\times$ water interaction was a result of a rank change with pines increasing TCC by 6.0% in response to DRT; whereas, for spruces DRT decreased TCC by 9.3% (Fig. 1b). The CO₂ $\times$ water interaction showed a magnitude effect, with TCC decreasing by 7.4 and 30.5% from aCO₂ to eCO₂, in response to well-watered and DRT, respectively (not shown), thus no DRT mitigation with eCO₂. Among the pines, WP had the greatest TCC followed by JP, and RP, which were significantly greater than PP (Fig. 1c). The spruce species TCC were not significantly different from each other (Fig. 1d).

For CAR, genus, species and CO₂ effects were significant, as well as genus $\times$ CO₂, genus $\times$ water, CO₂ $\times$ water, and genus $\times$ CO₂ $\times$ water interactions accounting for 23.7, 18.2, 7.9, 0.8, 0.9, 2.4, and 0.7% of the total variation, respectively (Table 2). Spruces had 40% greater CAR than pines and aCO₂ had 21% greater CAR than the eCO₂ treatment (not shown). The significant genus $\times$ CO₂ and genus $\times$ water interactions were magnitude effects. Pine and spruce CAR levels were reduced by 17 and 25%, respectively, in response to eCO₂. Pine CAR values did not change in response to DRT whereas spruces declined by 10% in response to DRT. Similar to TCC, The CAR CO₂ $\times$ water interaction was also a magnitude effect with no eCO₂ mitigation of DRT. Among the pines, WP was greater than RP and JP, which was greater than PP. Among spruces, RS had the greatest CAR followed by BS and NS.

For CHL_a:*b* ratio, species, CO₂ and water effects were significant and genus $\times$ CO₂, CO₂ $\times$ water and genus $\times$ CO₂ $\times$ water interactions were significant, accounting for 16.3, 6.7, 3.1, 2.0, 1.1 and 2.8% of the total variation (Table 2). The significant CHL_a:*b* genus $\times$ CO₂ effect was a result of magnitude change. The pines and spruces CHL_a:*b* declined by 1.9 and 6.7% from aCO₂ to eCO₂, respectively (Fig. 2a). CHL_a:*b* declined by approximately 3% for both pines and spruces in response to DRT (Fig. 2b). The CO₂ $\times$ water interaction was not due to rank change but a mitigation of the DRT effect under eCO₂. The genus $\times$ CO₂ $\times$ water interaction was a result of pines under eCO₂ mitigating the CHL_a:*b* decline brought on by DRT. WP and RS had the greatest CHL_a:*b*, BS was intermediate and the rest were significantly below WP and RS (Fig. 2c and d).

For TCC:CAR ratio, genus, species, CO₂, and water effects were the only significant sources of variation accounting for 27.5, 23.3, 2.2 and 2.0% of the total variation (Table 2). The TCC:CAR ratio was 6.1 and 5.3 for spruces

Table 2 Chlorophyll trait variance components and ANOVAs including the source of variation, degrees of freedom (*df*), mean square values (MS), variance component (VC), *P* values, and coefficient of determination (*R*²)

Source of variation	<i>df</i>	Chlorophyll <i>a</i> (mg/g)			Chlorophyll <i>b</i> (mg/g)			Total Chlorophyll (mg/g)		
		MS	VC (%)	<i>P</i> value	MS	VC (%)	<i>P</i> value	MS	VC (%)	<i>P</i> value
Block	1	2.357	3.7	< 0.001	0.313	3.7	< 0.001	4.39	3.7	< 0.001
Genus	1	25.954	41.1	< 0.001	3.493	41.6	< 0.001	48.49	41.7	< 0.001
CO ₂	1	3.967	6.3	< 0.001	0.35	4.2	< 0.001	6.67	5.7	< 0.001
Water	1	0.191	0.3	0.125	0.002	0.0	0.661	0.24	0.2	0.215
Genus*CO ₂	1	0.534	0.8	0.011	0.021	0.2	0.197	0.77	0.7	0.026
Genus*water	1	0.768	1.2	0.002	0.099	1.2	0.005	1.42	1.2	0.003
CO ₂ *water	1	1.032	1.6	< 0.001	0.198	2.4	< 0.001	2.14	1.8	< 0.001
Genus*CO ₂ *water	1	0.279	0.4	0.064	0.01	0.1	0.373	0.40	0.3	0.109
SP ¹ (genus)	6	1.470	14.0	< 0.001	0.153	10.9	< 0.001	2.54	13.1	< 0.001
SP(genus)*CO ₂	6	0.130	1.2	0.144	0.019	1.4	0.175	0.25	1.3	0.142
SP(genus)*water	6	0.110	1.0	0.231	0.015	1.1	0.304	0.20	1	0.244
SP(genus)*CO ₂ *water	6	0.002	0.0	1.000	0.001	0.1	0.998	0.01	< 0.1	1.000
Error	222	0.080	28.2		0.013	33.2		0.153	29.1	
<i>R</i> ²				0.717			0.667			0.723
Source of variation	<i>df</i>	Carotenoids (mg/g)			Chlorophyll <i>a</i> : <i>b</i> ratio			Chlorophyll: carotenoid ratio		
		MS	VC (%)	<i>P</i> value	MS	VC (%)	<i>P</i> value	MS	VC (%)	<i>P</i> value
Block	1	0.176	4.9	< 0.001	0.010	< 0.1	0.599	2.355	1.6	0.003
Genus	1	0.841	23.7	< 0.001	0.072	0.6	0.152	39.798	27.5	< 0.001
CO ₂	1	0.280	7.9	< 0.001	0.794	6.7	< 0.001	3.179	2.2	0.001
Water	1	0.019	0.5	0.071	0.363	3.1	0.001	2.936	2.0	0.001
Genus*CO ₂	1	0.028	0.8	0.031	0.239	2.0	0.009	0.392	0.3	0.226
Genus*water	1	0.031	0.9	0.022	< 0.001	< 0.1	0.933	0.143	0.1	0.464
CO ₂ *water	1	0.085	2.4	< 0.001	0.130	1.1	0.054	0.271	0.2	0.314
Genus*CO ₂ *water	1	0.024	0.7	0.043	0.334	2.8	0.002	0.264	0.2	0.320
SP(genus)	6	0.107	18.2	< 0.001	0.320	16.3	< 0.001	5.615	23.3	< 0.001
SP(genus)*CO ₂	6	0.008	1.3	0.239	0.008	0.4	0.961	0.180	0.7	0.670
SP(genus)*water	6	0.010	1.7	0.122	0.028	1.4	0.557	0.198	0.8	0.614
SP(genus)*CO ₂ *water	6	0.001	0.2	0.982	0.009	0.4	0.957	0.090	0.4	0.95
Error	222	0.006	36.8		0.035	64.9		0.266	40.7	
<i>R</i> ²				0.631			0.353			0.595

P values < 0.05 are in bold print

SP species

and pines, respectively (Fig. 3a). Both eCO₂ and DRT increased the TCC:CAR ratio for both genera by approximately 4% (Fig. 3a and b). The increase in TCC:CAR ratio in response to eCO₂ was observed across all eight species (Fig. 3c and d). WS had the greatest TCC:CAR ratio and pitch pine had among the lowest ratio.

Needle nitrogen

For TKN, genus, species, CO₂ and water effects were the only significant sources of variation accounting for 27.2,

21.2, 18.1 and 1.3% of the total variation (Table 3). Overall, spruces had greater TKN than pines with 2.2 and 1.8%, respectively, a 22% difference (Fig. 5a). Elevated CO₂ reduced TKN on average by 17% for both pines and spruces. Drought reduced TKN for both pines and spruces on average by 4.4% (Fig. 4b). Among pines, WP had the greatest needle TKN. Among spruces there were no significant differences among species (Fig. 4c, d). For needle N (LECO), genus, species, and CO₂ main effects and CO₂ × water were significant sources of variation accounting for 20.0, 36.1, 5.4, and 2.8% of the total variation (Table 3). Overall, spruces had

Fig. 1 Total chlorophyll concentration (mean $\pm$ SE) **a** by genus and CO_2 treatments, **b** by genus and soil moisture treatments, **c** by pine species and CO_2 treatments, and **d** by spruce species and CO_2 treatments. Species with no matching letters are significantly different

greater N than pines with 2.35 and 2.02%, respectively, (a 16% difference) and eCO_2 reduced N by an average of 9.3% (not shown). Drought reduced N for both pines and spruces on average by 4.7%. Among pines, WP had the greatest needle N, and among spruces NS had the greatest needle N. The water $\times \text{CO}_2$ interaction was a result of a magnitude change, under well-watered treatment, the N decrease in response to eCO_2 was smaller than in response to DRT thus there was no eCO_2 mitigation under DRT. Among individual samples, there was a positive correlation between N and TKN of 0.727. Among means for all eight species $\times$ two CO_2 levels $\times$ two water treatments correlation was 0.798.

Other needle macronutrients

For needle C, genus and species were the only significant effects accounting for 63 and 11% of the total variation (Table 3). Pines and spruces had 51.4 and 49.0% C,

using Tukey mean separation test ($P=0.05$). Species abbreviations WP, RP, JP and PP are for white pine, red pine, jack pine and pitch pine. Species abbreviations WS, RS, BS and NS are for white spruce, red spruce, black spruce and Norway spruce

respectively. The spruces had virtually the same foliage C; however, JP had greater foliage C than the other three pines (not shown). For needle C:N ratio, genus, species and CO_2 effects and genus $\times \text{CO}_2$, $\text{CO}_2 \times \text{water}$ interactions were significant (Table 3). Overall, pines and spruces had C:N ratios of 26.5 and 21.3, respectively. The significant genus $\times \text{CO}_2$ effect was due to a magnitude effect, as pines and spruces increased C:N ratio by 16% and 6.3%, respectively, in response to eCO_2 . Soil water had a significant effect with C:N ratios increasing with DRT for both genera. Among pines, PP had the greatest C:N ratio followed by RP, then JP, with WP having the lowest ratio. Among spruces, NS had the lowest C:N ratio. Genus and species dominated C:N variance accounting for 37.4% and 43.3% of the total variance.

Phosphorus was one of the more variable needle nutrients, with the ANOVA model accounting for almost 86% of the variation ($R^2=0.856$). Genus, species, CO_2 and water main effects were significant, as well as genus $\times \text{CO}_2$, genus

Fig. 2 Chlorophyll *a:b* ratio (mean ± SE) **a** by genus and CO₂ treatments, **b** by genus and soil moisture treatments, **c** by pine species and soil moisture treatments, and **d** by spruce species and soil moisture

treatments. Species with no matching letters are significantly different using Tukey mean separation test (*P* = 0.05). Species abbreviations are found in Fig. 1 caption

× water, genus × CO₂ × water and species × water interactions (Table 3). Genus and species accounted for 52.2% and 16.3% of the total variation. The genus × CO₂ × water, genus × CO₂ and genus × water interactions were all magnitude effects, not rank changes. Both genera showed an increase in *P* with eCO₂, though of different magnitudes (Fig. 5a); DRT caused a decrease in *P* in both genera, although to different degrees (Fig. 5b). Overall, pines and spruces had 0.21 and 0.31% *P*, respectively. Needle *P* across all species was consistently greater in eCO₂ than aCO₂ (Fig. 5c and d). Among pines, WP had greater needle *P* than the other three pines. The species × water interaction was the result of an NS rank change.

For needle *K*, species was the only significant effect accounting for 31.3% of the total variation (Table 3). Among pines, WP and RP had greater needle *K* compared to JP and PP, with 0.77% and 0.58% *K*, respectively (not shown). Among spruces, WS had lower needle *K* than the other spruces with 0.57% and 0.76% *K*, respectively. For needle *Ca*, genus and species were the only significant sources of

variation accounting for 68.8 and 8.7% of the total variation (Table 3). Spruce needle *Ca* was 2.2 times that of pines, with 1.25 and 0.56%, respectively. Among pines, WP had the greatest needle *Ca* followed by JP, RP and PP with 0.74, 0.57, 0.54 and 0.39, respectively. Among the spruces, RS had lower *Ca* (1.05%) than the other three spruces (1.31%). Variation in needle *Mg* was significant only for species accounting for 46% of the total variation (Table 3). Among pines, WP had the greatest *Mg* followed by RP, JP and PP with 0.145, 0.143, 0.138 and 0.086%, respectively. Among spruces BS had the greatest *Mg* concentrations followed by RS, NS and WS with 0.154, 0.146, 0.118 and 0.112%, respectively.

Relationship to other traits

Covariate analysis of pine mean needle *N* (TKN) in response to total biomass (covariate) and testing for CO₂ treatment effect showed no CO₂ treatment × total biomass interaction (*P* = 0.656). Further analysis showed no total biomass

Fig. 3 Chlorophyll to carotenoid ratio (mean $\pm$ SE) **a** by genus and CO_2 treatments, **b** by genus and soil moisture treatments, **c** by pine species and CO_2 treatments, and **d** by spruce species and CO_2 treat-

ments. Species with no matching letters are significantly different using Tukey mean separation test ($P=0.05$). Species abbreviations are found in Fig. 1 caption

covariate effect ($P=0.409$), but a significant CO_2 effect ($P=0.074$) resulting in two horizontal response lines with a CO_2 needle N above e CO_2 needle N (Fig. 6a). Covariate analysis of spruce mean needle N (TKN) in response to total biomass (covariate) and testing for CO_2 treatment effect showed no CO_2 treatment $\times$ total biomass interaction ($P=0.994$). Further analysis showed a significant slight positive total biomass covariate effect ($P=0.037$), and a significant CO_2 effect ($P<0.001$) resulting in two slight positive response lines with a CO_2 needle N above e CO_2 needle N (Fig. 6b).

Covariate analysis of pine mean needle TCC in response to total biomass (covariate) and testing for CO_2 treatment effect showed no CO_2 treatment $\times$ total biomass interaction ($P=0.367$). Further analysis showed no CO_2 effect ($P=0.845$) but a significant total biomass effect ($P=0.002$) resulting in one line sloping downward (Fig. 6c). Covariate analysis of spruce TCC in response to total biomass (covariate) and testing for CO_2 treatment effect showed no CO_2

treatment $\times$ total biomass interaction ($P=0.718$). Further analysis showed no total biomass effect ($P=0.501$) but a significant CO_2 effect ($P=0.003$) resulting in two horizontal response lines with a CO_2 TCC above e CO_2 TCC (Fig. 6d).

Covariate analysis of TCC in relation to needle TKN using means for all eight species $\times$ two CO_2 $\times$ two water treatments (32 means) and using mean needle TKN as a covariate and testing for genus showed no genus $\times$ needle N interaction ($P=0.990$), but significant genus ($P<0.001$) and needle N ($P<0.001$) effects. The result was a positive TCC response for both pines and spruces to needle TKN, with this relationship being greater for spruces than for pines (Fig. 7a). Similarly covariate analysis of TCC in relation to needle N (LECO) using means for all eight species $\times$ two CO_2 $\times$ two water treatments and using mean needle N as a covariate and testing for genus showed no genus $\times$ needle N interaction ($P=0.247$), but significant genus ($P<0.001$) and needle N ($P<0.001$) effects. The result was a positive TCC response for both pines and spruces to needle N, with

Table 3 Needle nutrient variance component and ANOVAs including source of variation, degrees of freedom (*df*), mean square values (MS), variance component (VC), *P* values, and coefficient of determination (R^2)

Source of variation	<i>df</i>	Nitrogen (%, TKN)			<i>df</i>	Nitrogen (%, LECO)			Carbon (%)		
		MS	VC (%)	<i>P</i> value		MS	VC (%)	<i>P</i> value	MS	VC (%)	<i>P</i> value
Block	1	0.123	0.7	0.119	1	0.012	<0.1	0.640	0.12	<0.1	0.670
Genus	1	4.459	27.2	<0.001	1	2.837	20.0	<0.001	138.94	63.0	<0.001
CO ₂	1	2.960	18.1	<0.001	1	0.764	5.4	<0.001	0.28	0.1	0.515
Water	1	0.219	1.3	0.039	1	0.187	1.3	0.070	0.15	<0.1	0.633
Genus*CO ₂	1	0.008	<0.1	0.683	1	0.206	1.5	0.058	0.41	0.2	0.433
Genus*water	1	<0.001	<0.1	0.990	1	0.094	0.7	0.197	0.33	0.1	0.481
CO ₂ *water	1	0.030	0.2	0.440	1	0.404	2.8	0.009	0.19	0.1	0.597
Genus*CO ₂ *water	1	0.011	<0.1	0.642	1	0.201	1.4	0.060	0.41	0.2	0.435
SP ¹ (genus)	6	0.579	21.2	<0.001	6	0.854	36.1	<0.001	4.01	10.9	<0.001
SP(genus)*CO ₂	6	0.059	2.2	0.319	6	0.037	1.6	0.672	0.31	0.9	0.825
SP(genus)*water	6	0.051	1.9	0.416	6	0.042	1.8	0.602	0.73	2.0	0.369
SP(genus)*CO ₂ *water	6	0.021	0.8	0.868	6	0.014	0.6	0.959	0.65	1.8	0.444
Error	87	0.050	26.4		69	0.055	26.8		0.66	20.7	
R^2				0.752				0.740			0.799

Source of variation	<i>df</i>	Carbon / nitrogen ratio (LECO)			<i>df</i>	Phosphorus (%)			<i>df</i>	Potassium (%)		
		MS	VC (%)	<i>P</i> value		MS	VC (%)	<i>P</i> value		MS	VC (%)	<i>P</i> value
Block	1	0.19	<0.1	0.883	0.006	1.3	0.014	0.026	1.0	0.252		
Genus	1	661.23	37.4	<0.001	0.246	52.2	<0.001	0.038	1.4	0.162		
CO ₂	1	113.83	6.4	0.001	0.017	3.6	<0.001	0.044	1.6	0.134		
Water	1	25.35	1.4	0.092	0.013	2.8	0.001	<0.001	<0.1	0.979		
Genus*CO ₂	1	44.23	2.5	0.027	0.004	0.8	0.038	0.049	1.8	0.115		
Genus*water	1	13.24	0.7	0.221	0.004	0.8	0.046	0.010	0.4	0.466		
CO ₂ *water	1	44.14	2.5	0.027	0.001	0.2	0.419	0.067	2.5	0.066		
Genus*CO ₂ *water	1	18.87	1.1	0.145	0.005	1.1	0.026	0.029	1.1	0.226		
SP ¹ (genus)	6	127.51	43.3	<0.001	0.013	16.3	<0.001	0.142	31.3	<0.001		
SP(genus)*CO ₂	6	4.64	1.6	0.780	0.001	0.6	0.766	0.014	3.2	0.610		
SP(genus)*water	6	6.92	2.4	0.575	0.003	3.6	0.017	0.007	1.5	0.903		
SP(genus)*CO ₂ *water	6	1.44	0.5	0.985	0.002	2.1	0.131	0.024	5.3	0.299		
Error	69	8.67	33.9		0.001	14.4		0.019	49.0			
R^2				0.754			0.856			0.518		

Source of variation	<i>df</i>	Calcium (%)			<i>df</i>	Magnesium (%)		
		MS	VC (%)	<i>P</i> value		MS	VC (%)	<i>P</i> value
Block	1	0.011	<0.1	0.625	2.51 × 10 ⁻⁴	0.3	0.499	
Genus	1	11.726	68.8	<0.001	5.02 × 10 ⁻⁴	0.5	0.341	
CO ₂	1	<0.001	<0.1	0.946	19.17 × 10 ⁻⁴	2.0	0.065	
Water	1	0.002	<0.1	0.828	1.93 × 10 ⁻⁴	0.2	0.554	
Genus*CO ₂	1	0.018	0.1	0.538	0.74 × 10 ⁻⁴	0.1	0.713	
Genus*water	1	0.012	0.1	0.616	4.69 × 10 ⁻⁴	0.5	0.357	
CO ₂ *water	1	0.006	<0.1	0.727	10.75 × 10 ⁻⁴	1.1	0.165	
Genus*CO ₂ *water	1	0.001	<0.1	0.907	9.64 × 10 ⁻⁴	1.0	0.188	
SP(genus)	6	0.248	8.7	<0.001	71.64 × 10 ⁻⁴	46.0	<0.001	
SP(genus)*CO ₂	6	0.020	0.7	0.869	3.84 × 10 ⁻⁴	2.5	0.646	
SP(genus)*water	6	0.034	1.2	0.633	5.44 × 10 ⁻⁴	3.5	0.433	
SP(genus)*CO ₂ *water	6	0.033	1.2	0.645	3.09 × 10 ⁻⁴	2.0	0.755	
Error	69	0.047	19.2		5.45 × 10 ⁻⁴	40.2		
R^2				0.815			0.618	

P values <0.05 are in bold print

SP species

Fig. 4 Needle nitrogen (mean $\pm$ SE) **a** by genus and CO₂ treatments, **b** by genus and soil moisture treatments, **c** by pine species and CO₂ treatments, and **d** by spruce species and CO₂ treatments. Species with

no matching letters are significantly different using Tukey mean separation test ($P = 0.05$). Species abbreviations are found in Fig. 1 caption

spruces greater than pines (not shown). Covariate analysis of TCC in relation to Mg using the same mean TCC values and mean Mg as a covariate, and testing for genus showed no genus $\times$ Mg interaction ($P = 0.581$), but significant genus ($P < 0.001$) and Mg ($P < 0.001$) effects. The result was a positive TCC response for both pines and spruces to Mg, with spruces greater than pines (Fig. 7b).

Covariate analyses of mean photosynthetic quantum efficiency in relation to mean TCC using TCC as a covariate and testing for genus showed no genus $\times$ TCC interaction ($P = 0.864$), but significant genus ($P = 0.062$) and TCC ($P < 0.054$) effects. The result was a positive quantum efficiency response for both pines and spruces to TCC, with pines responding to a greater extent than spruces (Fig. 8a). Covariate analyses of photosynthetic light convexity in relation to TCC, again using 32 treatment $\times$ species means, and using TCC as a covariate and testing for genus showed no genus $\times$ TCC interaction ($P = 0.813$), but a significant genus ($P = 0.008$) and TCC ($P = 0.028$) effects. The result was a positive photosynthetic light convexity response for both pines and spruces to TCC, with a greater response in pines than spruces (Fig. 8b).

Discussion

Needle N

Was there a pot size limitation on root biomass, total biomass, N uptake or TCC? There was no pot size limitation evident for spruces (Fig. 6b and d). For pines, there appears to be one for TCC but not for N (Fig. 6a and c). This has to be taken in the context of the totality of results. First, the largest species, PP, had the greatest species total biomass response to eCO₂ at 59%, almost twice the total biomass response of the other three pines and four times the average spruce response (Major and Mosseler 2019). Second, in response to eCO₂, PP had the greatest increase in root mass (65%) This was greater than the aboveground biomass increase of 58%. Thus, there is no evidence of pot size limitation on growth above- or belowground. Third, root-bound seedlings reduce carbohydrate sink demand for root growth; however, net photosynthesis and light response traits did not decline in PP, but, in fact, increased the most in response to eCO₂ due to the greater biomass sink (Major et al. 2018;

Fig. 5 Needle phosphorus (mean $\pm$ SE) **a** by genus and CO₂ treatments, **b** by genus and soil moisture treatments, **c** by pine species and CO₂ treatments, and **d** by spruce species and CO₂ treatments. Species

with no matching letters are significantly different using Tukey mean separation test ($P = 0.05$). Species abbreviations are found in Fig. 1 caption

Major and Mosseler 2020). Fourth, 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” (Major and Mosseler 2019). There appears to be a negative effect of total biomass on TCC; however, photosynthesis and biomass are direct measures of source and sink metrics, respectively, and neither had pot size limitations. So the negative correlation in this instance is not necessarily causation. Therefore, what could be driving this relationship? PP was the largest species with the lowest TCC and this could be driving this correlation. However, TCC was related to needle N and needle N is not negatively affected by biomass size. Needle N is a species-driven effect, evident by PP and RP having similar needle N while being at the opposite ends of the biomass size scale, RP was the smallest pine and the second smallest of the eight species, whereas PP was overall the largest species.

Needle TKN with LECO N gave very similar results, judging by the strong correlation and with both methods having significant effects of genus, species and CO₂. The one difference between the two methods was a significant CO₂ $\times$ water interaction for the LECO N, which was a magnitude effect, not a rank change. Thus, there was not much difference with respect to plant response as a result of this difference. Total Kjeldahl N digestion has always been the gold standard by which other N measurement methods were judged. The Dumas combustion procedure using LECO’s CNS analyzer has also given N values comparable to TKN for soil and plant products (Etheridge et al. 1998). As in our case, LECO N results are often slightly greater than TKN due to TKN difficulty in capturing some organic N (Munoz-Huerta et al. 2013).

Fig. 6 Relationship between needle nitrogen to total biomass by CO_2 treatments for **a** pines, and **b** spruces. Relationship between total chlorophyll to total biomass by CO_2 treatments for **c** pines and **d** spruces

Chlorophyll traits

Similar to our findings, Croser et al. (2001) found that spruces had greater TCC than pines: specifically, WS and BS TCC were greater than JP TCC. Minocha et al. (2009) found that NS, but not RS, had greater TCC than RP. In contrast, comparing *Pinus sylvestris* and *Picea obovata*, there were no significant differences in TCC between the species (Suvorova et al. 2011). In response to increasing CO_2 concentrations (10 atmospheric CO_2 treatments) TCC declined for both *P. sylvestris* and *Betula pendula*, but less severely for *P. sylvestris* (Juurola 2003). Tissue et al. () reported a 24% decrease in *Pinus ponderosa* TCC in response to eCO_2 , resulting in a decreased maximum rate of carboxylation and thus photosynthesis. Photosynthetic traits significantly downregulated in *Pinus sylvestris* after one year in

response to eCO_2 treatments, while TCC also decreased but not significantly (Jach and Ceulemans 2000). In our study, spruces downregulated TCC more than pines in response to eCO_2 . Photosynthetic traits also followed the same trend with maximum rates of carboxylation downregulated by 25.2 and 12.6% and maximum assimilation downregulated 23.4 and 8.5% in response to eCO_2 , for spruces and pines, respectively (Major et al. 2018). The TCC and needle N downregulation in response to eCO_2 is a response to a greater supply of a resource (CO_2). More on why this might appear later in relationship to other traits section.

CHL $a:b$ ratio declined more in spruces than pines in response to eCO_2 due to spruces having a greater decline in CHL a . Similar to our findings, Bigras and Bertrand (2006) reported a decrease in CHL $a:b$ ratio for black spruce in response to eCO_2 . Total chlorophyll to carotenoid ratio

Fig. 7 Relationship between total chlorophyll concentration and **a** needle nitrogen, and **b** needle magnesium using 2 CO₂ × 2 water treatment means for all eight species

(TCC:CAR) increased in response to eCO₂ due to the greater relative decline in CAR in response to eCO₂. In examining BS, RS and their hybrids, Major et al. (2007a) also found that TCC:CAR ratio increased in response to eCO₂ and this was also due to the greater relative decline in CAR under eCO₂.

Wallin et al. (2002) found NS had a 15% decrease in TCC in response to DRT. Ditmarova et al. (2009) found NS had no change in TCC under mild DRT but a significant, 25% decrease in TCC in response to severe DRT. Comparing *Pinus sylvestris* and *Picea obovata* responses to a dry year compared to a wet year, the spruce had a greater decline in TCC than the pine (Suvorova et al. 2011). Elevated CO₂ did not mitigate the drought effect for TCC as has been found in other studies (Sekhar et al. 2017; Lahive et al. 2021). In our study, TCC:CAR ratio increased in response to DRT due to the greater relative decline in CAR under DRT. By using their numbers, Yang et al. (2007) also found that for *Picea asperata* TCC:CAR ratio increased in response to DRT and

Fig. 8 Relationship between **a** photosynthetic quantum efficiency and **b** photosynthetic light convexity and total chlorophyll concentration using 2 CO₂ × 2 water treatment means for all eight species

this was due to the greater relative decline in CAR under DRT.

Other needle macronutrients

In an assessment of four reviews of foliar nutrient thresholds using a database of almost 3100 records for four European tree species including *Pinus sylvestris* and *Picea abies*, Mellert and Gottlein (2012) found that similar to our results, the “normal range” for P, K, Ca, and to some extent Mg were greater for Norway spruce than Scots pine. Unlike our findings, Mellert and Gottlein (2012) found that the normal range for N was similar for both species: between 1.40 and 1.83% for Scots pine and 1.4 and 1.75% for NS. Similar to our findings, some of the largest genus differences in needle macronutrients reported by Mellert and Gottlein (2012) were needle Ca and to a lesser extent needle P. Their ‘normal’ mean for needle Ca for NS and Scots pine were 5 and

3 mg/g, respectively; whereas our average was $2.2\times$ greater needle Ca for spruces than pines. For needle P the mean for NS and Scots pine were 1.85 and 1.60 mg/g, respectively; our genus difference was 50% greater needle P for spruces than pines.

Similar to our findings, specific needle nutrient analyses of *Pinus sylvestris* showed that N significantly declined in response to eCO_2 while K, Ca and Mg remained the same (Luomala et al. 2005). Our needle P significantly increased in eCO_2 while Luomala et al. (2005) found that needle P declined. Similar to our findings, needle K and Mg remained largely unaffected in eCO_2 (Utriainen et al. 2000). Our results showed that under DRT, macronutrients were either reduced or remained the same for both pines and spruces. This is consistent with feedback due to water shortage, which leads to stomatal regulation and reduction in transpiration-driven water flow, which is required for nutrient uptake (Salazar-Tortosa et al. 2018). In a study examining the impact of water stress on *Pinus palustris* Mill. (longleaf pine), Jose et al. (2003) found that needle N and P significantly declined and that Mg was unchanged, similar to our results. They also found that needle K and Ca declined in response to DRT whereas our values did not.

Components of variance

By examining not just statistical significance but also the variance component, we can determine the relative importance of genetic (genus and species) and environmental (CO_2 and water) factors in trait control. Chlorophyll and carotenoids are under strong genetic control with between 42 and 55% of the total variation attributable to the effects of genus and species. The environmental effect for chlorophyll and carotenoids accounted for a lower but significant 4–8% of the total variation. There was an even larger genetic control of macronutrients and carbon, accounting for 48–78% of the total variation. There was one lower exception, K, for which genetic components accounted for a lower 33% of the total variation.

The genus effect often accounted for the largest amount of the total variation, particularly for chlorophyll pigment, which accounted for between 24 and 42%, and macronutrients TKN, C, P and Ca, accounting for between 27 and 69%. Characteristically spruces are found on well-developed, wet organic (e.g. moss) sites or well-drained, moist organic sites (Blum 1990; Nienstaedt and Zasada 1990; Viereck and Johnston 1990). Whereas pines are characteristically found on coarser-textured, well drained soils with low to very low fertility (Little and Garrett 1990; Rodoff 1990; Rudolph and Laidly 1990; Wendel and Smith 1990). Inherent site type adaptations undoubtedly influenced the large genus differences in chlorophyll pigment and macronutrient concentrations detected in our common garden experiment.

Species often accounted for the next greatest component of the total variation. Overall, species variation is largely due to the greater variation detected among pines, notably WP, which always had greater chlorophyll and macronutrient concentrations than RP, JP and PP. White pine belongs to the subgenus *Strobus*, containing the five-needle pines, also known as “soft” pines. The other pines studied (RP, JP and PP) belong to the subgenus *Pinus*, and have either two or three needles per needle bundle and are known as “hard” pines. The hard pines are characteristically found on coarser-textured sandy and gravelly sites or shallower, less fertile soils (Little and Garrett 1990; Rodoff 1990; Rudolph and Laidly 1990). In comparison, WP are often found on more fertile sandy, fine sandy loam or silt loam soils (Wendel and Smith 1990). There is certainly site type overlap between these subgenus groups as WP is often associated with RP and even PP in some instances (Little and Garrett 1990; Rodoff 1990; Wendel and Smith 1990).

Interestingly, in spruces there was little differentiation among spruce species for chlorophyll pigment and nutrient traits; only minor chlorophyll pigment and nutrient differences were detected, with species rank changes depending on the trait. Generally, pines are early-successional species with WP reaching mid-successional status in some forest types where WP regenerates itself under the more open established forest canopy. Nevertheless, the large differentiation we see for chlorophyll and nutrient traits are important in pine species distinguishing characteristics for site type adaptation. What differentiates the spruce species ecologically appears more related to their successional ecology. Red spruce is a late-successional species (Blum 1990), WS mid- to late-successional species depending on the ecosystem (Nienstaedt and Zasada 1990), BS and NS early- to mid-successional species (Viereck and Johnston 1990).

Relationships to other traits

Similar to our findings, Mandre et al. (2010) observed that Scots pine had positive TCC relationships to needle N and Mg. Each chlorophyll *a* and *b* molecule requires four N and one Mg; whereas, carotenoids do not require N or Mg. In our study, the greater slope of TCC to needle N than Mg probably reflects the four to one ratio difference in the chlorophyll requirement. An experiment with a fertilized N $\times$ CO_2 factorial using *Betula platyphylla*, Zhao et al. (2010) found that within each N treatment, leaf N decreased in response to eCO_2 , while CHLa, CHLb and TCC increased with increasing N treatments (Zhao et al. 2010). Our TCC downregulation and thus the N downregulation is probably in response to the accumulation of nonstructural carbohydrates in an eCO_2 environment and is usually associated and is commonly interpreted as evidence of a lack of sink activity (Ainsworth et al. 2004)

This supports the theory of sink regulation (biomass growth or lack thereof) of photosynthetic traits, in this case TCC (Ainsworth et al. 2004; Major and Mosseler 2020).

For QE, the initial slope of the light response curve is greater for pines per unit TCC, and QE increases with increased TCC concentration. Quantum efficiency is also positively related to needle N (Major and Mosseler 2020) and thus illustrates how these traits are tightly related. Needle N is an essential component of chlorophyll and photosynthetic proteins, which account for approximately 75% of the total leaf N (Evans 1989). Similar to our findings for chlorophyll pigments, there was a significant genus $\times$ CO₂ interaction for which the pine QE did not downregulate in response to eCO₂ but the spruce QE significantly downregulated in response to eCO₂ (Major and Mosseler 2020). Furthermore, by examining QE change (%) from aCO₂ to eCO₂ in relation to biomass stimulation (%), there was a significant positive relationship to biomass growth stimulation (%) across the eight species. Thus, the greater the biomass stimulation to eCO₂, the less the photosynthetic traits downregulated. This again supports the theory of sink regulation of photosynthetic traits. Another important LRC trait, assimilation at light saturation, declined for both pines and spruces in response to eCO₂ (Major and Mosseler 2020), corresponding to needle N declining for both genera in response to eCO₂.

Spruces have greater TCC than pines and covariate analysis showed that when controlling for needle N, spruce had greater TCC. However controlling for needle N (Major and Mosseler 2020) and TCC (this manuscript), pines had greater QE than spruces. Thus, it would appear that pines are more efficient for each additional unit of light for a given unit of N or TCC up to the beginning of the light response curvature. Spruces have greater assimilation at light saturation than pines, which is consistent with their greater TCC. Photosynthetic light convexity represents the ratio of the physical to the total resistances to CO₂ diffusion, describing the sharpness of the transition from light limitation to light saturation (de Lobo et al. 2013). Pines had greater convexity or curve sharpness compared to spruces, and per unit of chlorophyll. This may be because the pines had a greater SNA than spruces, which means thinner needles (Major and Mosseler 2020). Using shade treatments with *Pinus nigra*, (Gomez-Aparicio et al. 2006) found that increased shade increased SNA, resulting in greater convexity/curve sharpness.

Application

Records show that for 2018 and 2019, spruces (WS, RS, BS and NS) made up 99.4 and 97.3% of tree planting programs

in the province of Nova Scotia (Pers. Comm. Jane Kent, NS Dept. of Lands and Forestry) and New Brunswick (Pers. Comm., Andrew Conn, NB Department of Natural Resources), respectively, with the balance of tree planting being in pines. This is a very heavy bet on spruces for harvest rotations that would occur near the end of this century. Interestingly, pollen records from the Holocene in an environment of comparatively low atmospheric CO₂ (280 ppm) from various northern New England locations, showed that spruces, particularly RS, WS and some BS, have been abundant for the last 1000 years, a period of relatively cool and wet climatic conditions (Schauffler and Jacobson 2002; Lindbladh et al. 2003). However, for the previous 1000–7000 years, when it was warmer and drier during the Holocene maximum, the pollen record indicated more pines than spruces on the landscape, with WP having a particularly strong presence on the landscape. With anticipated increases in CO₂, the atmosphere is predicted to become warmer, and hence potentially drier (as in the recent geological past), with an associated increase in fire frequency. When taken together with the historical pollen record, the superior relative performance of pines versus spruces in biomass production (Major and Mosseler 2019), and the lower acclimation or downregulation of pines in response to eCO₂ and DRT, suggests that a shift from dominantly favoring spruces in tree planting programs to at least a greater admixture of pines in forest management planning in northeastern North America may be warranted.

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

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Declarations

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

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