Carbon Sequestration Efficiencies of Six Early-Successional Deciduous Species in Response to Atmospheric CO_2 and Soil Moisture Treatments

by

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Abstract

Early successional deciduous trees are an important component of many ecological restoration projects in Canada and are now often used in fire prone areas. As rising atmospheric CO_2 concentrations drive changes in climate, the long-term success of these projects depends on our ability to select and propagate species that can successfully adapt. We examined ecophysiological responses of six early successional deciduous tree species from three genera in response to a 2 x 2 factorial of CO_2 and soil moisture treatments. One year-old seedlings of gray birch (*Betula populifolia*), white birch (*B. papryifera*), pin cherry (*Prunus pennsylvanica*), choke cherry (*P. virginiana*), red oak (*Quercus rubra*) and bur oak (*Q. macrocarpa*) were planted in a low-nutrient medium, and subjected to ambient (400ppm) or elevated (800ppm) CO_2 and irrigated (10-15% moisture content) or droughted (3-8% moisture content) soil moisture treatments for two years in a greenhouse environment. Various growth traits were examined along with biochemical efficiency and gas exchange traits including assimilation at 600 ppm CO_2 (A_{600}), stomatal conductance (Gwv_{600}) intrinsic water use efficiency ($iWUE_{600}$), and maximum carboxylation rate (V_{cmax}). A subset of seedlings was administered additional fertilizer treatments during the study's 2nd year. After two years, elevated CO_2 did not affect growth traits but resulted in a downregulation of V_{cmax} and A_{600} for most species. Drought resulted in lower heights, biomasses and Gwv_{600} values but increased V_{cmax} and $iWUE_{600}$. Significant interactions with genus and or species were commonly observed. Additional fertilizer increased V_{cmax}, A_{600}, and stomatal conductance, with growth responses differing by genus. Differing responses to elevated CO_2 between years one and two indicate that trees become acclimatized overtime.

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List of Symbols, Nomenclature, or Abbreviations

A:	Carbon assimilation (μmol $m^{-2}s^{-1}$)
A_{600}:	Carbon assimilation at carbon dioxide concentrations of 600 parts per million (μmol $m^{-2}s^{-1}$)
A/C_i:	Ratio of carbon assimilation to intercellular carbon dioxide
aCO_2:	Ambient carbon dioxide (400 parts per million)
ANOVA:	Analysis of Variance
BO:	Bur oak (*Quercus macrocarpa* Michx.)
CC:	Choke cherry (*Prunus virginiana var. virginiana* L.)
C_i:	Intercellular carbon dioxide
cm:	Centimeters
CO_2:	Carbon dioxide
$\delta^{12}C$:	Carbon isotope with 12 neutrons
$\delta^{13}C$:	Carbon isotope with 13 neutrons
DAT:	Dynamic assimilation technique
df:	Degrees of Freedom
DMF:	Dimethyl-formamide
eCO_2:	Elevated carbon dioxide (800 parts per million for this experiment)
FERT:	Fertilizer treatment
Fig:	Figure
g:	Grams
GB:	Gray birch (*Betula populifolia* Marsh)
GLM:	General Linear Model

G_{wv}:	Stomatal conductance to water vapour (µmol $m^{-2}s^{-1}$)
Gwv_{600}:	Stomatal conductance to water vapour at carbon dioxide concentrations of 600 parts per million (µmol $m^{-2}s^{-1}$)
iWUE:	Intrinsic water use efficiency
$iWUE_{600}$:	Intrinsic water use efficiency at carbon dioxide concentrations of 600 parts per million
J_{max}:	Maximum rate of photosynthetic electron transport (µmol $m^{-2}s^{-1}$)
m:	Meters
meq/100g:	Milliequivalents per 100 grams
mL:	Millilitres
MS:	Mean Square
N:	Foliar nitrogen
p-value:	Probability of observing data as extreme assuming null hypothesis.
PC:	Pin cherry (*Prunus pennsylvanica* L. f.)
PPFD:	Photosynthetic photon flux density
ppm:	Parts per million
R^2:	Coefficient of determination
RO:	Red oak (*Quercus rubra* L.)
rpm:	Revolutions per minute
SMT:	Soil Moisture Treatment
TCC:	Total chlorophyll content, measured as sum of chlorophyll a and chlorophyll b
µmol·m^{-2}·s^{-1}:	Micromoles per square meter per second

VC: Variance components

V_{cmax}: Maximum rate of carboxylation

VPD: Vapour pressure deficit (kPa)

WB: White birch (*Betula papyrifera* Marsh.

Introduction

Ecological restoration is becoming an increasingly important practice within applied ecology as governments and other authorities seek to remediate the adverse ecological impacts of industrial activity. Restoration aims to restore the ecosystem services of degraded or damaged natural systems and often requires the reintroduction of native plant species. Conditions on restoration sites vary immensely based upon the original cause of degradation and therefore require site-specific considerations. The success of these projects will depend largely on our ability to select and propagate species that can successfully adapt to severe site conditions. Deciduous species are now often used in fire prone areas as they have greater moisture content, absence of volatile oils, lack ladder fuels (dead branches common with conifers) and landscape cooling with broad shade with more humid microclimate. Climate change is playing an increasingly vital role in project planning, as elevated levels of atmospheric carbon dioxide (CO_2) have been shown to increase the frequency and severity of drought events (Jain, 2022; Trenberth et al., 2014). The long-term success of any ecological restoration initiative, therefore, is contingent on a thorough understanding of how the chosen plant species will adapt to the predicted effects of climate change. This adaptation will depend largely on the ecophysiological and morphological responses of plant species to a variety of environmental factors. Chief among these are atmospheric CO_2 levels, soil moisture levels, and nutrient availability (Major et al., 2022).

The combined effects of drought and elevated CO_2 (eCO_2) concentrations on plants have been studied, though the great majority of prior research has focused on species with commercial importance to the forestry and agricultural sectors (van der Kooi

et al., 2016; Thiec and Dixon, 1996; Pérez-Jiménez et al., 2018; Kellner and Swihart, 2016; Major and Mosseler, 2021). In northeastern North America, many species used in ecological restoration are small trees or shrubs of little commercial value, and research into these species has been limited as a result. Moreover, responses to these factors have been found to vary considerably by species, with soil nutrient levels and length of exposure to treatment conditions resulting in different outcomes (Major et al., 2022).

Most studies on early successional deciduous trees have found that eCO_2 significantly affect their growth, physiology, and morphology, often resulting in increased biomass accumulation (Ainsworth and Long, 2005; Bazzaz et al., 1990; Atkinson et al., 1997; Bazzaz et al., 1993; Brisebois and Major, 2024; Centritto, 2002). Biochemical efficiencies including the maximum rates of carboxylation (V_{cmax}) and photosynthetic electron transport (J_{max}), as well as gas exchange traits including carbon assimilation (A), have been shown to upregulate in response to eCO_2 over the short term (Centritto et al., 1999; Heath and Kerstiens, 1997; Riikonen et al., 2008) while downregulating over longer periods. This has been observed in European species of birch and cherry, as well as in red oak (Bazzaz et al., 1993; Rey and Jarvis, 1997; Centritto et al., 1999; Pérez-Jiménez et al., 2018; Pérez-Jiménez et al., 2017a; Pérez-Jiménez et al., 2017b; Anderson and Tomlinson, 1998). Downregulation generally results due to a build up of non-structural carbohydrates in the leaves that occur due to low sink activity (Rey and Jarvis, 1997; Kitao et al., 2007; Byeon et al., 2021; Sanz-Sáez, 2010; Sugiura et al., 2019; Mosseler and Major, 2022).

The magnitude and duration of these responses, however, is often influenced by the availability of water and nutrients. Because soil nitrogen (N) is frequently a limiting

factor for plant growth, the addition of N fertilizers has been shown to reduce the downregulation of *A* (Sanz-Sáez, 2010; Sage, 1994; Reich et al., 2006; Pettersson et al., 1993; Brisebois and Major, 2024). Soil water availability is also a limiting factor, with drought conditions leading to decreased biomass and other physiological changes. Decreases in shoot-to-root ratio in response to drought, and especially decreases in fine root biomass, have been well documented (Joslin et al., 2000; Konôpka et al., 2007; Eldhuset et al., 2013; Brunner et al., 2015) and are generally a consequence of reduced transpiration and respiration rates. Interactions between drought and eCO_2 can also occur, and in at least one study, eCO_2 had stronger effects on *A* in droughted seedlings (Anderson and Tomlinson, 1998). Intrinsic water-use efficiency (*iWUE*) often increases under eCO_2, with reduced stomatal opening. (Eamus, 1991; Medlyn et al., 2001; Ainsworth and Rogers, 2007). This reduces transpiration and thus water loss, potentially allowing eCO_2 to mitigate some drought effects (Anderson and Tomlinson, 1998; Catovsky and Bazzaz, 1999; Centritto et al., 1999). Another important consideration for restoration sites is the ability of plants to resprout following damage. Previous studies conducted on oaks have shown increased biomass following coppicing as a result of increased shoot-to-root ratios (Mosseler and Major, 2022; Mosseler et al., 2019). The resulting reduction in foliage can limit leaf transpiration and increase root water absorption; potentially allowing seedlings to adapt better to dry soils (Farquhar et al., 2002; Mosseler et al., 2019). Understanding plant responses to each of these factors can be critical on restoration sites where water and soil nutrients are key limiting factors that influence seedling growth and survival.

To investigate and compare these relationships in different plants, we examined six species from three different phylogenetic families. White birch (WB, *Betula. papyrifera* Marsh.) and gray birch (GB, *B. populifolia* Marsh.) of the family *Betulaceae*, are both shade intolerant, early-successional, deciduous trees. White birch is a forest tree with a transcontinental range in North America. It is a site generalist, growing to 25 m and living up to 140 years (Safford et al., 1990). Gray birch is restricted to northeastern North America, where it occurs on sandy or gravelly sites, and is smaller and shorter lived than WB, growing to 10 m over a 50-year lifespan (Beauregard et al., 2020). Pin cherry (PC, *Prunus pennsylvanica* L. f.) and choke cherry (CC, *P. virginiana var. virginiana* L.) of the family Rosaceae, are small, short-lived, early successional, deciduous trees or tall shrubs (Welch, 2004; Wendel, 1990). Pin cherry grows to approximately 10–15 m while CC is smaller, growing to 5 m. Both species are shade intolerant, have a lifespan between 20–40 years and grow on a variety of soils (Welch, 2004; Wendel, 1990). The two species of oak; red oak (RO, *Quercus rubra* L.) and bur oak (BO, *Q. macrocarpa* Michx.) of the family Fagaceae, are larger and longer lived, growing to 25 m over their 200–300-year lifespans (Tardif, 2006; Nicolescu et al., 2020; Craft and Ashley, 2007). Both species are more moderately shade intolerant, with RO preferring well drained sandy or loamy soils, while BO is more common on clays (Johnson, 1990; Cogliastro et al., 1997). Bur oak ranges further west to the North American Midwest, whereas RO ranges further east, well into the Canadian Maritimes (Craft and Ashley, 2007; Nicolescu et al., 2020). All the above species are native to northeastern North America, exhibit rapid early growth, and can coppice following harvesting (Safford et al., 1990; Beauregard et al., 2020; Welch, 2004; Wendel, 1990;

Nicolescu et al., 2020; Craft and Ashley, 2007; Brisebois and Major, 2024; Mosseler et al., 2019). Many of these species are commonly used in ecological restoration projects but have limited commercial value in the forest sector (Brisebois and Major, 2024; Lavoie and Pellerin, 2015; Mosseler et al., 2019). As a result, there is limited literature available concerning their adaptation to the predicted effects of climate change.

The two chapters of this thesis will be presented in paper format. In chapter one, our goal was to examine and compare interspecies differences in ecophysiological traits and functional carbon efficiencies. To this end, bulk seed sources from central NB, Canada, were used to grow seedlings of each of the six species. These were planted in a low-nutrient, sandy soil medium and grown in growth chambers under environmentally controlled conditions between June 2023 and October 2024. The experiment utilized a 2×2 factorial design of CO_2 and soil moisture treatments. Seedlings were subject to either an ambient (aCO_2, 400 ppm) or elevated CO_2 (eCO_2, 800 ppm) treatment and a well-watered (10-15% soil moisture content) or droughted (3-8% soil moisture content) soil moisture treatment (SMT). In the second year, a subset of well-watered seedlings was also administered fertilizers to examine their effects. We examined biochemical efficiency traits including V_{cmax} and J_{max}, while gas exchange traits including carbon assimilation (A_{600}), stomatal conductance (G_{wv600}), and intrinsic water use efficiency ($iWUE_{600}$) were measured and compared at a common CO_2 concentration of 600 ppm. Foliar concentrations of nitrogen (N), total foliar chlorophyll content (TCC) and the heavy carbon isotope $\delta^{13}C$ were also examined. We hypothesized that eCO_2 treatments would result in greater $iWUE_{600}$ under drought conditions, and that differences in functional carbon efficiencies would appear at the genus and species levels. To test our

hypothesis, we quantified these carbon efficiencies and the variation attributed to genus and species. We also examined the ecophysiological responses and interactions relative to the CO_2 and SMTs. In addition, we assessed if additional fertilizer treatment (FERT) resulted in an enhancement of the CO_2 effect in the second year under well-watered conditions. By analyzing these traits, we aim to better understand the impact of eCO_2 on drought response at the metabolic level and how this varies by species.

In chapter two, we aim to understand how these metabolic responses translate into growth and morphological characteristics. Our goal was to examine and compare interspecies differences in growth traits for each of the six species. The experiment utilized the same design and factorial of CO_2 and SMTs as chapter one and took place over the same time period. Additionally, we coppiced half of replicates at end of year one to examine responses to damage in ecological restoration scenarios. We hypothesized that well-watered and eCO_2 treatments would result in increased biomass under all conditions and that eCO_2 might mitigate some effects of drought. We expected the effect of fertilizer to be similar, potentially enhancing the CO_2 response. We also expected differences in growth traits to appear at the genus and species levels. To test these hypotheses, we quantified growth traits and variation attributed to genus and species and examined the possible interactions relative to the CO_2 and SMTs. As in chapter one, we also assessed if additional fertilization resulted in increased morphological gains in the second year under well-watered conditions.

The intent of this experiment was to expand the current scientific knowledge base on North American early-successional deciduous tree species by investigating the interactive relationships between eCO_2, soil moisture stress, and available N. By

examining their effects on ecophysiology and morphology, this research will allow future scientists and ecologists to engineer restorative solutions that better incorporate the predicted effects of global climate change.

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Chapter 1: Physiological variation of Six Early-Successional Deciduous Species in Response to Atmospheric CO_2 and Soil Moisture Treatments

Abstract

Physiological genetic variation of six early-successional deciduous tree species was examined over two years under a 2×2 factorial design of CO_2 and soil moisture treatments. One year old seedlings were planted in a low-nutrient, sandy soil medium, and were subjected to ambient (aCO_2, 400 ppm) or elevated CO_2 (eCO2, 800 ppm) treatments and well-watered (10–15% soil moisture content) or droughted (3–8% soil moisture content) soil moisture treatments. We examined biochemical efficiencies, gas exchange traits, TCC, and foliar N. A subset of seedlings was administered additional fertilizer treatments during the study's 2nd year. Two species from three genera were used, namely gray birch (GB, *Betula populifolia* Marsh.) white birch (WB, *B. papyrifera* Marsh.), pin cherry (PC, *Prunus pennsylvanica* L. f.), choke cherry (CC, *P. virginiana var. virginiana* L.), red oak (RO, *Quercus rubra* L.), and bur oak (BO, *Q. macrocarpa* Michx.). In the first year of study, V_{cmax} was upregulated, but A_{600} was lower in response to eCO_2. Oaks and birches were equal, and cherries had the lowest values. There were few drought effects in the first year. In the second year of treatments, biochemical efficiency traits, A_{600}, G_{wv600}, TCC, and foliar N were downregulated in most species except PC. Drought decreased G_{wv600} and A_{600} as expected, but upregulated V_{cmax}, J_{max}, TCC, foliar N, and $iWUE_{600}$ while also increasing concentrations of the heavy $\delta^{13}C$ isotope in plant tissues. Additional fertilizer increased biochemical efficiencies, gas

exchange, TCC, and N but $iWUE_{600}$ response was dependent on species. Significant interactions with species and or genus occurred in many cases mostly due to magnitude effects. Birches generally showed greater responses to treatment variables than cherries in the second year, which showed greater responses than oaks. In most cases, both species of birch responded similarly to the same treatments. The same was true of oaks. Means for most traits were often slightly greater in GB and BO than in WB and RO respectively. Conversely, cherry species often responded differently with PC means for most traits often much greater than those of CC. Upregulation of V_{cmax} and J_{max} under eCO_2 in year one of the study followed by downregulation under the same treatments in year two may indicate that these species become acclimatized to eCO_2 overtime. This appears to be due to limitations in soil N, thus, studying the progression of these traits in response to longer term fertilizer additions may be worthwhile.

Introduction

Early-successional deciduous trees are becoming a critical component of many ecological restoration projects. Deciduous species are now often used in fire prone areas as they have a greater moisture content, lack volatile oils and ladder fuels (dead branches common with conifers), and aid in landscape cooling as broad shade creates more humid microclimates. As rising atmospheric CO_2 concentrations continue to drive changes in climate and precipitation patterns, the long-term success of these projects will depend on our ability to select and propagate species that can successfully adapt to these harsh conditions. Such successful adaptation will depend on the physiological and morphological responses of plant species to a variety of environmental factors including CO_2 levels, soil moisture, and nutrient availability (Major et al., 2022). Differential adaptive responses to these factors at both the genera and species level imply potential for altered competitive relationships that may result in further impacts to plant community composition (van Breugel et al., 2012). Understanding these relationships will thus have a significant impact on the planning and design of restoration projects in the future.

To investigate these relationships, we examined six species from three different phylogenetic families. White birch (WB, *Betula. papyrifera* Marsh.) and gray birch (GB, *B. populifolia* Marsh.) of the family Betulaceae, are both early successional, deciduous trees. White birch is a forest tree with a transcontinental range in North America. It is a site generalist, growing to 25 m and living up to 140 years (Safford et al., 1990). Gray birch is restricted to northeastern North America, where it occurs on sandy or gravelly sites (Beauregard et al., 2020). It is smaller and shorter lived than WB, living approximately 50 years and growing to 10 m. Pin cherry (PC, *Prunus pennsylvanica* L.

f.) and choke cherry (CC, *P. virginiana var. virginiana* L.) of the family *Rosaceae*, are short-lived, early successional, small deciduous trees or tall shrubs (Welch, 2004; Wendel, 1990). Pin cherry grows to approximately 10–15 m while CC is smaller, growing to 5 m. Both species have a lifespan between 20-40 years and grow on a variety of soils (Welch, 2004; Wendel, 1990). The two species of oak; red oak (RO, *Quercus rubra* L.) and bur oak (BO, *Q. macrocarpa* Michx.) of the family *Fagaceae* are larger and longer lived, growing to 25 m over their 200–300-year lifespans (Tardif, 2006; Nicolescu et al., 2020; Craft and Ashley, 2007). Both species are more moderately shade intolerant (Tardif, 2006; Nicolescu et al., 2020). Red oak prefers well-drained sandy or loamy soils while BO is more common on clays (Craft and Ashley, 2007; Nicolescu et al., 2020). Bur oak ranges further west to the North American Midwest, whereas RO ranges further east, well into the Canadian Maritimes (Craft and Ashley, 2007; Nicolescu et al., 2020). All the above species are native to northeastern North America, exhibit rapid early growth, coppice following harvesting, and have been used in ecological restoration projects (Safford et al., 1990; Beauregard et al., 2020; Welch, 2004; Wendel, 1990; Nicolescu et al., 2020; Craft and Ashley, 2007; Brisebois and Major, 2024; Mosseler et al., 2019).

Previous studies on early successional hardwoods have detected short term increases in A and $iWUE$ in response to eCO_2, while G_{wv} has generally been shown to decrease (Centritto et al., 1999; Li et al., 2008; Ponton et al., 2002; Medlyn et al., 1999). The magnitude and time span of these changes also vary, with soil nutrients, water availability, length of exposure to eCO_2, and species significantly affecting these relationships (Brisebois and Major, 2024; Medlyn et al., 2001; Maillard et al., 2001). Due

to their limited commercial importance, comparatively little scientific literature exists for the specific species examined in this study. The authors of Morse et al. (1993) found that GB seedlings exposed to drought exhibited increased tissue elasticity under eCO_2 (700 ppm) allowing them to maintain positive turgor pressure at lower water potentials. In WB, eCO_2 has been shown to increase net photosynthesis and enhance growth in times of drought (Catovsky and Bazzaz, 1999; Riikonen et al., 2008). Long-term exposure to eCO_2 has also been shown to downregulate photosynthesis, including V_{cmax} and J_{max}, in the European species *Betula pendula* (Rey and Jarvis, 1997). In a study of RO, eCO_2 increased A and $iWUE$ but decreased G_{wv}. These effects were found to be stronger when seedlings were experiencing drought stress (Anderson and Tomlinson, 1998). Although very few studies exist for PC or CC, the literature revealed either similar results (Centritto et al., 1999; Pérez-Jiménez et al., 2018; Pérez-Jiménez et al., 2017a; Pérez-Jiménez et al., 2017b) or no change (Centritto, 2002; Centritto, 2005; Centritto et al., 1999) in these gas exchange parameters for European *Prunus* species in response to eCO_2.

Our goal was to examine and compare interspecies differences in ecophysiological traits and functional carbon efficiencies in response to a 2 x 2 factorial of soil moisture and CO_2 treatments. We examined biochemical efficiency traits including V_{cmax} and J_{max}, while gas exchange traits including A_{600}, G_{wv600}, and $iWUE_{600}$ were examined at a common CO_2 concentration of 600 ppm. Foliar concentrations of N, TCC, and the heavy carbon isotope $\delta^{13}C$ were also examined. Each of the six birch, cherry and oak species was examined under either an aCO_2 (400 ppm) or eCO_2 (800 ppm) CO_2 treatment and a well-watered (10–15% soil moisture content) or droughted (3–

8% soil moisture content) SMT. A subset of seedlings was also administered additional fertilizer treatments during the study's 2nd year to examine any effects on the above traits. We hypothesize that eCO_2 treatments would result in greater *iWUE* under drought conditions, and that differences in functional carbon efficiencies would appear at the genus and species levels. To assess our hypothesis, we quantified functional carbon efficiencies and variation attributed to genus and species and examined the ecophysiological responses and interactions relative to the CO_2 and SMTs.

Materials and Methods

Plant Material Growing and Preparation

All seedlings used in this experiment were grown from bulk seed sources collected in western and central NB, Canada (Table 1). Seedlings were grown in hard-sided multi-pot trays of 45 (90 cm^3) cells at the Atlantic Forestry Center (AFC) greenhouse in Fredericton, NB (45◦ 52′ N, 66◦ 31′ W). Seeds were sown in early summer 2022. After entering winter dormancy in late 2022, each stem was trimmed to a height of 10 cm to reduce leaf transpiration-to-root water absorption ratio, a technique recently common in restoration (Farquhar et al., 2002). Following trimming, all seedlings were removed from the trays, boxed, and stored in AFC freezers at -4ºC until June 18th, 2023.

Table 1. Species and seed sources of *Betula*, *Prunus* and *Quercus* seedlings utilized in the experiment.

Species	Seed Source	Approximate Latitude	Approximate Longitude	# Per Chamber	Total Seedlings
Betula populifolia	CFB Gagetown, NB	45.570410	-66.375810	11	88
Betula papyrifera	CFB Gagetown, NB	45.570410	-66.375810	11	88
Prunus pensylvanica	CFB Gagetown, NB	45.570410	-66.375810	11	88
Prunus virginiana	New Maryland, NB	45.891030	-66.684795	11	88
Quercus rubra	Oromocto, NB	45.837485	-66.422915	11	88
Quercus macrocarpa	Jemseg, NB	45.811415	-66.095165	11	88
Totals				66	528

Treatment Delivery 2023

Two identical greenhouses were used for the experiment. Within each greenhouse, four treatment-chambers were constructed: each measuring 4.2 × 2.4 × 2.4 m and consisting of a wooden frame covered by 1 cm thick sheets of corrugated transparent plastic. Each treatment-chamber was randomly assigned one of two CO_2 treatments; aCO_2 (400 ppm) or eCO_2 (800 ppm), and one of two SMTs; well-watered (10–15% soil moisture content) or droughted (3–8% soil moisture content). Thus, the experiment contained four treatment conditions, each replicated twice, once in each of the two greenhouses (blocks). Within each treatment chamber, 84 fabric root control bags (Smart Pot Pro 5 Gallon, High Caliper Growing Inc., Oklahoma City, Oklahoma, USA) were arranged in a 7 × 12 grid pattern on plastic pallets to ensure adequate drainage. Each bag contained approximately 18 L of screened sand (M.W. Price & Sons, Charter's Settlement, NB, CA). Bulk soil samples were collected on June 23rd and sent for nutrient and soil texture analysis at the University of New Brunswick in Fredericton. The soil texture properties were, on average, 0.7% clay, 2.1% silt, and 97.2% sand. Overall, soils contained 0.35% organic matter, 0.06% N, 0.21% carbon (C), and 0.02% sulfur

(S),sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) averaged 0.03, 0.04, 0.38 and 0.08 meq/100 g, respectively. On June 18th, 2023, seedlings were removed from cold storage and allowed to thaw in refrigerators at 4ºC until planting on June 21st. Root control bags were watered to field capacity immediately before planting to minimize transplant shock, and each seedling was planted randomly in its own root control bag. Eleven replicates of each species were planted, totaling 66 experimental seedlings per chamber. Water was delivered to each root control bag in equal amounts using a drip irrigation system at a rate of approximately 33 mL/minute. Spigots were placed centrally in each bag. Sensors (5TM, Decagon Devices, Pullman, WA, USA) were used to monitor soil moisture content in each treatment chamber, while water was administered manually by switching on and off the drip irrigation system for each chamber. This was done as needed based on the soil moisture readings such that treatment levels were maintained. The frequency and dosage of waterings varied by treatment, chamber, and in response to external weather conditions, which influenced humidity, temperature, and evaporation rates. Fertilizer, when administered using this system, was added at a rate of 1 L per 50 L of water using a D14MZ10 Coolant Mixer (Dosatron, Clearwater, FL, USA). Air temperatures within each chamber were controlled by two air conditioning units set to engage if temperatures reached or exceeded 27°C. Air circulation was facilitated by a series of ducts which drew air from the outside environment using fans. Air temperature and humidity were monitored and logged using a separate system of wireless sensors (Tempstick, Ideal Sciences, Bountiful, UT, USA). Carbon dioxide was supplied from compressed gas canisters and introduced to eCO_2 chambers via air intake ducts. Atmospheric CO_2 concentrations in each chamber were measured centrally using Li-

840A CO_2/H_2O Gas Analyzers (LI-COR Inc., Lincoln, Nebraska). Carbon dioxide concentration was automatically maintained at consistent levels in the eCO_2 treatments using an electronic solenoid manifold, with CO_2 being released automatically if the concentration was ≤ 800 ppm as detected by the analyzer. Concentrations in aCO_2 treatments were allowed to fluctuate naturally but averaged approximately 400 ppm. Carbon dioxide treatments began immediately after seedlings were planted on June 21st.

To ensure proper seedling establishment, droughted and well-watered chambers were administered equal amounts of water until July 19th, 2023, when a 400 mL dose of grower fertilizer mixture (Plant-Prod "Forestry Special" 20:8:20, 625 g/50 L, +1250 mL of MgNiFeCal) was administered to each bag at half strength using the drip irrigation system (Table 2). Additional doses (450 mL) of grower fertilizer mixture were also administered to each bag on August 17th and 24th (Table 2). On October 30th, 2023, five trees per species per chamber were harvested to examine biomass growth. Stems were cut approximately three cm above the soil surface to allow for coppice regrowth the following spring. Treatments continued until November 8th, 2023, at which point, shade curtains were installed to induce winter dormancy, and all experimental treatments were paused. Seedlings overwintered within the growth chambers, with all treatment groups receiving equal amounts of water and aCO_2. To prevent freezing of greenhouse infrastructure, temperature in the chambers was maintained at slightly above 0ºC.

Table 2. Schedule of fertilizer additions applied to experimental seedlings during the 2023 growing season.

Year	Date	Brand	Product Name	NPK Ratio	Mix Ratio	Volume Mixed (mL)	Mix Volume Administered (mL)
2023	19-Jul	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	8	400
	17-Aug	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	9	450
	24-Aug	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	9	450

Treatment Delivery 2024

Carbon dioxide treatments resumed on April 18th, 2024, at which point shade curtains were removed. To ensure proper recovery of the coppiced trees, all trees were administered equal amounts of water until May 7th. Starter fertilizer (Plant-Prod "Forestry Starter" 10:52:10, 312.5 g/25 L) was administered to all trees on May 1st and May 7th, as outlined in Appendix B. Drought treatments were resumed the following day. Plants were further fertilized with Grower fertilizer (Plant-Prod "Forestry Special" 20:8:20, 1250g/100L) followed by MagNifiCal (REMO "MagNifiCal" 3:0:0, 1.5 mL/25 L) on May 17th and 23rd, and on June 7th (Appendix B.). In subsequent treatments, fertilizer was added before and after MagNifiCal to prevent reactions between the different fertilizer chemicals (Appendix B.).

Experimental Fertilizer Treatments, 2024

On September 6th, 2024, four saplings of each species were selected at random in each well-watered treatment chamber. After removing their drip-irrigation spigots, the root bag of each sapling was lifted to sever any root connections with neighbouring bags. To prevent water and fertilizer from being absorbed by neighboring saplings, a plastic bag was perforated on its underside to allow for drainage and placed over each root bag. Two of the seedlings from each species were selected at random and assigned to the

fertilized treatment group, with the remaining two seedlings assigned to the unfertilized treatment group. All fertilized saplings received 1 L of fertilizer mixture, which was 50 parts water, and one part grower fertilizer (Plant-Prod "Forestry Special" 20:8:20, 1250 g/100 L). Unfertilized saplings received 1 L of water only. Fertilizer treatment (FERT) began on September 10th, 2024, and occurred thrice weekly, until October 15th (Appendix B).

Biochemical efficiencies and gas exchange traits, 2023

Between September 18th and October 6th, 2023, assimilation to internal CO_2 (A/C_i) curves were created *in situ* using an LI-6800 portable gas-exchange system (LI-COR, Lincoln, NE, USA). Biochemical efficiencies were then determined from the results using graphical presentation and curve-fitting algorithms (Sharkey et al., 2007). Two individuals of each species were measured per treatment-chamber in both blocks for a total of 96 curves. The species, chamber and specimen were randomly selected immediately prior to curve creation. The instrument was programmed to maintain an air flow of 400 µmol $m^{-2}s^{-1}$, and a saturating photosynthetic photon flux density (PPFD) of 1000 µmol $m^{-2}s^{-1}$. Leaf temperature was set at 25ºC in order to maintain a vapor pressure deficit (VPD) of 1.0–1.8 kPa. Relative humidity was also adjusted situationally to maintain this, but was generally set at about 60%. The fan speed was 9000 rpm, while light (colour spec R90B40) was set to 500 µmol $m^{-2}s^{-1}$. Carbon assimilation was measured at 400, 330, 260, 190, 120, 50, 400, 400, 600, 800, 1200, 1700, and 2200 ppm in that order. Multiple measurements were taken at 400 ppm to ensure the plant had acclimated to changing CO_2 concentrations. To generate each curve, a fully expanded healthy leaf selected from near the top of a major shoot. Chamber conditions were

allowed a period of five to ten minutes to stabilise before curve creation began. A settling time of at least two minutes also occurred once each CO_2 level was reached before measurements.

Following data collection, each A/C_i response curve was fitted using "A/C_i curve fitting utility" *v.* 2007.1, a commercially available Microsoft Excel-based calculator developed by the authors of Sharkey et al. (2007). Estimates for V_{cmax} and J_{max} were fit using the program's algorithms. A least-squares fit was used to generate estimations and fit the model via the solver add-in for Microsoft Excel. Measurements of gas exchange parameters taken at an intermediate CO_2 level of 600 ppm were used to compare results between treatments and examine physiological downregulation. Intrinsic water use efficiency at 600 ppm was calculated as A_{600} divided by G_{wv600}.

Biochemical efficiencies and gas exchange traits, 2024

Assimilation to internal CO_2 curves were also created during the second growing season between August 6th and August 2nd 2024 using the Dynamic Assimilation Technique ™ (DAT) as described in (LI-COR, Inc, n.d.; Tejera-Nieves et al., 2024). This method was used as a time saving measure as it does not require chamber conditions to stabilise prior to curve creation. The same sample design described in the preceding section was used to create 96 A/C_i curves, though random selection resulted in a new set of replicate saplings. Machine settings for PPFD, leaf temperature, VPD, relative humidity, fan speed and light were the same as those used in 2023. For the DAT, start and end CO_2 were set at 10 and 2200 ppm, respectively. Ramp rate was set to 300 ppm/minute, with a pre-ramp wait time of one minute, and log interval of five seconds.

Because the DAT does not routinely take internal CO_2 (C_i) measurements at exactly 600 ppm, the nearest C_i outputs below and above 600 ppm (about 590 ppm and 610 ppm, respectively) were averaged and used to compare results between treatments. The previous model (Sharkey et al., 2007) could not be used during the 2024 season due to the substantial number of data points generated by the DAT. The R-based curve fitting routine "Plantecophys" (Duursma, 2015) was used to estimate V_{cmax} and J_{max} as an alternative as recommended by LI-COR Inc. Any curves that could not be autofit by this model were assessed visually and fitted using "msuRACifit" (McClain et al., 2025), another R-based curve fitting routine, when appropriate.

Foliar Chlorophyll

Foliar chlorophyll samples were taken on October 10th in 2023 and September 4th and October 22nd in 2024 with the same methodology used in both years. The same sample design used to measure biochemical efficiencies and gas exchange traits was applied, with each of the same 96 saplings that had been measured with the Li-6800 having two leaves harvested. These included the previously marked leaf and one other leaf chosen at random. The leaves harvested were mature, of healthy appearance, and taken from the top one-third of each plant. A standard hole-punch (3 mm radius) was used to collect two small samples (discs) from the central blade of both leaves. These were placed in labeled microcentrifuge tubes, while the remaining leaf tissue was placed into sealed, labeled plastic bags. Following collection, the tubes and bagged leaves were immediately placed on ice in opaque coolers to avoid degradation of the chlorophyll during transport. Upon return to the laboratory, all samples were stored at -80ºC to preserve them for analysis. On December 4th, 2023, and January 7th, 2024, the discs were

removed from the freezer, weighed to the nearest 0.00001 g (AT261 DeltaRange, Mettler Toledo Inc., Columbus, OH, USA), and placed back in the microcentrifuge tubes. To each tube, 1.5 mL of dimethyl-formamide (DMF) was added to extract the chlorophyll. The tubes were then sealed, covered in aluminum foil to prevent light degradation, and placed on an orbital shaker set to 125 rpm (Innova 2000 platform shaker, New Brunswick Scientific, Edison, NJ, USA) for 12 hours at room temperature to ensure proper mixing and extraction. On the following day, the microplates used for absorbance (Corning COSTAR 3364 Storage Plate, ETC) were scanned using a spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd., Holliston, MA, USA) at 480, 647, and 664 nm wavelengths for the later correction of absorbance readings. Each microtube was mixed by inversion before 40 µL of liquid was extracted from each tube and added to each microplate cell. Each cell was then diluted with a further 80 µL of DMF. On each microplate, eight cells were left empty to act as control readings. Absorbance readings were then taken at 480, 647, and 664 nm wavelengths. All blank readings were < 0.003 for each wavelength after the plate correction was made. Concentrations (mg/g) of chlorophyll a, chlorophyll b, TCC (chlorophyll a + chlorophyll b), were then calculated using the previously weighed fresh weight of each sample.

Foliar Nutrients and Stable Isotope Analysis

In 2023, total foliar N content was analysed by the Laboratory for Forest Soils and Environmental Quality at the University of New Brunswick. The bagged tissue samples were submitted to the laboratory and analysed using an elemental analyzer (CNS-2000, LECO Corporation, St. Joseph, MI, USA).

Carbon stable isotope ratios were also examined in 2024 to test whether differences in drought stress existed between trees in the well-watered and droughted soil moisture regimes. The ratio of $\delta^{12}C$-to-$\delta^{13}C$ can be used as an analogue to analyse drought stress, as stressed plants accumulate greater amounts of the heavier $\delta^{13}C$ isotope because of decreased discrimination at lower C_i (Hentschel et al., 2016; Gouveia, 2019). $\delta^{13}C$ percentages from the 2024 season were reported by the Stable Isotopes in Nature Laboratory (SINLab) at the University of New Brunswick. Foliar samples were analysed for $\delta^{13}C$ and using a DeltaPlus XP – Conflo IV mass spectrometer (Thermo Finnigan; Bremen, Germany). Total foliar N concentration of the 2024 samples was also conducted and reported by SINLab as part of the same testing service. It should be noted that while the CO_2 effect accounts for the vast majority of total variation in $\delta^{13}C$ content (Table 10), its significance can be attributed to the lack of heavy isotopes in the gas supplied to the eCO_2 chambers. As such, the CO_2 effect and its interactions were not included in our analysis, as its main purpose was to analyse differences between soil moisture regimes.

Statistical Analyses

This experiment utilized a randomized block design. The fixed effects in the physiological model were genus, species, CO_2 treatment, and SMT. Growth chamber (block) and replicate seedling were random effects. A general linear model (GLM) was used to conduct mixed-effects analyses of variance (ANOVA) with nested factors, using SYSTAT version 13.2.01 (Palo Alto, CA, USA). The following model was used for biochemical efficiency, gas exchange, and foliar nutrient parameters in the main experiment:

$$Y_{ijklm} = \mu + B_i + G_j + C_k + H_l + GC_{jk} + GH_{jl} + CH_{kl} + GCH_{jkl} + S_{m(j)} + SC_{m(j)k} + SH_{m(j)l} + SCH_{m(j)kl} + e_{ijklm},$$

where term Y_{ijklm} denotes the dependent seedling of the i^{th} growth chamber, the j^{th} genus, the k^{th} CO_2 treatment, the l^{th} SMT, the m^{th} species, with μ as the overall mean. Term 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), and H_l is the effect of the l^{th} SMT (l = 1, 2). Term GC_{jk} is the interactive effect between genus j and CO_2 treatment k. Term GH_{jl} is the interactive effect of genus j and SMT l. Term CH_{kl} is the interactive effect of CO_2 treatment k and SMT l. Term GCH_{jkl} is the interactive effect of genus j, CO_2 treatment k and SMT l. Term $S_{m(j)}$ is the effect of species m (m = 1,…, 6) nested in genus j. Term $SC_{m(j)k}$ is the interactive effect of species m nested in genus j with CO_2 treatment k. Term $SH_{m(j)l}$ is the interactive effect of species m nested in genus j with SMT l. finally, Term $SCH_{m(j)kl}$ is the interactive effect of species m nested in genus j with CO_2 treatment k and SMT l. Term e_{ijklm} represents the random error component, which incorporates interactions with the growth chamber factor and any variation among seedlings. The fertilizer sub-experiment used the same GLM, with SMT treatment (H) substituted for fertilizer treatment (F).

Effects were considered statistically significant at the $p = 0.05$ level, although individual p-values are listed for the reader's interpretation. The data satisfied assumptions of normality and equal variance. Any source of variation, if significant was post-hoc tested using the Tukey mean separation test ($p = 0.05$). The variance component analysis in the ANOVA tables was conducted using the sum of squares as outlined in Hicks (1964).

Results

Biochemical Efficiency Traits

V_{cmax} – Maximum rate of carboxylation

Genus and CO_2 effects were the only significant factors affecting V_{cmax} during the first growing season in 2023. These accounted for 12 and 6% of the total variation, respectively (Table 3). The maximum rate of carboxylation was upregulated in eCO_2 in all species by 23% on average (Fig. 1A). In addition, it was significantly greater in birches and oaks than in cherries, with average values of 53.4, 52.7 and 38.0 μmol $m^{-2}s^{-1}$, respectively (Fig. 1A).

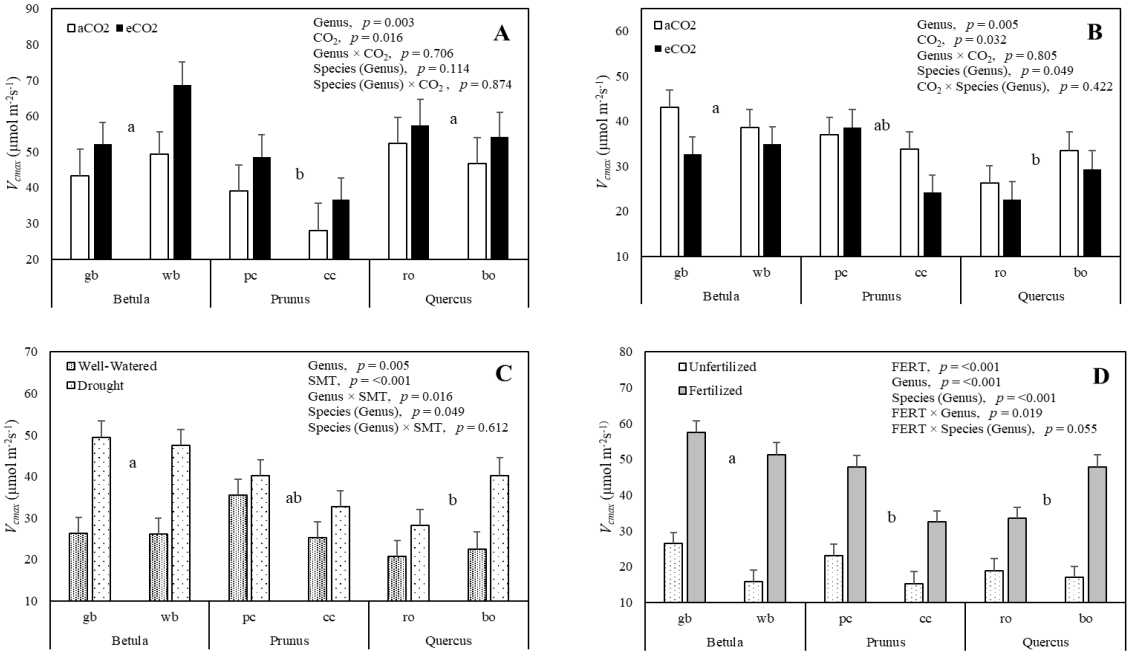

Figure 1. Maximum rate of carboxylation (V_{cmax}) in µmol $m^{-2}s^{-1}$ by species, genus and (A) CO_2 treatment in the 2023 growing season, (B) CO_2 treatment in the 2024 growing season, (C) soil moisture treatment (SMT) in the 2024 growing season, and (D) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2023, 2024 or fertiliser experiment. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with different lower-case letters.

Table 3. ANOVA outputs for Maximum rate of carboxylation (V_{cmax}), and maximum rate of electron transport (J_{max}) for seedlings in the 2023 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	V_{cmax} (μmol $m^{-2}s^{-1}$)			df	J_{max} (μmol $m^{-2}s^{-1}$)		
		MS	VC (%)	*p*-Value		MS	VC (%)	*p*-Value
Block	1	623.0	1.78	0.166	1	480.4	1.45	0.152
Genus	2	2076.3	11.87	**0.003**	1	836.9	2.53	0.060
CO_2	1	1933.8	5.53	**0.016**	1	201.2	0.61	0.352
SMT	1	6.1	0.02	0.890	2	3809.4	23.05	**<0.001**
Genus × CO_2	2	111.5	0.64	0.706	1	21.1	0.06	0.762
Genus × SMT	2	373.8	2.14	0.315	2	134.8	0.82	0.558
CO_2 × SMT	1	233.5	0.67	0.395	2	236.1	1.43	0.362
Genus × CO_2 × SMT	2	69.8	0.40	0.804	2	169.8	1.03	0.480
Species (Genus)	3	653.8	5.61	0.114	3	554.5	5.03	0.073
Species (Genus) × CO_2	3	73.8	0.63	0.874	3	118.1	1.07	0.673
Species (Genus) × SMT	3	291.8	2.50	0.438	3	172.1	1.56	0.525
Species (Genus) × CO_2 × SMT	3	526.7	4.52	0.185	3	375.3	3.41	0.188
Error	70	318.5	63.71		70	229.0	48.50	
R^2			0.357				0.508	

In the second growing season, genus, species CO_2, and SMT, had significant effects on V_{cmax} accounting for 8, 6, 3, and 25% of total variation, respectively (Table 4). There was also a significant Genus × SMT interaction accounting for 6% of the total variation. Elevated CO_2 downregulated V_{cmax} in 2024, with mean values approximately 18% lower for all species except PC, which was about the same (Fig. 1B). The Genus × SMT interaction was due to magnitude effects. That is, all genera upregulated but to varying magnitudes as there was no rank changes (Fig. 1C). Birches displayed the greatest response (85%), followed by oaks and cherries, at 59 and 20%, respectively. Mean V_{cmax} was significantly greater in birches (37.3 μmol $m^{-2}s^{-1}$) than oaks (27.9 μmol $m^{-2}s^{-1}$), though neither was significantly different from cherries (33.4 μmol $m^{-2}s^{-1}$; see Fig. 1C). By species, both PC, and GB displayed the greatest mean mean with 37.8 μmol $m^{-2}s^{-1}$. These were followed by WB (36.8 μmol $m^{-2}s^{-1}$), which was significantly greater

than RO (24.4 µmol $m^{-2}s^{-1}$). Bur oak and CC, with values of 31.3 and 28.9 µmol $m^{-2}s^{-1}$ were not significantly different from WB or RO. Overall, average V_{cmax} values were 32% lower in 2024 than in 2023 (Fig. 1A, 1B).

Table 4. ANOVA outputs for Maximum rate of carboxylation (V_{cmax}), and maximum rate of electron transport (J_{max}) for seedlings in the 2024 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	V_{cmax} (µmol $m^{-2}s^{-1}$)			df	J_{max} (µmol $m^{-2}s^{-1}$)		
		MS	VC (%)	*p*-Value		MS	VC (%)	*p*-Value
Block	1	36.0	0.20	0.587	1	64.7	0.08	0.735
Genus	2	686.8	7.78	**0.005**	2	1796.0	4.46	**0.047**
CO_2	1	577.3	3.27	**0.032**	1	21.1	0.03	0.847
SMT	1	4359.4	24.68	**<0.001**	1	12233.0	15.20	**<0.001**
Genus × CO_2	2	26.3	0.30	0.805	2	103.8	0.26	0.832
Genus × SMT	2	527.9	5.98	**0.016**	2	4319.3	10.74	**0.001**
CO_2 × SMT	1	47.4	0.27	0.533	1	60.0	0.07	0.745
Genus × CO_2 × SMT	2	63.9	0.72	0.592	2	158.3	0.39	0.755
Species (Genus)	3	331.9	5.64	**0.049**	3	3498.9	13.05	**0.001**
Species (Genus) × CO_2	3	114.5	1.94	0.422	3	936.3	3.49	0.182
Species (Genus) × SMT	3	73.5	1.25	0.612	3	425.2	1.59	0.522
Species (Genus) × CO_2 × SMT	3	47.1	0.80	0.760	3	463.8	1.73	**0.485**
Error	69	120.8	47.18		70	562.2	48.91	
R^2			0.528				0.511	

In 2024, results compared fertilized and unfertilized subsets of well-watered seedlings. The main effects of genus, FERT and species were significant, and accounted for 5, 57, and 7% of the total variation, respectively (Table 5). There were also significant FERT × Genus and FERT × CO_2 × Species interactions, both accounting for 3% of the total variation. Both were magnitude effects, no rank change (Fig. 1D). The maximum rate of carboxylation was always upregulated in fertilized seedlings by 133% on average, though the effect of CO_2 varied by species.

Table 5. ANOVA outputs for Maximum rate of carboxylation (V_{cmax}), and maximum rate of electron transport (J_{max}) for seedlings in the fertilizer experiment, including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). FERT denotes fertilizer treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	V_{cmax} (μmol $m^{-2}s^{-1}$)			df	J_{max} (μmol $m^{-2}s^{-1}$)		
		MS	VC (%)	*p*-Value		MS	VC (%)	*p*-Value
Block	1	51.7	0.19	0.425	1	1501.7	0.42	0.412
Genus	2	699.2	5.27	**<0.001**	2	962.3	0.54	**0.648**
CO_2	1	18.4	0.07	**0.634**	1	2073.3	0.59	0.336
FERT	1	15143.3	57.05	**<0.001**	1	130850.1	36.99	**<0.001**
Genus × CO_2	2	80.7	0.61	0.372	2	4010.8	2.27	0.17
Genus × FERT	2	336.7	2.54	**0.019**	2	2518.2	1.42	0.326
CO_2 × FERT	1	223.3	0.84	0.100	1	4634.7	1.31	0.152
Genus × CO_2 × FERT	2	10.1	0.08	0.882	2	4981.6	2.82	0.113
Species (Genus)	3	624.3	7.06	**<0.001**	3	8625.6	7.32	**0.012**
Species (Genus) × CO_2	3	27.8	0.31	0.792	3	175.3	0.15	0.971
Species (Genus) × FERT	3	214.0	2.42	0.055	3	1812.7	1.54	0.487
Species (Genus) × CO_2 × FERT	3	262.4	2.97	**0.027**	3	3336.6	2.83	**0.219**
Error	68	80.4	20.60		67	2207.3	41.81	
R^2			0.793				0.587	

J_{max} - Maximum rate of photosynthetic electron transport

Responses of the maximum rate of photosynthetic electron transport to treatments were mostly similar to those of V_{cmax}, though the effects of CO_2 were not significant (not shown). In 2023, Genus was the only significant effect and accounted for approximately 20% of total variation (Table 3). Oaks displayed the greatest mean J_{max} values at 74.86 μmol $m^{-2}s^{-1}$, followed by birches and cherries with values of 69.69 and 52.16 μmol $m^{-2}s^{-1}$ respectively.

In 2024, Genus, Species and SMT were significant for J_{max}, accounting for 5, 13 and 15% of the total variation (Table 4). There was also a significant Genus × SMT effect accounting for 11%. This interaction was largely a magnitude effect. In birches and oaks,

drought increased J_{max} by 79 and 63% respectively (not shown). In cherries, minor change was observed. This was due to the response of PC, which was the only species to upregulate J_{max} in the well-watered treatment. By species, PC displayed the greatest mean J_{max} values at 89.0 µmol $m^{-2}s^{-1}$. This was significantly greater than CC and RO, with means of 62.7 and 51.6 µmol $m^{-2}s^{-1}$. White birch, GB, and BO, with values of 79.4, 77.6 and 72.6 µmol $m^{-2}s^{-1}$ were not significantly different from any other species. Average J_{max} values were approximately 10% higher in 2024 than they were in 2023.

In the fertilizer experiment, only species and FERT had significant effects on J_{max}, accounting for 7 and 37% of total variation, respectively (Table 5). The maximum rate of photosynthetic electron transport was upregulated by 153% on average when fertilized. Pin cherry had the highest mean J_{max} value at 114.5 µmol $m^{-2}s^{-1}$. This was followed by GB, BO, WB, CC, and RO with means of 102.1, 95.0, 77.8, 69.1, and 67.4 µmol $m^{-2}s^{-1}$, respectively (not shown). Pin cherry was near significantly greater than RO at the p = 0.074 level, while all other species showed no significant differences.

Gas Exchange Traits

A_{600} - Carbon assimilation at 600 ppm

Genus, species and CO_2 had significant effects on A_{600} in 2023. These accounted for 25, 20 and 5% of the variation, respectively (Table 6). On average, A_{600} was downregulated by 16% in eCO_2. Carbon assimilation at 600 ppm was downregulated by 10 and 32% in GB and WB. Both oaks downregulated by 22%, on average, while cherries displayed little to no response (Fig. 2A). Oak and birch had significantly greater

mean A_{600} values than cherries at 11.0, 9.8, and 6.7 µmol $m^{-2}s^{-1}$, respectively, though they were not significantly different from one another (Fig. 2A).

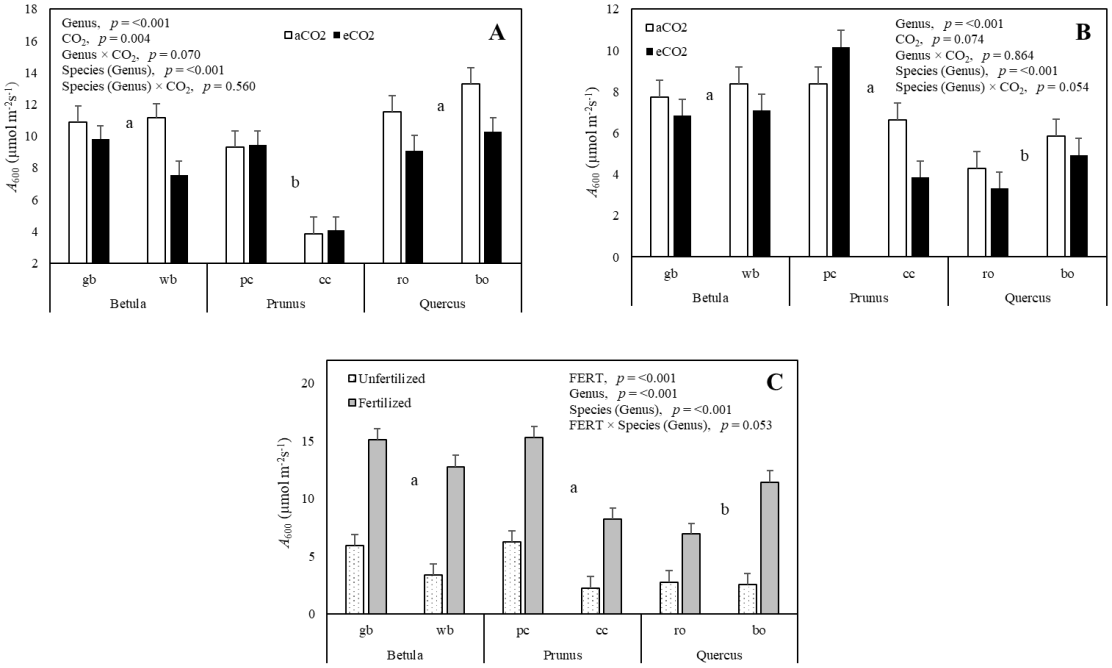

Figure 2. Assimilation at 600 ppm (A_{600}) in µmol $m^{-2}s^{-1}$ by species, genus and (A) CO_2 treatment in the 2023 growing season, (B) CO_2 treatment in the 2024 growing season, and (C) fertilizer (FERT) treatment in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2023, 2024 or FERT experiment. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

Table 6. ANOVA outputs for Assimilation at 600 ppm (A_{600}), stomatal conductance at 600 ppm (G_{wv600}), and intrinsic water-use efficiency at 600 ppm ($iWUE_{600}$) for seedlings in the 2023 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	A_{600} (µmol $m^{-2}s^{-1}$)			df	$G_{wv\,600}$ (µmol $m^{-2}s^{-1}$)			df	$iWUE_{600}$		
		MS	VC (%)	*p*-Value		MS	VC (%)	*p*-Value		MS	VC (%)	p-Value
Block	1	9.3	0.82	0.223	1	0.005	1.03	0.196	1	7978.1	6.72	**0.004**
Genus	2	139.7	24.59	**<0.001**	2	0.024	9.92	**0.001**	2	3428.9	5.78	**0.029**
CO_2	1	54.3	4.78	**0.004**	1	0.006	1.24	0.164	1	432.0	0.36	0.496
SMT	1	0.1	0.01	0.898	1	0.025	5.17	**0.006**	1	15734.7	13.26	**<0.001**
Genus × CO_2	2	17.1	3.01	0.070	2	0.01	3.93	**0.049**	2	274.9	0.46	0.744
Genus × SMT	2	6.9	1.22	0.331	2	0.004	1.45	0.323	2	620.9	1.05	0.514
CO_2 × SMT	1	3.6	0.32	0.448	1	0.001	0.21	0.644	1	567.3	0.48	0.436
Genus × CO_2 × SMT	2	4.5	0.79	0.487	2	0.002	0.62	0.584	2	2.8	0.00	0.997
Species (Genus)	3	75.1	19.82	**<0.001**	3	0.044	27.48	**<0.001**	3	5058.9	12.79	**0.002**
Species (Genus) × CO_2	3	4.3	1.13	0.560	3	0.001	0.62	0.768	3	90.8	0.23	0.961
Species (Genus) × SMT	3	5.5	1.44	0.455	3	0.003	1.65	0.462	3	969.9	2.45	0.376
Species (Genus) × CO_2 × SMT	3	10.7	2.83	0.169	3	0.002	1.24	0.609	3	128.9	0.33	0.936
Error	72	6.2	39.23		72	0.003	45.45		72	924.0	56.08	
R^2			0.651				0.566				0.459	

In 2024, genus and species significantly affected A_{600} while CO_2 was nearly significant ($p = 0.074$) for A_{600}. These accounted for 20, 18 and 2% of the total variation, respectively (Table 7). There was also significant Genus × SMT and Genus × CO_2 × SMT interactions accounting for 5 and 4%. The three-way Genus × CO_2 × SMT effect was a rank change. In this case, A_{600} downregulated or remained unchanged in eCO_2 in all combinations except well-watered cherries, which upregulated by 12% (Fig. 2B). Again, CO_2 rank change was primarily due to the response of PC. The Genus × SMT interaction was arank change.. Birches and oaks upregulated mean A_{600} values by 6 and 7%, while cherries downregulated by 28% (not shown). Overall, values were 30% lower in 2024 then they were in 2023 (Fig. 2A, 2B). By species, PC had the highest mean at 9.3 µmol $m^{-2}s^{-1}$. It was significantly greater than BO, CC and RO with 5.4, 5.2 and 3.8 µmol

$m^{-2}s^{-1}$ respectively. White birch and GB at 7.7 and 7.3 µmol $m^{-2}s^{-1}$ respectively, and were not significantly different from any other species.

Table 7. ANOVA outputs for Assimilation at 600 ppm (A_{600}), stomatal conductance at 600 ppm (G_{wv600}), and intrinsic water-use efficiency at 600 ppm ($iWUE_{600}$) for seedlings in the 2024 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	A_{600} (µmol $m^{-2}s^{-1}$)			df	$G_{wv\,600}$ (µmol $m^{-2}s^{-1}$)			df	$iWUE_{600}$		
		MS	VC (%)	*p*-Value		MS	VC (%)	*p*-Value		MS	VC (%)	p-Value
Block	1	0.4	0.05	0.783	1	0.005	0.68	0.183	1	190.9	0.10	0.638
Genus	2	83.4	19.64	**<0.001**	2	0.034	9.20	**<0.001**	2	283.4	0.30	0.719
CO_2	1	17.3	2.04	0.074	1	0.004	0.54	0.235	1	1671.7	0.88	0.167
SMT	1	7.4	0.87	0.241	1	0.226	30.58	**<0.001**	1	88825.6	46.63	**<0.001**
Genus × CO_2	2	0.8	0.18	0.864	2	0.005	1.49	0.173	2	140.7	0.15	0.849
Genus × SMT	2	20.1	4.74	**0.026**	2	0.022	6.09	**0.001**	2	6668.6	7.00	**0.001**
CO_2 × SMT	1	1.2	0.14	0.631	1	0.006	0.81	0.179	1	1756.4	0.92	0.156
Genus × CO_2 × SMT	2	17.5	4.11	**0.042**	2	0.011	3.11	**0.028**	2	36.4	0.04	0.958
Species (Genus)	3	50.6	17.86	**<0.001**	3	0.024	9.61	**<0.001**	3	364.5	0.57	0.735
Species (Genus) × CO_2	3	14.0	4.94	0.054	3	0.003	1.35	0.342	3	2359.5	3.72	**0.049**
Species (Genus) × SMT	3	1.8	0.63	0.797	3	0.013	5.41	**0.007**	3	3371.7	5.31	**0.012**
Species (Genus) × CO_2 × SMT	3	2.5	0.87	0.706	3	0.006	2.30	0.133	3	1574.5	2.48	0.148
Error	71	5.3	43.93		71	0.003	28.82		71	855.7	31.90	
R^2			0.561				0.711				0.681	

In the fertilizer experiment, genus, species, and FERT were significant, accounting for 7, 12, and 5% of the total variation (Table 8). The species × FERT interaction was significant ($p = 0.053$) and a result of magnitude effects. Carbon assimilation at 600 ppm upregulated across species when fertilized by 201% on average. Bur oak upregulated most by 341%, followed by WB (276%), and CC (265%).Upregulation was by 154% in RO and GB, and by 146% in PC. By genus, birches and cherries had significantly greater averages than oaks, at 9.3, and 8.0 µmol $m^{-2}s^{-1}$ compared to 5.9 µmol $m^{-2}s^{-1}$, respectively (Fig. 2C). By species, PC and GB, at 10.78 and 10.52 µmol $m^{-2}s^{-1}$, had significantly greater mean A_{600} than BO, CC and RO, at 7.0,

5.25 and 4.85 µmol $m^{-2}s^{-1}$, respectively. White birch (8.1 µmol $m^{-2}s^{-1}$) was not significantly different from any other species.

Table 8. ANOVA outputs for Assimilation at 600 ppm (A_{600}), stomatal conductance at 600 ppm (G_{wv600}), and intrinsic water-use efficiency at 600 ppm ($iWUE_{600}$) for seedlings in the fertilizer experiment including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). FERT denotes fertilizer treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	A_{600} (µmol $m^{-2}s^{-1}$)			df	$G_{wv\,600}$ (µmol $m^{-2}s^{-1}$)			df	$iWUE_{600}$		
		MS	VC (%)	*p*-Value		MS	VC (%)	*p*-Value		MS	VC (%)	p-Value
Block	1	21.7	0.84	0.088	1	0.000	0.00	0.760	1	990.2	1.28	0.190
Genus	2	88.0	6.79	**<0.001**	2	0.172	22.54	**<0.001**	2	4978.3	12.84	**<0.001**
CO_2	1	1.5	0.06	0.652	1	0.005	0.33	0.225	1	313.7	0.40	0.459
FERT	1	1384.5	53.45	**<0.001**	1	0.311	20.38	**<0.001**	1	4102.3	5.29	**0.009**
Genus × CO_2	2	13.1	1.01	0.174	2	0.030	3.87	**<0.001**	2	381.7	0.98	0.513
Genus × FERT	2	14.6	1.13	0.142	2	0.025	3.34	**0.001**	2	2321.7	5.99	**0.021**
CO_2 × FERT	1	25.5	0.98	0.066	1	0.027	1.77	**0.006**	1	66.5	0.09	0.733
Genus × CO_2 × FERT	2	6.6	0.51	0.407	2	0.008	1.05	0.103	2	62.2	0.16	0.896
Species (Genus)	3	104.8	12.14	**<0.001**	3	0.126	24.71	**<0.001**	3	1828.5	7.07	**0.028**
Species (Genus) × CO_2	3	2.4	0.28	0.806	3	0.021	4.19	**0.001**	3	1869.1	7.23	**0.025**
Species (Genus) × FERT	3	19.6	2.27	0.053	3	0.007	1.31	0.119	3	1776.8	6.87	**0.031**
Species (Genus) × CO_2 × FERT	3	12.5	1.45	0.172	3	0.007	1.44	0.102	3	573.7	2.22	0.392
Error	68	7.3	19.10		68	0.003	15.07		68	565.6	49.59	
R^2			0.81				0.851				0.51	

G_{wv600} – Stomatal Conductance at 600 ppm

Genus, species and SMT had significant effects on G_{wv600} in 2023, accounting for 10, 28, and 13% of the total variation, respectively (Table 6). There was also a significant Genus × CO_2 interaction due to a rank change, which accounted for 4% of the total variation. In birches and oaks, G_{wv600} downregulated in eCO_2 by 23 and 21%, respectively, while cherries upregulated by 23% (Fig. 3A). Stomatal conductance values at 600 ppm were downregulated 22% under drought on average. Means were significantly greater in birches (0.17 µmol $m^{-2}s^{-1}$) than in oaks (0.14 µmol $m^{-2}s^{-1}$) and cherries (0.12 µmol $m^{-2}s^{-1}$), which were not significantly different from each other (Fig. 3A).

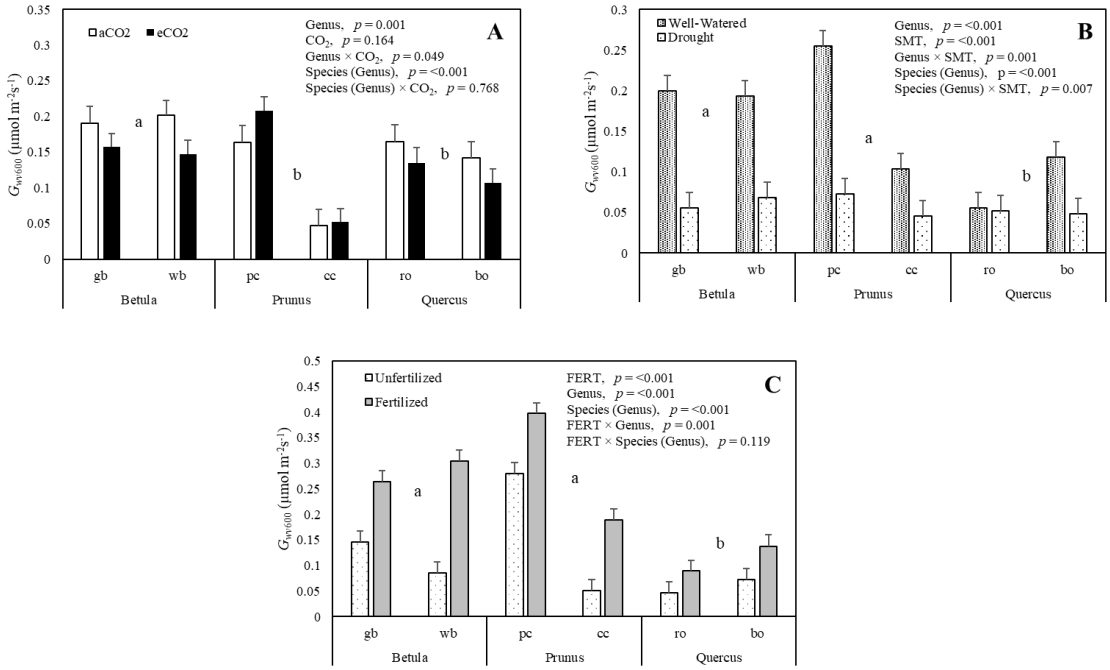

Figure 3. Stomatal conductance (G_{wv600}) in µmol $m^{-2}s^{-1}$ by species, genus and (A) CO_2 treatment in the 2023 growing season, (B) soil moisture treatment (SMT) in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2023, 2024 or fertilizer experiment. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

The main effects of genus, species, and SMT were significant in 2024 accounting for 9, 10, and 31% of total variation (Table 7). There was significant Genus × SMT × CO_2, Genus × SMT, and Species × SMT interactions, which accounted for 3, 6, and 5%.. The three-way interaction was due to rank change. In most cases, G_{wv600} downregulated under eCO_2. Cherries upregulated in the well-watered SMT, being the only exception. The SMT interactions with genus and species were magnitude effects. Stomatal conductance values at 600 ppm were downregulated under drought in all species. Birches downregulated by 69%, followed by cherry and oak, at 66 and 4% respectively (Fig. 3B). By species, the greatest downregulated response was observed in GB, (72%), followed by

PC (71%), WB (65%), BO (59%), CC (56%) and RO (7%). On average, birches (0.20 μmol $m^{-2}s^{-1}$) and cherries (0.23 μmol $m^{-2}s^{-1}$) had significantly greater G_{wv600} levels than oaks (0.09 μmol $m^{-2}s^{-1}$). By species, PC displayed the greatest mean (0.16 μmol $m^{-2}s^{-1}$), significantly higher than BO, CC and RO at 0.08, 0.08 and 0.05 $m^{-2}s^{-1}$, respectively. White birch and GB (0.13 and 0.13 μmol $m^{-2}s^{-1}$) were not significantly different from any other species. Overall, G_{wv600}-values were 26% lower in 2024 compared to 2023.

In the fertilizer experiment, the main effects of genus, species and FERT were significant, accounting for 23, 25, and 20% of the total variation, respectively. Species × CO_2 (4%), Genus × CO_2 (4%), Genus × FERT (3%), and FERT × CO_2 (2%) were also significant (Table 8). The FERT and genus interactions with CO_2 were both rank changes. Stomatal conductance at 600 ppm upregulated by 19% in eCO_2 but downregulated by the same amount when fertilizer was administered. Under eCO_2, G_{wv600} downregulated by 34% in birches, while oaks showed minor change (2% decrease) and cherries upregulated by 19% (Fig. 3C). At the species level, these results were due to RO and PC, which upregulated by 27 and 37% in eCO_2 while all other species downregulated by 25% on average (Fig. 3C). The Genus × FERT interaction occurred due to magnitude effects. Fertilized trees always had mean G_{wv600} values greater than unfertilized trees, though the amount varied by genus, with birches, oaks and cherries increasing by 145, 90 and 77% on average (Fig. 3C).

iWUE$_{600}$ – Intrinsic water use efficiency at 600 ppm

Genus, species and SMT significantly affected *iWUE*$_{600}$ in 2023. Genus accounted for 6% of total variation, while species and SMT both accounted for 13%. (Table 6). Intrinsic water-use efficiency at 600 ppm was upregulated in the drought treatment by 49% on average, while oaks had significantly greater *iWUE*$_{600}$ values (86) than birches (64). Cherries (73) were not significantly different from either of the other genera (Fig. 4A).

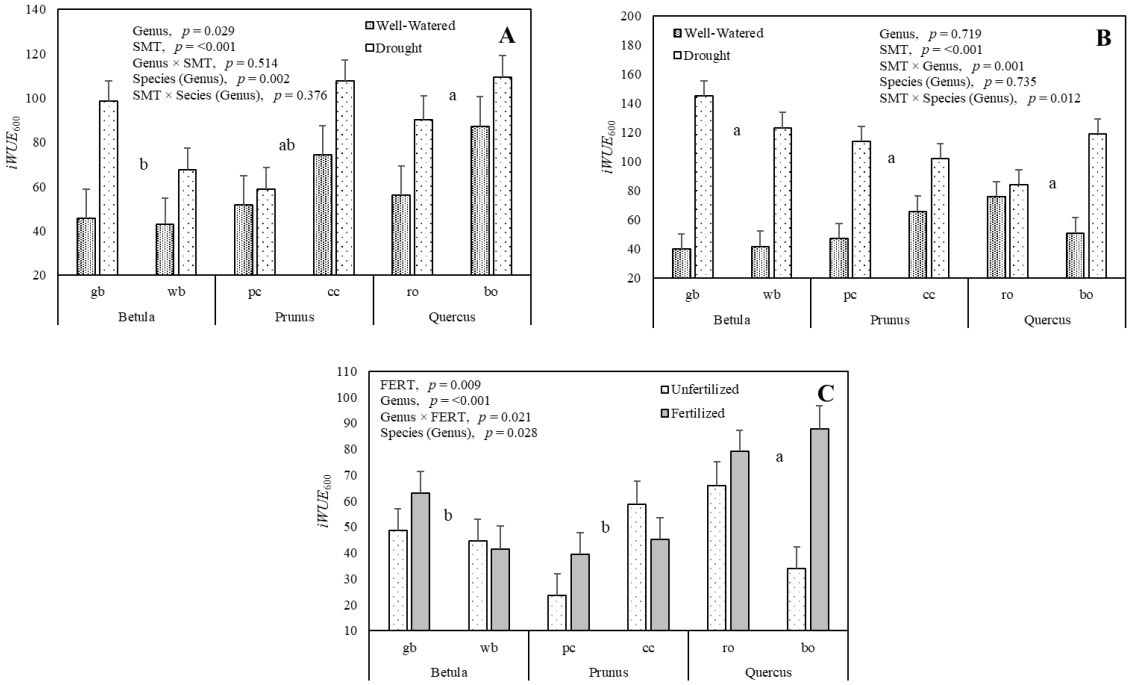

Figure 4. Intrinsic water use efficiency (*iWUE*$_{600}$) by species, genus and (A) soil moisture treatment (SMT) in the 2023 growing season, (B) soil moisture treatment in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2023, 2024 or FERT experiment. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

In 2024, interaction terms for Genus × SMT, Species × CO_2, and Species × SMT were significant, accounting for 7, 4 and 5% of total variation respectively (Table 7). SMT was the only significant main effect and was responsible for 47% of the total variation. On average, $iWUE_{600}$ was upregulated in the drought treatment by 113%, with specific amounts depending on genus and species. SMT interactions with Genus and species were due to magnitude effects. Birches upregulated by 228%, followed by cherries (91%) and oaks (59%). Gray birch responded most strongly (263%), followed by WB (194%), PC (140%), BO (133%), CC (55%), and RO at 11% (Fig. 4B). The Species × CO_2 interaction was a rank change. For GB, CC and RO, eCO_2 downregulated $iWUE_{600}$ by 11, 35, and 28%, while WB, PC and BO upregulated by 4, 15, and 10% (not shown). On average, $iWUE_{600}$ values were 13% higher in 2024 than in 2023 (Fig. 4A, 4B).

In the fertilizer experiment, genus, species and FERT were significant, accounting for 13, 7 and 5% of the total variation (Table 8). There were significant Genus × FERT, Species × CO_2 and Species × FERT interactions, accounting for 6, 7, and 7% respectively (Table 8). The effect of CO_2 depended on species and occurred due to rank changes. Gray birch, CC and BO upregulated $iWUE_{600}$ in eCO_2 by 73, 27, and 25% respectively, while WB, PC and RO downregulated by 19, 11 and 25%. The effect of fertilizer depended on both genus and species. The FERT × Species interaction was due to a rank change. When fertilized, GB, PC, RO and BO upregulated $iWUE_{600}$ by 29, 68, 20, and 158% respectively (Fig. 4C). White birch and PC downregulated by 7 and 23%. The Genus × FERT interaction was a magnitude effect. When fertilized, $iWUE_{600}$ upregulation was by 3% in cherries, 12% in birches, and 67% in oaks (Fig. 4C). At the genus level, the $iWUE_{600}$ for oaks (66) were significantly greater than cherries (4) and birches (49)

respectively. Red oak displayed the highest mean $iWUE_{600}$ of 72 which was significantly greater than WB and PC with mean s of 43 and 32. Bur oak, GB and CC (61, 56, and 52), were not significantly different from any other species.

Foliar Nutrients

Foliar Nitrogen

Genus, Species and CO_2 had significant effects on foliar nitrogen (N) concentrations in 2023, accounting for 49, 4, and 4% of total variation (Table 9). Overall, foliar N was 17% lower in eCO_2 (not shown). Oaks displayed the highest mean concentrations at 1.77% and were significantly greater than birches and cherries at 1.35 and 1.12% (not shown). By species, BO and RO displayed the highest foliar N means at 1.91 and 1.62%. These were significantly greater than GB (1.60%), which was significantly greater than PC (1.34%). White birch and CC were significantly lower with means of 1.10 and 0.91%, respectively (not shown).

In 2024, Genus, Species, CO_2, and SMT, were significant and accounted for 4, 8, 3, and 28% of total variation respectively (Table 10). The Genus × SMT interaction was due to magnitude effects and accounted for 7% of the total variation. Foliar N increased under drought by 47% on average but depended on genus (Fig. 5B). Oaks increased by 71% followed by birches, (56%) and cherries (15%). Foliar N was 17% lower in eCO_2 (Fig. 5A). At the genus level, oaks (1.21%) were significantly greater than cherries (1.01%), though birches (1.17%) were not significantly different from either. By species, BO (1.35 %) and GB (1.29%) were significantly greater than CC at 0.94%. Pin cherry

(1.09%), RO (1.08%), and WB (1.04%) were not significantly different from either group. Foliar N was 20% lower in 2024 than in 2023 (Fig. 5A, 5B).

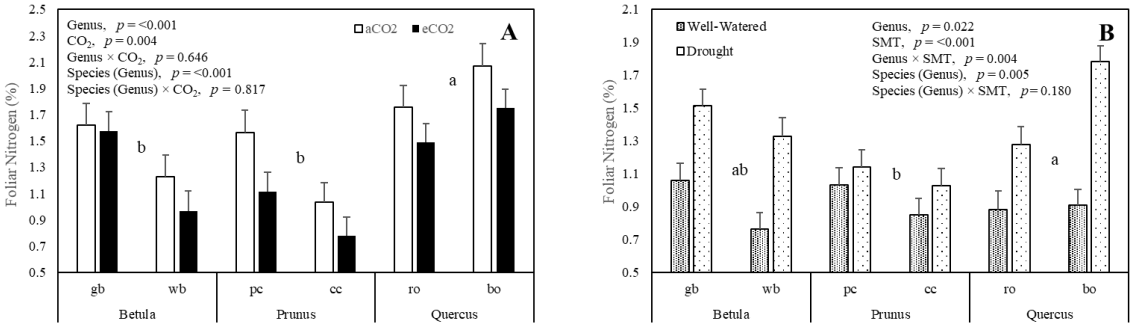

Figure 5. Foliar nitrogen in percent by species, genus and (A) CO_2 treatment in the 2024 growing season, and (B) soil moisture treatment (SMT) in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2023, 2024 or FERT experiment. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

Table 9. ANOVA outputs for Total chlorophyl content (TCC) and Total Nitrogen (N) percentages for seedlings in the 2023 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	TCC (mg/g)			df	Total N (%)		
		MS	VC (%)	*p* -Value		MS	VC (%)	*p* -Value
Block	1	0.468	0.67	0.179	1	0.44	1.04	**0.035**
Genus	2	15.675	44.90	**<0.001**	2	10.48	49.41	**<0.001**
CO_2	1	2.361	3.38	**0.003**	1	1.53	3.61	**<0.001**
SMT	1	0.208	0.30	0.370	1	0.22	0.51	0.137
Genus × CO_2	2	0.288	0.82	0.328	2	0.06	0.29	0.533
Genus × SMT	2	0.288	0.83	0.328	2	0.06	0.29	0.527
CO_2 × SMT	1	0.925	1.32	0.061	1	0.32	0.75	0.072
Genus × CO_2 × SMT	2	0.337	0.96	0.273	2	0.03	0.12	0.774
Species (Genus)	3	3.988	17.13	**<0.001**	3	0.55	3.89	**0.001**
Species (Genus) × CO_2	3	0.101	0.43	0.756	3	0.06	0.39	0.634
Species (Genus) × SMT	3	0.172	0.74	0.570	3	0.12	0.83	0.306
Species (Genus) × CO_2 × SMT	3	0.611	2.62	0.075	3	0.18	1.27	0.140
Error	71	0.255	25.88		164	0.10	37.59	
R^2			0.788				0.585	

Table 10. ANOVA outputs for Total chlorophyl content (TCC), Total Nitrogen (N), and the carbon 13 isotope ratio ($\delta^{13}C$) percentages for seedlings in the 2024 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R_2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	TCC (mg/g)			df	Total N (%)			df	$\delta^{13}C$		
		MS	VC (%)	*p* -Value		MS	VC (%)	*p* -Value		MS	VC (%)	*p* -Value
Block	1	1.61	0.21	0.644	1	0.239	1.51	0.098	1	3.0	0.05	0.072
Genus	2	17.03	4.48	0.110	2	0.344	4.34	**0.022**	2	12.0	0.39	**<0.001**
CO_2	1	43.10	5.67	**0.019**	1	0.461	2.91	**0.023**	1	5794.1	94.72	**<0.000**
SMT	1	12.13	1.59	0.207	1	4.355	27.45	**<0.001**	1	149.1	2.44	**<0.001**
Genus × CO_2	2	12.64	3.32	0.192	2	0.199	2.51	0.104	2	0.7	0.02	0.480
Genus × SMT	2	3.42	0.90	0.635	2	0.512	6.46	**0.004**	2	4.2	0.14	**0.012**
CO_2 × SMT	1	5.54	0.73	0.393	1	0.009	0.06	0.748	1	39.2	0.64	**<0.001**
Genus × CO_2 × SMT	2	0.79	0.21	0.900	2	0.169	2.13	0.145	2	3.7	0.12	**0.020**
Species (Genus)	3	2.05	0.81	0.844	3	0.396	7.50	**0.005**	3	1.3	0.06	0.245
Species (Genus) × CO_2	3	2.13	0.84	0.836	3	0.143	2.70	0.180	3	1.9	0.09	0.102
Species (Genus) × SMT	3	14.82	5.85	0.125	3	0.160	3.02	0.142	3	2.5	0.12	**0.048**
Species (Genus) × CO_2 × SMT	3	19.00	7.49	0.064	3	0.069	1.30	0.492	3	3.5	0.17	**0.012**
Error	69	7.49	67.91		71	0.085	38.13		71	0.9	1.03	
R^2			0.297				0.633				0.99	

In the fertilizer experiment, only species and FERT had significant effects and were responsible for 8 and 60% of total variation (Table 11). Overall, fertilizer increased foliar N by 149% (not shown). Gray birch displayed the highest mean foliar N concentration at 1.74, and was significantly greater than RO, CC, and WB at 1.25, 1.20 and 1.06% respectively. Bur oak and PC, with means of 1.54 and 1.37%, were not significantly different from any other species.

Table 11. ANOVA outputs for Total chlorophyl content (TCC) and Total Nitrogen (N) percentages for seedlings in the fertilizer experiment including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). FERT denotes fertilizer treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	TCC (mg/g)			df	Total N (%)		
		MS	VC (%)	*p* -Value		MS	VC (%)	*p* -Value
Block	1	0.071	0.07	0.622	1	0.12	0.24	0.390
Genus	2	1.246	2.59	**0.017**	2	0.11	0.41	0.530
CO_2	1	0.362	0.38	0.267	1	0.09	0.17	0.467
FERT	1	56.057	58.37	**<0.001**	1	31.66	60.82	**<0.001**
Genus × CO_2	2	0.027	0.06	0.910	2	0.17	0.66	0.360
Genus × FERT	2	0.032	0.07	0.897	2	0.30	1.16	0.168
CO_2 × FERT	1	0.810	0.84	0.099	1	0.14	0.27	0.359
Genus × CO_2 × FERT	2	0.081	0.17	0.756	2	0.40	1.55	0.094
Species (Genus)	3	2.998	9.36	**<0.001**	3	1.40	8.08	**<0.001**
Species (Genus) × CO_2	3	0.280	0.87	0.412	3	0.30	1.72	0.154
Species (Genus) × FERT	3	1.384	4.32	**0.004**	3	0.23	1.34	0.247
Species (Genus) × CO_2 × FERT	3	0.785	2.45	0.051	3	0.18	1.01	0.371
Error	68	0.289	20.44		71	0.17	22.56	
R^2			0.795				0.782	

Chlorophyl Content

Genus, species and CO_2, had significant effects on TCC in 2023, accounting for 45, 17, and 3% of total variation respectively (Table 9). Mean chlorophyl values were 17% lower in eCO_2 on average. Oaks displayed the greatest concentration at 2.60 %,

followed by birches (1.63 %) and cherries (1.13 %). Each was significantly different from the others (Fig. 6A). By species, BO (3.16 %) was significantly greater than RO (2.05 %), which was significantly greater than all other species except GB (1.89 %). Pin cherry and WB (1.37 %), and CC (0.89%) displayed the lowest concentrations.

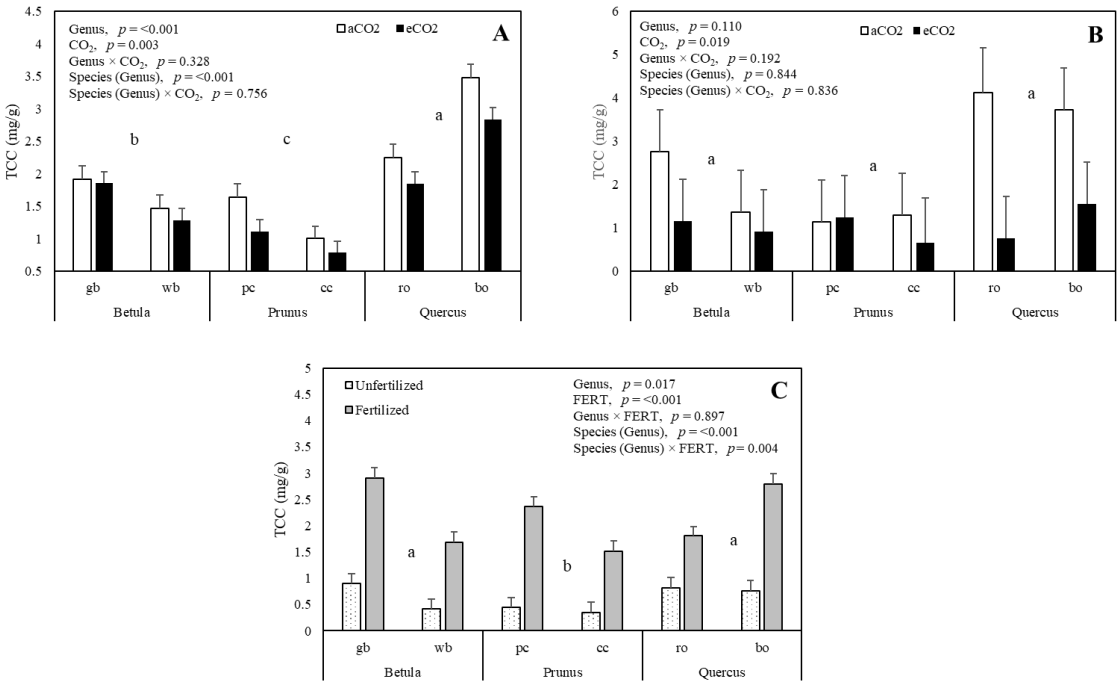

Figure 6. Total foliar chlorophyl concentration (TCC) in mg/g by species, genus and (A) CO_2 treatment in the 2023 growing season, (B) CO_2 treatment in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2023, 2024 or FERT experiment. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

In 2024, only CO_2 had a significant effect on TCC, accounting for 6% of total variation (Table 10). Concentrations decreased in eCO_2 by 57% on average, and were 4% lower in 2024 than in 2023 (Fig. 6A, 6B)

In the fertilizer experiment, genus, species and FERT were significant, accounting for 3, 9, and 58% of total variation (Table 11). The Species × FERT interaction was also significant and accounted for 4% of the total variation and was due to magnitude effects. Fertilizer increased TCC by 255% on average, though this depended on species (Fig. 6C). PC displayed the strongest response upregulating by 436%. This was followed by CC (342%), WB (303%), BO (264%), GB (222%), and RO (122%) respectively. At the genus level, cherries displayed the greatest mean (1.17%) followed by oak and birch with 1.55 and 1.48% respectively. By species, GB contained the most chlorophyl (1.91%) followed by BO (1.78%), PC (1.40%), RO (1.31%), WB (1.05%) and CC (0.93%) (Fig. 6C).

Carbon Stable Isotope Analysis

In 2024, Genus had significant effects on $\delta^{13}C$ content as well as Genus × H_2O and Species × H_2O interactions (Table 11). Drought increased overall mean $\delta^{13}C$concentrations by 7%, though exact amounts depended on both genus and species. Both the Genus × SMT and Species × SMT interactions were due to magnitude effects. By genus, drought increased $\delta^{13}C$ concentrations by 8% in cherries, and birches, and 5% in oaks. At the species level, the largest increase was observed in PC (10%) followed by WB and GB (8%), BO (7%), CC (6%), and RO (3%) (Fig. 7). By overall means, RO's $\delta^{13}C$ content (-34.18) was significantly greater than all other species except BO (-34.80), which was not significantly different from any species. Means for WB, CC, PC and GB were -35.45, -35.46, -35.67, and -35.69, respectively.

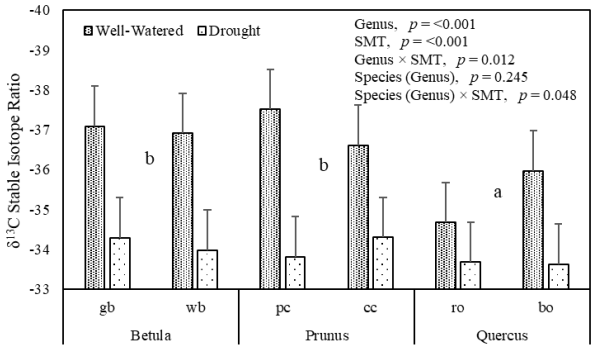

Figure 7. Carbon 13 stable isotope ratio ($\delta^{13}C$) (in delta notation) by species, genus and soil moisture treatment in the 2024 growing season. Each bar displays mean ± SE (n = 96 seedling replicates) of the associated trait in the 2024 growing season. gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

Discussion

Genus and Species Patterns

Consistent genus and species patterns in biochemical efficiency and gas exchange traits were observed in both growing seasons. By the end of the experiments, genus and species were significant factors for all traits except $iWUE_{600}$ and TCC. These were usually between the 2nd and 4th most influential factors, with species often more influential than genus. Across all traits, genus, when significant, accounted for between 4 and 20% of the total variation, while species accounted for between 6 and 18%. In the main experiment, these often followed SMT as the most influential factor, while FERT was usually most influential in the fertilizer experiment. These results indicate different patterns between birches, cherries and oaks in terms of biochemical efficiency and gas exchange traits. After two growing seasons, birches displayed the greatest mean for all

but one of the studied traits. Birches possessed the greatest V_{cmax}, and J_{max}, as well as the greatest A_{600}, G_{wv600}, $iWUE_{600}$, and $\delta^{13}C$ concentrations (Fig. 1, 2B, 2C, 3, 4B, 4C, 7). Birches were followed by cherries and oaks respectively, with the only exception being foliar N, for which oaks had the greatest mean. Interestingly, in 2023 and 2024 the birches had the greatest height and total aboveground (leaf and stem) dry mass and in 2024 the greatest total dry mass (stem and root) (Tennant and Major, 2026). In 2023 cherry had the second greatest height and aboveground (leaf and stem) mass. In 2024 cherry had the second greatest height but, equal stem dry mass with oaks.

Species trends existed as well, though with few exceptions, species within a genus were not significantly different from one another. For most traits, GB was greater or equal with WB, PC was almost always greater than CC, and BO was generally greater than RO. Despite birches being greatest at the genus level, PC had the greatest means for V_{cmax}, J_{max}, A_{600} and G_{wv600} (Fig. 1B, 1C, 1D, 2B, 2C, 3B, 3C). It was followed by GB, WB, BO, CC and RO for the same traits. This comparatively significant differences in means for PC and CC caused their overall mean to be lower than birches and likely result due to life history differences between the species. Choke cherry's lower mean biochemical efficiencies are likely attributable to lower sink activity in the form of limited growth. Generally, CC only grows half to one third the size of PC (Welch, 2004; Wendel, 1990). Slower growth rates in CC could be inferred from its mean size and lifespan. The same would be true for birches and oaks, with the former showing greater height and biomass growth in Chapter Two of the study while also displaying greater mean biochemical efficiencies here (Tennant and Major, 2026). Because they are not sink driven, N and $\delta^{13}C$ concentrations followed different trends (Fig. 6, 7), while species was

not a significant factor for either $iWUE_{600}$ or TCC. Overall, trends were similar in the fertilizer experiment, with minor changes to genus and species rankings for certain traits due to interactions with FERT. Birches had the greatest values for V_{cmax}, J_{max}, A_{600} and foliar N, while cherries were greater for G_{wv600} and TCC (Fig. 1D, 2C, 3C, 6C). Gray birch rather than WB, had greater biochemical efficiencies in the fertilizer experiment, though only N and TCC were significantly greater.

Biochemical Efficiency Traits

In the initial growing season (2023), upregulation of V_{cmax} was observed in response to eCO_2 for all species (Fig. 1A). At the time of measuring, seedlings had been exposed to eCO_2 for approximately 3 months. By 2024, however, V_{cmax} had been downregulated in all species except PC by approximately 18%, suggesting a fairly rapid (within one year) acclimation to eCO_2 (Fig. 1B). This is consistent with previous studies finding short term increases in either V_{cmax} or photosynthetic capacity (Centritto et al., 1999; Heath and Kerstiens, 1997; Riikonen et al., 2008). Other sources have observed decreases over the longer term (Rey and Jarvis, 1997; Kitao et al., 2007; Sanz-Sáez, 2010; Byeon et al., 2021; Mosseler and Major, 2022). Downregulation is generally due to a buildup of non-structural carbohydrates in the leaves, resulting from a lack of sink activity (Byeon et al., 2021; Sanz-Sáez, 2010; Sugiura et al., 2019). In our experiment, this lack of sink activity was likely due to N induced limitations on growth. The growing media used in the experiment was 97% screened sand, was purposely nutrient poor to mimic field conditions at field restoration sites, and only contained 0.06% N. Low soil N availability could limit leaf area leading to decreased light interception, limit photosynthetic capacity, or limit other physiological processes involving N (Schneider et

al., 2004; Reich, et al., 2006). With more CO_2 available than could be utilized, downregulation of V_{cmax} would occur as a response. Numerous studies (e.g., Sáez, 2010; Sage, 1994; Reich, et al., 2006; Pettersson et al., 1993; Brisebois and Major, 2024) have demonstrated amelioration of this response with the addition of N fertilizers. We also report this, with a 133% increase in V_{cmax} observed in the fertilizer experiment (Fig. 1D).

Interestingly, we also observed a consistent upregulation in V_{cmax} under drought (Fig. 1C). The difference was only significant in birches, but the effect was consistent across all species studied. Generally, V_{cmax} is downregulated under such conditions as stomatal closure causes decreases in C_i and thus RuBP regeneration (Vaz et al., 2010; Xu and Baldocchi, 2003; Bihmidine et al., 2010). Few sources report V_{cmax} upregulation under drought, though it has been observed (Mosseler and Major, 2022). We postulate that this upregulation is an adaptation to maintain rates of photosynthesis with lower C_i as stomata close. Consistent with biochemical upregulation, foliar N and TCC also increased under drought (Fig. 5B, 6B), possibly signaling an increase in Rubisco. This would result in increased V_{cmax}, though the cause remains unclear (Tennant & Major, 2026). This increase was larger in birches than in other genera.

The response of J_{max} was similar to that of V_{cmax}, though less pronounced, as the effect of CO_2 was not significant. Despite this, upregulation did still occur in eCO_2 followed by downregulation the following year, as has been found in previous studies (Rey and Jarvis, 1997). Because photosynthesis was likely N limited in unfertilized treatments, J_{max}, like V_{cmax}, was upregulated significantly when fertilized (153%). In addition, J_{max} was also upregulated under drought. This occurred in all species except PC, though only in GB was this difference significant.

Gas Exchange Traits

A_{600} - Carbon assimilation at 600 ppm

The rate of carbon assimilation can depend on the biochemical capacity of the photosynthetic system environmental conditions and sink demands (Quebbeman and Ramirez, 2016). Assimilation at 600 ppm was mostly downregulated in response to eCO_2 depending on species in both 2023 and 2024 (Fig. 2A, 2B). Assimilation downregulation has regularly been observed in response to eCO_2 (Major et al., 2022; Van Oosten and Besford, 1996; Sanz-Sáez, 2010) and is common when atmospheric CO_2 remains elevated longer than a few weeks. Like V_{cmax} and J_{max}, the mechanism is a buildup of non-structural carbohydrates in the leaves that results due to low sink activity (Byeon et al., 2021; Sanz-Sáez, 2010; Sugiura et al., 2019). In 2023, downregulation occurred in birches and oaks, while cherries showed no change (Fig. 2A). Similar trends were apparent in 2024 (p = 0074). Studies have observed the stimulation of plant growth under eCO_2 (Xu et al., 2013; Brisebois and Major, 2024). The lack of response under well-watered condition indicate that N limitation (N = 0.06%) affected A_{600} but also growth. Often there is a compensatory effect of eCO_2 on drought (Li et al., 2007; Eamus, 1991). We did not see that here, as the severe N limitation restricted assimilation. Soil moisture treatment had no significant effect in 2024, with A_{600} in well-watered plants only 8% greater than in droughted plants. Such results likely imply that sink activity was limited by factors other than water or CO_2. As discussed previously, this was likely soil N. Corroborating this hypothesis is the significance of FERT, with plants upregulating A_{600} by 201% on average when fertilizer was administered. In similar experiments, A_{600} has been observed to be highly corelated with foliar N (Brisebois and Major, 2024).

Fertilization and a resulting increase in sink activity would thus drive higher demand for C and result in increased A_{600}

G_{wv600} – Stomatal Conductance at 600 ppm

Downregulation of G_{wv} is a common response by plants in drought conditions because stomatal closing reduces water loss to evapotranspiration (Jiang et al., 2021). This downregulation was by 22% in 2023 but decreased further by 63% in 2024 (Fig. 3B). This may have occurred for two main reasons. First, the simulated drought period began later in 2023 to ensure proper establishment of the seedlings. As trees were already established, the drought period was about 2 months longer in 2024. Second, the 2023 growing season was also wetter than average for the experimental location which slowed drying of soils in the growth chambers. Sunnier conditions improved drying considerably in 2024. Genus and species interactions with SMT were due to magnitude effects. Birches and PC downregulated G_{wv600} more than CC and oaks, likely because oaks and CC had greater shoot-to-root ratios (Tennant and Major 2026) and are adapted to more xeric conditions (Welch, 2004; Nicolescu et al., 2020; Craft and Ashley, 2007).

Many previous studies have also observed downregulation of G_{wv} in response to eCO_2, as these conditions lead to greater C_i (Ainsworth and Rogers, 2007; Medlyn et al., 2001). We observed this in both years of the study (Fig. 3A), though the main effect was not significant and varied by genus. Birches and oaks downregulated by 22% in 2023 while cherries upregulated by the same amount. This response was mostly due to PC (Fig. 3A). With PC also displaying among the greatest means for V_{cmax} and J_{max} (Fig. 1B, 1C), greater sink activity was the likely cause. Results were similar in 2024, with eCO_2

resulting in downregulation by between 18 and 34% in GB, WB, CC and BO. Despite this, the main effect of CO_2 was not significant as PC and RO upregulated by 26 and 18% respectively (not shown).

Increased G_{wv600} was observed in fertilized trees, as fertilizer stimulated sink activity and drove higher demand for carbon. In unfertilized trees, demand was unaltered and plants in eCO_2 downregulated G_{wv600}. The effect of FERT was greatest in birches, followed by oaks and cherries (Fig. 3C), following the trends for V_{cmax} and J_{max}.

$iWUE_{600}$ – Intrinsic water use efficiency at 600 ppm

Intrinsic water use efficiency (*iWUE*) measures a plant's efficiency of carbon uptake per unit of water lost to evapotranspiration. The main effect of SMT was significant in both years of the study, with $iWUE_{600}$ significantly greater under drought conditions. Drought-induced increases in *iWUE* have been commonly reported (Ponton et al., 2002; Maseyk et al., 2011; Hentschel et al., 2016; Vickers et al., 2012) and relate to the saturating relationship between A and C_i (Jiang et al., 2021; Farquhar et al., 1980; Yi et al., 2019). Drought can result in increases to *iWUE* if stomatal closing to reduce water stress occurs and A is not reduced by a comparable fraction (Yi et al., 2019). We observed this (Fig. 4A, 4B), with SMT having no significant effect on A_{600} due to soil N limitations. Upregulation of *iWUE* has also commonly been observed under eCO_2 as rising atmospheric CO_2 levels lead to increased photosynthetic rates while G_{wv} rates remainstable or decline (Hentschel et al., 2016; Linares and Camarero, 2012; Mathias and Thomas, 2021; Poorter and Pérez-Soba, 2001; Waterhouse et al., 2004; Peñuelas, et al.,

2011). The severe limitation by soil N and the downregulation of A_{600} along with G_{wv600} rendered $iWUE_{600}$ unchanged.

In the first year of the study, genus and species trends followed those for shoot-to-root ratio, with oaks and CC being the most water use efficient under both well-watered and droughted treatments. In 2024 however, this trend was maintained only in the well-watered treatment. In fact, the least water use efficient species in well-watered conditions (GB, WB and PC) became the most efficient under drought (Fig. 4B). These same species displayed the highest values of A_{600} in 2024, which was not significantly affected by SMT (Fig. 2B, 2C). As a result, decreasing G_{wv600} in the same species resulted in a greater increase in $iWUE_{600}$.

The main effect of FERT was also significant, though the response of $iWUE_{600}$ varied by species. Upregulation occurred in all species but WB and CC when fertilizer was applied, with BO and PC upregulating the most, and RO upregulating the least (Fig. 4C). Fertilizer resulted in upregulation of both A_{600} and G_{wv600}, thus increases in $iWUE_{600}$ imply greater FERT influence over G_{wv600} than A_{600}. As SMT was not a factor in the fertilizer experiment, changes in $iWUE_{600}$ can likely be attributed to a combination of fertilizer induced changes to V_{cmax}, J_{max}, A_{600} and G_{wv600}.

Foliar Nitrogen and Chlorophyll Content

The responses of foliar N and TCC to eCO_2 and drought conditions were largely similar, and previous studies have found strong correlations between the two (Brisebois and Major, 2024; Wood et al., 1992). Downregulation of both foliar N and chlorophyll content have been commonly observed in response to eCO_2 (Ainsworth and Long, 2005;

Centritto and Jarvis, 1999; Farquhar et al., 2002; Major and Mosseler, 2021) and occur as photosynthesis downregulates in response to increased C_i. We report similar responses, which were consistent in both years of the study. For TCC, the downregulation was stronger in 2024 (Fig. 6A, 6B), reflecting the greater downregulation of associated photosynthetic parameters such as V_{cmax}, J_{max} and A_{600} in that year (Fig. 1B, 2B). On the other hand, foliar N was downregulated by a smaller amount in 2024, as N had started to become limiting. In both cases, genera with the greatest TCC or N content showed the strongest responses to CO_2, though this trend was not as consistent at the species level (Fig. 5A. 6B). Other than genus and species, CO_2 was the only significant factor affecting TCC in the main experiment (Table 10). Foliar N however, was also significantly affected by SMT, though as with most other traits, only in 2024. Decreases in leaf N under drought conditions have been observed in previous studies and may aid in optimization of the N economy under soil moisture limitations (Farquhar et al., 2002: Weih et al., 2011). Foliar N concentrations averaged 47% greater under drought, with the response of oaks, the most drought adapted species, being significantly stronger than that of cherries (Fig. 5B).

In the fertilizer experiment, species responded similarly to eCO_2. Both TCC and foliar N were downregulated, though the results were not significant (Table 11). This was likely due to the much stronger FERT effect, which accounted for 58 and 61% of the total variation for TCC and N respectively. Unsurprisingly, the addition of fertilizers led to an increase in foliar N, of about 149% on average. As previously discussed, fertilizer additions increased photosynthesis via V_{cmax}, J_{max} and A_{600}. This increase in photosynthesis drives greater demand for chlorophyll which was increased by 255%.

With chlorophyl often accounting for over 70% of foliar N, it too increases. (Wood et al., 1992). These increases were consistent across species and genera, though specific magnitudes varied, with PC and RO showing the strongest and weakest responses to fertilizer respectively (Fig. 6C).

Carbon Stable Isotopes

The stable isotope composition of plant organic matter is often used to assess the leaf level acclimation of A and G_{wv} to environmental conditions (Hentschel et al., 2016). The heavy carbon isotope ($\delta^{13}C$) in particular, can be used to quantify the relative *iWUE* between different plants (Gouveia, 2019). In most plants, water limitation results in reduced G_{wv}. This was observed in our study and generally leads to lowered leaf internal CO_2 concentration. Greater enrichment with $\delta^{13}C$ thus results, as plants are less discriminatory to $\delta^{13}C$ under lower C_i (Hentschel et al., 2016; Hubick and Farquhar, 1989; Gouveia, 2019). We also observed this response, with plants grown in the drought treatments having concentrations 7% greater on average than those in the well-watered SMT (Fig. 7). Overall, responses were consistent across species and genera, though oaks had significantly higher $\delta^{13}C$ concentrations thus greater $iWUE_{600}$ than cherries and birches (Fig. 7, 4B). By species PC and WB exhibited the greatest differences between soil moisture treatments, while CC and RO exhibited the least (Fig. 7).

Conclusion

The six species of birches cherries and oaks studied are early-successional deciduous trees from three different phylogenetic families used in ecological restoration of degraded sites. Birches possessed the greatest V_{cmax} and J_{max} values, as well as the A_{600}, Gwv_{600}, $iWUE_{600}$, and TCC. For most traits, birches were generally greater than cherries and oaks respectively, though oaks had the highest means for foliar N concentrations. Species within a given genera rarely displayed significantly different responses from one another. After two growing seasons, eCO_2 generally resulted in downregulation of V_{cmax}, J_{max}, A_{600}, G_{wv600}, TCC and foliar N in most species. Acclimation to eCO_2 occurred within one year and was likely the result of severe soil N limitations. Late-season fertilizer additions partially ameliorated most of these effects. Contrary to our predictions, $iWUE_{600}$ was not affected by eCO_2, likely for the same reason. Drought treatments increased $iWUE_{600}$, foliar N and $\delta^{13}C$, while G_{wv600} decreased. Interestingly, drought treatments also resulted in upregulation of V_{cmax} and J_{max} while A_{600} was unaffected. Understanding the variability in responses by species can help inform restoration prescriptions by allowing land managers to select and propagate the species best adapted to specific sites.

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Chapter 2: Morphological variation of Six Early-Successional Deciduous Species in Response to Atmospheric CO_2 and Soil Moisture Treatments

Abstract

Genetic variation of morphological traits for six early-successional deciduous tree species were examined under a 2×2 factorial design of atmospheric CO_2 and soil moisture treatments. The tree species investigated include gray birch (GB, *Betula populifolia* Marsh.), white birch (WB, *B. papyrifera* Marsh.), pin cherry (PC, *Prunus pennsylvanica* L. f.), choke cherry (CC, *P. virginiana var. virginiana* L.), red oak (RO, *Quercus rubra* L.) and bur oak (BO, *Q. macrocarpa* Michx.). One-year-old seedlings were planted in a low-nutrient, sandy soil medium, and were subjected to aCO_2 (400 ppm) or eCO_2 (800 ppm) and well-watered (10–15% soil moisture content) or droughted (3–8% soil moisture content) soil moisture treatments over two years in a greenhouse environment. Subsets of seedlings were coppiced at the root collar and or administered additional fertilizer treatments during the second year of the study to examine any effects on growth traits including height, basal diameter and dry biomass. Genus and species were strongest effects accounting for up to 60% and 23% of the total variation, respectively. Birches had the greatest stem dry mass followed by cherries and oaks with 14.8, 5.9 and 4.8 g, respectively, whereas oaks had the greatest root dry mass followed by birches and cherries with 23.1, 17.1 and 12.1 g, respectively. The result was very large differences in shoot-to-root ratio by genus accounting for almost half of the total variation. Birches, cherries, and oaks had shoot-to-root ratios of 0.89, 0.48 and 0.20,

respectively, with birches affected most strongly by drought treatments followed by cherries and oaks. By species, height, basal diameter, and dry mass results generally found that GB was greater than WB, PC was greater than CC, and BO was greater than RO. Under drought, heights and basal diameters were lower by 10 and 18% respectively, while overall biomass traits were more strongly affected, being reduced on average by 33%. Late season fertilizing increased total biomass by 40% in birches and 17% in cherries while oaks showed no change. One year of coppice regrowth resulted in 13 to 40% decreases for every trait other than stem number which increased by 40%. Biomass traits were not significantly altered in response to eCO_2, likely in response to nitrogen limitations in the nutrient-poor growing medium used to mimic restoration site conditions.

Introduction

Early-successional deciduous trees are a vital component of ecological restoration, particularly in areas that are prone to wildfires. As rising atmospheric CO_2 concentrations continue to drive changes in climate and precipitation patterns, the long-term success of these projects will depend on our ability to select and propagate species that can successfully adapt. Such successful adaptation will depend on the ecophysiological and morphological responses of plant species to a variety of environmental factors including CO_2 levels, soil moisture, and nutrient availability (Major et al., 2022). Differential adaptive responses to these factors at the genera and species levels implies potential for altered competitive relationships and may result in further impacts to plant community composition (van Breugel et al., 2012). The ability to predict these relationships will thus have significant impacts on the planning and design of restoration projects in the future.

To investigate these relationships, we examined six species from three different phylogenetic families. White birch (WB, *Betula papyrifera* Marsh.) and gray birch (GB, *B. populifolia* Marsh.) of the family Betulaceae, are both early-successional, deciduous trees. WB is a forest tree with a transcontinental range in North America. It is a site generalist, growing to 25 m and living up to 140 years (Safford et al., 1990). Gray birch is restricted to northeastern North America, where it occurs on sandy or gravelly sites (Beauregard et al., 2020). It is smaller and shorter lived than WB, living approximately 50 years and growing to 10 m. Pin cherry (PC, *Prunus pennsylvanica* L. f.) and choke cherry (CC, *P. virginiana var. virginiana* L.) of the family Rosaceae, are short-lived, early successional, small deciduous trees or tall shrubs (Welch, 2004; Wendel, 1990). PC

grows to approximately 10–15 m while CC is smaller, growing to 5 m. Both species have a lifespan between 20–40 years and grow on a variety of soils (Welch, 2004; Wendel, 1990). The two species of oak; red oak (RO, *Quercus rubra* L.) and bur oak (BO, *Q. macrocarpa* Michx.) of the family *Fagaceae* are larger and longer lived, growing to 25 m over their 200–300-year lifespans (Tardif et al., 2006; Nicolescu et al., 2020; Craft and Ashley, 2007). Both species are more moderately shade intolerant (Nicolescu et al., 2020; Craft and Ashley, 2007). Red oak prefers well-drained sandy or loamy soils, while BO is more common on clays (Johnson, 1990; Cogliastro et al., 1997). Bur oak ranges farther west to the North American Midwest, whereas RO ranges farther east, well into the Canadian Maritimes (Johnson, 1990; Craft and Ashley, 2007). All the above species are native to northeastern North America, exhibit rapid early growth, coppice following harvesting, and have been used in ecological restoration projects (Safford et al., 1990; Beauregard et al., 2020; Welch, 2004; Wendel, 1990; Nicolescu et al., 2020; Craft and Ashley, 2007; Brisebois and Major, 2024; Mosseler et al., 2019).

Previous studies on early-successional hardwoods have generally observed short term increases in growth and biomass production in response to elevated CO_2 (eCO_2) (Bazzaz et al., 1990; Atkinson et al., 1997; Centritto, 2002). The magnitude and duration of these increases is dependant on species, soil nutrients, and water availability (Bazzaz et al., 1990; Atkinson et al., 1997; Bazzaz et al., 1993; Brisebois and Major, 2024). Although research on the species featured in this experiment has been limited, studies on the European species *Betula pendula* and *Quercus robur* have revealed increased dry biomass and carbon allocation to fine roots when grown in eCO_2. Shoot-to-root ratios either declined or were unchanged (Rey and Jarvis, 1997; Pettersson et al., 1993;

Atkinson et al., 1997; Maillard et al., 2001). In other experiments, the European species *Prunus avium* has also displayed dry mass increases, though these generally did not apply to the roots and were short term in nature (Kerstiens and Hawes, 1994; Centritto et al., 1999; Atkinson et al., 1997). A similarly designed study on WB and GB also found decreased growth under drought and a slight mitigation of drought effects under eCO_2 (Brisebois and Major, 2024).

Our goal was to examine and compare interspecies differences in growth traits in response to a 2 x 2 factorial of soil moisture and CO_2 treatments. We examined traits including height, basal diameter, dry biomass, and shoot/root ratio, for each of the six species under an aCO_2 (400 ppm) or eCO_2 (800 ppm) treatment and a well-watered (10–15% soil moisture content) or droughted (3–8% soil moisture content) SMT. We hypothesize that eCO_2 treatments would result in increased biomass under all conditions and may mitigate some effects of drought. We also expect that any differences in growth traits will appear at the genus and species levels. To test our hypothesis, we quantified growth traits and variation attributed to genus and species and examined the possible interactions relative to the CO_2 and drought treatments. At the end of the first year of treatments, we coppiced half of the replicates to examine responses to mimic potential damage sustained in restoration scenarios. In addition, we tested if additional fertilization in the second year resulted in increased morphological gains under well-watered conditions.

Materials and Methods

Plant Material Growing and Preparation

All seedlings used in this experiment were grown from bulk seed sources collected in western and central NB, Canada (Table 12). Seedlings were grown in hard-sided multi-pot trays of 45 90 cm^3 cells at the AFC greenhouse in Fredericton, NB (45◦52′ N, 66◦31′ W). After entering winter dormancy in late 2022, each stem was trimmed to a height of 10 cm to reduce leaf transpiration to root water absorption ratio to mimic prescriptions for restoration planting on harsh, degraded sites (Galea and Major, 2024). Following trimming, all seedlings were removed from the trays, boxed, and stored in AFC freezers at -4ºC until June 18th, 2023.

Table 12. Species and seed sources of Betula, Prunus and Quercus seedlings utilized in the experiment.

Species	Seed Source	Approximate Latitude	Approximate Longitude	# Per Chamber	Total Seedlings
Betula populifolia	CFB Gagetown, NB	45.570410	-66.375810	11	88
Betula papyrifera	CFB Gagetown, NB	45.570410	-66.375810	11	88
Prunus pensylvanica	CFB Gagetown, NB	45.570410	-66.375810	11	88
Prunus virginiana	New Maryland, NB	45.891030	-66.684795	11	88
Quercus rubra	Oromocto, NB	45.837485	-66.422915	11	88
Quercus macrocarpa	Jemseg, NB	45.811415	-66.095165	11	88
Totals				66	528

Treatment Delivery 2023

Two identical greenhouses were used for the experiment. Within each greenhouse, four treatment-chambers were constructed: each measuring 4.2 m × 2.4 m × 2.4 m and consisting of a wooden frame covered by 1 cm thick sheets of corrugated transparent plastic. Each treatment-chamber was randomly assigned one of two CO_2

treatments; aCO_2 (400 ppm) or eCO_2 (800 ppm), and one of two SMTs; well-watered (10–15% soil moisture content) or droughted (3–8% soil moisture content). Thus, the experiment contained four treatment conditions, each replicated once in each of the two greenhouses (blocks). Within each treatment chamber, 84 fabric root control bags (Smart Pot Pro 5 Gallon, High Caliper Growing Inc., Oklahoma City, Oklahoma, USA) were arranged in a 7 × 12 grid pattern on plastic pallets to ensure adequate drainage. Each bag contained approximately 18 L of screened sand (M.W. Price & Sons, Charter's Settlement, NB, CA). Bulk soil samples were collected and sent for nutrient and soil texture analysis at the University of New Brunswick in Fredericton. The soil texture properties were, on average, 0.7% clay, 2.1% silt, and 97.2% sand. Overall, soils were 0.35% organic matter, and contained 0.06% N, 0.21% C and 0.02% S, Na, K, Ca), and Mg averaged 0.03, 0.04, 0.38 and 0.08 meq/100 g, respectively. On June 18th, 2023, seedlings were removed from cold storage and allowed to thaw in refrigerators at 4^{o}C until planting on June 21st. Root control bags were watered to field capacity immediately before planting to minimize transplant shock, and each seedling was planted randomly in its own root control bag. 11 replicates of each species were planted, totaling 66 experimental seedlings per chamber. Water was administered to the root control bags using a drip irrigation system that delivered equal amounts of water to each bag at a rate of approximately 33 mL/min. Spigots were placed centrally in each bag. Fertilizer, when administered using this system, was added at a rate of 1 L per 50 L of water using a D14MZ10 Coolant Mixer (Dosatron, Clearwater, FL, USA). Soil moisture probes (5TM, Decagon Devices, Pullman, WA, USA) were used to measure moisture content (%) and water was administered manually to keep treatments within the desired soil moisture

ranges. The frequency and dosage of waterings varied in response to external weather conditions, which influenced humidity, temperature, and evaporation rates within the treatment-chambers. Air temperatures within each chamber were controlled by two air conditioning units set to engage if temperatures reached or exceeded 27°C. Air circulation was facilitated by a series of ducts which drew air from the outside environment using fans. Air temperature and humidity were monitored and logged using a separate system of wireless sensors (Tempstick, Ideal Sciences, Bountiful, UT, USA). Carbon dioxide was supplied from compressed gas canisters and introduced to eCO_2 chambers via air intake ducts. Atmospheric CO_2 concentrations in each chamber were measured centrally using Li-840A CO_2/H_2O Gas Analyzers (LI-COR Inc., Lincoln, Nebraska). Carbon dioxide concentration was automatically maintained at consistent levels in the eCO_2 treatments using an electronic solenoid manifold, with CO_2 being released automatically if the concentration was ≤ 800 ppm as detected by the analyzer. In ambient treatments, CO_2 concentrations were allowed to fluctuate naturally but averaged approximately 400 ppm. Atmospheric CO_2 treatments began immediately after seedlings were planted on June 21st.

To ensure proper seedling establishment, droughted and well-watered chambers were administered equal amounts of water until July 19th, 2023. Grower fertilizer (Plant-Prod “Forestry Special” 20:8:20, 1250 g/100 L, +1250 mL of MgNiFeCal) was administered to each bag using the drip irrigation system on three occasions as outlined in Table 13. Seedlings were allowed to grow in their respective treatments for the remainder of the growing season, receiving water as needed to maintain desired soil moisture levels. On October 30th, 2023, five trees per species per chamber were harvested to examine

aboveground biomass growth. Stems were cut approximately three cm above the soil surface to allow for coppice regrowth the following spring. Treatments continued until November 8th, 2023, at which point, shade curtains were installed to induce winter dormancy, and the CO_2 and soil moisture treatments were paused. Seedlings overwintered within the growth chambers, with all treatment groups receiving equal amounts of water and aCO_2. To prevent freezing of greenhouse infrastructure, temperature in the chambers was maintained at just above 0ºC.

Table 13. Schedule of fertilizer additions applied to experimental seedlings during the 2023 growing season.

Year	Date	Brand	Product Name	NPK Ratio	Mix Ratio	Volume Mixed (mL)	Mix Volume Administered (mL)
2023	19-Jul	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	8	400
	17-Aug	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	9	450
	24-Aug	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	9	450

Treatment delivery 2024

Carbon dioxide treatments resumed on April 18th, 2024, at which point shade curtains were removed. To ensure proper recovery of the coppiced trees, all trees were administered equal amounts of water until May 7th. Starter fertilizer (Plant-Prod "Forestry Starter" 10:52:10, 312.5 g/25 L) was administered to all trees on May 1st and May 7th, as outlined in Appendix B. Drought treatments were resumed the following day. Plants were further fertilized with Grower fertilizer (Plant-Prod "Forestry Special" 20:8:20, 1250g/100L) followed by MagNifiCal (REMO "MagNifiCal" 3:0:0, 1.5 mL/25 L) on May 17th and 23rd, and on June 7th (Appendix B.) In subsequent treatments, fertilizer was added before and after MagNifiCal to prevent reactions between the different fertilizer chemicals (Appendix B.).

Experimental Fertilizer Treatments, 2024

On September 6th, 2024, four coppiced saplings of each species were selected at random in each well watered treatment-chamber and randomly assigned a fertilizer treatment (FERT). After removing their drip-irrigation spigots, the root bag of each sapling was lifted to sever any root connections with neighbouring bags. To prevent water and fertilizer from being absorbed by neighboring saplings, a plastic bag was perforated on its underside to allow for drainage and placed over each root bag. The bags were then lowered it back into place. Two of the seedlings from each species were selected at random and assigned to the fertilized treatment group, with the remaining two seedlings assigned to the unfertilized treatment group. The treatment group of each sapling was recorded and marked using colored flagging tape. All fertilized saplings received 1L of fertilizer mixture, which was 50 parts water, and one part grower fertilizer (Plant-Prod "Forestry Special" 20:8:20, 1250 g/100 L). Unfertilized saplings received 1 L of water only. Treatments began on September 10th, 2024, and occurred thrice weekly until October 15th (Appendix B).

Morphological measurements

After two years of treatments, height of the tallest stem from the soil surface to its highest point was measured to the nearest 0.1 cm using a meter stick. Electronic calipers were used to measure basal diameter at the soil surface to the nearest 0.1 mm. The number of stems were also recorded. A stem was defined as being any woody growth at least five cm in length that originated from below the coppice cut, or any other leader with a length longer than half the total height of the tree and originated at or below the 10

cm trim line. Leaf abscission had begun to occur in some species by the time of the harvest, so any remaining leaves were stripped from stems and discarded. All stems were then cut at the soil surface placed in labeled paper bags to be oven dried at 65ºC for a minimum of 72h. Root control bags were dumped to exhume the roots, which were washed with garden hoses to remove all soil and foreign organic matter. These were then placed in labeled paper bags and dried using the same procedure as the stems. Following drying, the root and stem tissues were weighed separately to within 0.01 g using a precision scale (accu-4102, Fisher Scientific,Waltham, MA, USA).

Statistical analyses

This experiment utilized a randomized block design. The fixed effects in the physiological model were genus, species, CO_2 treatment, and soil moisture treatment. Growth chamber (block) and replicate seedling were random effects. A general linear model (GLM) was used to conduct mixed-effects analyses of variance (ANOVA) with nested factors, using SYSTAT version 13.2.01 (Palo Alto, CA, USA). The following model was used:

$$Y_{ijklmn} = \mu + B_i + G_j + C_k + H_l + GC_{jk} + GH_{jl} + CH_{kl} + GCH_{jkl} + S_{m(j)} + SC_{m(j)k} + SH_{m(j)l} + SCH_{m(j)kl} + e_{ijklmn},$$

Where term Y_{ijklmn} denotes the dependent seedling of the i^{th} growth chamber; of the j^{th} genus; of the k^{th} CO_2 treatment; of the l^{th} SMT treatment; of the m^{th} species; of the n^{th} replicate with μ as the overall mean. Term B_i refers to the effect of the i^{th} growth chamber (i = 1, 2), term G_j is the effect of the j^{th} genus (j = 1, 2, 3), term C_k is the effect of the k^{th} CO_2 treatment (k = 1, 2), and term H_l is the effect of of l^{th} SMT (l = 1, 2). Term GC_{jk} is

the interactive effect between genus j and CO_2 treatment k. Term GH_{jl} is the interactive effect of genus j and SMT l. Term CH_{kl} is the interactive effect of CO_2 treatment k and SMT l. Term GCH_{jkl} is the interactive effect of genus j, CO_2 treatment k and SMT l. Term $S_{m(j)}$ is the effect of species m (m = 1. . .6) nested in genus j. Term $SC_{m(j)k}$ is the interactive effect of species m nested in genus j with CO_2 treatment k. Term $SH_{m(j)l}$ is the interactive effect of species m nested in genus j with SMT l. finally, Term $SCH_{m(j)kl}$ is the interactive effect of species m nested in genus j with CO_2 treatment k and SMT l. Term e_{ijklm} represents the random error component.

To analyze separate datasets of coppiced and non-coppiced trees in 2024, the CO_2 factor was removed as it had no significant effect. Preliminary results found the main effect of CO_2 was not significant for any traits and accounted for a maximum of 1.1% of total variation. Interactions containing a CO_2 effect were rarely significant and only accounted for maximum 2.6% of total variation when they were. As such, CO_2 was replaced with a coppice factor using the same model for more simplified interpretation. The fertilizer experiment utilized the same GLM, with FERT substituting SMT, as only watered seedlings were administered FERT.

Results

Height

In 2024, genus, species, coppice and SMT were significant accounting for 39, 14, 8, and 1% of total variation. Genus × Coppice, Genus × SMT, and Species × Coppice interactions were also significant, accounting for 3, 1, and 1% (Table 14). The Genus ×

SMT interaction was a result of magnitude effects. Mean heights were consistently shorter in the drought treatment by 10% on average, though exact differences were 14, 8, and 5% in birches, oaks, and cherries, respectively (Fig. 8A). The Genus × Coppice and Species × Coppice interactions were also magnitude effects. Coppiced trees were 22% shorter on average, though differences of 30, 20, and 11% were observed in birches, oaks and cherries, respectively (Fig. 8B). By species, the greatest decreases (35%) were observed in RO, followed by GB (32%), WB (27%), PC (12%), CC (11%), and BO (4%). By overall means, birches had the greatest height at 90.0 cm, followed by cherries and oaks at 61.8 and 45.0 cm. Each was significantly different from one another (Fig. 8B). By species, GB were tallest at 96.2 cm. This was significantly greater than WB and PC (83.8 and 79.3 cm). Significantly smaller were BO, CC and RO, at 46.9, 44.3 and 43.2 cm respectively. There were no significant FERT effects or interactions for height (Table 15).

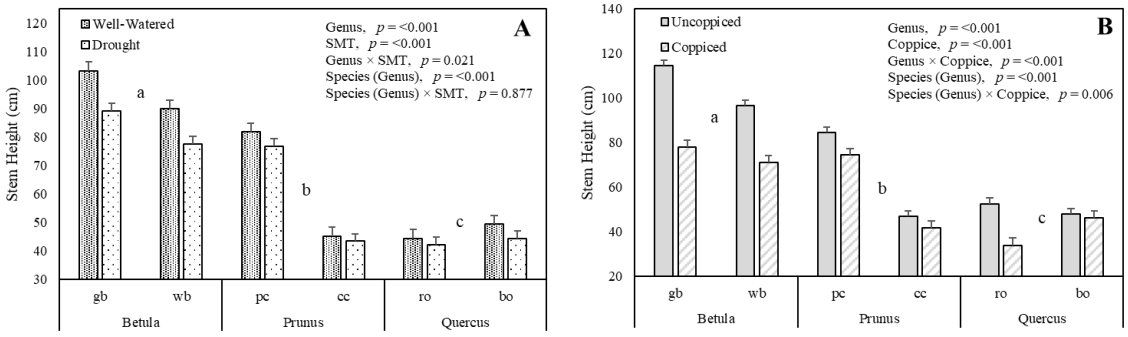

Figure 8. Stem height (cm) by species, genus and (A) soil moisture treatment (SMT) in the 2024 growing season, and (B) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE of the associated trait in the, 2024 (n = 462 seedling replicates) or fertilizer experiment (n = 95 seedling replicates). gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

Table 14. Analysis of variance (ANOVA) outputs for stem height, stem diameter, and stem number for seedlings in the 2024 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	Height (cm)			df	Diameter (mm)			df	Stem Number		
		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*
Block	1	375.8	0.10	0.248	1	13.6	0.60	**0.009**	1	0.11	0.02	0.700
Genus	2	70506.1	38.52	**<0.001**	2	274.9	24.48	**<0.001**	2	30.73	14.36	**<0.001**
Coppice	1	27566.5	7.53	**<0.001**	1	435.8	19.40	**<0.001**	1	30.81	7.20	**<0.001**
SMT	1	4668.0	1.28	**<0.001**	1	172.2	7.67	**<0.001**	1	0.01	0.00	0.915
Coppice × Genus	2	6005.4	3.28	**<0.001**	2	46.1	4.10	**<0.001**	2	10.53	4.92	**<0.001**
Genus × SMT	2	1101.8	0.60	**0.021**	2	12.0	1.07	**0.002**	2	0.78	0.37	0.334
Coppice × SMT	1	27.2	0.01	0.756	1	2.1	0.09	0.303	1	0.26	0.06	0.545
Coppice × Genus × SMT	2	89.1	0.05	0.729	2	2.3	0.21	0.307	2	0.14	0.07	0.819
Species (Genus)	3	16769.2	13.74	**<0.001**	3	23.7	3.17	**<0.001**	3	0.54	0.38	0.518
Coppice × Species (Genus)	3	1176.2	0.96	**0.006**	3	3.1	0.41	0.197	3	0.08	0.06	0.950
SMT × Species (Genus)	3	64.2	0.05	0.877	3	3.0	0.40	0.209	3	0.52	0.36	0.536
Coppice × SMT × Species (Genus)	3	375.2	0.31	0.263	3	1.9	0.25	0.417	3	0.59	0.41	0.479
Error	437	281.3	33.57		437	2.0	38.16		430	0.71	71.77	
R^2			0.700				0.662				0.279	

Table 15. Analysis of variance (ANOVA) outputs for stem height, stem diameter, and stem number for seedlings in the fertilizer experiment including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). FERT denotes fertilizer treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	Height (cm)			df	Diameter (mm)			df	Stem Number		
		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*
Block	1	74.5	0.12	0.544	1	5.03	1.76	0.077	1	4.74	2.356	0.117
Genus	2	14278.7	46.28	**<0.001**	2	46.73	32.59	**<0.001**	2	26.59	26.426	**<0.001**
CO_2	1	0.0	0.00	0.99	1	1.07	0.37	0.410	1	0.06	0.029	0.860
FERT	1	73.8	0.12	0.545	1	0.23	0.08	0.700	1	0.09	0.043	0.831
Genus × CO_2	2	418.7	1.36	0.131	2	1.14	0.79	0.487	2	0.05	0.051	0.973
Genus × FERT	2	502.4	1.63	0.088	2	2.21	1.54	0.250	2	0.10	0.094	0.951
FERT × CO_2	1	0.6	0.00	0.957	1	0.04	0.01	0.877	1	2.50	1.240	0.254
FERT × Genus × CO_2	2	136.0	0.44	0.51	2	0.68	0.47	0.650	2	1.15	1.146	0.546
Species (Genus)	3	4668.3	22.70	**<0.001**	3	18.48	19.34	**<0.001**	3	0.43	0.638	0.878
CO_2 × Species (Genus)	3	429.6	2.09	0.102	3	2.65	2.77	0.177	3	0.11	0.163	0.982
FERT × Species(Genus)	3	379.8	1.85	0.138	3	0.97	1.02	0.604	3	0.27	0.403	0.934
FERT × CO_2 × Species (Genus)	3	80.8	0.39	0.751	3	0.45	0.47	0.833	3	1.18	1.759	0.601
Error	71	200.0	23.02		71	1.57	38.78		70	1.89	65.650	
R^2			0.774				0.615				0.346	

Basal diameter

Similar to height, basal diameter was significant for genus, species, coppice and SMT, accounting for approximately 24, 3, 19, and 8% of total variation, respectively (Table 14). Coppice × Genus and Genus × SMT interactions were also significant, accounting for 4 and 1% and were also magnitude effects. On average, mean diameters were 27% smaller in coppiced trees, and 18% smaller under drought, though this depended on genus. Coppiced diameters were 33, 27 and 16% smaller in birches oaks and cherries, respectively. A similar trend existed in the droughted SMT, with birches displaying the greatest decrease (21%), followed by oaks and cherries at 18 and 12%. By overall means, birches had the largest diameters (8.29 mm) and were significantly larger than cherries and oaks at 5.90 and 5.84 mm, respectively (not shown). By species, GB and WB had the greatest diameters at 8.30 and 8.7 mm and were significantly greater than PC (6.60 mm). Significantly smaller was BO (5.89 mm), which was significantly greater than CC (5.20 mm) but not red oak (5.78 mm). There were no significant FERT effects or interactions for basal diameter (Table 15).

Stem number

Following the 2024 growing season, genus, coppice, and their interaction, had significant effects on stem number, accounting for 14, 7, and 5% of total variation, respectively (Table 14). Coppicing increased mean stem number by 40% on average, though this depended on genus. The Genus × Coppice interaction was due to magnitude effects. Stem count increased by 75% in birches, by 23% in cherries, and 14% in oaks (Fig. 9). By means, birches had the most stems at 2.18, a significantly greater number

than cherries and oaks at 1.48 and 1.27, respectively (Fig. 9). There were no significant FERT effects interactions for stem number (Table 15).

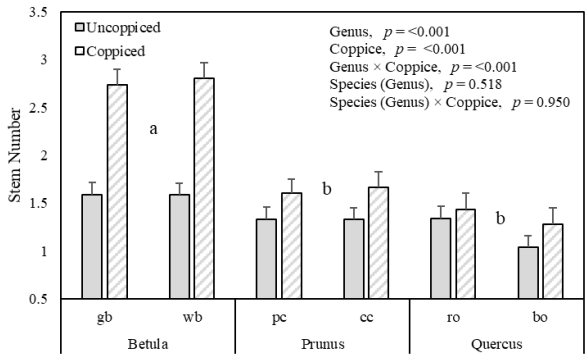

Figure 9. Stem number by species, genus and coppice treatment in the 2024 growing season Each bar displays mean ± SE of the associated trait in the, 2024 (n = 462 seedling replicates). gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for the analysis with significance levels are denoted with lower case letters.

Stem dry mass

In 2024, genus, species, coppice and SMT had significant impacts on stem dry biomass, accounting for 33, 4, 8, and 5% of total variation respectively (Table 16). There were significant Genus × Coppice, Genus × SMT, and Coppice × SMT interactions accounting for 4, 2, and 1% (Table 16). The Genus × SMT interaction was due to magnitude effects with dry mass 33% lower in drought treatments on average (Fig. 10A). The largest reductions (37%), were observed in birches, followed by oaks (29%) and cherries (24%). Both the Genus × Coppice and Coppice × SMT interactions were a result ofmagnitude effects. Overall, coppiced trees had dry masses 40% lower than uncoppiced trees, though this depended on Genus and SMT (Fig. 10B). Birches showed the largest

reduction in stem dry mass (45%), closely followed by oaks (44%), while the smallest reduction was observed in cherries (26%). Drought always reduced stem dry mass, though the reduction was greater in uncoppiced trees (34%), than in coppiced trees (29%). By overall means, birches (14.78 g), were significantly greater than cherries and oaks with means of 5.92 and 4.83 g. At the species level, GB and WB were greatest at 14.84 and 14.72 g, respectively. These were significantly greater than PC (8.50 g), which was significantly greater than RO (4.85 g), BO (4.80 g) and CC (3.33 g) respectively.

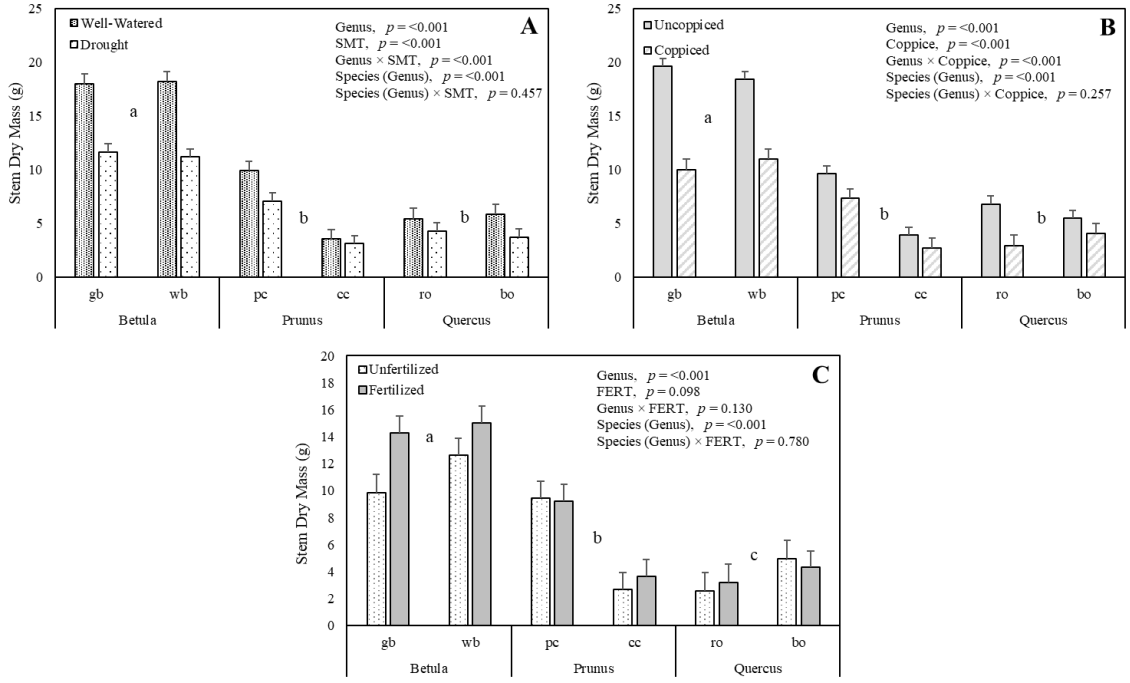

Figure 10. Stem dry mass (g) by species, genus and (A) soil moisture treatment (SMT) in the 2024 growing season, (B) coppice treatment in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE of the associated trait in the, 2024 (n = 462 seedling replicates) or fertilizer experiments (n = 95 seedling replicates). gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

Table 16. Analysis of variance (ANOVA) outputs for stem dry biomass, root dry biomass, total dry biomass, and shoot to root ratio for seedlings in the 2024 growing season including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). SMT denotes soil moisture treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	df	Stem Dry Biomass (g)			df	Root Dry Biomass (g)			df	Total Dry Biomass (g)			df	Shoot Root Ratio		
		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*		MS	VC (%)	*p-Value*
Block	1	171.5	0.68	**0.008**	1	18.2	0.05	0.505	1	307.9	0.37	0.091	1	0.731	1.07	**<0.001**
Genus	2	4117.4	32.85	**<0.001**	2	3705.1	22.25	**<0.001**	2	6694.4	15.95	**<0.001**	2	16.072	47.26	**<0.001**
Coppice	1	1915.5	7.64	**<0.001**	1	633.5	1.90	**<0.001**	1	4747.4	5.66	**<0.001**	1	3.124	4.59	**<0.001**
SMT	1	1132.0	4.52	**<0.001**	1	5265.9	15.81	**<0.001**	1	11209.9	13.36	**<0.001**	1	0.428	0.63	**0.003**
Coppice × Genus	2	472.9	3.77	**<0.001**	2	301.4	1.81	**0.001**	2	1462.3	3.48	**<0.001**	2	0.223	0.66	**0.011**
Genus × SMT	2	295.3	2.36	**<0.001**	2	154.3	0.93	**0.023**	2	775.8	1.85	**0.001**	2	0.134	0.39	0.065
Coppice × SMT	1	129.5	0.52	**0.022**	1	161.8	0.49	**0.047**	1	561.9	0.67	**0.023**	1	0.014	0.02	0.592
Coppice × Genus × SMT	2	47.1	0.38	0.147	2	77.8	0.47	0.149	2	212.3	0.51	0.140	2	0.016	0.05	0.727
Species (Genus)	3	322.1	3.86	**<0.001**	3	212.5	1.91	**0.002**	3	383.5	1.37	**0.014**	3	2.902	12.80	**<0.001**
Coppice × Species (Genus)	3	33.2	0.40	0.257	3	73.6	0.66	0.145	3	194.3	0.69	0.145	3	0.095	0.42	0.120
SMT × Species (Genus)	3	21.3	0.25	0.457	3	4.0	0.04	0.961	3	43.2	0.15	0.752	3	0.020	0.09	0.752
Coppice × SMT × Species (Genus)	3	3.2	0.04	0.943	3	25.6	0.23	0.598	3	19.7	0.07	0.908	3	0.189	0.83	**0.009**
Error	437	24.5	42.74		437	40.7	53.45		436	107.6	55.87		436	0.049	31.19	
R^2		0.625				0.510				0.502				0.722		

In the fertilizer experiment, only genus and species had significant effects on stem dry biomass, accounting for 49 and 13% of total variation (Table 17). There were no significant FERT effects or interactions, though fertilized birches had greater stem mass than unfertilized birches (Fig. 10C). Birches had the greatest stem dry biomass at 12.95 g, followed by cherries and oaks at 6.24 and 3.78 g. Each was significantly different from the others (Fig. 10C). By species, WB had the greatest stem biomass at 13.83 g. This was significantly greater than PC at 9.33 g. GB (12.07 g), was not significantly different from either. Bur oak, CC and RO, had significantly lower means at 4.65, 3.15, and 2.89 g respectively.

Table 17. Analysis of variance (ANOVA) outputs for stem dry biomass, root dry biomass, total dry biomass, and shoot to root ratio for seedlings in the fertilizer experiment including source of variation, degrees of freedom (df), mean square values (MS), variance components (VC), *p*-values, and coefficient of determination (R^2). FERT denotes fertilizer treatment. Significant *p*-values (< 0.05) are listed in bold print.

Source of Variation	Stem Dry Biomass (g)				Root Dry Biomass (g)				Total Dry Biomass (g)				Shoot Root Ratio			
	df	MS	VC (%)	*p-Value*	df	MS	VC (%)	*p-Value*	df	MS	VC (%)	*p-Value*	df	MS	VC (%)	*p-Value*
Block	1	28.9	1.034	0.135	1	2.6	0.05	0.813	1	50.6	0.48	0.468	1	0.006	0.09	0.494
Genus	2	680.1	48.569	**<0.001**	2	316.8	12.80	**0.002**	2	799.4	15.07	**0.001**	2	1.910	60.12	**<0.001**
CO_2	1	0.3	0.010	0.883	1	0.9	0.02	0.891	1	0.3	0.00	0.956	1	0.015	0.24	0.295
FERT	1	35.4	1.265	0.098	1	223.5	4.51	**0.029**	1	438.6	4.13	**0.035**	1	0.020	0.31	0.224
Genus × CO_2	2	13.1	0.934	0.361	2	44.7	1.80	0.376	2	87.3	1.65	0.404	2	0.048	1.51	**0.035**
Genus × FERT	2	26.5	1.894	0.130	2	169.9	6.86	**0.028**	2	317.6	5.99	**0.041**	2	0.009	0.30	0.503
FERT × CO_2	1	5.5	0.196	0.512	1	1.0	0.02	0.880	1	10.3	0.10	0.743	1	0.000	0.00	0.990
FERT × Genus × CO_2	2	11.7	0.836	0.401	2	28.8	1.16	0.530	2	73.2	1.38	0.467	2	0.001	0.02	0.947
Species (Genus)	3	117.1	12.544	**<0.001**	3	117.3	7.11	0.059	3	311.2	8.80	**0.026**	3	0.347	16.38	**<0.001**
CO_2 × Species (Genus)	3	13.9	1.485	0.356	3	19.3	1.17	0.733	3	31.8	0.90	0.801	3	0.059	2.78	**0.007**
FERT × Species (Genus)	3	4.6	0.491	0.780	3	26.6	1.61	0.622	3	20.6	0.58	0.884	3	0.074	3.51	**0.002**
FERT × CO_2 × Species (Genus)	3	0.9	0.094	0.976	3	2.3	0.14	0.985	3	0.6	0.02	0.999	3	0.006	0.27	0.734
Error	68	12.6	30.650		69	45.0	62.73		68	95.1	60.92		68	0.014	14.47	
R^2			0.699				0.384				0.396				0.858	

Root dry mass

Genus, species, coppice and SMT had significant effects on root dry biomass, accounting for 22, 2, 2, and 16% of total variation in 2024 (Table 16). There were significant Genus × Coppice, Genus × SMT and Coppice × SMT interactions responsible for 2, 1, and 1% (Table 16). Both interactions with SMT were due to magnitude effects. Drought treatment reduced root dry mass 34% on average, though the amount depended on genus and coppice. Birches showed the largest reduction (41%), followed by cherries (32%) and oaks (28%) (Fig. 11A). In the drought treatment, coppiced trees were reduced less (30%) than uncoppiced trees (36%) (not shown). The Genus × Coppice interaction was the result of a magnitude effect. On average, coppicing reduced 2024 root dry mass by 13%, though this depended on genus. Birches and oaks were 27 and 11% lower respectively while cherries showed no significant difference between treatments (Fig. 11B). Oaks had the greatest mean root biomass (23.1 g), followed by birches and cherries at 17.1 and 12.7 g respectively (Fig. 11A, 11B). Each was significantly different. By species, BO (23.2 g) and RO (23.1 g) were significantly greater than GB (19.1 g), which was significantly greater than WB (15.1 g). White birch was significantly greater than PC (12.0 g), though CC (13.4 g) was not significantly different from either of these.

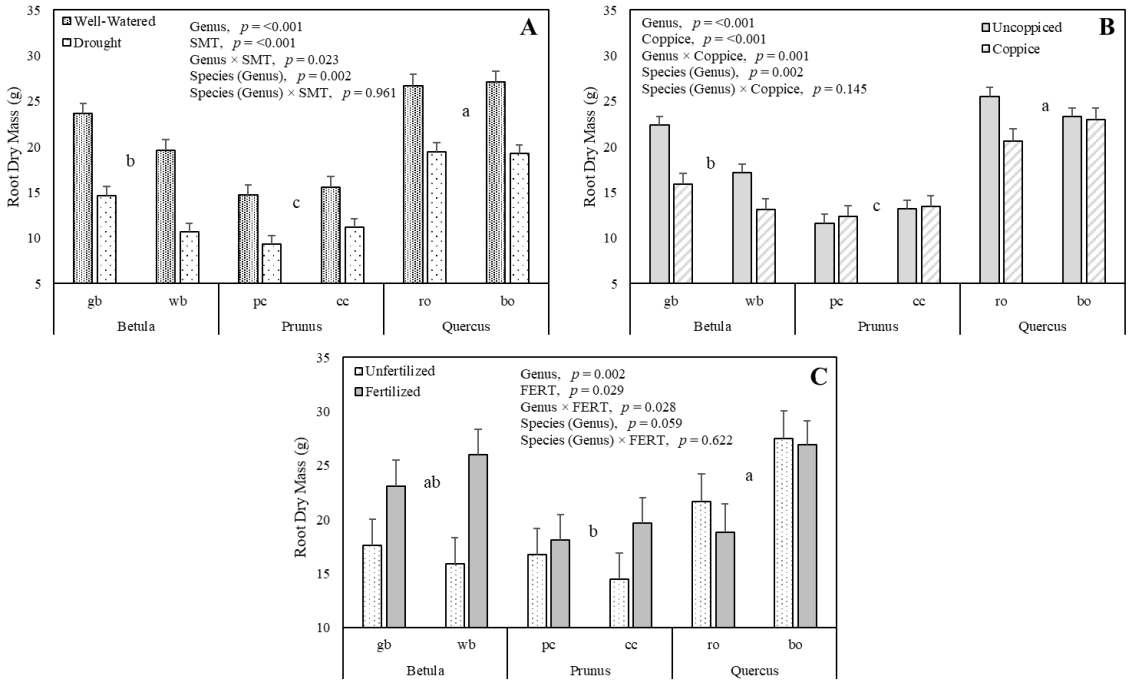

Figure 11. Root dry mass (g) by species, genus and (A) soil moisture treatment (SMT) in the 2024 growing season, (B) coppice treatment in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE of the associated trait in the, 2024 (n = 462 seedling replicates) or FERT experiments (n = 95 seedling replicates). gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

Genus, FERT, and the Genus × FERT interaction had significant effects on root biomass in the fertilizer experiment, accounting for 13, 5, and 7% of total variation (Table 17). The interaction was the result of a minor rank change. Root dry mass increased for birches and cherries 46 and 21% when fertilized, while oaks were 7% lower. Red oak had slightly lower values for fertilized compared to unfertilized seedlings while BO were equal (Fig. 11C). By genus, oaks showed the greatest mean biomass at 23.7 g, significantly greater than cherries at 17.3 g. Birches (20.7 g), were not significantly different from either. By species, BO (27.2 g) was significantly greater than

RO, PC, and CC at 20.3, 17.5 and 17.1 g respectively. White birch and GB (21.0 and 20.4 g) were not significantly different from any species.

Total dry mass

Genus, species, FERT, coppice and SMT all had significant effects on total dry biomass, accounting for 16, 1, 6, and 13% of the total variation (Table 16). The Genus × Coppice, Genus × FERT, Genus × SMT and Coppice × SMT interactions were also significant and accounted for 3, 2, and 1% (Table 16). Both the Genus × SMT and Coppice × SMT interactions were magnitude effects. On average, dry biomass was reduced by 33% under drought, though this depended on genus and coppice. The largest decrease was observed in birches (39%), followed by cherries and oaks at 28, and 10% (Fig. 12A). Drought had a greater effect (36%) on uncoppiced trees, than in coppiced trees (30%). Coppicing reduced dry biomass by 23%, on average though this depended on Genus. Birches were reduced by 36%, followed by oaks (25%), and cherries (7%) (Fig. 12B). Birches had the greatest total biomass at 3.0 g, followed by oaks (27.9 g). and cherries (18.6 g.) Each was significantly different. By species, GB at 34.1 g, was significantly greater than BO and RO, at 28.0 and 27.9 g. White birch (29.9 g) was not significantly different from any of these. Pin cherry and CC displayed significantly lower means (20.5 and 16.7 g) than all other species.

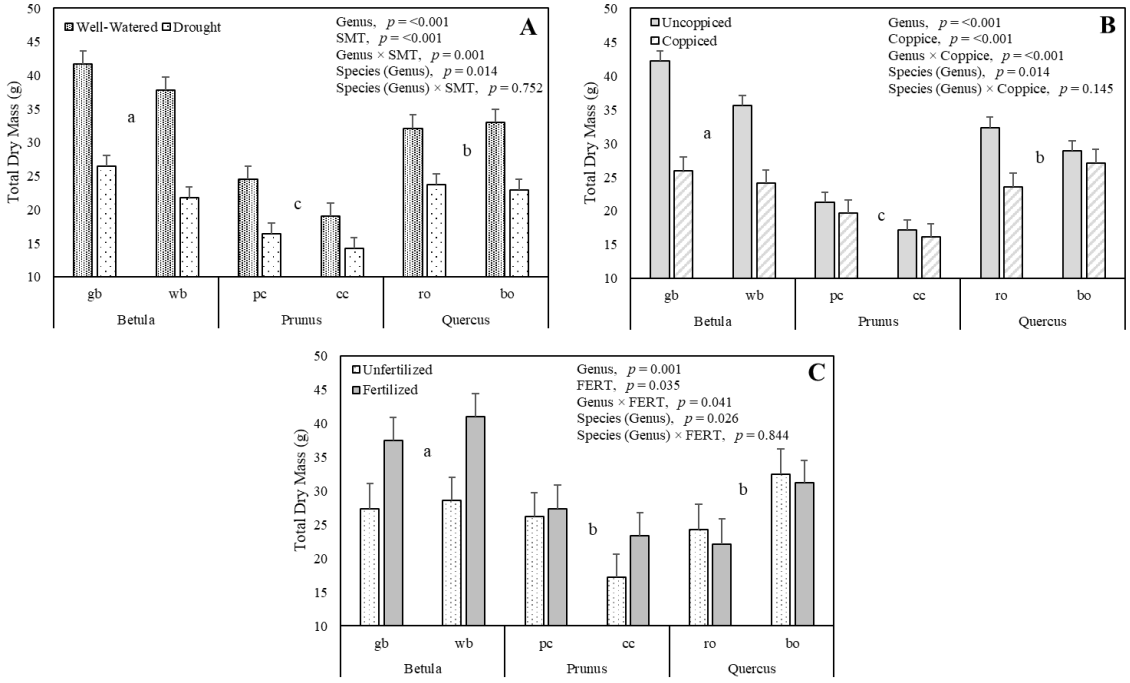

Figure 12. Total dry mass (g) by species, genus and (A) soil moisture treatment (SMT) in the 2024 growing season, (B) coppice treatment in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE of the associated trait in the, 2024 (n = 462 seedling replicates) or FERT experiments (n = 95 seedling replicates). gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

In the fertilizer experiment, Genus, Species, and FERT were significant along with the Genus × FERT interaction, accounting for 15, 9, 4 and 6% of total variation (Table 17). The interaction was the result of a small rank change. Dry biomass was 40 and 17% greater for birches and cherries in the fertilized treatment. Following the pattern of root dry mass, oaks were the only genera that had less total dry mass (6% decrease) under fertilized rather than unfertilized treatments (Fig. 12C).

Shoot-to-root ratio

The shoots to roots ratio was significantly affected by Genus, Species, Coppice, and SMT in 2024. These accounted for 47, 13, 5, and 1% of total variation respectively (Table 16). There were also significant Species × Coppice × SMT and Genus × Coppice interactions both accounting for 1%, both due to magnitude effects. On average, shoot-to-root ratio was 13% greater under drought though this depended on coppice and species (Fig. 13A, 13B). In GB and PC, shoot root ratio increased more under drought when coppiced, while the other species increased more in the uncoppiced treatment. Coppicing decreased shoot-to-root ratio by 29% on average, though amounts depended on SMT (described above) and species (Fig. 13B). Oaks decreased by 39%, followed by cherries (30%) and birches (25%). Birches had the greatest shoot-to-root ratio (0.89) followed by cherry (0.48) and oak (0.20). Each was significantly different from the others. White birch had the greatest shoot-to-root ratio, at 0.98. This was significantly greater than GB and PC at 0.79 and 0.71, which in turn were significantly greater than CC (0.25), BO (0.20), and RO (0.20), respectively.

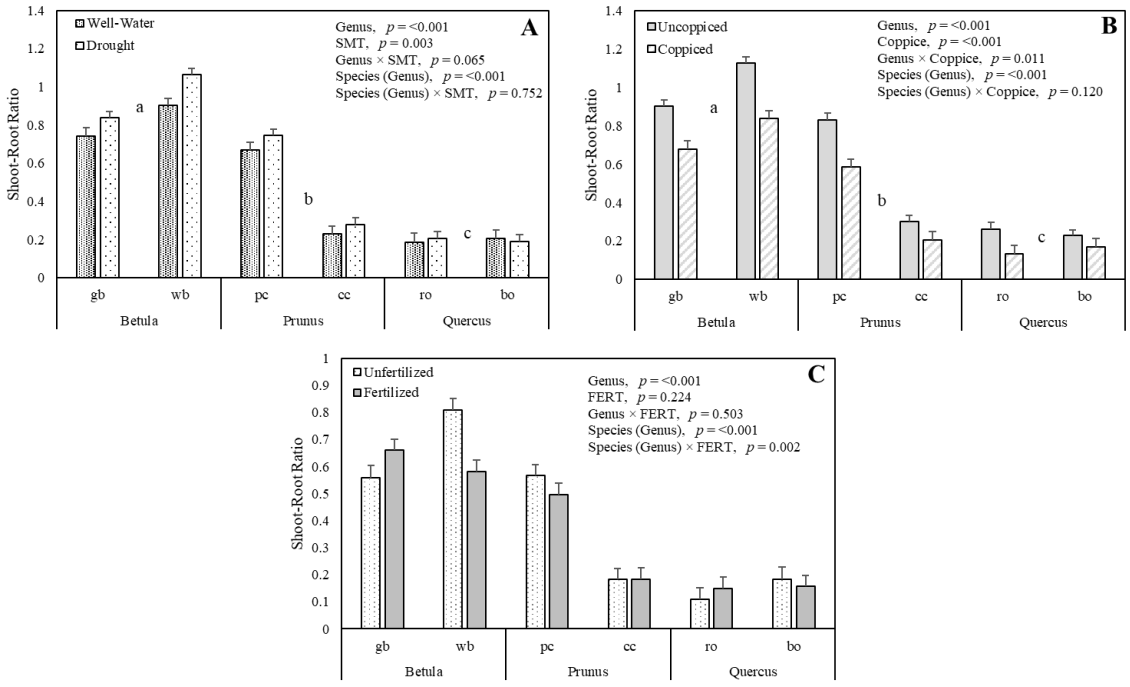

Figure 13. Shoot to root ratio by species, genus and (A) soil moisture treatment (SMT) in the 2024 growing season, (B) coppice treatment in the 2024 growing season, and (C) fertilizer treatment (FERT) in the 2024 growing season. Each bar displays mean ± SE of the associated trait in the, 2024 (n = 462 seedling replicates) or FERT experiments (n = 95 seedling replicates). gb refers to gray birch, wb to white birch, pc to pin cherry, cc choke cherry, ro to red oak, and bo to bur oak. Tukey's mean separation test was performed on genus (p = 0.05) for each analysis. Significance levels are denoted with lower case letters.

In the fertilizer experiment, shoot-to-root ratios were significantly affected by Genus and Species, which accounted for 60 and 16% percent of variation respectively. Species × CO_2, Species × FERT, and Genus × CO_2 interactions were also significant, accounting for 3, 4, and 2% (Table 17). The Genus× CO_2 and Species × FERT interactions both occurred due to rank changes. In eCO_2, birches and oaks saw increases of 15 and 34% in shoot root ratio while cherries decreased by 15%. By species, CC, GB, RO and BO increased by 37, 35, 82, and 7% in eCO_2, while WB showed no change, and PC decreased by 29%. The Species × FERT interaction was also the result of a rank change, with fertilizer increasing ratios by 38% and 18% in red oak and gray birch.

Fertilizer had minimal effects on CC, while WB, BO and PC, decreased by 28, 14, and 12%, respectively (Fig. 13C). By genus means, birches displayed the greatest shoot root ratio (0.65), followed by cherries (0.36) and oaks (0.15). Each mean was significantly different from the others. By species, White birch (0.70) was significantly greater than PC (0.53), while GB (0.61) was not significantly different from either. Choke cherry, BO and RO were all significantly lower, with means ratios of 0.18, 0.17 and 0.13.

Discussion

Genus and Species Differences

Consistent genus and species patterns were observed in growth traits. Genus was the most significant factor for every variable we examined and was responsible for 26-77% of the total variation (Tables 14-17). These results indicate considerably different patterns between birches, cherries and oaks in all aspects of growth including height, biomass and overall structure. Adaptation explains the majority of these differences, with each genus evolving to exploit different ecological niches and thus exhibiting considerable differences in size and form. When coppice was not a factor (in 2023 and the fertilizer experiment) species was generally the second most significant and accounted for between 2-22% of variation from defined sources (Tables 14-17).

Birches displayed the greatest value for every trait except root biomass (Fig. 8, 9, 10, 12, 13). They possessed greater heights, diameters, total and stem biomasses, had the greatest number of stems and had the greatest ratio of shoots to roots. In the main experiment, GB typically had greater means for these traits than did WB. In addition to

its faster growth rate and shorter lifespan, GB often colonises more open and marginal sites than WB (Safford et al., 1990; Beauregard et al., 2020) possibly explaining the greater observed growth on the low nutrient medium used in this experiment. In another studies (Berntson et al., 1995), GB did not respond as strongly to nutrient additions compared to another birch species (B. allegheniensis). White birch generally had a greater response with greater heights, diameters and biomasses than GB in the fertilized treatments (Fig. 10C, 11C, 12C, 13C).

Cherries generally followed birches in biomass and growth metrics, except for total dry biomass, where they followed oaks (Fig. 8-13). Pin cherry significantly outperformed CC in most traits with the exception of root biomass and stem number. Silvics and adaptations can also explain these differences, as unlike PC, CC generally produces multiple stems and spreads via root suckers (Welch, 2004). The significantly greater shoot-to-root ratios and heights of PC indicate greater allocation to stem growth while CC responded with greater allocation below ground. Although metrics are not easily available for these non-merchantable species, faster growth rates in PC could be inferred from the species mean sizes and lifespan.

Oaks generally displayed the lowest values for every trait except root dry mass, where they exhibited the greatest values and total dry mass, where they ranked second to cherries (Fig. 11-12). With the greatest mean root biomass and lowest stem biomass, oaks displayed the lowest shoot-to-root ratios indicating the greater resource allocation to root growth (Fig. 11). In fact, the only species with a shoot-to-root ratio not significantly greater was CC; this being the only studied species that regularly produces root suckers. Both oak species examined here are adapted to more xeric soil conditions than the other

genera (Johnson, 1990; Cogliastro et al., 1997). Lower shoot-to-root ratios at the seedling stage are likely an adaptation allowing greater access to limited water resources during crucial growth and development periods. For height, diameter, dry mass, and shoot root ratio, BO generally produced greater mean values than RO, though these differences were usually not significant. However, it would appear that BO's natural range farther west (dryer) and RO's farther east (maritime) indicates BO is naturally more drought tolerant. Red oak produced more stems than BO when coppiced, but not by a significant margin.

CO_2 Response

Most studies on early-successional deciduous trees have observed short term increases in growth and biomass production in response to eCO_2 (Bazzaz et al., 1990; Atkinson et al., 1997; Centritto, 2002). In 2024 the CO_2 effect did not have a significant influence on any traits in either coppiced or uncoppiced trees, nor was it significant in the fertilizer experiment. Significant interactions involving CO_2 did sometimes occur for certain traits, though these cumulatively accounted for a maximum of 6% of the total variation. In other words, we observed eCO_2 as having little or no effect on growth or biomass. A lack of CO_2 induced change on traits like shoot-to-root ratio has been documented (Kerstiens and Hawes, 1994; Centritto et al., 1999), though most studies do find significant eCO_2 effects on growth (Bazzaz et al., 1990, Bazzaz and Miao, 1993; Bazzaz et al., 1993; Cao et al., 2008; Centritto et al., 1999; McConnaughay et al., 1996) We hypothesize that critical N limitations were the cause. Numerous studies have demonstrated soil N availability constraining positive plant biomass responses to eCO_2 (Oren et al., 2001; Reich et al., 2006; Major et al., 2022). Multiple mechanisms for this have been proposed. (Reich et al., 2006). Low soil N availability could limit leaf area

leading to decreased light interception, limit photosynthetic capacity, or limit other physiological processes involving N. (Schneider et al., 2004; Reich et al., 2006) Growing media used in the experiment was purposely nutrient poor to mimic field conditions at typical restoration sites. The media was 97% screened sand and contained only 0.06% N, likely resulting in severe N limitations which drove downregulation in sink activity. Thus, with C uptake limited by N, any benefit of eCO_2 would be negated. Previous studies found significant fertilizer-induced enhancement of previously limited eCO_2 responses for both WB and GB, as well as shrub willows grown on similar media (Brisebois and Major, 2024; Major et al., 2022). The fertilizer experiment was thus designed to replicate this effect in birches and investigate it in cherries and oaks. Time and resource constraints meant that the fertilizer experiment could not be conducted until fairly late in the season (September 6th – October 15th) when growth of the seedlings had likely slowed considerably. As a result, a lack of sink activity in the form of limited shoot growth likely prevented any N additions from having large effects on biomass accumulation via a stronger CO_2 response.

Fertilizer Response

The main effect of FERT was only significant for total dry biomass and root dry biomass, though Genus × FERT interactions were significant for these traits as well indicating cross genus or species effects (Table 17). The genus interactions were magnitude effects, and were mostly due to oaks, which did not display consistent biomass increases when fertilized unlike birches and cherries. Species interactions affected shoot-to-root ratio and were the result of CC having significantly lower shoot root ratios than PC due to its suckering habit. Examining overall biomass and root shoot ratios suggests

GB allocated more of the added nutrients to shoot growth, while WB allocated more to root growth (Fig. 10-12). PC allocated all nutrients toward root growth while CC showed no change in shoot-to-root ratio (Fig. 13C). Oaks showed no significant biomass differences between fertilized and unfertilized treatments (Fig. 10C, 11C, 12C). As mentioned, the fertilizer experiment could not be conducted until fairly late in the season (September 10th – October 15th) when growth of the seedlings may have been limited. Temperate climate deciduous trees generally produce root growth later in the season than shoot growth (Makoto et al., 2020) possibly explaining the increases for WB and PC. Anecdotally, active shoot growth was observed later into the season in GB compared to the other species studied. It was also the only species that grew more shoots than roots in the fertilized treatment, perhaps as a result. Red oak, BO, and CC had likely ceased shoot growth entirely by the time of the fertilizer additions explaining the lack significant effects on these biomass traits. Despite this, trees were still metabolizing, and significant changes to biochemical efficiency and other related traits were reported in response to fertilizer in the study's first chapter (Tennant & Major 2026).

Soil Moisture Response

Apart from stem number, SMT significantly affected every trait analyzed (Tables 14-17). For biomass traits, main effect of SMT was often the 2nd to 4th most influential variable after genus, species and coppice, having smaller, but still significant, effects on height, basal diameter, and shoot-to-root ratio (Tables 14-17). All interactions were magnitude effects, with birches responding most negatively to drought in every case, likely as a result of high shoot-to-root ratios (Fig. 8-13). Water is among the most limiting factors for plant growth, and unsurprisingly, trees grown in the drought treatment

showed significantly less growth than those in well-watered conditions. Under drought, heights and basal diameters were lower by 10 and 18% respectively (Fig. 8A), while overall biomass traits were more strongly affected, being reduced by 33% on average. This was due to the significant effect of drought on shoot-to-root ratio, which surprisingly, was about 13% higher in droughted conditions. Decreases in shoot-to-root ratio in response to drought, and especially decreases in fine root biomass, have been well documented (Joslin et al., 2000; Konôpka et al., 2007; Eldhuset et al., 2013; Brunner et al., 2015) and are generally a consequence of reduced transpiration and respiration rates. As such, this finding was somewhat surprising. We postulate one potential explanation for this. One greenhouse study of ten tree and shrub species reported that while overall shoot-to-root ratios had decreased under drought, only the biomass of roots less than <0.5 mm in diameter increased significantly (Olmo et al., 2014). While we took care to preserve as much biomass as possible, the exhuming and washing processes required to obtain dry weights did lead to the loss of some percentage of fine roots. If such roots represented a higher proportion of biomass in the drought treatment, it is possible that droughted trees lost more biomass to the root washing process than did well watered trees. If this difference was greater than 13%, it may be possible that any decreases to the shot root ratio were not detected.

Coppice Response

Coppicing had significant effects on all growth and biomass trait examined, usually being the second or third most influential trait after Genus and or species, and often more influential than SMT (Tables 14-17). Coppice accounted for an average of 8% of the total variation, being as great as 19% (stem diameter) (Table 41) and as little as 2%

(root biomass) (Table 16). Previous studies of temperate deciduous trees have identified rapid recovery of shoot biomass and or growth following coppicing for certain species including RO, BO, and PC, willows and others (Kruger and Reich, 1993; Willis et al., 2021; Mosseler et al., 2019; Shabel and Peart, 1994; Major et al., 2022). In these studies, growth of coppiced or browsed shoots often exceeded that of uncoppiced controls in as little as one to two years, though the size and age of studied seedlings varied and this was not the case for all species (Willis et al., 2021). Such compensatory growth generally results from reduced shoot-to-root ratios, which give sprouts greater access to water, soil nutrients and root carbohydrate reserves allowing for faster regrowth (Matoušková et al., 2022). While we did observe shoot-to-root ratios decline by 29% and greater negative drought responses in uncoppiced trees (Fig. 13B), coppicing still resulted in 13 to 40% decreases for every trait other than stem number (Fig. 8B-13B). While the regrowth described in previous studies (Mosseler et al., 2019; Willis et al., 2021; Shabel and Peart, 1994) did occur over longer durations than analysed here, the growth rate of coppiced seedlings in our study, was 37% lower than that of uncoppiced seedlings. This may also be attributable to N induced growth limitations as described in preceding sections. The only biomass metric that did increase due to coppicing was stem number, which increased by 40% due to the production of stump sprouts. The Genus × Coppice interactions that occurred for all traits were due to magnitude effects. Despite possessing the greatest pre-coppicing biomasses, birches showed the least regrowth relative to their original biomass after coppicing occurred (Fig. 10B-12B). This was likely due to their greater shoot-to-root ratios. While oaks had the lowest shoot to root ratios, cherries recovered more relative biomass, likely owing to their faster growth rates.

Conclusion

The species of birches, cherries and oaks studied here are early successional deciduous trees from three different phylogenetic families used in ecological restoration on degraded sites. Birches displayed the greatest height and biomass growth and had the highest shoot-to-root ratios resulting in the strongest negative response to drought treatments. Oaks grew the least but possessed the lowest shoot-to-root ratios making them the least affected by low soil moisture. Growth and biomass traits were not significantly altered in response to eCO_2, likely in response to severe N limitations in the nutrient-poor growing medium. While fertilizer additions may mitigate this effect to some extent, the timing of such additions is crucial and must coincide with periods of growth. Coppicing resulted in lower overall biomasses, with stem biomass and shoot-to-root ratios smaller than control seedlings after one year of regrowth. Both species of birches and oaks generally behaved similarly, though the responses of PC and CC were often significantly different due to their differing silvics and adaptations.

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Summary

The findings presented in the first and second chapters of this thesis have expanded the body of knowledge on ecophysiological and morphological differences in early successional deciduous trees. The results from both chapters have significant implications for restoration efforts and management decisions, as they provide valuable insights into potential plant responses to predicted climate conditions.

The findings of the first chapter revealed that birches possessed the greatest V_{cmax}, J_{max}, A_{600}, G_{wv600}, $iWUE_{600}$, TCC and $\delta^{13}C$ concentrations. Cherries and oaks respectively generally followed birches for these traits. With few exceptions, species within a genus were not significantly different from one another, however, in most cases GB was greater than or equal to WB, PC was almost always greater than CC, and BO was greater than RO. In the initial growing season (2023), upregulation of V_{cmax} was observed in response to eCO_2 for all species. By 2024 however, V_{cmax} had been downregulated in all species except PC, suggesting a fairly rapid acclimation to eCO_2. This was consistent with the findings of previous studies (Centritto et al., 1999; Heath and Kerstiens, 1997; Riikonen et al., 2008; Rey and Jarvis, 1997; Kitao et al., 2007; Sanz-Sáez et al., 2010; Byeon et al., 2021; Mosseler and Major, 2022), with the results for J_{max} being similar. Downregulation was also observed in A_{600} during both years of the study and likely resulted due to soil N limitations on growth and other sink activities (Yi et al., 2019). Elevated CO_2 did not result in an increase to $iWUE_{600}$ as we had predicted, because stomatal conductance (G_{wv600}) was downregulated by a similar fraction (Yi et al., 2019). The addition of N fertilizers did alleviate some of the downregulation effects, though additions either

occurred too late in the season or were insufficient to completely ameliorate the responses.

The results of the second chapter showed similar species and genus trends for growth. Birches displayed the greatest mean values for every trait except root biomass, and developed greater heights, diameters, and total and stem biomasses than cherries or oaks. Birches also displayed the highest shoot-to-roots ratio, indicating greater resource allocation to stem growth. Cherries generally followed birches in growth metrics other than total dry biomass, where they followed oaks. Oaks displayed the lowest means for every trait except root dry mass. Here, they exhibited the greatest values indicating greater energy investment into root growth at the seedling stage. By species, GB, PC and BO, typically outperformed WB, PC and RO respectively. Contrary to our hypothesis, CO_2 had little or no effect on growth or biomass and had no significant influence on any traits in either coppiced or uncoppiced trees. We hypothesize that nitrogen limitations are the cause, as numerous studies have demonstrated soil nitrogen availability constraining positive biomass responses to eCO_2 (Oren et al., 2001; Reich et al., 2006; Major et al., 2022). Interestingly however, CO_2 effects were mostly insignificant in the fertilizer experiment as well, with only root and total biomass measurements affected. We theorize that fertilizer additions were administered too late in the season when only roots were still actively growing. Soil moisture was the most significant factor in the experiment with droughted trees showing significantly less growth. Unlike some previous studies (Kruger and Reich, 1993; Willis et al., 2021; Mosseler et al., 2019; Shabel and Peart, 1994; Major et al., 2022), we did not observe compensatory regrowth within one season following coppicing. This, again, was likely the result of soil N induced growth limitations.

Overall, these experiments provide valuable insights into the ecophysiological and morphological differences between our six species, expanding the limited knowledge base on these non-commercial trees. Gaps in the literature still exist however, and future studies will still be necessary to fully resolve our research questions. Future studies should incorporate earlier or more intensive fertilization regimes to fully assess the impact of nutrient limitations on CO_2 and drought response. Studies could also better assess real-world implications by examining growing mediums with differing nutrient profiles. Longer term studies could also be beneficial to further examine compensatory regrowth following coppicing. While the research presented in this thesis provides a foundation for expanding the collective knowledge of the studies species, further experimentation and replication will be essential steps to advance the understanding of deciduous tree physiology in the face of rapid climate change.

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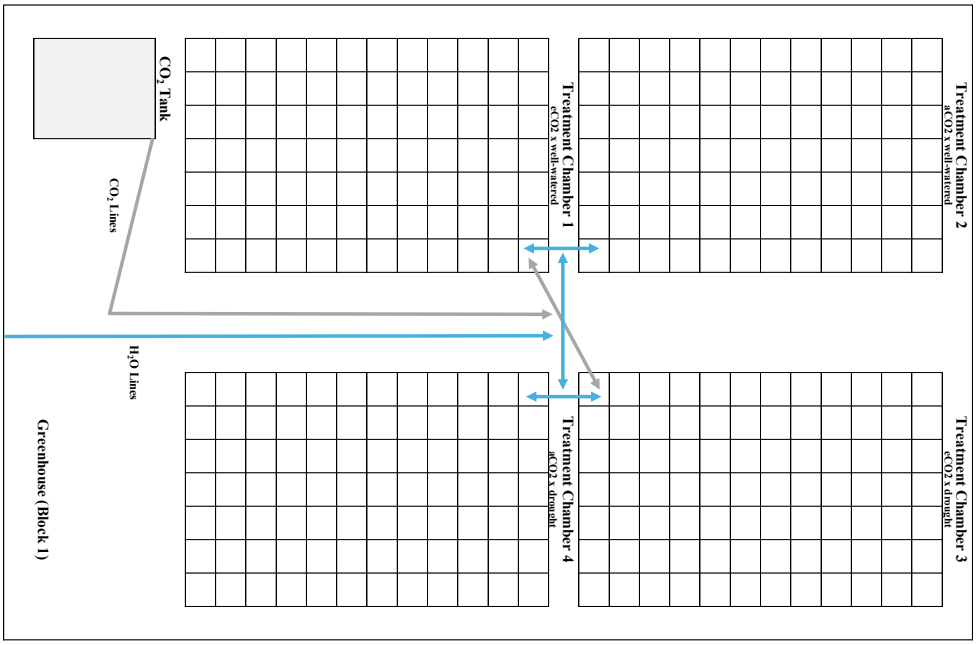
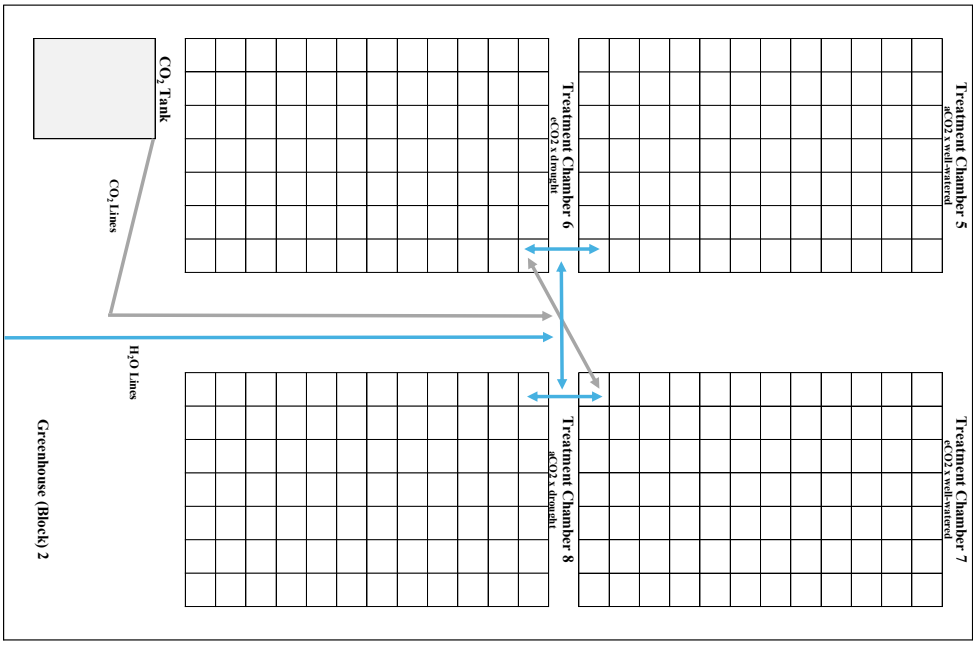
Appendix A

Schematic diagram of experiment design, illustrating placement of greenhouses, growth chambers, CO_2 tank and distribution, as well as irrigation distribution. Not shown are the individual irrigation lines to each root-control bag.





Appendix B

Schedule of fertilizer treatments applied to experimental seedlings during the 2024 growing season. Dates and volumes administered as part of the fertilizer experiment are denoted with asterisks.

Year	Date	Brand	Product Name	NPK Ratio	Mix Ratio	Volume Mixed (mL)	Mix Volume Administered (mL)
2024	01-May	Plant-Prod	*Forestry Starter*	10:52:10	625g/50L	20	1000
	07-May	Plant-Prod	*Forestry Starter*	10:52:10	625g/50L	20	1000
	17-May	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	26	1333
	17-May	REMO	*MagNifiCal*	3:0:0	1.5mL/25L	7	333
	23-May	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	13	667
	23-May	REMO	*MagNifiCal*	3:0:0	1.5mL/25L	5	267
	07-Jun	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	7	333
	07-Jun	REMO	*MagNifiCal*	3:0:0	1.5mL/25L	3	167
	11-Jul	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000
	11-Jul	REMO	*MagNifiCal*	3:0:0	1.5mL/25L	10	500
	27-Jul	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	7	333
	27-Jul	REMO	*MagNifiCal*	3:0:0	1.5mL/25L	5	267
	27-Jul	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	7	333
	10-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	12-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	18-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	20-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	23-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	25-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	27-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	30-Sep*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	02-Oct*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	04-Oct*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	07-Oct*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	09-Oct*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*
	11-Oct*	Plant-Prod	*Forestry Special*	20:8:20	625g/50L	20	1000*

Glossary

Carbon assimilation: The process of incorporating carbon dioxide into organic molecules during photosynthesis.

Carboxylation: Incorporation of carbon dioxide into organic compounds.

Chlorophyll: Green pigment in plants that captures light for photosynthesis.

Early successional species: Plants adapted for colonization and rapid early growth following a disturbance.

Magnitude Effect: A type of interaction wherein a treatment effect results in a consistent response across multiple groups (i.e. genera), while the magnitude of these responses varies among the groups.

Photosynthesis: The process by which plants convert sunlight into chemical energy.

Photosynthetic downregulation: An adaptive response to reduce photosynthesis under stress or unfavorable conditions.

Photosynthetic upregulation: An adaptive response to increase photosynthesis under favorable conditions.

Rank Change: A type of interaction wherein one treatment effect results in opposite response in different groups (i.e. genera).

Sink effect: Increased availability of a resource results in increased demand for the resource.

Stomata: Small openings on the surface of leaves and stems that regulate gas exchange.

Evapotranspiration: Exhalation of water vapor through the stomata

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