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Effects of CO_2 and Soil Moisture Treatments on Morphological and Allometric Trait Variation in Coppiced Seedlings: A Study of Four Early-Successional Deciduous Species

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Abstract: Atmospheric CO_2 levels have been increasing, and likewise, increasing drought events have been following increasing temperatures. There is very little literature on the effects of climate change factors on early-successional deciduous species used for ecological restoration. Thus, morphological and allometric variation in four coppiced early-successional deciduous species was examined in response to a 2 × 2 factorial of ambient CO_2 (aCO_2, 400 ppm) and elevated CO_2 (eCO_2, 800 ppm), as well as well-watered and drought treatments with 15%–20% and 5%–10% volumetric moisture content, respectively, grown in sandy soil with low soil nitrogen (N) under greenhouse conditions. The four species examined were as follows: green alder (*Alnus viridis* subsp. *crispa* (Ait.) Turrill), speckled alder (*A. incana* subsp. *rugosa* (Du Roi) R.T. Clausen), gray birch (*Betula populifolia* (Marshall)), and white birch (*B. papyrifera* (Marshall)), and all are from the same phylogenetic family, *Betulaceae*. Genus differences in morphological and growth traits were large, especially in response to the environmental treatments used. Alders upregulated all growth traits under eCO_2 because of the strong coppicing sink effect and the additional foliar N provided by the actinorhizal ability of the genus, whereas birches remained the same or slightly decreased under eCO_2. As a result, alders have a significantly greater foliar N than birches, with 2.8 and 1.0%, respectively. All species reduced growth under drought, and green alder had the greatest stem dry mass growth, followed by speckled alder and then the birches. Under drought, eCO_2 not only mitigated the alder drought dry mass but, in fact, doubled the stem dm, whereas eCO_2 only just mitigated the birches drought response. When corrected for size using stem height, alders allocated more to stem and leaf and less to root dry mass than birches. Atmospheric CO_2 and soil moisture treatments changed organ biomass allocation. The tallest stem height was the best predictor of total (above and below) dry mass. With increasing atmospheric CO_2, particularly on low nutrient sites, the results show alders are capable of sequestering far more carbon than birches. In addition, with more atmospheric CO_2, alders can mitigate against drought conditions better compared to birches.

Keywords: alders; birches; elevated CO_2; drought; allometry; dry mass; foliar N; soil moisture treatments

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1. Introduction

The Earth's climate is changing, and there will be many implications for natural ecosystems if these changes continue the same trajectory. Levels of atmospheric carbon dioxide (CO_2) have been increasing [1], and drought events are expected to increase in frequency and severity [2]. Both environmental factors have been shown, in most cases, to affect plant physiology and subsequent morphology [3]. The differences in species-specific responses to these environmental factors will likely result in new interspecific competitions and ensuing changes to plant community composition within our forests and natural areas.

In this study, we examined growth and allometry among four early-successional, deciduous tree species utilized in land restoration efforts. These species include green alder (*Alnus viridis* subsp. *crispa* (Ait.) Turrill), speckled alder (*A. incana* subsp. *rugosa* (Du

Roi) R.T. Clausen), gray birch (*Betula populifolia* (Marshall)), and white birch (*B. papyrifera* (Marshall)), and all are from the same phylogenetic family, Betulaceae. Green alder and speckled alder are deciduous shrubs native to northeastern North America. First, these species were chosen as they are native North American species that are used for ecological restoration [4,5]. Second, the four species used are found in surrounding areas where we conduct land restoration research. Third, very little information is published using these species, and being phylogenetically similar to each other, we can explore differences between genera and among species better. Lastly, spatial limitations limit how many species we can study at any one time, as one of our objectives is to utilize various seed sources (provenances) to better represent the species while maintaining suitable replication. Alders are characterized by short lifespans, early successional nature, low shade tolerance, exhibit rapid early growth, and are capable of coppicing [6]. Additionally, they are capable of actinorhizal symbiosis, forming mutualistic relationships with *Frankia alni* bacteria, which enables them to fix atmospheric nitrogen (N) through root nodules [7]. While green alder can grow in moist soils, it is competitively better adapted to drier upland sites compared to speckled alder, which is more restricted to wetter areas such as stream banks [6,8,9]. Both species are tolerant of nutrient-poor soils [8,9]. Gray birch and white birch are deciduous trees that are intolerant of shade, exhibit fast early growth, and are early-successional species [10,11]. Gray birch primarily occurs in the eastern region of North America, possesses a relatively short lifespan of approximately 50 years, and is commonly found in sandy or gravelly soils. In contrast, white birch is widely distributed (transcontinental) and can survive for up to 120 years [6]. It can be found growing in a diverse range of soil types.

Elevated carbon dioxide (eCO_2) has a notable impact on tree growth and morphology, often leading to increased dry mass accumulation [3,12–14]. However, responses to eCO_2 vary significantly across different tree species, and assimilation downregulation (A_{dr}) has emerged as an often-observed phenomenon whereby plants reduce their photosynthetic processes, consequently impeding further growth responses. The underlying mechanisms driving this effect are not entirely understood, although it has been proposed that nutrient limitations, primarily nitrogen, and the intricate dynamics of source–sink relationships play pivotal roles in determining a plant's response to eCO_2 [10,11]. One study found in three coppiced species of poplar trees that eCO_2 further shifted allometry to enhance above-ground growth and decrease below-ground growth [14]. Coppicing has independently been observed to influence allometry in a similar way, increasing the shoot-to-root ratio [15]. Additionally, soil moisture availability significantly influences dry mass growth, allocation, and species distribution, as plants exhibit varying tolerances to different moisture levels. Low soil moisture decreasing shoot-to-root ratio has previously been found in the literature for *Alnus*, likely being an environmental stress strategy to lower transpiration and thus water loss [16]. Although the interactive effects between CO_2 and soil moisture lack comprehensive understanding, and limited literature exists on the subject, some experiments have indicated that exposure to eCO_2 sometimes enhances water-use efficiency (iWUE) [17,18] and thus can mitigate drought effects. This relationship is of importance as both environmental factors, CO_2 levels and precipitation, are projected to undergo substantial changes in the future. Understanding morphological and allometric responses to these changes will further our understanding of a species' value in restoration, reforestation, and carbon sequestration.

The goal of this study was to examine and compare the growth and allometry of four North American alder and birch species under the interactive effects of CO_2 and soil moisture treatments (SMT). We examined growth (dry mass production and height), foliar N, and allometric (stem, leaf, and root) relationships of the four species under different atmospheric CO_2 levels and SMT. We hypothesized that adaptive genetic differences occur at a genus, species, and possibly provenance level regarding dry mass growth and allocation in response to a 2 $\times$ 2 factorial of atmospheric CO_2 and SMT. To test this hypothesis, we (1) quantified the variation in morphological growth traits as well as foliar N for green alder, speckled alder, gray birch, and white birch; (2) examined their morphological responses and interactions to CO_2 and SMT; and (3) examined species and genus dry mass allometry corrected for seedling size, using greatest stem height, in response to CO_2 and SMT.

2. Material and Methods

Stems were removed in October 2021, and the remaining stumps were left to re-grow via coppicing for this experiment, which took place from 20 April to 21 September 2022.

2.1. Plant Material, Growing Conditions, and Treatment Delivery: 2021

Three to five seed sources (provenances) for each of the four deciduous species, green alder, speckled alder, white birch, and gray birch, were used in this study (Table 1. Seed lots were taken from natural populations across three provinces of Canada, including New Brunswick, Nova Scotia, and Prince Edward Island. Please see [19] for a description of growing and cold-storing seedlings prior to planting in the experiment.

Table 1. Species and provenance seed sources (coordinates and elevation) and replicates used of *Betula* and *Alnus* species used in the 2022 CO_2 × soil moisture experiment.

Species	Provenances	* Reps. Per Chamber	Latitude	Longitude	Elevation (m)
Gray Birch (*Betula populifolia* (Marshall))	Afton Road, PEI, CA	4	46.383	−62.933	25
	Bishop Mountain, NS, CA	4	45.033	−64.983	175
	Coles Island, NB, CA	4	45.900	−65.717	30
	Mount Albian, PEI, CA	4	46.233	−62.950	40
	Newmarket, NB, CA	4	45.805	−66.956	130
White Birch (*Betula papyrifera* (Marshall))	Fredericton, NB, CA	4	45.962	−66.627	28
	Lincoln, NB, CA	4	45.833	−66.600	25
	Mount Pleasant, NB, CA	4	45.417	−66.833	250
	Oromocto Lake, NB, CA	4	45.700	−66.650	40
	Wayerton, NB, CA	4	47.217	−65.933	300
Green alder (*Alnus viridis* subsp. *Crispa* (Ait). Turrill)	Dipper Harbour West, NB, CA	4	45.100	−66.433	10
	Jouriman Island, NB, CA	4	46.150	−63.833	10
	Indian Falls Depot, NB, CA	4	47.383	−66.333	225
	Lower Prince William, NB, CA	4	45.867	−67.000	20
	West Quaco, NB, CA	4	45.333	−65.533	65
Speckled alder (*Alnus incana* subsp. *rugosa* (Du Roi) R.T Clausen))	McDougall Lake, NB, CA	4	45.333	−66.733	80
	Shediac, NB, CA	4	46.233	−64.600	15
	Bulk: Millvale, PE, CA	12	46.400	−63.400	70
	Bulk: Valleyfield, PE, CA		46.130	−62.720	45
	Bulk: View Lake, NS, CA		44.530	−65.350	168

* Abbreviation: "Reps." = seedling replicates.

Four replicates per provenance were randomly planted in each of eight chambers in individual fabric, root-control bags that measure 30 cm in diameter and 23 cm in height (Smart Pot PRO 5 Gallon, High Caliper Growing Systems, Oklahoma City, OK, USA) filled with a sand medium. Bulk soil samples were taken on 31 August 2022 by removing the top 1 cm of soil (to remove any incidental organic matter) and taking a sample of 500 mL to a depth of ~8 cm. Further depth was not taken to preserve the root system of each plant. The samples were then sent for nutrient and texture analysis at the Laboratory for Forest Soils and Environmental Quality, University of New Brunswick, Fredericton. The soil texture properties were, on average, 1.8% clay, 7.8% silt, and 90.4% sand, with no significant nutrient or pH differences between blocks (Table 2). Please see [19] for a description of the experimental chambers used and environmental monitoring.

Table 2. Soil properties (mean ± SE, n = 16) during the 2022 CO_2 × soil moisture treatments.

Organic Matter (%)	Carbon (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (meq/100 g)	Calcium (meq/100 g)	Magnesium (meq/100 g)
0.568 ± 0.049	2.775 ± 0.213	0.119 ± 0.002	6.375 ± 1.002	0.049 ± 0.007	0.568 ± 0.049	0.104 ± 0.01
pH	C: N Ratio	Sand (%)	Silt (%)	Clay (%)		
6.045 ± 0.079	23.389 ± 1.658	90.4 ± 3.09	7.8 ± 2.91	1.8 ± 0.75		

The eight chambers were housed across two separate greenhouses (considered blocks) located at the Canadian Forest Service—Atlantic Forestry Center (CFS-AFC) in Fredericton, NB, Canada (45°52′ N, 66°31′ W). The four main treatment types (a 2 × 2 factorial of two CO_2 levels and two irrigation levels) were randomly assigned in each greenhouse chamber. The two CO_2 treatments were ambient (aCO_2—No CO_2 added, ~400 ppm) and elevated (eCO_2—CO_2 regulated at ~800 ppm). Elevated CO_2 chambers were regulated through the opening or closing of solenoid valves to control CO_2 being delivered via the air stream entering the chamber. Irrigation treatments were maintained at well-watered (WW—at ~15%–20% volumetric moisture content (VMC)) and drought levels (DRT—at ~5%–10% VMC).

The experimental design is summarized as follows: there were four species × five provenances (seed sources, three for speckled alder) × two CO_2 treatments × two irrigation treatments × two chambers (blocks) × four random replicates in each chamber for each seed source (see Table 1).

2.2. Plant Material Growing Conditions and Treatment Delivery: 2022

On 20 April 2022, blackout curtains were opened, and CO_2 treatment resumed on 22 April. Coppiced plants were then allowed to grow. Soil moisture treatment started on 13 May. Starter fertilizer (Plant-Prod "Forestry Starter" 11:41:8, 287 g/25 L, +250 mL of MgNiFeCa) was supplied to plants at half-strength to all treatment combinations in equal proportions via the dripper system (30 min = 1 L) on 17 May (5 min), 25 May (5 min), 10 June (5 min), and 17 June (5 min), 2022. Grower fertilizer (Plant-Prod "Forestry Special" 20:8:20, 312 g/25 L, +37.5 g of Plant-Prod "Micronutrients") was supplied to plants at half-strength to all treatment combinations in equal proportions via the dripper system (30 min = 1 L) on 21 June (5 min), 8 July (10 min), and July (10 min), 2022.

2.3. Growth Assessment and Foliar Nitrogen Concentrations

Foliar N samples were taken in September 2022 by harvesting two leaves from one individual of four provenances of each species (3 for speckled alder) from each growth chamber (n = 120). The leaves harvested were mature and taken from the top one-third of each plant. Total foliar N was determined using an elemental analyzer (CNS-2000, LECO Corporation, St. Joseph, MI, USA) service provided by the Laboratory for Forest Soils and Environmental Quality at the University of New Brunswick.

The above-ground dry mass was harvested in October 2022 by cutting each plant at the base, flush with the soil level. Heights and diameters were taken for the tallest stems. The stems and branches were then stripped of all leaves which were placed in labeled paper bags alongside the stems, and dried in ovens at 65 °C for a minimum of 72 h. Roots were harvested shortly afterward by pouring out the soil from each bag, retrieving the root system, and washing all remaining soil off before placing the roots in a labeled paper bag and drying under the same procedure as the stems and leaves. Once dry, leaf, stem, and root mass were weighed separately using a precision scale capable of measuring to the nearest 0.01g (accu-4102, Fisher Scientific, Waltham, MA, USA).

2.4. Statistical Analysis

This experiment utilized a randomized block design. Genus, species, provenance, CO_2 treatment, and SMT were considered fixed effects. The growth chamber (block) and replicates were random factors. We utilized a general linear model (GLM) to conduct mixed-effects analyses of variance (ANOVA) with nested factors, using Systat version No.13.00.05 (San Jose, CA, USA). The first model was used for height and all dry mass measurements above-to-below-ground ratio and stem number data.

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

Y_{ijklmn} denotes the dependent seedling of the *i*th greenhouse (block), of the *j*th genus, of the *k*th CO_2 treatment, of the *l*th soil moisture treatment, of the *m*th species, or *o*th provenance, of seedling n, with μ being the overall mean. B_i refers to the effect of the *i*th greenhouse (i = 1, 2), G_j is the effect of the *j*th genus (j = 1, 2), C_k is the effect of the *k*th CO_2 treatment (k = 1, 2). W_l refers to the effect of *l*th soil moisture treatment (l = 1, 2). GC_{jk} is the interaction effect between genus *j* and CO_2 treatment *k*. GW_{jl} is the interaction effect between genus *j* with soil moisture treatment *l*. $S_{m(j)}$ is the effect of species *m* (m = 1, . . ., 4) nested in genus *j*. $SC_{m(j)k}$ is the interactive effect of species *m* nested in genus *j* with CO_2 treatment *k*. $SW_{m(j)l}$ is the interactive effect of species *m* nested in genus *j* with soil moisture treatment *l*. $SCW_{m(j)kl}$ is the three-way interactive effect of species *m* nested in genus *j* with soil CO_2 treatment *k* and soil moisture treatment *l*. $P_{o(m)}$ is the effect of provenance *o* (o = 1, . . ., 3 or 1, . . ., 5 depending on species) nested in species *m*. $PC_{o(m)k}$ is the interactive effect of provenance *o* nested in species *m* with CO_2 treatment *k*. $PW_{p(m)l}$ is the interactive effect of provenance *o* nested in species *m* with soil moisture treatment *l*. $PCW_{o(m)kl}$ is the three-way interactive effect of provenance *o* nested in species *m* with soil CO_2 treatment *k* and soil moisture treatment *l*. Lastly, $e_{ijklmno}$ is the random error component incorporating interactions with the growth chamber factor and the variation among seedlings.

Model (2) was used for foliar N concentrations. This model is the same as Model (1) with provenance removed, as the dataset was smaller:

$$Y_{ijklmn} = \mu + B_i + G_j + C_k + W_l + GC_{jk} + GW_{jl} + CW_{kl} + GCW_{jkl} + S_{m(j)} + SC_{m(j)k} + SW_{m(j)l} + SCW_{m(j)kl} + e_{ijklmn} \quad (2)$$

Y_{ijklmn} denotes the dependent seedling of the *i*th growth chamber (block), of the jth genus, of the kth CO_2 treatment, of the lth soil moisture treatment, of the mth species, of seedling n, with μ being the total mean. B_i refers to the effect of the *i*th growth chamber (i = 1, 2), G_j is the effect of the *j*th genus (j = 1, 2), C_k is the effect of the *k*th CO_2 treatment (k = 1, 2). W_l refers to the effect of *l*th soil moisture treatment (l = 1, 2). GC_{jk} is the interaction effect between genus *j* and CO_2 treatment *k*. GW_{jl} is the interaction effect between genus *j* with soil moisture treatment *l*. GCW_{jkl} is the three-way interactive effect of genus *j* with CO_2 treatment *k*, and soil moisture treatment *l* $S_{m(j)}$ is the effect of species *m* (m = 1. . .4) nested in genus *j*. $SC_{m(j)k}$ is the interactive effect of species *m* nested in genus *j* with CO_2 treatment *k*. $SW_{m(j)l}$ is the interactive effect of species *m* nested in genus *j* with soil moisture treatment *l*. $SCW_{m(j)kl}$ is the three-way interactive effect of species *m* nested in genus *j* with CO_2 treatment *k* and soil moisture treatment *l*. Lastly, e_{ijklmn} is the random error component incorporating interactions with the growth chamber factor and the variation among seedlings. Significant interactions are referred to as either rank change interaction or magnitude effect interaction. A rank change interaction occurs when one effect has a greater mean under one scenario and a lower mean under another scenario. A magnitude effect would refer to when the effect mean is always greater than under both scenarios but of different magnitudes.

Effects were considered statistically significant at the p = 0.05 level, although all p-values are listed for the reader's interpretation. Variance component analysis was conducted for the ANOVA tables using the sums of squares in accordance with methods outlined in "Variance Component Analysis" in [20]. The statistical assumptions of data normality and equal variance were satisfied prior to running either model. The general linear model from Systat No.13.00.05 (Chicago, IL, USA) was used for these analyses, and if the source of variation for species was significant ($p = 0.05$), the Tukey mean separation test was used for post hoc analysis. In analyses where species is referred to, it is nested within the genus, and when provenance is referred to, it is nested within species.

Covariate analysis was used to evaluate the relationships among species organ dry mass allocation (stem, leaf, and roots) changes with size (tallest stem height) and to test if CO_2 treatment, SMT, or species affected the allocation of dm resources. In these analyses, the dependent trait (i.e., stem dry matter allocation (%)) was examined in relation to three sources of variation studied: (1) covariate (i.e., stem height), (2) independent effect (i.e., CO_2 treatment), and (3) independent effect × covariate (i.e., CO_2 treatment × stem height). The analyses were performed based on the following model:

$$Y_{ij} = B_0 + B_{0i} + B_1 X_{ij} + B_{1i} X_{ij} + e_{ij} \tag{3}$$

where Y_{ij} is the dependent trait of the ith species of the jth genus treatment. B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are the treatment-specific coefficients, X_{ij} is the independent variable, and e_{ij} is the error term. Results were considered statistically significant at $p < 0.050$ so that they were different enough to warrant their own allocation line, although individual P values were provided for all traits so that readers could make their own interpretations of significance.

3. Results

3.1. Morphological Results, CO_2 × Soil Moisture

With respect to a number of morphological traits, i.e., stem, leaf, and root, dm had similar ANOVA responses, and thus, we tried to avoid repetition as much as possible but provide the quantitative response results.

For tallest stem height, in order of impact, SMT, genus, species, genus × SMT, CO_2, provenance, genus × CO_2, SMT × CO_2, and genus × SMT × CO_2 were significant, accounting for 34.8, 18.0, 7.6, 5.0, 2.2, 1.5, 1.0, 0.4, and 0.4% of total variation, respectively (Table 3). It is important to note, for ease of interpretation, that for the tallest stem height, two- and three-way interactions involving genus, CO_2, and SMT were all magnitude effects, not rank changes. Thus, the main effects become important, as well as the different magnitude findings. Key results were as follows: First, alders were always significantly taller than birches, with an average stem height of 103.2 cm, compared to birches at 68.9 cm, close to a 50% difference (Figure 1A,B). The average stem height of green alder, speckled alder, gray birch, and white birch were 119.0, 87.3, 73.3, and 64.5 cm, respectively. Second, under well-watered (WW) treatments, stem heights were always greater than DRT with an average of 110 and 62 cm, respectively, an almost 100% difference (Figure 1B). Third, the tallest stem heights were always greater or equal under eCO_2 than aCO_2, with 92 and 80 cm, respectively (Figure 1A). Fourth, the genus × CO_2 interaction was a result of alders positively responding to eCO_2, but birches did not. Fifth, eCO_2 mitigated the DRT treatment for alders but not for birches.

Table 3. Tallest stem height, stem dry mass, and leaf dry mass variance components and ANOVAs, including the source of variation, degrees of freedom (*df*), mean square values (MS), variance components (*VC*), *p*-values, and coefficient of determination (R^2). *p*-values < 0.05 are in bold. Source of variation abbreviations are soil moisture treatments (SMT), provenance (prov.), and species (Spp.).

Source of Variation	*df*	Tallest Stem Height (cm)			Stem Dry Mass (g)			Leaf Dry Mass (g)		
		MS	*VC* (%)	*p*-Value	*MS*	*VC* (%)	*p*-Value	*MS*	*VC* (%)	*p*-Value
Block	1	736.2	0.1	0.174	978.9	0.2	0.058	1641.0	0.5	**<0.001**
Genus	1	120,932.6	18.0	**<0.001**	159,971.2	30.3	**<0.001**	88,603.4	29.5	**<0.001**

Table 3. *Cont.*

Source of Variation	*df*	Tallest Stem Height (cm)			Stem Dry Mass (g)			Leaf Dry Mass (g)		
		MS	*VC* (%)	*p*-Value	*MS*	*VC* (%)	*p*-Value	*MS*	*VC* (%)	*p*-Value
SMT	1	233,178.5	34.8	**<0.001**	95,182.1	18.0	**<0.001**	42,959.9	14.3	**<0.001**
CO_2	1	14,942.0	2.2	**<0.001**	18,155.1	3.4	**<0.001**	15,459.7	5.2	**<0.001**
SMT × CO_2	1	2917.5	0.4	**0.007**	4504.1	0.9	**<0.001**	7560.7	2.5	**<0.001**
Genus × SMT	1	33,538.2	5.0	**<0.001**	64,951.7	12.3	**<0.001**	32,922.7	11.0	**<0.001**
Genus × CO_2	1	6636.8	1.0	**<0.001**	25,385.8	4.8	**<0.001**	18,142.2	6.0	**<0.001**
Genus × SMT × CO_2	1	2630.4	0.4	**0.01**	10,135.7	1.9	**<0.001**	9637.4	3.2	**<0.001**
Species (Genus)	2	25,529.6	7.6	**<0.001**	1719.3	0.7	**0.002**	3830.5	2.6	**<0.001**
Species (Genus) × SMT	2	46.1	0.0	0.891	5.9	0.0	0.978	240.4	0.2	0.162
Species (Genus) × CO_2	2	569.9	0.2	0.239	82.6	0.0	0.737	203.4	0.1	0.215
Species (Genus) × SMT × CO_2	2	30.2	0.0	0.927	188.6	0.1	0.498	68.8	0.0	0.594
Prov. (Spp.)	14	708.7	1.5	**0.038**	661.8	1.8	**0.002**	486.4	2.3	**<0.001**
Prov. (Spp.) × SMT	14	396.6	0.8	0.454	580.6	1.5	**0.009**	332.0	1.5	**0.002**
Prov. (Spp.) × CO_2	14	152.0	0.3	0.98	138.0	0.4	0.927	130.0	0.6	0.465
Prov. (Spp.) × SMT × CO_2	14	431.5	0.9	0.368	312.9	0.8	0.305	138.2	0.6	0.403
Error	450	397.4	26.7		270.1	23.0		131.7	19.8	
R^2			0.767			0.782			0.809	

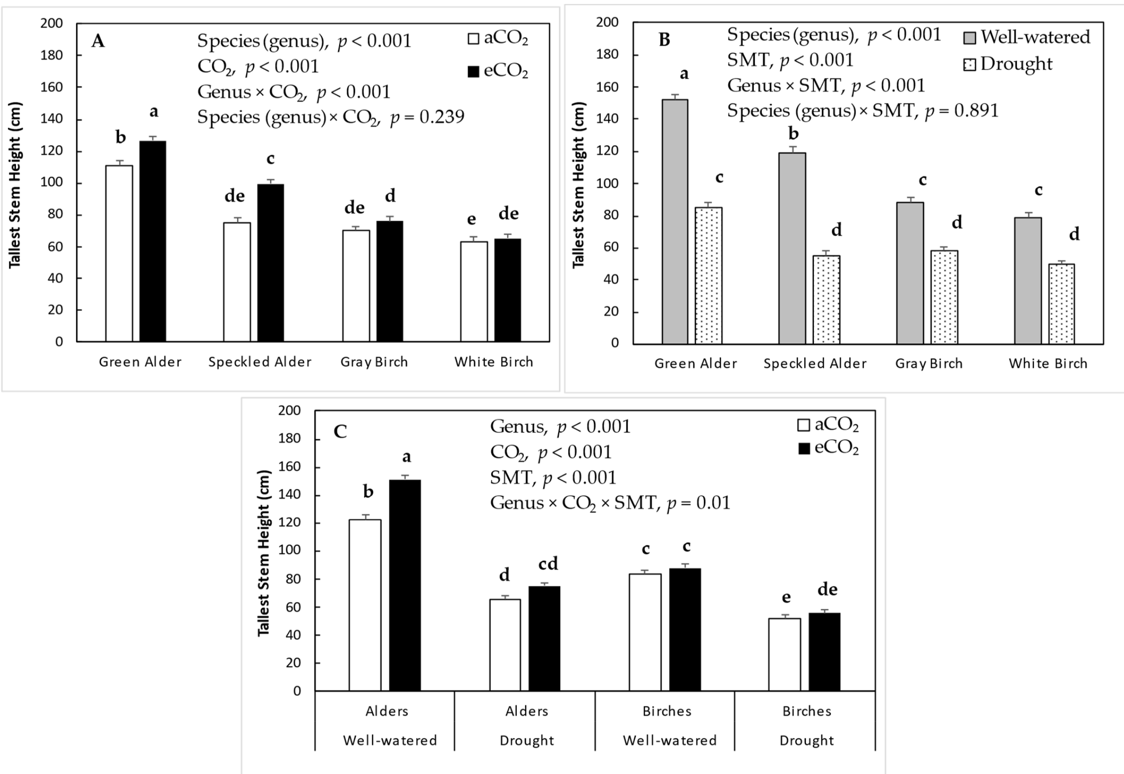

Figure 1. Tallest stem height (mean ± *SE*, *n*~65): (**A**) species and CO_2 treatments (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) species and soil moisture treatments (SMT); (**C**) genus, SMT and CO_2 treatments. Figure-relevant *p*-values are presented in each figure from the full ANOVA model (Table 3). Post hoc Tukey's mean separation tests were performed on the species × treatment interactions (**A**,**B**) and on the three-way genus × CO_2 × SMT interaction (**C**). Bars within each figure with the same letter were not significantly different ($p = 0.050$). Total sample size: $n = 523$.

An additional significant source of variation for stem dm to that of tallest stem height was provenance × SMT and some change in order. Significant sources of variation and order of impact are as follows: genus, SMT, genus × SMT, genus × CO_2, CO_2, genus × SMT × CO_2, provenance, provenance × SMT, and SMT × CO_2, accounting for 30.3, 18.0, 12.3, 4.8, 3.4, 1.9, 1.8, 1.5, 0.9, and 0.7% of total variation, respectively (Table 3).

The key results are as follows: first, stem dm two- and three-way interactions involving genus, CO_2, and SMT were all magnitude effects but one small rank change. Under WW treatment, birches had less stem dm under eCO_2 than aCO_2 (Figure 2C). Second, alders always had greater stem mass than birches, with an average stem dm of 48.1 and 8.7 g, respectively, a 550% difference (Figure 2A,B). The average stem dm of green alder, speckled alder, gray birch, and white birch were 52.4, 43.8, 9.2, and 8.3 g, respectively. Third, under WW treatments, stem mass was always greater than DRT with, on average, 43.6 and 13.2 g, respectively, a 330% difference (Figure 2B). Fourth, under eCO_2, stem mass was always greater, with the exception noted above, than aCO_2, with an average of 35.1 and 21.8 g, respectively (Figure 2A). Fifth, the genus $\times$ CO_2 interaction was a result of alders doubling stem dm in response to eCO_2, but birches did not respond. Sixth, eCO_2 mitigated DRT for alders with 14.2 g under aCO_2 and 26.6 g under eCO_2, but no mitigation for birches (Figure 2C). Seventh, the significant provenance variation was due to alders; the five gray birch provenances were very uniform and had a maximum and minimum stem dm of 29.8 and 26.7 g (not shown). The five white birch provenances were even more uniform and had a maximum and minimum stem mass of 28.8 and 28.0 g. The five green alder provenances had a large range in stem mass: Dipper Harbour West and Jouriman Island had 35.5 and 38.3 g, whereas Indian Falls Depot and Lower Prince William had 18.4 and 18.2 g, respectively. Eighth, the provenance $\times$ SMT interaction was due to a magnitude effect in which the same greater green alder provenances' stem dm responded to WW with greater stem dm than the other less productive provenances (not shown).

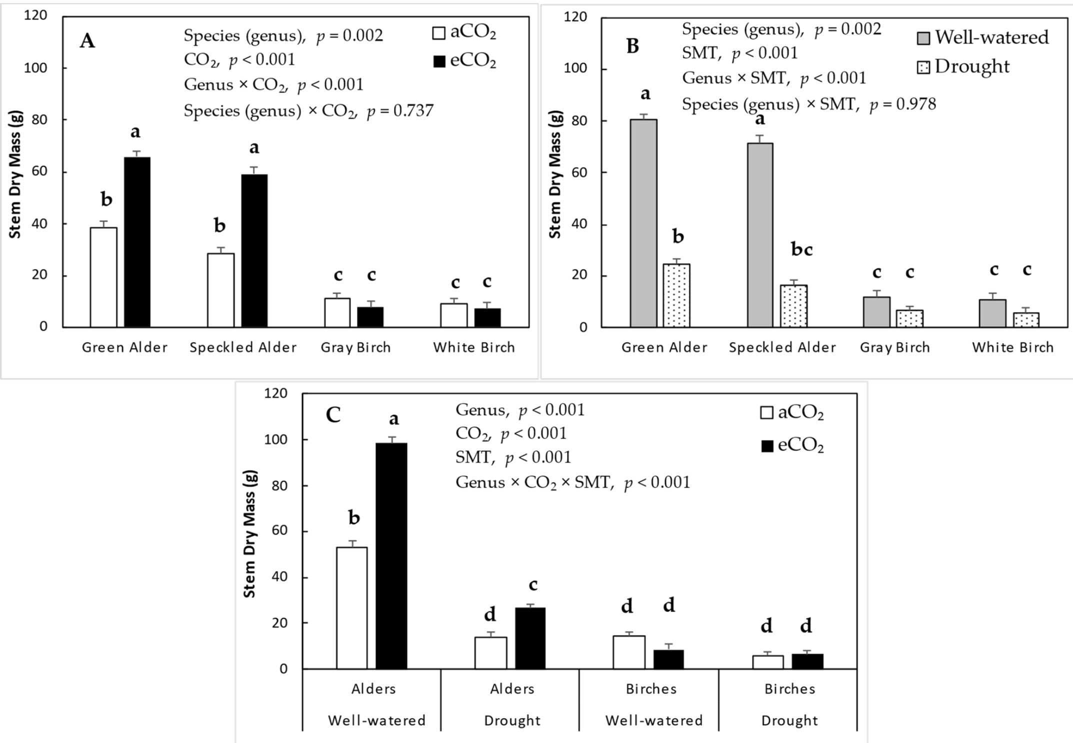

Figure 2. Stem dry mass (mean $\pm$ SE, n~65): (**A**) species and CO_2 treatment (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) soil moisture treatment (SMT); (**C**) genus and interactive CO_2 and SMT. *P*-values presented in each figure come from the full ANOVA model (Table 3). A post hoc Tukey's mean separation test was performed on the species interaction with (**A**) CO_2, (**B**) SMT, or (**C**) on the three-way genus $\times$ CO_2 $\times$ SMT interaction (p = 0.050) for each analysis where species or genus was significant. Bars within each figure with the same letter are not significantly different. Total sample size: n = 523.

Leaf dry mass (leaf dm) had the same ANOVA results, order, and coefficient of determination (R^2) as with stem dry mass (Table 3), and thus the ANOVA results description is not repeated here. Key results are as follows: first, alders always had greater leaf dm than birches with an average leaf dm of 36.0 and 6.5 g, respectively, a similar 550% difference (Figure 3A,B). The average leaf dm of green alder, speckled alder, gray birch, and white birch were 42.2, 29.4, 6.2, and 6.9 g, respectively. Third, under WW treatments, leaf dm was always greater than DRT with, on average, 31.4 and 11 g, respectively, a 280% difference (Figure 3B). Fourth, the genus × CO_2 interaction was a result of alders also doubling leaf dm in response to eCO_2, but birches did not respond. Fifth, eCO_2 mitigated DRT treatment for alders with 13.1 g under aCO_2 and 20.3 g under eCO_2 but not for birches (Figure 3C). Sixth, leaf dm provenance variation was driven by alders, as exemplified by stem dm.

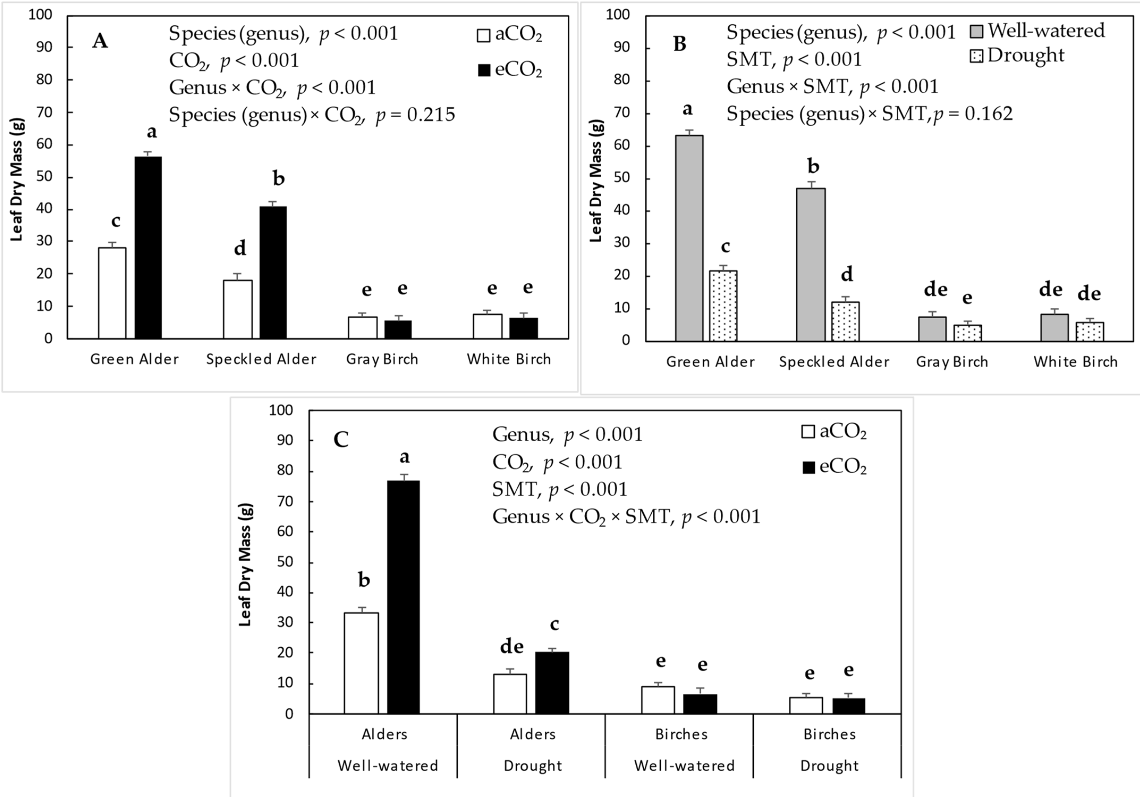

Figure 3. Leaf dry mass (mean ± *SE*, *n*~65): (**A**) species and CO_2 treatment (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) soil moisture treatment (SMT); (**C**) genus and interactive CO_2 and SMT. *p*-values presented in each figure come from the full ANOVA model (Table 3). A post hoc Tukey's mean separation test was performed on the species interaction with (**A**) CO_2, (**B**) SMT, or (**C**) on the three-way genus × CO_2 × SMT interaction (p = 0.050) for each analysis where species or genus was significant. Bars within each figure with the same letter are not significantly different. Total sample size: n = 523.

Root dm had almost the same ANOVA results and order but a lower coefficient of determination (R^2 = 0.56, Table 4) and no significant provenance × SMT interaction as stem dry mass; thus, the ANOVA results description is not repeated. Key quantitative results are as follows: first, alders always had greater root dm than birches with an average dm of 36.1 and 16.8 g, respectively, a 100% difference (Figure 4A,B). The average root dm of green alder, speckled alder, gray birch, and white birch were 32.7, 39.6, 18.2, and 15.5 g, respectively. Third, under WW treatments, root dm was always greater than DRT with, on average, 33.4 and 19.5 g, respectively, a 70% difference (Figure 4B). Fourth, under eCO_2, root dm was always greater than aCO_2 with an average of 29.1 and 23.9 g, respectively, a 22% increase. Fifth, the genus × CO_2 interaction was a result of alders increasing root dm by 50% in response to eCO_2, but birches did not respond. Sixth, eCO_2 mitigated DRT treatment for alders with 18.5 g under aCO_2 and 32.3 g under eCO_2 but not for birches (Figure 4C). Seventh, provenance was significant, as described for stem dm.

Table 4. Root dry mass, above-to-below-ground dry mass, and stem number variance components and ANOVAs, including the source of variation, degrees of freedom (*df*), mean square values (*MS*), variance components (*VC*), *p*-values, and coefficient of determination (R^2). *p*-values < 0.05 are in bold. Note that the number of stems was arcsine square root transformed for normality. Sources of variation abbreviations are soil moisture treatments (SMT), provenance (prov.), and species (Spp.).

Source of Variation	*df*	Root Dry Mass (g)			Above/Below Dry Mass			Number of Stems		
		MS	*VC* (%)	*p*-Value	*MS*	*VC* (%)	*p*-Value	*MS*	*VC* (%)	*p*-Value
Block	1	41.5	0.0	0.621	2.2	0.3	**0.021**	0.2	0.0	0.851
Genus	1	38,315.5	22.4	**<0.001**	194.3	30.0	**<0.001**	1644.3	34.4	**<0.001**
SMT	1	19,869.4	11.6	**<0.001**	71.3	11.0	**<0.001**	190.4	4.0	**<0.001**
CO_2	1	2795.3	1.6	**<0.001**	13.5	2.1	**<0.001**	8.3	0.2	0.199
SMT × CO_2	1	9902.3	5.8	**<0.001**	14	2.2	**<0.001**	52.4	1.1	**0.001**
Genus × SMT	1	5861.9	3.4	**<0.001**	43.6	6.7	**<0.001**	90.0	1.9	**<0.001**
Genus × CO_2	1	627.3	0.4	0.055	6.3	1.0	**<0.001**	90.6	1.9	**<0.001**
Genus × SMT × CO_2	1	1434.7	0.8	**0.004**	6.3	1.0	**<0.001**	0.1	0.0	0.873
Species (Genus)	2	1323.4	1.5	**<0.001**	33.3	10.3	**<0.001**	13.9	0.6	0.062
Species (Genus) × SMT	2	475.1	0.6	0.062	1.9	0.6	**0.011**	3.6	0.2	0.484
Species (Genus) × CO_2	2	196.9	0.2	0.315	0.3	0.1	0.525	1.0	0.0	0.824
Species (Genus) × SMT × CO_2	2	267.8	0.3	0.208	0.6	0.2	0.246	2.0	0.1	0.670
Prov. (Spp.)	14	313.5	2.6	0.030	0.9	2.0	**0.007**	17.1	5.0	**<0.001**
Prov. (Spp.) × SMT	14	165.3	1.4	0.481	0.7	1.6	**0.041**	4.3	1.3	0.597
Prov. (Spp.) × CO_2	14	146.7	1.2	0.600	0.3	0.7	0.66	3.3	1.0	0.806
Prov. (Spp.) × SMT × CO_2	14	178.7	1.5	0.401	0.4	0.9	0.473	5.7	1.7	0.308
Error	450	170.0	44.7		0.4	29.3		5.0	46.8	
R^2			0.56			0.742			0.572	

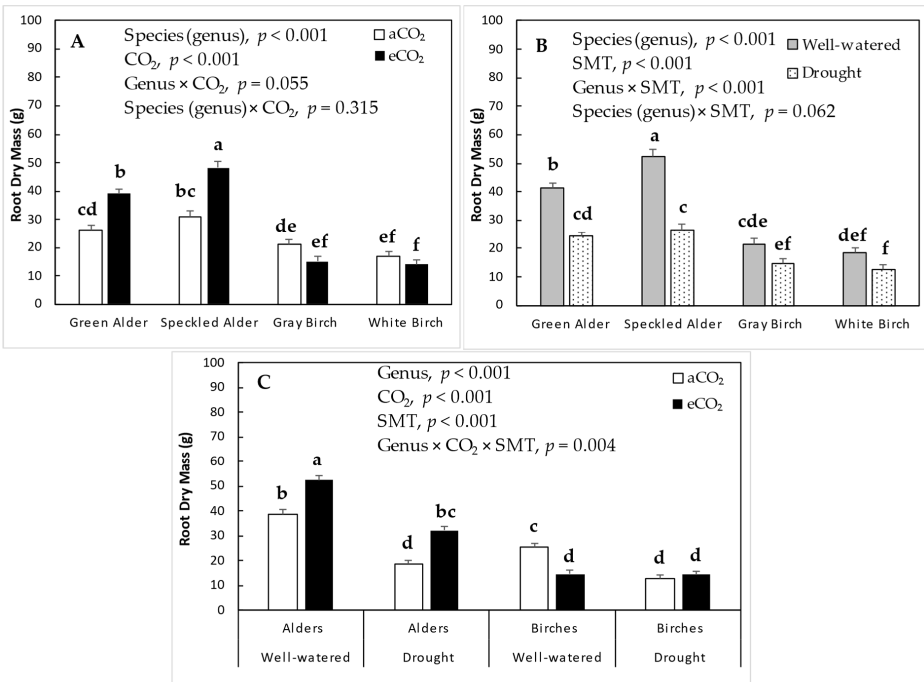

Figure 4. Root dry mass (mean ± *SE*, *n*~65): (**A**) species and CO_2 treatment (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) soil moisture treatment (SMT); (**C**) genus and interactive CO_2 and SMT. *p*-values presented in each figure come from the full ANOVA model (Table 4). A post hoc Tukey's mean separation test was performed on the species interaction with (**A**) CO_2, (**B**) SMT, or (**C**) on the three-way genus × CO_2 × SMT interaction (*p* = 0.050) for each analysis where species or genus was significant. Bars within each figure with the same letter are not significantly different. Total sample size: *n* = 523.

The above-to-below-ground dry mass ratio (ABV/BLW dm) had almost the same ANOVA results, order, and coefficient of determination (Table 4) as with stem dry mass, except there was one additional source of variation that was significant species × SMT. Key results are as follows: First, stem dm two- and three-way interactions involving genus, $CO_{2,}$ and SMT were all magnitude effects that did not rank change. Thus, the main effects become important, as well as the magnitude differences. Second, alders always had greater shoot-to-root dm than birches, with an average stem mass of 2.31 and 0.94, respectively, a 250% difference (Figure 5A,B). The average shoot/root ratio of green alder, speckled alder, gray birch, and white birch were 2.91, 1.72, 0.88, and 1.00, respectively. Third, under WW treatments, the shoot-to-root ratio was always greater than DRT with, on average, 2.04 and 1.21, respectively, a 68% reduction (Figure 5B). Third, under eCO_2, shoot-to-root dry mass ratios were always greater than aCO_2, with an average of 1.81 and 1.45, respectively, a 25% increase. Fourth, the genus × CO_2 interaction was a result of alders and birches increasing shoot-to-root ratio by 30 and 18%, respectively, in response to eCO_2. Fifth, provenance was significant, and this was driven by alders and largely by green alders. Speckled alder had a greater relative shoot-to-root dm reduction than green alder in response to DRT.

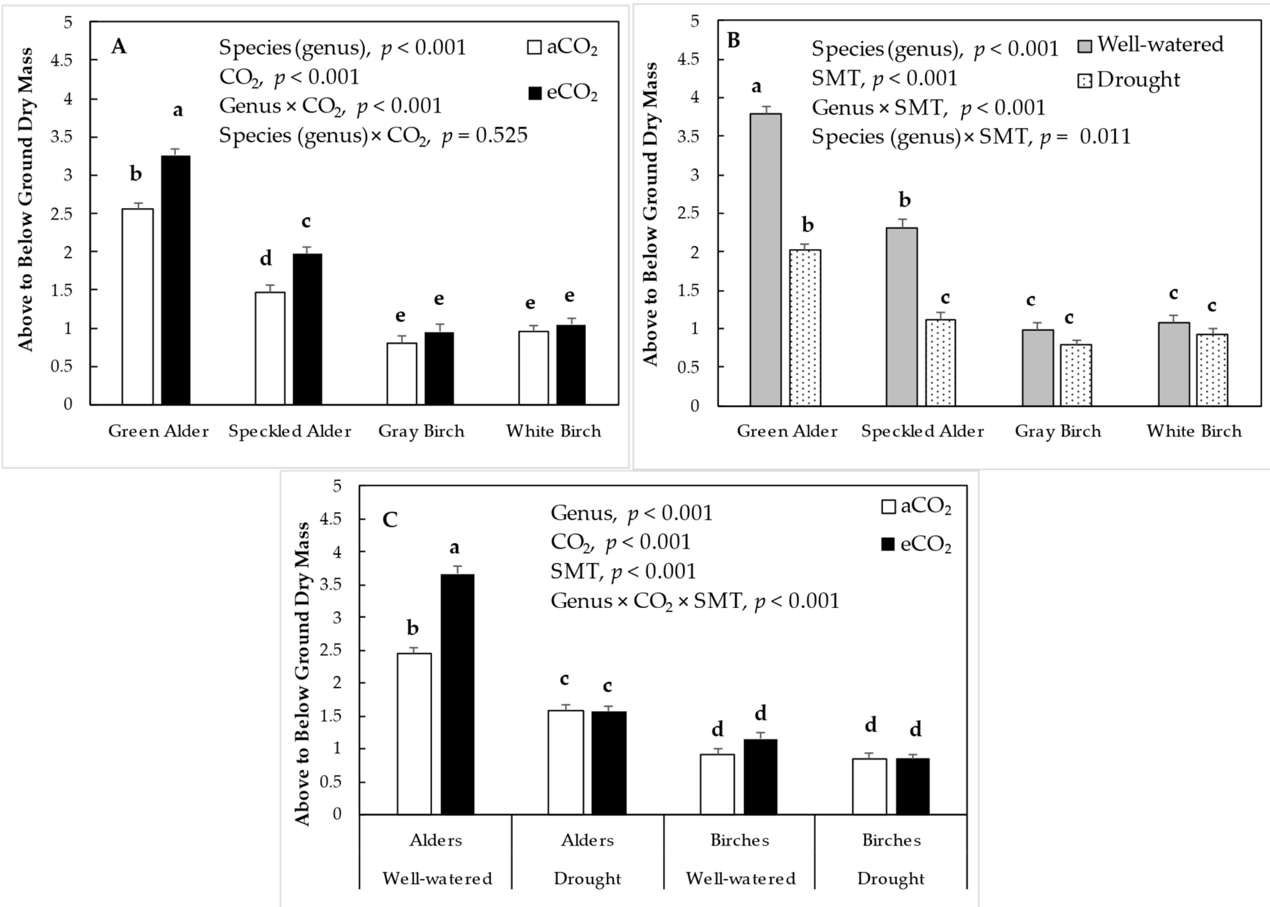

Figure 5. Above-to-below-ground dry mass ratio (mean ± *SE*, *n*~65): (**A**) species and CO_2 treatment (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) soil moisture treatment (SMT); (**C**) genus and interactive CO_2 and SMT. *p*-values presented in each figure come from the full ANOVA model (Table 4). A post hoc Tukey's mean separation test was performed on the species interaction with (**A**) CO_2, (**B**) SMT, or (**C**) on the three-way genus × CO_2 × SMT interaction (p = 0.050) for each analysis where species or genus was significant. Bars within each figure with the same letter are not significantly different. Total sample size: n = 523.

The coppiced stem number ANOVA was slightly different in that it had no three-way interaction. Significant sources of variation in the order of impact were genus, provenance, SMT, genus × CO_2, genus × SMT, SMT × CO_2, and species accounting for 35.1, 4.8, 3.0, 2.0, 1.3, 1.2, and 0.9% of total variation, respectively (Table 4). The genus × CO_2 interaction (Figure 6C) was the result of a small rank change wherein alder stem numbers increased from 7.1 to 8.7, and birches decreased from 4.2 to 3.5 under aCO_2 and eCO_2, respectively. The genus × SMT interaction was a magnitude effect wherein both alders and birches stem numbers decreased in response to drought; alders decreased from 9.2 to 6.6, and birches decreased from 4.2 to 3.5 under well-watered and drought, respectively. The SMT × CO_2 interaction was a magnitude effect: under WW treatment, stem numbers were the same for both CO_2 treatments at approximately 6.7; under DRT, aCO_2 and eCO_2 had 4.6 and 5.6 average stem numbers, respectively. Overall, alders had a significantly greater coppiced number of stems than birches, with an average of 7.7, compared to birches at 3.7, a 106.7% difference. Most of the provenance variation was found within green alder and gray birch (not shown).

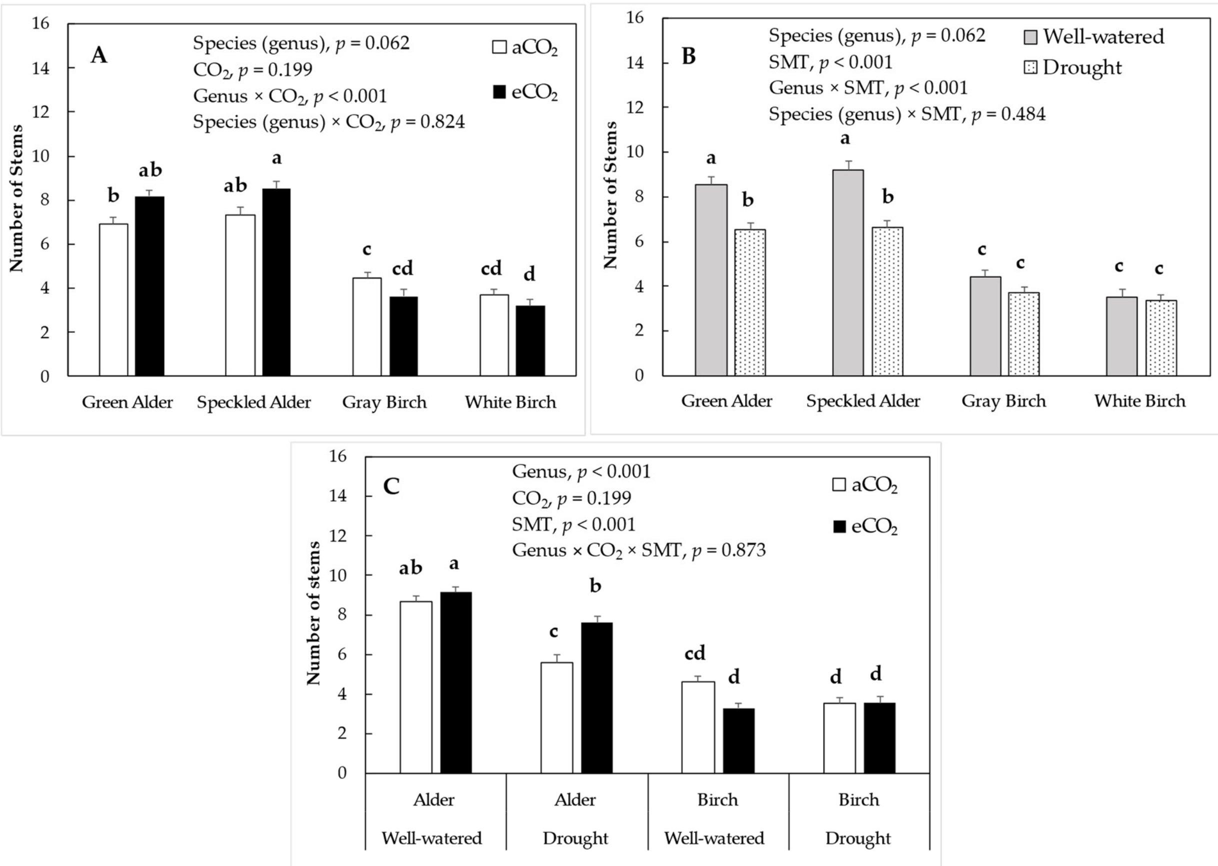

Figure 6. Coppiced number of stems (mean ± *SE*, *n*~65): (**A**) species and CO_2 treatment (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) soil moisture treatment (SMT); (**C**) genus and interactive CO_2 and SMT. *p*-values presented in each figure come from the full ANOVA model (Table 4). A post hoc Tukey's mean separation test was performed on the species interaction with (**A**) CO_2, (**B**) SMT, or (**C**) on the three-way genus × CO_2 × SMT interaction (p = 0.050) for each analysis where species or genus was significant. Bars within each figure with the same letter are not significantly different. Total sample size: n = 523.

The total dm had similar ANOVA results, order, and coefficient of determination as with stem dry mass (Table 3); thus, the results are only presented as text here. As the individual components of total dry mass are presented separately, figures for total dm are therefore also not presented. The key results are as follows: first, alders always had greater total dm than birches with an average stem mass of 120.1 and 32.1 g, respectively, an approximate 400% difference. The average total dm of green alder, speckled alder, gray birch, and white birch were 127.3, 112.8, 33.6, and 30.6 g, respectively. Third, under WW treatments, total dm was always greater than DRT, with, on average, 108.4 and 43.7 g, respectively, a 250% difference. Fourth, under eCO_2, total dm was always greater, with the exception noted above, than aCO_2, with an average of 91.4 and 60.7 g, respectively. Fifth, eCO_2 mitigated DRT treatment for alders with 45.8 g under aCO_2 and 79.2 g under eCO_2, but not for birches. Sixth, in a reduced model for total dm, the five gray birch provenances ranged from 69.6 to 77.1 g, and for the five white birches, the range was 74.5 to 78.5 g (not shown). In the same model analyzing alders total dm, the provenance variation was significant (p = 0.006) and ranged from 42.07g to 62.2 g for green alder and 40 g to 48.0 g for speckled alder. It is important to note for interpretation, however, that green alder had five provenances, and speckled alder had three, including a bulk provenance. Nevertheless, for birches total dm, the provenance variation was not significant (p = 0.186) (not shown). Thus, it appears that the overall provenance variation is driven by alders. The provenance × SMT was as described in stem dm above.

For foliar N, the sources of variation that were significant in order of impact, genus, CO_2, species, genus × CO_2, and SMT, accounted for a remarkable 88.6, 1.6, 1.6, and 0.3% of the total variation, respectively (Table 5). The genus × CO_2 interaction was a magnitude effect alders downregulated N more than birches in response to eCO_2 (Figure 7B). Alders had a significantly greater N at 2.8%, compared to birches at 1.0%, a 185.6% difference. The average N of green alder, speckled alder, gray birch, and white birch were 2.6, 2.9, 1.1, and 0.9%, respectively. All species downregulated N under drought, on average, by 0.1% N (Figure 7A).

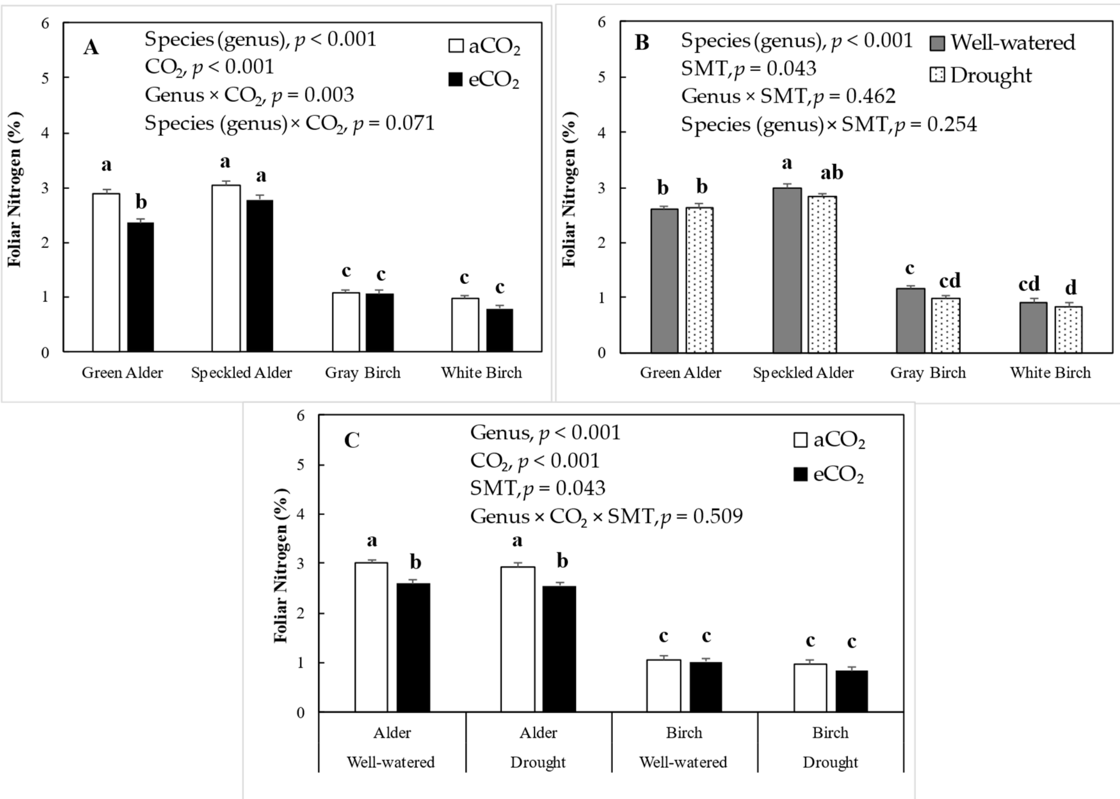

Figure 7. Foliar nitrogen concentration (%) (mean ± *SE*, n~15): (**A**) species and CO_2 treatment (aCO_2 = ambient CO_2, eCO_2 = elevated CO_2); (**B**) soil moisture treatment (SMT); (**C**) genus and interactive CO_2 and SMT. p-values presented in each figure come from the full ANOVA model (Table 5). A post hoc Tukey's mean separation test was performed on the species interaction with (**A**) CO_2, (**B**) SMT, or (**C**) on the three-way genus × CO_2 × SMT interaction (p = 0.050) for each analysis where species or genus was significant. Bars within each figure with the same letter are not significantly different. Total sample size: n = 121.

Table 5. Foliar nitrogen ANOVA, including the source of variation, degrees of freedom (*df*), mean square values (*MS*), variance components (*VC*), *p*-values, and coefficient of determination (R^2). *p*-values < 0.05 are in bold. Source of variation abbreviation, soil moisture treatments (SMT).

Source of Variation	*df*	Foliar Nitrogen (%)		
		MS	*VC* (%)	*p*-Value
Block	1	0.101	0.1	0.23
Genus	1	96.328	89	**<0.001**
SMT	1	0.292	0.3	**0.043**
CO_2	1	1.784	1.6	**<0.001**
SMT × CO_2	1	0.006	<0.1	0.764
Genus × SMT	1	0.038	<0.1	0.462
Genus × CO_2	1	0.654	0.6	**0.003**
Genus × SMT × CO_2	1	0.03	<0.1	0.509
Species (Genus)	2	0.863	1.6	**<0.001**
Species (Genus) × SMT	2	0.097	0.2	0.254
Species (Genus) × CO_2	2	0.188	0.3	0.071
Species (Genus) × SMT × CO_2	2	<0.001	<0.1	0.999
Error	104	0.069	6.6	
R^2			0.933	

3.2. Covariate Analysis

Covariate analyses were tested at a genus level and species level for stem dm (dependent variable) in relation to the tallest stem height, tallest stem diameter, and stem number (independent variable), testing either CO_2 or soil moisture treatment. Stem height consistently had the highest coefficient of determination (R^2) and thus will be the only independent variable presented herein. The covariate analyses were used to examine if organ (stem, leaf, and root) allocation was statistically the same or different among species × CO_2 and soil moisture treatments, correcting for inherent species size differences while examining allocation changes with size.

3.3. Covariate Analysis Testing CO_2 Effect on Allocation

Covariate analysis of green alder and speckled alder examining stem *dm* allocation (%) in relation to size using tallest (used in subsequent covariance) stem height found a significant CO_2 × stem height interaction for both green alder, $p = 0.004$ and speckled alder, $p < 0.001$. Further analysis, testing alder species, found a significant alder species × stem height interaction under aCO_2 ($p = 0.008$) but no significant interaction under eCO_2 ($p = 0.281$) and no significant species effect ($p = 0.688$). This results in positive differential alder species responses under aCO_2 but a single positive genus response line under eCO_2 (Figure 8A) (Table 6). Covariate analysis of gray birch examining stem dm allocation (%) in relation to size using stem height found no significant CO_2 × stem height interaction ($p = 0.054$); mean aCO_2 and eCO_2 stem allocations were 26.2 and 26.4 (%), and, thus, hardly large; and no CO_2 effect ($p = 0.729$). Covariate analysis of white birch found no significant height × CO_2 interaction ($p = 0.523$) and no CO_2 effect ($p = 0.971$). Both gray birch and white birch stem allocation (%) had a positive relationship with stem height (gray birch and white birch, $p < 0.001$). Covariate analysis testing birch species found no significant species × height interaction ($p = 0.096$) or species ($p = 0.085$), resulting in a single genus—CO_2 stem allocation response line (Figure 8A).

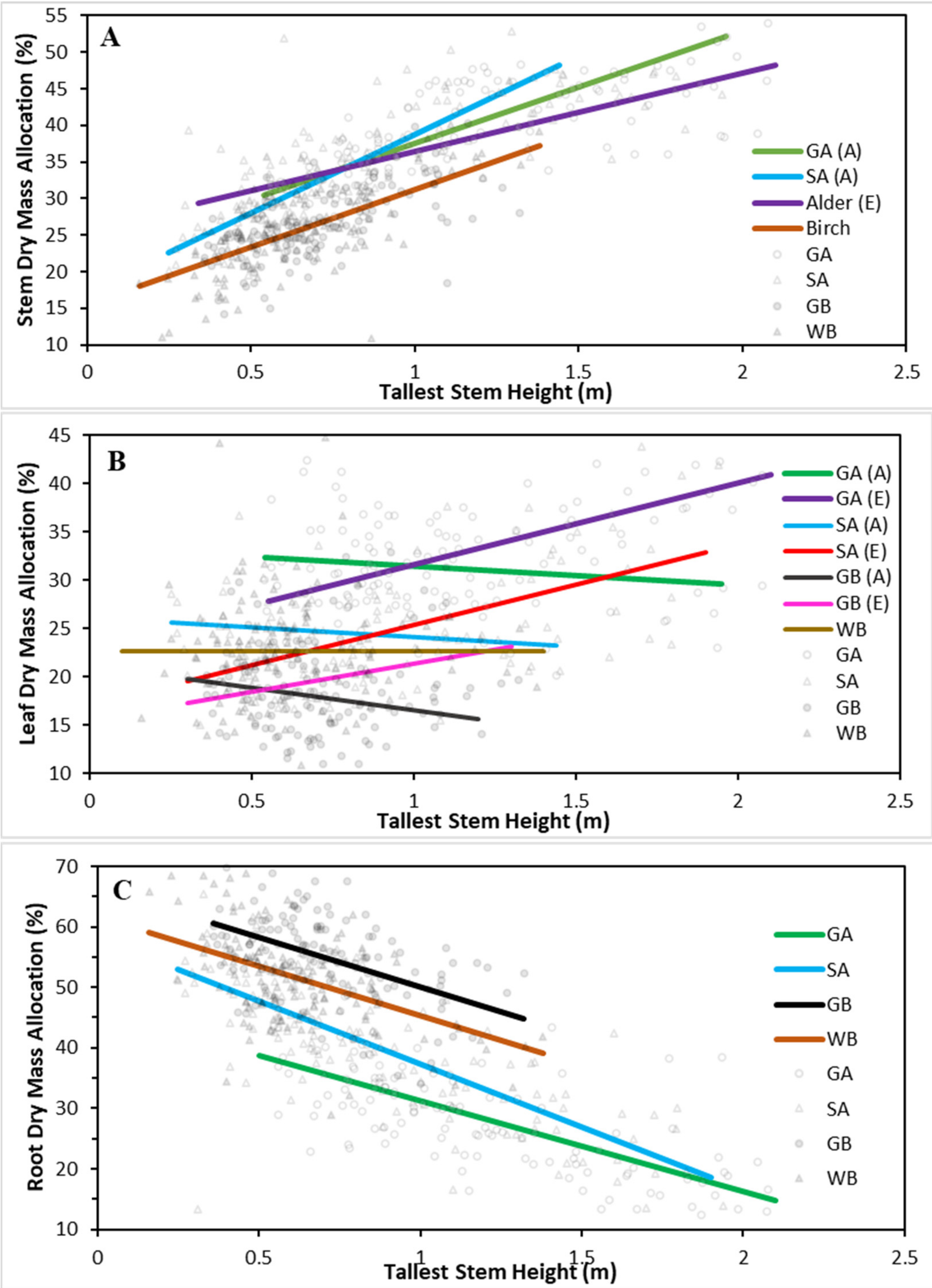

Figure 8. Allometric relationships between (**A**) stem dry mass allocation (%), (**B**) leaf dry mass allocation (%), and (**C**) root dry mass allocation (%) and tallest stem height (m), testing CO_2 effect (ambient CO_2 (A) vs. elevated CO_2 (E)). Species abbreviated: GA = green alder; SA = speckled alder; GB = gray birch; WB = white birch.

Table 6. Allometric relationships between stem, leaf, or root dry mass (dm) allocation and tallest stem height testing either CO_2 (ambient CO_2 (A) vs. elevated CO_2 (E)) or soil moisture treatment (well watered (W) vs. drought (D)), including *p*-value, regression equation, coefficient of determination (R^2), and equation range (stem height range) for two-year-old coppiced plants found in accompanied Figures 8 and 9.

Stem Dry Mass Allocation (%) in Relation to Tallest Stem Height (m) Testing CO_2 Treatment (Figure 8A)				
Species	***p*-Value**	**Equation**	R^2	**Stem Height Range (m)**
Green alder (A) Speckled alder (A)	0.008	y = 22.21 + 15.39x y = 17.13 + 21.65x	0.73	0.54–1.95 0.25–1.44
Alder (E)	<0.001	y = 25.79 + 10.67x	0.556	0.34–2.08
Birch	<0.001	y = 15.59 + 15.67x	0.436	0.23–1.38
Leaf dry mass Allocation (%) in Relation to Tallest Stem Height (m) Testing CO_2 Treatment (Figure 8B)				
Species	***p*-Value**	**Equation**	R^2	**Stem Height Range (m)**
Green alder (A) Speckled alder (A)	<0.001	y = 33.37 − 1.93x y = 26.05 + 1.93x	0.334	0.54–1.95 0.25–1.44
Green alder (E) Speckled alder (E)	<0.001	y = 23.27 + 8.38x y = 17.01 + 8.38x	0.504	0.55–2.08 0.34–1.94
Gray birch (A) Gray birch (E)	0.006	y = 21.11 − 4.52x y = 15.61 + 5.74x	0.089	0.36–1.21 0.37–1.32
White birch	0.975	WB y = 22.7	<0.001	0.16–1.38
Root Dry Mass Allocation (%) in Relation to Tallest Stem Height (m) Testing CO_2 Treatment (Figure 8C)				
Species	***p*-Value**	**Equation**	R^2	**Stem Height Range (m)**
Green alder Speckled alder	0.006	y = 46.20 − 14.96x y = 58.22 − 20.84x	0.654	0.54–2.08 0.23–1.94
Gray birch White birch	<0.001	y = 66.51 − 16.42x y = 61.75 − 16.42x	0.269	0.36–1.32 0.16–1.38
Stem Dry Mass Allocation (%) in Relation to Tallest Stem Height (m) Testing Soil Moisture Treatment (Figure 9A)				
Species	***p*-Value**	**Equation**	R^2	**Stem Height Range (m)**
Alder (W) Alder (D)	<0.001	y = 32.46 + 7.35x y = 20.88 + 15.91x	0.655	0.68–2.08 0.25–1.38
Birch	<0.001	y = 15.59 + 15.67x	0.436	0.16–1.38
Leaf Dry Mass Allocation (%) in Relation to Tallest Stem Height (m) Testing Soil Moisture Treatment (Figure 9B)				
Species	***p*-Value**	**Equation**	R^2	**Stem Height Range (m)**
Green alder (W) Speckled alder (W)	<0.001	y = 22.50 + 7.26x y = 18.14 + 7.26x	0.308	0.89–2.08 0.68–1.94
Green alder (D) Speckled alder (D)	<0.001	y = 35.28 + 4.70x y = 25.48 − 4.70x	0.425	0.54–1.38 0.23–0.82
Gray birch (W) Gray birch (D)	0.044	y = 14.86 + 4.67x y = 22.68 − 6.71x	0.033	0.38–1.32 0.36–0.87
White birch (W) White birch (D)	0.029	y = 21.31 y = 23.78	0.036	0.23–1.38 0.16–0.77
Root Dry Mass Allocation (%) in Relation to Tallest Stem Height (m) Testing Soil Moisture Treatment (Figure 9C)				
Species	***p*-Value**	**Equation**	R^2	**Stem Height Range (m)**
Green alder (W) Green alder (D)	<0.001	y = 37.34 − 9.90x y = 42.72 − 9.90x	0.523	0.89–2.08 0.54–1.38
Speckled alder	<0.001	y = 58.22 − 20.83x	0.576	0.23–1.94
Gray birch White birch	<0.001	y = 66.51 − 16.42x y = 61.75 − 16.42x	0.269	0.36–1.32 0.16–1.38

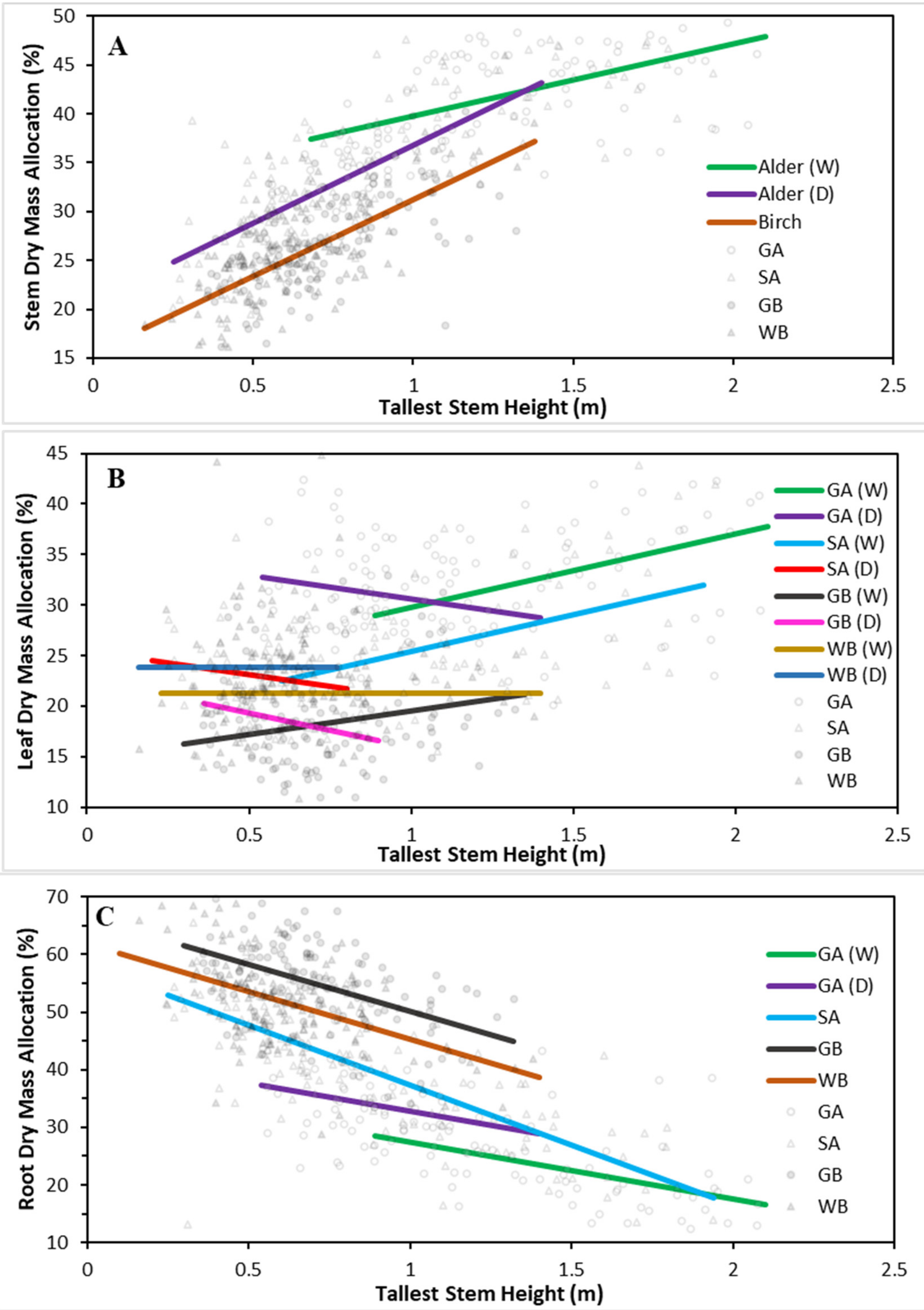

Figure 9. Allometric relationships between (**A**) stem dry mass allocation (%), (**B**) leaf dry mass allocation (%), and (**C**) root dry mass allocation (%) and tallest stem height (m), testing soil moisture treatment (well watered (W) vs. drought (D)). Species abbreviated: GA = green alder; SA = speckled alder; GB = gray birch; WB = white birch.

Covariate analysis of green alder testing leaf dm allocation (%) in relation to size using stem height found a significant CO_2 treatment × height interaction ($p < 0.001$), resulting in differential response lines with a positive and negative slope for eCO_2 and aCO_2, respectively (Figure 8B) (Table 6). Covariate analysis of speckled alder leaf dm also found a significant CO_2 treatment × height interaction ($p < 0.001$), resulting in differential response lines with a positive and negative slope for eCO_2 and aCO_2, respectively

(Figure 8B). Further analysis of leaf dm testing alder species under aCO_2 found no significant species × height interaction ($p = 0.167$) but confirmed the species were significantly different ($p < 0.001$), resulting in two parallel lines with negative slopes (Figure 8B). Further analysis of leaf dm allocation testing alder species under eCO_2 found no significant species × height interaction ($p = 0.322$) but confirmed the species were significantly different ($p < 0.001$), resulting in two parallel lines with positive slopes (Figure 8B). Covariate analysis of gray birch found a significant CO_2 × height interaction ($p = 0.007$), resulting in differential response lines with different slopes. The slope for gray birch under the aCO_2 slope was negative, and the slope under the eCO_2 slope was positive (Figure 8B). Analysis of white birch found no significant CO_2 treatment × height interaction ($p = 0.946$), CO_2 effect ($p = 0.282$), nor height correlation ($p = 0.991$), resulting in a single flat response line (Figure 8B).

Covariate analysis of green alder examing root dm allocation in relation to size using stem height found no CO_2 × height interaction ($p = 0.076$) and no CO_2 effect ($p = 0.883$). Analysis of speckled alder found no significant CO_2 × height interaction ($p = 0.859$) and no significant CO_2 effect ($p = 0.389$). Covariate analysis of root dm allocation in relation to stem height, testing alder species, found a significant species × height interaction ($p = 0.006$) (Table 6), resulting in differential negative slopes for alder species root allocation responses (Figure 8C). Covariate analysis of gray birch testing root dm allocation in relation to stem height found no CO_2 × height interaction ($p = 0.389$) and no CO_2 effect ($p = 0.077$). Further analysis testing the CO_2 effect for white birch found no significant interaction ($p = 0.729$) and no significant CO_2 effect ($p = 0.435$). Further analysis of root dm in relation to stem height, testing birch species, found no significant species × height interaction ($p = 0.467$), but further analysis found significant species effects ($p < 0.001$), resulting in two parallel negative birch species response lines (Table 6) (Figure 8C).

3.4. Covariate Analysis Testing SMT Effect on Allocation

Covariate analysis of green alder testing stem dm allocation in relation to size using stem height found a significant SMT × height interaction ($p < 0.001$). Covariate analysis of speckled alder also found a significant SMT × height interaction ($p < 0.043$). Further analysis of stem dm allocation in relation to stem height, testing alder species, found no significant interaction under well-watered nor drought ($p = 0.074$, $p = 0.495$, respectively), and further analysis found no species effect ($p = 0.209$, $p = 0.989$, respectively), indicating no species difference. Covariate analysis of alder, testing stem dm allocation in relation to stem height, found a significant interaction between SMT × height ($p < 0.001$) (Table 6), resulting in positive response lines with different slopes (Figure 9A). Covariate analysis of gray birch testing stem dm allocation in relation to stem height found no significant SMT × height interaction ($p < 0.896$) nor SMT effect ($p = 0.270$). Covariate analysis of white birch testing stem dm allocation in relation to height found no significant SMT × height interaction ($p < 0.890$) nor SMT effect ($p = 0.967$). Covariate analysis of stem dm allocation in relation to height, testing birch species, found no significant height interaction under well-watered nor drought ($p = 0.198$, $p = 0.280$, respectively), and further analysis found no species effect ($p = 0.210$, $p = 0.325$, respectively). Further analysis of birches found no significant species × height interaction ($p = 0.096$) nor species effect ($p = 0.085$) but a significant correlation with height ($p < 0.001$), resulting in a single genus response line (Figure 9A).

Covariate analysis of green alder testing leaf dm allocation in relation to height found a significant SMT × height interaction ($p = 0.038$) (Table 6). Analysis of speckled alder found a significant SMT × height interaction ($p = 0.003$). Further analysis of alders under well-watered treatments found non-significant alder species × height interaction ($p = 0.286$) but a significant species effect ($p = 0.001$), resulting in two positive parallel slopes (Figure 9B). Further analysis of alders under drought found non-significant alder species × height interaction ($p = 0.556$) but a significant species effect ($p < 0.001$), resulting in two parallel negative slopes (Figure 9B). Covariate analysis of gray birch testing leaf dm allocation found a significant drought treatment × height interaction ($p = 0.044$). Gray birch irrigated and drought treatments had positive and negative leaf dm allocation slopes in relation to height, respectively. Analysis of white birch found no significant height × drought treatment interaction (p = 0.589) but a significant drought effect (p = 0.029) (Table 6) and no significant height covariate (p =0.185), resulting in two horizontal lines with well-watered leaf dm allocation above drought (Figure 9B).

Covariate analysis of green alder testing root dm allocation in relation to size using stem height found no significant height × drought interaction (p = 0.697) but a significant drought effect (p = 0.004) (Table 6). Covariate analysis of speckled alder found no significant height × drought interaction (p = 0.347) and no significant drought effect (p = 0.053), resulting in a single negative response line (Figure 9C). Covariate analysis of root dm allocation for gray birch found no significant height × SMT treatment interaction (p = 0.160) nor SMT effect (p = 0.631). Covariate analysis of white birch found no significant height × drought treatment interaction (p = 0.626) nor drought effect (p = 0.109). Further analysis of root dm allocation in relation to height, testing birch species, found no significant species × height interaction (p = 0.467), but further analysis found significant species effects ($p < 0.001$), resulting in two negative parallel species response lines (Table 6) (Figure 9C).

Covariate analysis for total (above and below) dry mass was examined in response to the tallest stem height, basal diameter, and stem number. In all cases, the tallest stem height was the best predictor of total dry mass. We present the findings from covariate analysis of total (above and below) dry mass in relation to height testing alder species found no species × height interaction, but further analysis found a significant species effect (p = 0.006) (Table 7). This resulted in two parallel response lines wherein speckled alder was greater in total dry mass for a given stem height (Figure 10). Covariate analysis of total dry mass in relation to height testing birch found a significant species × height interaction (p = 0.049), resulting in two response lines of different slopes, with gray birch having slightly greater total dry mass for a given stem height (Figure 10).

Table 7. Species-specific equations, coefficient of determination (R^2) values, and ranges for total dry mass accumulation under aCO_2 in relation to tallest stem height, testing species for 2-year-old coppiced plants.

Total Dry Mass in Relation to Tallest Stem Height, Testing Species (Figure 10)				
Species	***p*-Value**	**Equation**	**R^2**	**Height Range (m)**
Green alder	0.006	y = −25.09 + 106.88x	0.625	0.54–1.95
Speckled alder		y = −7.61 + 106.88x		0.25–1.44
Gray birch	0.049	y = −16.11 + 77.96x	0.717	0.36–1.21
White birch		y = −6.31 + 62.62x		0.16–1.21

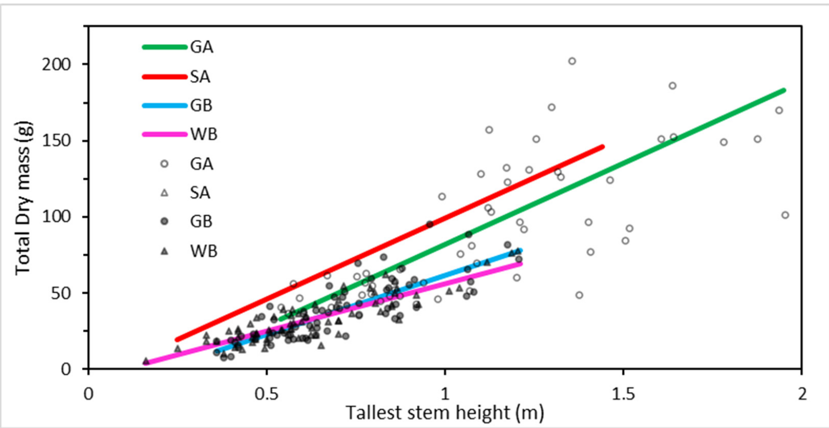

Figure 10. Simple linear regression analyzing total dry mass (g) in relation to tallest stem height (m) testing species. Species abbreviated: GA = green alder; SA = speckled alder; GB = gray birch; WB = white birch.

4. Discussion

4.1. Genetic Structure

The morphological growth differences between species and genera used in this experiment were large, driven mostly by genera accounting for between 18 and 34.4% of the total variation. In examining the genetic structure further, species accounted for between 0.6 and 7.6% of the total variation. Provenance accounted for between 1.5 and 5.0% of the total variation. What is interesting is that the provenance structure of alders and birches were different. For total dm, the full model showed that provenance variation was significant, yet when run separately by genus, alder provenance variation was significant, whereas birches' was not, with high uniformity among provenances. Most seed sources were from New Brunswick and some from surrounding provinces, which are relatively close to Eastern Canada. Birches have crowns in the canopy and thus have greater wind pollen mixing potential, whereas alders are shrub species, and the pollen mixing potential would be lower and from shorter distances. This is consistent with the greater alder provenance genetic structure. Very little literature exists on alders; a study published in 1988 [21] using only nine enzyme variants suggested that for speckled alder and green alder, diversity primarily existed within populations, and among-population gene diversity was low, somewhat contrary to what we found here with total dm. However, our findings are not that there is no population mixing in alders but that alders and birches' provenance variation differed significantly, which indicates that birches had more population mixing compared to alders.

4.2. Morphological Responses to eCO_2

Elevated CO_2 did not result in additional growth in birches, consistent with previous findings [19], and it was determined to be a result of (1) foliar N limitation and (2) a lack of sink activity, which are often observed limitations under eCO_2 [3,22–24]. These factors have previously resulted in birch assimilation downregulation (A_{dr}) of −29%, but not in alders (+3%) [19]. Although from the same biological family, birches are not actinorhizal and thus cannot fix atmospheric N. Positive linear relationships between photosynthetic efficiency and foliar N have been found [25,26], and we found that foliar N was three times greater in alders than in birches (Figure 7). The lack of additional dry mass growth under eCO_2 in birch is usually also indicative of a build-up of non-structural carbohydrates caused by a lack of sink activity [3]. Dry mass allocation in birches was different from alders in that CO_2 had no effect on birches for stem dm allocation, also appearing to be explained by a lack of sink activity and available foliar N. Correcting for size (height), stem dm allocation was lower for birches than alders, which is likely because of less coppiced stems. Nearly all dm values, regardless of treatment, were greater for alders. Alders produced greater coppiced stem number, stem, leaf, and root dm and had a greater positive response to eCO_2 for all growth parameters. Many studies have found a positive relationship between dry mass growth and eCO_2 [3,22]. A study using red alder (*Alnus rubra* Bong.) found significantly greater height; stem diameter; and root, stem, and leaf dm under eCO_2 (700 ppm) compared to aCO_2 (350 ppm) [27]. Another study using speckled alder (*Alnus incana* subsp. *rugosa*) and green alder (*Alnus viridis* subsp. *crispa*) grown under eCO_2 (1000 ppm) and aCO_2 (400 ppm) found that dry mass production was only significantly stimulated under eCO_2 in the presence of *Frankia* sp., and not when absent [28]. Furthermore, dry mass allocation (nodule, root, leaf, and stem partitioning) was not significantly affected by eCO_2 [28]. It was visually apparent in our experiment that infection and nodulation by *Frankia alni* had occurred on all individuals of alder upon harvesting the root systems, which confirms that infection influenced growth success under eCO_2. Although [29] found no significant dry mass allocation differences, our alder allocation differences occurred at the species, CO_2, and SMT levels.

Coppiced plants sometimes prioritize vigorous above-ground growth over root growth, although this can be species-dependent, with some species being described as more "resource-demanding" or "resource-saving" [29,30]. One study testing three poplar species under eCO_2 found that eCO_2 induced above-ground sink upregulation and root dm downregulation [14]. Our findings indicate that alders have a strong sink effect not only for above-ground dry mass production but likely initially for root growth, too, indicating a "resource-demanding" strategy, which was likely enhanced by eCO_2. Although the slope of the response line of root dm allocation to size was negative, the initial allocation to root dm was high (~51%). Alders have been successful in soil stabilization projects before [31,32], and other studies have noted the importance of root structure to riparian species [33], which is representative of the ecological niche these alder species inhabit. For green alder, a strong root structure would be important in stabilization on sloped, upland sites, and like speckled alder, to ensure continuous water supply, as is important for the genera. Root allocation declined with increasing height, however, reaching ~10%–25% of allocation at 1.5–2 m height for both alder species. Root restriction has been found to lower the root/shoot ratio in black alder [16]; however, our observations did not indicate root restriction inside the root control bag. It is likely that a larger initial root system and the coppicing effect additionally played a role in these root allocation patterns.

Leaf allocation increased with size under eCO_2 for green alder, speckled alder, and gray birch but decreased in response to aCO_2. This suggests a strategy to maximize growth potential under eCO_2 by investing in foliage. The literature on this is sparse and often species- or functional-group-dependent, but leaf number has also been observed to increase under eCO_2 [3]. Birches had lower leaf allocation than alders, and white birch had no response to CO_2 treatments nor a significant correlation with size, instead maintaining the same leaf dm allocation. Ref. [34] found that white birch had no leaf dm difference under CO_2 treatments, which we report here. It is not clear why gray birch had a significant height $\times$ CO_2 interaction, considering most growth traits are similar to white birch. Nevertheless, like alders, eCO_2 did not affect root dm allocation, although root allocation was greater for birches than alders and greater for gray birch than white birch.

Speckled alder allocated more to root dm than green alder, with no significant response to CO_2 treatments observed in either species, and the allocation decreased as height increased (Figure 8C). The greater above-ground growth in alders is due to a greater coppice stem number with perhaps a larger initial root system, as alders were larger than birches after one year [19]. Multiple studies have found that alders lack initial inhibitory competitive advantages but obtain these in later growth stages [4,35]. A restoration field study by [4] found that first-year height growth between alders and birches was similar; however, after the second year, alders had magnitude height and stem dry mass differences compared to birches. Second-year height growth and stem dm for alders were an average of 5.6 times and 13 times greater than birches, respectively [4].

It should be noted that the growth rate of alders used in this study can be quick, and in natural sites, alders are known to quickly dominate and outcompete other species on early successional sites, with the mechanisms being (1) shading out competition and (2) the physical and allelopathic nature of the leaf litter [35,36]. Ref. [36] hypothesized that initial spruce growth was facilitated by alder planted in conjunction with increased N availability, leaf litter and soil organic material, reduced soil pH (via increased organic matter input), and shading that reduces leaf temperature and transpiration. It was found after six years of establishment, however, that the alders were outcompeting the spruce above and below ground [36]. Although nutrient competition was not a factor in our experiment, as each plant was contained within a root bag, competition for light did occur. It is likely that the vigorous growth of some alders resulted in the shading of some birches in well-watered chambers, perhaps enhancing the differences between the two genera. As alders produced significantly more leaves, were either taller or the same height, and produced more stems, our findings provide more evidence for alders being aggressively competitive, even when grown with other early successional species that reside in similar ecological niches.

4.3. Morphological Responses to Soil Moisture and Interactive Effects of CO_2 $\times$ Soil Moisture

Large impacts to dry mass were found across all growth traits for all four species in response to DRT. SMT often accounted for the second-most variation (behind genus), ranging from 4.0% to 34.8%. Although alders had a greater decrease in dry mass than birches, alders had significantly greater growth under DRT conditions than birches. Species exhibiting different drought stress responses are common, especially given the differing silvics of the species used in this experiment. Speckled alder is hydrophilic, often growing along stream banks in periodically flooded areas [6], and although green alder is also often found in wet soils along stream banks or other wetland habitats, green alder can also tolerate upland rocky sites [6]. Alders can exhibit strong below-ground competition [35,36]. Strong root systems would additionally allow alders to anchor themselves in areas where soils are less stable, like wetlands and rocky areas (in the case of green alder), and have been noted for other alder species [31]. In addition, it has been suggested that alders are anisohydric and will maintain high stomatal conductance even when water availability becomes increasingly sparse [37]. This strategy would have negative implications under DRT; however, the greater root dm probably mitigated this response.

White birch grows on a wide variety of soils, whereas gray birch often grows in nutrient-poor, dry sites [10,11]. It appears that both birch species have a more precautionary soil moisture strategy than alders in nutrient- and water-limited habitats, preferring slow growth over riskier, vigorous growth that could potentially lead to drought harm such as cavitation, which has been found for white birch [38]. Ref. [39] ranked white birch as the lowest drought tolerant of 22 common Canadian tree species across several drought resiliency traits. Gray birch is potentially more drought tolerant, as one study found moderate tolerance under very dry conditions [40], although little literature exists to confirm this. Our findings indicate a very similar but slightly better tolerance than white birch based on dm, which was also found in the first year [19]. Since neither birch species are actinorhizal, they are unable to access atmospheric N like alders, creating another limiting growth factor. A study on white birch seedlings subjected to different soil N and soil moisture treatments found that nitrogen use efficiency (NUE) was negatively correlated with water use efficiency (WUE), suggesting that a greater level of WUE or NUE results in a decreased efficiency in the use of other resources [41]. Were the birches in this experiment prioritizing WUE, thus limiting growth through poor utilization of other limited resources, such as N? It is possible, although, that to understand this relationship further, another study would need to be conducted under differing nutrient and water regime combinations.

Coppiced plants behave morphologically different than non-coppiced plants, which could explain why genus accounted for more variation than it did in the first-year experiment [19]. Coppiced plants often exhibit vigorous above-ground growth by using stored non-structural carbohydrates from the roots and any remaining stems [30] to quickly restore and maximize light capture and carbon fixation. This can lead to greater above-to-below-ground dry mass, which is what we found. It is probable that the root systems of birches were smaller after the first year of growth, magnifying the decreased growth in comparison to alders as coppicing vigor would be stunted due to less reserved non-structural carbohydrates. This would additionally result in a more evenly distributed above/below-ground dry mass ratio, which was also found and supported by the root allocation analysis, in which birches allocated more to roots than alders, at ~40–60% (Figures 8C and 9C).

It was previously found that the actinorhizal ability of alders, paired with the additional sink activity, resulted in greater photosynthetic efficiency under eCO_2 [19]. The interactive effects between SMT $\times$ CO_2 showed that eCO_2 more than mitigated the drought effect and, in fact, doubled the stem and root dm. Measured only under well-watered conditions, alders had significantly greater assimilation and iWUE than birches [19]. This showed that alders were able to better take advantage of eCO_2 under DRT, while for birches, eCO_2 had little to no mitigating effect. Although the above-ground-to-below-ground dry mass ratio (ABV/BLW dm) was upregulated for both alders and birches in response to eCO_2, under drought, the ratio was unaffected for both genera.

Interestingly, stem dm allocation increased at a greater rate under drought for alders than under well-watered. Leaf dm allocation for green alder, speckled alder, and gray birch increased with size under well-watered but decreased with increasing size under DRT.

This is likely a strategy to maximize carbon sequestration under favorable conditions and minimize water loss under water limitation. White birch leaf allocation did not change with size with either SMT, like the unresponsive findings above to CO_2 treatments. Green alder had the greatest leaf allocation and is the only species of the four to increase root allocation, when corrected for size difference, in response to DRT, which is most likely because of green alder's tolerance of dry conditions [8] compared to the other species. This was confirmed by green alder having the greatest above-to-below-ground dm ratio and the greatest total dm under DRT. Ref. [37] found that the shoot-to-root ratio decreased when *Alnus glutinosa* was exposed to drought, which is like what we found for green alder and speckled alder and, marginally so, for gray and white birch.

Allometric relationships for birches did not often differ significantly in response to drought, remaining the same for both stem and root dm allocation. Gray birch and white birch did not differ in stem dm allocation, and allocation simply increased with height, whereas root dm allocation decreased with height, but root allocation was greater in gray birch than white birch. For leaf dm allocation, gray birch followed the same trend as alders, wherein leaf dm was increased under irrigation but decreased under drought. White birch had no correlation with height, but leaf dm allocation was greater under drought than well-watered.

5. Conclusions

Genera differences in morphological and growth traits were large, especially in response to the environmental treatments used. Alders upregulated all growth traits under eCO_2 because of the strong coppicing sink effect and the additional foliar N provided by the actinorhizal ability of the genus. Visual confirmation of infection by *Frankia alni* was determined upon harvesting of the alder root systems. Aldershad a significantly greater foliar N with 2.8%, compared to birches with 1.0%. In contrast, birches downregulated or remained the same for all dry mass traits in response to eCO_2. This was a result of low soil nitrogen (0.12%) and low sink demand. In our previous paper [19], alders increased in dry mass and height as a result of greater photosynthetic efficiencies under eCO_2 but were greatly downregulated in birches. Both genera had large decreases in dry mass when exposed to drought; however, alders were still much larger on average under drought than birches. It has been proposed that alders are anisohydric, which would indicate that soil moisture levels within the experiment were high enough not to result in more severe consequences of this strategy, whereas birches were much more conservative, indicated by a lower percent decrease in dry mass and growth under drought. Stem height was determined to be the best predictor of total dry mass, which offers estimations for above and belowground dry mass production. Although both genera are viable considerations for use in site restoration and soil stabilization projects, alders demonstrate that the actinorhizal ability and strong sink effect make it a very desirable choice in projects that are nutrient-limited or aim to colonize a site as quickly as possible. With increasing atmospheric CO_2, particularly on low nutrient sites, the results show alders are capable of sequestering far more carbon than birches. In addition, with more atmospheric CO_2, alders can better mitigate against drought conditions compared to birches.

Author Contributions: J.E.M. designed and co-analyzed the experiment and co-authored the manuscript. A.B. managed the experiment, co-analyzed the data, and co-authored the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

Nomenclature

ABV/BLW dm	Above-to-below-ground dry mass ratio
aCO_2	Ambient CO_2
A_{dr}	Assimilation downregulation
CFS—AFC	Canadian Forest Service—Atlantic Forestry Center
DRT	Drought (treatment)
Dm	Dry mass
eCO_2	Elevated CO_2
iWUE	Intrinsic water-use efficiency
N	Nitrogen
SMT	Soil moisture treatment
VMC	Volumetric moisture content
WW	Well-watered (treatment)

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