PHENOLOGY, MORPHOLOGY AND PHYSIOLOGY OF EASTERN DECIDUOUS
SEEDLINGS UNDER INCREASED TEMPERATURE AND PRECIPITATION
TREATMENTS

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ABSTRACT

I examined phenology, morphology and physiology of 11 early successional eastern deciduous tree species grown under increased temperature and irrigation treatments over three years in Central Pennsylvania. The relationship between climate manipulation and the phenology, growth rate and biomass allocation was explored for species predicted to experience changes in habitat suitability over the next century as climate changes. Seedling physiology was used to test the biological responses to manipulated climate conditions through gas exchange and plant-water relations during the final year of the experimental study. Climate manipulation treatments were established in a four-hectare area that was clear-cut and fenced in August 2007. Sixteen plots were established in a 2x2 factorial design with four treatments; ambient (control), irrigated (20% increase), warmed (2°C increase), and warmed+irrigated (20% + 2°C increase). Seeds of 9 northeastern and 2 southeastern species were planted in the fall of 2007-2009 and were allowed to germinate and grow under treatment conditions until August 2010. Both germination and leaf out advanced under warmed treatment conditions (10-20 days and 7-11 days respectively). Increasing temperature stimulates biomass production and growth of young, deciduous seedlings, with the greatest proportional increase found in above ground woody tissue. Seedlings in warmed treatments displayed lower leaf area ratios and altered leaf morphology. Climate treatments did not appear to alter leaf-level measures of foliar carbon and nitrogen. Warming also resulted in strong positive correlations between relative growth rate and photosynthetic capacity, suggesting that the increase in temperature facilitated an increase in assimilation and growth for the study species. Warming decreased seedling water potentials, however these decreases did not correspond to declines in biomass allocation (root:shoot), photosynthetic rates or stomatal conductance responses. Instead, early seedling growth was not limited by changes in plant water potential and the increased rates of photosynthesis are a
potential cause for the decreases in predawn water potentials observed over the course of the 2010 growing season. My results highlight the importance of examining both observable morphological responses, as well as the mechanistic physiological responses that young trees exhibit in response to changing climate. Increasing the temperature and precipitation experienced by developing seedlings in this study showed that the responses of individuals and species may be limited by the developmental constraints of small tree morphology and physiology. The implications of the results suggest that temperate forests systems experiencing moderate climate change may have a greater resilience at the seedling stage.
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Chapter 1

Past, present and future: global climate change patterns and the effects on plant phenological, morphological and physiological responses.

Climate change

Global circulation models (GCMs) predict increases in mean global temperature from 1.4- 5.8°C by the year 2100, while predictions for precipitation patterns show a wider range of responses from 0-20% (Melillo et al. 1993, Stainforth et al. 2005, Solomon 2007). Downscaled global circulation models have predicted warmer and wetter conditions throughout the northeastern United States and the potential impact of these climate changes on forest ecosystems could be dramatic (Kattenberg et al. 1996, Hanson and Weltzin 2000). As global and regional climate conditions change, plant communities must migrate or adjust their morphological and physiological behaviors to survive under new temperature and precipitation regimes. The historical patterns in climate that forest systems have developed under must be considered to better understand how these systems may respond to changing future climate.

Long-term global climate patterns have varied widely, forming distinct periods over geologic time. These long-term patterns have had lasting impacts on the evolution of the terrestrial biosphere. Biological populations, such as plants, respond to climate change rapidly with respect to geologic time, with adaptation to new conditions occurring over mere centuries (Birks and Ammann 2000, Tinner and Lotter 2001, Williams et al. 2002). Long-term changes in climate can be determined by examining terrestrial vegetation records, such as remnant populations and pollen records across time periods (Shumana et al. 2002). In fact, long-term climate patterns, reconstructed from paleoecological records, are primary drivers of vegetation

Global climate cycles follow orbital changes, known as Milankovitch cycles, which occur approximately every 100,000 years due to the eccentricity of orbital patterns (Heinrich 1987). Slight changes in the Earth’s tilt are responsible for intra-annual fluctuation in climate, but as the degree of change becomes more drastic, large scale changes in global climate can occur (Short et al. 1989). The Milankovitch theory also predicts periodic cooling during the 100,000 year cycle, which occurs every 40,000 years as the earth’s axial tilt oscillates (Imbrie and Imbrie 1980, Bradley 1985, Bennett 1990). These cooling cycles are considered to be contributing factors in the development of glacial cycles. However, the last four glacial cycles lasted between 80,000 and 130,000 years, indicating the major changes in global climate were aperiodic and could not be fully explained by orbital variation alone (Winograd et al. 1992, Heimann and Reichstein 2008).

The Quaternary Period, or the last 2.5 million years, is defined by the presence of a permanent ice sheet, which continues to exist over Antarctica today. Quaternary climate has been generally viewed as glacial and interglacial, or periods of cold and warm global climates (Webb 1986). The cyclic nature of the climate during this time created distinct periods of warming and cooling controlled by a range of factors including: orbital changes (the Younger Dryas), ice-sheet changes and the corresponding changes in sea-surface temperatures and sea ice (the Little Ice Age) and volcanic activity (the Medieval Warm Period) (Bartlein and Prentice 1989, Campbell et al. 1998). Analysis of glacial ice cores for the last 250,000 years have seen frequent and abrupt climate changes. The current interglacial period has lasted approximately 10,000-15,000 years with warm, stable temperatures and is commonly referred to as the Holocene epoch (Prentice et al. 1991). This period is defined by pronounced increases in global temperature with the longest interval of stable global climate and sea level in the last 400,000 years (Zalasiewicz et al. 2008).
Long term climate reconstructions, like those used to determine climate changes over the past 400,000 years, are created using climate proxies. Climate proxies are the preserved natural records of past climate and include deep ice cores (Huang et al. 2000), tree rings (Fritts et al. 1979, Briffa et al. 2001, Esper et al. 2002), isotope analysis (Winograd et al. 1992) and lake sediment pollen cores (Davis 1983, Davis et al. 1991, Pederson et al. 2005). Through the use of proxy-based climate reconstructions, long-term relationships between past and current climate can be used to model predictions of future climate variability. Understanding natural climate variation can aid in the development of models that incorporate mechanistic factors controlling climate change on regional and global scales (Jones et al. 2001). Previous interglacial periods last approximately 10,000 years and climatologists predict that if the pattern is maintained, the Earth is approaching another glacial period (Loutre and Berger 2000). Within the current natural cycle, modern warming seems to be occurring at the same degree as that of the Medieval Warm Period, which on average was -0.35°C cooler than the last 30 years of the 20th century (Bradley et al. 2003). However, when natural cycles are combined with anthropogenic changes in atmospheric CO₂ emissions the warming trend may be amplified and prolonged (Campbell et al. 1998).

The response of species to changing global climate is a major ecological concern, particularly for long-lived sedentary species, such as trees. In the post-glacial Holocene epoch the pattern of migration was an upward shift, both in latitude and elevation, as tree-lines advanced under warming climate (Grace et al. 2002). Historically, climate-driven migration took place over hundreds of years and current climate predictions for migration would require North American trees to extend their range northward from 100-1000 meters per year over the span of the next century (Davis 1981, Davis and Zabinski 1992, Iverson and Prasad 2002, McLachlan et al. 2005). Palynological records suggest the rate of Holocene migration was generally faster than the actual rate of seed dispersal, particularly for heavy seeded species such as *Quercus* and *Carya*, although human activity in the late Holocene may have aided northward migration and
persistence of these species (McLachlan et al. 2005, Nowacki and Abrams 2008). Current anthropogenic activity resulting in habitat fragmentation may make natural long distance migration more difficult for current forest populations to achieve. Understanding the impact of the rapid global warming that occurred during the Holocene can highlight the potential responses of species to global climate change.

**Forests and Climate Change**

As global climate continues to change, either by the perpetuation of natural cycles, anthropogenic forcing, or a combination of both, a major concern remains regarding the potential alteration of temperature and precipitation regimes on both global and regional ecosystems (Hanson and Weltzin 2000). Global circulation models provide a basis for estimating many aspects of climate over the next 100 years. Additionally, these GCMs indicate that the intensity and frequency of climatic events (i.e. droughts, floods and heat waves) are likely to increase over the next century (IPCC 2007 ). Regional climate predictions have suggested that warmer and wetter conditions are likely to occur throughout the northeastern United States, with increases in temperature from 2-6°C and increases in precipitation ranging from 0-20% (Ollinger et al. 2007). The potential impacts that these regional climatic changes will have on forest ecosystems is the subject of intense ecological study. As climate conditions change, plant communities must migrate or adjust their biological responses to survive under new temperature and precipitation regimes (Norby and Luo 2004)

Forest models predict major changes in species composition and growth in response to climate change (Solomon 1986, Pastor and Post 1988, Shugart and Smith 1996, Iverson and Prasad 2001). The potential impacts of climate change on the eastern forests of the United States are characterized by an expected northward shift in distribution of most major trees species. Iverson and Prasad (2001) based their predictions of changes in habitat suitability on DISTRIB, a
deterministic regression tree analysis model. Regression tree analysis was used to establish predictable rules to evaluate the relationship of environmental variables to eastern tree species importance values (USDA Forest Service Inventory and Analysis Data) from current distributions and then use them to predict potential suitable habitat across the eastern United States. Environmental variables included attributes of soil (total water-holding capacity, pH, percent organic matter, percent clay, percent slope, and percent weight of rock fragments 8–25 cm), land use/land cover, elevation, landscape pattern, and climate (Iverson and Prasad 2001). Under the modeled climate scenarios of the DISTRIB model, the Maple-Beech-Birch forest type showed a large reduction in area, while Oak-Hickory was modeled to increase significantly across all potential climate scenarios. However, species redistribution is dependent on factors beyond basic responses to climate and environmental variables. Additional information on species response to climate change that needs to be incorporated into these regression models includes the rate of climate change, herbivory, intra- and inter-specific competition, ecophysiological responses and the ability to move propagules across fragmented habitat. Regression tree analysis, such as DISTRIB generally does not incorporate biological attributes or interactions and the role that disturbance may play on the impact of future species assemblages.

In the Northeast, forest harvesting removes mature trees and opens the canopy to allow opportunity for species changes. Pennsylvania forests, like much of the northeastern United States, are accumulating biomass following recovery from harvesting (Bormann and Likens 1979). The use of harvesting in forest systems resulted in eastern ecosystems existing in a state of transition from abandoned agricultural lands to forest during the last century. However, recent land-use has resulted in a net loss of 4% of the eastern mature forest cover since 1973 (Drummond and Loveland 2010). The opportunity and resources in recently cleared stands for the establishment of new species through migration can be facilitated by changing climate conditions. In the absence of a dominant overstory, species with the ability to establish quickly and capture
available resources have the potential to outcompete the locally adapted species that dominated
the pre-harvest overstory.

**Plant Responses to Climate Change**

As sedentary organisms, plants must possess a wide range of genetic and phenotypic
variability to cope with changing climate. Biological responses of tree species to altered
temperature and precipitation may be moderated by morphological and physiological acclimation,
or short-term, non-heritable changes caused by altered environmental conditions (Abrams 1994 ).
The potential acclimation of eastern tree species to altered climate is not well known, although
broadly distributed species may have the capacity to tolerate moderate changes in climate and
habitat suitability (Gunderson et al. 2000). The ability of species to respond to increasing
temperature and precipitation through changes in their phenology, physiology and morphology
provides a better understanding of the potential of eastern species to adjust to predicted climate
changes.

**Phenological responses**

Advances in of the onset of spring warming can induce changes in plant phenology, such
as earlier bud burst and leaf out, providing competitive advantages to species that can capitalize
on this early growing period (Chmielewski and Rötzer 2001). Increases in temperature and
precipitation over the last century have been associated with lengthened growing season for
northeastern species and advancement in the timing of spring “green-up,” or the emergence of
leaves, has been recorded at the community-level throughout the eastern United States (Schwartz
1998, Iverson and Prasad 2001, Price et al. 2001). Seedling germination and leaf out are sensitive
to environmental cues, making them highly responsive to changing climate and potentially
altering the dynamics of species establishment and growth (Hanson et al. 2005). Species with the
capacity to respond to earlier phenological cues for bud burst and leaf out may have a competitive
advantage in early spring growth when compared to species that are adapted for later emergence,
since early emergence may provide additional time for photosynthetic activity in the absence of
other competition (Badeck et al. 2004). Additionally, changes in timing of phenological events
and their corresponding morphological and physiological responses can also impact competitive
abilities of individuals as they continue to develop in changing climate conditions.

**Morphological Responses**

Plant carbon gains and biomass allocation are influenced by a variety of factors including
climate, disturbance and species tolerance to environmental conditions. Changes in mean
temperature and precipitation can alter the allocation of carbon to roots, shoots and leaves,
particularly in young seedlings that lack the stored reserves to withstand prolonged drought or
et al. 2005, King et al. 2006). Plants respond to variation in the environment by allocating
resources to plant organs that increase the acquisition of limiting resources, such as water,
nitrogen or light (Reich 2002). In high light environments, young trees with the ability to limit
belowground biomass allocation in favor of shoot growth have the potential to grow quickly and
become dominant within the developing forest canopy. However, increased above ground growth
must be able to be supported both by increases in photosynthetically active tissue and by adequate
belowground development for water and nutrient acquisition (van der Werf et al. 1993). As
changing precipitation patterns are coupled with increasing temperatures, the structure and
function of root systems must have the ability to effectively maximize soil resource acquisition
and total belowground biomass allocation (Wullschleger et al. 1994). Similar morphological
changes can be observed in the development of foliar tissues in seedlings grown under resource
limited conditions. Leaf structure is generally a strong indicator of changes in resource availability and changes in leaf morphology can be used to measure plant responses to changing environmental conditions (Abrams and Kubiske 1990, Abrams et al. 1994). Increased irradiance, temperature or limited water availability can cause seedlings to produce thicker or denser leaves. Leaf morphology has important implications in the understanding the physiology of leaf function and the ability to predict whole tree level responses to changing environmental conditions (Reich et al. 1998b).

**Physiological Responses**

In the eastern United States, mean annual temperatures and the length of the growing season are correlated with primary production, or the production of organic compounds through photosynthesis, as plants experience an increase in the time available for photosynthetic activity (Lieth and Whittaker 1975). In young stands, growth is strongly influenced by the length of time that the foliage is photosynthetically active, the total amount of biomass allocated to photosynthetic activity, as well as the biochemical processes involved in the photosynthetic pathways (Kozlowski et al. 1991). At the leaf-level, rates of photosynthesis are controlled by internal CO$_2$ concentrations, plant water potentials and enzyme-catalyzed reactions, which are influenced by environmental conditions like air temperature and soil water availability. For some species, increasing temperatures can results in thermal acclimation, or the temperature-mediated changes in photosynthesis and respiration, which results in changes in the rate of enzyme-catalyzed reactions and increasing gas exchange until a photoinhibition threshold is reached and gas exchange is halted. Rubisco is highly temperature sensitive and can become inactivated at even moderately leaf high temperatures (>35°C) leading to a loss in the ability of plants to fix carbon (Daas et al. 2008). Approximately half of leaf nitrogen is invested in the structure of chloroplasts and the enzyme, Rubisco. There is a strong correlation between a
species’ capacity for photosynthetic activity and the foliar N concentration of its leaves (Field and Mooney 1986, Reich et al. 1995b, Reich et al. 1997, Hikosaka 2004, Montgomery 2004). For individuals that are incapable of thermal acclimation, increased temperatures reduce the solubility of CO₂ within the leaf, impeding the ability of Rubisco to fix carbon and resulting in increased photorespiration rates and water loss (Yamori et al. 2006). However, leaf-level gas exchange may also be altered in response to increasing temperature and precipitation as plant-water limitations are alleviated and increasing temperatures stimulate photosynthetic activity.

Water loss through transpiration is an unavoidable tradeoff during photosynthesis, as water vapor escapes to the atmosphere through stomatal openings during gas exchange. Changes in the frequency and amount of precipitation events can alter soil water availability and can provide plants that are unable to adequately respond to increasing atmospheric temperatures with the additional water resources needed to maintain functional internal leaf temperatures. Plant-atmospheric conductance is controlled through stomatal regulation and internal soil-root-leaf signals in response to water availability (Abrams et al. 1990, Kozlowski et al. 1991, Ni and Pallardy 1992, Givnish 1995, Hanson and Weltzin 2000). Evapotranspiration, or water lost to the atmosphere during gas exchange, also serves as a mechanism to lower internal leaf temperatures to protect against biochemical and cellular breakdown that can occur at high temperatures (Ludlow and Björkman 1984, Loreto and Sharkey 1990, Chaves 1991, Ort et al. 1994, Cornic and Massacci 1996). If water is limiting, species that are incapable of acclimating to changes in temperature suffer breakdowns in enzyme-catalyzed reactions and membrane processes that can restrict or cease photosynthetic activity (Schwinning and Ehleringer 2001). Increasing air and leaf temperatures can also alter cell membrane fluidity, reducing mesophyll conductance and thylakoid function, both of which can lead to cellular leaking, with a loss of ions from within cellular organelles (Sharkey 2005). This breakdown in the physical structure of plant cells can lead to a loss of turgor, leaf abscission and eventually growth inhibition (Kozlowski et al. 1991). In the highly competitive post-harvest environment, this loss of physiological function and
growth can result in a failure to successfully recruit into the developing canopy and alter the species composition of the future mature stand.

For the species composition of mature stands to change in response to altered climate and habitat suitability, migration and competition for space in the post-harvest environment can shift the course of stand development and species composition. To test model predictions of changing habitat suitability, research needs to bring together climate change manipulations with intensive studies that examine the morphology, physiology and phenology of the species that are predicted to change. Understanding the biological mechanisms that may influence species responses to climate change will enable the development of more detailed habitat distribution models, which for Pennsylvania means looking at post-harvest establishment, growth and physiology.

Objectives

The “small stature,” early successional phase of forest development provides the opportunity to use whole ecosystem climate manipulation to test predictions of forest regeneration response to climate change. This study was conducted as a field-based ecosystem manipulation experiment that simulated predicted increases in temperature and precipitation in a northeastern forest. I studied the responses of northeastern tree species to predicted climate changes with a field manipulation of temperature (~ +2°C increase) and precipitation (+20%). The study took place in a recently harvested forest area in central Pennsylvania, which lies at the interfaces of the maple-beech-birch and oak-hickory forest types through the Northeast and Mid-Atlantic regions. This work represents one portion of a collaborative project looking at the plant and biogeochemical responses to simulated climate change. My objective was to measure tree seedling phenology, morphology and physiology to determine if a species’ ability to respond to increased temperature
and moisture could lead to changes in seedling phenology, physiology and growth in a recently disturbed stand within northeastern forests.

Tree seedling responses to a climate change experiment will be discussed in the following chapters: Chapter 3: Advances in eastern deciduous tree species germination and seedling leaf phenology in response to increased temperature and precipitation treatments; Chapter 4: The effects of increased temperature and precipitation on relative growth rate and biomass allocation of eastern deciduous tree seedlings; Chapter 5: The effects of seedling morphology and leaf characteristics on the photosynthetic responses of temperate deciduous tree seedling grown under increased temperature and precipitation treatments; Chapter 6: Water relations, gas exchange and morphology of deciduous tree seedlings after three years of increased temperature and precipitation manipulations.
Chapter 2

Experimental Design and Methods

Materials and Methods

The Forest Regeneration and Climate Experiment (FORCE) is an \textit{in situ} climate manipulation experiment located in Pennsylvania State University’s Stone Valley Forest in Huntingdon County, PA (40°N 85’, 77°W 83’). The two hectare site was established on a southeast slope in August 2007. Prior to initiating the experiment, the forest community was a second growth oak-hickory forest of approximately 80 years in age. The dominant overstory species included \textit{Quercus alba, Quercus rubra, Acer saccharum, Fraxinus americana} and \textit{Carya} species. The understory was dominated by seedlings and small stature tree species including: \textit{Fraxinus americana, Acer saccharum, Cornus florida, Prunus serotina}, and \textit{Acer rubrum}. The soils in the site are Clarksburg series with shale, siltstone, and fine grain sandstone parent materials. Central Pennsylvania is a temperate region with an average mean temperature of 9°C. The average temperature ranges from 27°C in July to -8°C in January. Precipitation is distributed throughout the year falling primarily as rain or snow, with a mean of approximately 100 cm year$^{-1}$. The two-hectare study site was cleared of above ground biomass through a whole tree harvest in August 2007. The whole tree harvest removed standing biomass from the overstory, although some advanced regeneration was left on the site. Following the harvest, a utility line was buried in a trench from a power line located 100 m from the site to a transformer at the center of the slope to provide electricity to the experimental equipment. A two m high large animal exclosure was installed to prevent deer and other large mammals from entering the study site. Within the fence boundaries, 16 - 2x4 m treatment plots were established (Figure 2.1). Plots were spaced >10 m apart to limit the influence of the treatments on neighboring plots. Each plot was
surrounded by a 0.5-m tall plastic and wire mesh fence, which was erected in 2009 to reduce the grazing impact of small mammals.

Figure 2.1: FORCE site layout of 16 experimental treatment plots in 4 randomized complete blocks. Treatment plots (rectangles) are labeled with their treatment and block identification. Five precipitation catchments (circles) are distributed across the site.

Each 2x4 m plot was established immediately after harvest and was divided into two 2x2 m subplots that were randomly assigned one of two types: “natural” and “planted” (Figure 2.2). Vegetation was allowed to regenerate in the “natural” subplot, while the “planted” subplot was planted with selected tree seeds and weeded of other plants. The natural subplots are not the focus of this dissertation and all response measurements were taken from the planted subplots, and from this point forward will be referred to as the “planted” plots. Each plot was divided into 1) a harvested side where seedlings were removed at the end of each growing season, and 2) a seedling side where germinated seedlings were allowed to grow for the duration of the
experiment. This initial design was revised after the first year’s harvest, and trees that germinated on the harvested side were allowed to remain and grow in years 2 and 3 of the experiment. This yearly planting resulted in data collected for seedlings that were 1, 2 or 3 years old at the end of the 3 year study period. The species that were selected for planting will be described in detail later.

Figure 2.2: Example of experimental plot set up. Location of nested subplots was determined through random placement of 2x2m natural versus planted and then 1x2m seedling versus germination within the planted subplot. One-year-old seedlings were harvested from the Germination subplot in the fall of 2008. All seedlings (1, 2 and 3 year-old) were harvested from the Planted subplot in August 2010.

The 16 plots were laid out in four randomized complete blocks and each was assigned one of the following four treatments: ambient, warmed, irrigated, or warmed+irrigated. The two factorial design was established to determine the effects of manipulated temperature and precipitation and their interactions on regeneration of temperate forest species. The treatments were selected to simulate change within the predictions of global circulation models (GCMs) for temperature (2-6°C) and precipitation (0-20%) for the northeastern United States. The temperature and precipitation treatment experimental design will be discussed in further detail and the effectiveness of each will be reported in the following sections.
**Temperature treatment**

The experiment was designed to heat 1°C during the day and 3°C at night for an average increase of 2°C above air temperatures, to simulate GCM temperature predictions for central Pennsylvania by the end of the next century. The temperature increase selected as a target warming treatment was within both the GCM predictions and the known experimental limitations of open plot warming (Kimball 2005). Ambient site conditions were monitored and recorded using a climate station with a thermocouple (CR107-L Campbell Scientific) and anemometer to record air temperature and windspeed. Plot-level increases in temperature were accomplished through the use of two encased 165 cm x 15 cm infrared heaters with 100 watts m⁻² output (Kalglo Electronics Inc., Bethlehem, Pennsylvania, USA) suspended 1.5 meter above the ground on a steel cable supported by 2.4 meter tall steel posts (Figure 2.3).

![Infrared heater suspension system](image)

**Figure 2.3:** Schematic of a treatment plot layout with subplot dimensions and the infrared heater suspension system. The planted subplot is identifiable by the PVC seed protectors which are visible on the right side of the image.
Elevated temperatures were maintained on all 8 warmed plots through constant monitoring and feedback with infra-red radiometers (IRR) (IRR-P, Apogee Instruments Inc., Logan, UT, USA) connected to a central datalogger (CR10X Campbell Scientific) running a real-time proportional-integrative-derivative (PID) feedback system (Kimball 2005). The PID system maintained a constant temperature increase on the warmed plots of 1°C during the day and 3°C at night when compared to unwarmed plots. This diurnal difference in heating allowed warmed plots to receive an effective temperature increase of 1.8°C ±0.2. Warmed plots had 30% fewer IRR readings below freezing temperatures (<0°C) when compared to unwarmed plots from May 2008-August 2010. Warmed plots also had 23% more readings between 20-25°C, which encompasses an optimal leaf temperature window (19.2-23.6°C) for plant photosynthetic activity (Helliker and Richter 2008) (Figure 2.5). Temperature of warmed plots was measured every 15 seconds using IRR sensors and referenced against the temperature of unwarmed plots to maintain the target warming. The warming feedback system was based on plots paired by treatment and across blocks (1 and 2, 3 and 4). For example, warmed plots from block 1 and 2 were referenced together with ambient plots from block 1 and 2 and controlled by the same source in the PID system. Depending on the temperature difference between paired plots, a voltage signal was sent from a multiplexer (SDM-CV04 Campbell Scientific, Logan, UT, USA) output modules to 1 of 4 incandescent dimmers (LCED 2484, Kalglo Electronics Inc., Bethlehem, Pennsylvania, USA) that send voltage signals to the lamps of the respective warmed plots. SDM-CV04 output modules increase or decrease voltage signals to maintain the programmed temperature difference through dimmers that operate on a signal scale of 0-10 to the infrared heaters. The temperature readings recorded from each IRR were averaged on one hour increments and stored by a datalogger (CR-1000, Campbell Scientific, Logan, UT, USA)
Plots were warmed from May 2008 through August 2010 (Figure 2.4). The shading and dripline effects of the lamps were simulated on unwarmed plots through the use of dummy lamps that were constructed using aluminum sheeting to replicate the shape and size of the heaters, and weighted with a pvc and rebar frame for stability. Dummy lamps were suspended over unwarmed plots with the same steel post and cable structure as functioning heaters. Each IRR’s wiring was also encased in pvc conduit to protect wires from rodent damage. Soil temperature and moisture probes (Decagon ECH2O EC-TM soil probes (Decagon Devices, Inc, Pullman, WA, USA) were installed in the natural subplots at the start of the experiment to monitor soil (3.5 cm to 6.5 cm depth) and temperature effects of the treatments year round.

![Graph showing historical mean monthly air temperature from State College, PA, maximum and minimum, and ambient Forest Response to Climate Change (FORCE) air temperature from 2008 to 2010.]

Figure 2.4: Historical (1899-2007) mean monthly air temperature from State College, PA (dashed line), maximum and minimum (dotted line) and ambient Forest Response to Climate Change (FORCE) air temperature (solid black line), FORCE warmed temperature (gray line) from 2008 to 2010.
Precipitation treatment

Precipitation patterns in the mid-Atlantic region are predicted to vary in both amount and frequency over the next 100 years. GCM predict that precipitation received in Pennsylvania is expected to increase by 0-20% increase over the current amounts. Predicted changes in precipitation were simulated in this experiment by adding 20% of the long-term average precipitation weekly; increasing both the amount and the frequency of manipulated precipitation events experienced by irrigated plots. The effective increase in irrigation over the three years of study was 23% above ambient precipitation (Figure 2.6). The volume of weekly irrigation amounts was calculated as 20% of the monthly average for State College, Pennsylvania.
(approximately 10 km from the study location in Huntingdon County) from 1899-2006 using data from the Pennsylvania State Climatologist database (http://climate.met.psu.edu/data/state.php). Irrigation treatment water was collected in five precipitation catchments constructed around the perimeter of the study site (Figure 2.1). Rainfall was collected and stored in opaque 208 liter barrels wrapped in reflective insulation to limit solar heating and algal growth (Figure 2.7). Eight open topped winter catchments were also installed across the site to capture snow, ice and precipitation that were applied weekly from November through April. Winter catchments consisted of six 68.13-liter rubber totes designed to capture 20% of the plot area for snow and ice accumulation (Figure 2.7).

![Figure 2.6: Historical (1899-2007) mean monthly precipitation from State College, PA (dashed line), ambient and warmed FORCE average monthly precipitation (black line) from 2008 to 2010 and average monthly precipitation with irrigation on irrigated and warmed+irrigated treatment plots (gray line).]
Figure 2.7: Five growing season catchments (white cisterns) were distributed around the perimeter of the site, while 12 winter catchments (tubs) were set out from November-April throughout the perimeter of the site.
Ambient precipitation was measured with a tipping-bucket rain gauge installed in the study area to measure ambient precipitation. Manual application of precipitation took place during weekly irrigation of all plots receiving wetted treatments during all three years of the study. The quantity of water (liquid, snow or ice) applied weekly by hand to the irrigated plots was recorded and compared to ambient precipitation to calculate the total percent effective irrigated treatment by week, month, year, and experiment (Table 2.1). Growing season irrigation was applied during the early morning hours to limit evaporative losses during irrigation treatments. Winter irrigation was calculated, converted to depth and recorded weekly as either water volume (pre-determined) or snow/ice weight and used to calculate the total precipitation applied (irrigated amount/actual precipitation amount). When snow and ice were present, the contents of the catchments were distributed evenly across the plot area during weekly irrigation, however when only melt-water was present it was applied with a constant value of 22.7 liters per plot. This constant value was the calculated liquid volume of the average amount of precipitation received during the winter months based on the long term average precipitation patterns for the study area.
Tree species selection

Eleven early successional eastern tree species were selected to be planted within experimental plots. These species were chosen based on their current or future importance in the central PA forests based on Iverson and Prasad’s regression tree analysis (RTA) of habitat suitability model, DISTRIB (2001) (Table 2.2a). This species distribution model uses forest inventory and analysis (FIA) data, coupled with a suite of environmental variables to model habitat suitability changes in response to climate change. DISTRIB uses five common GCM’s, run, both separately and averaged across all five: Geophysical Fluid Dynamics Laboratory (GFDL), Goddard Institute of Space Studies (GISS) model, United Kingdom Meteorological Office (UKMO) model, Hadley Centre for Climate Prediction and Research (Hadley) model, and Canadian Climate Centre (CCC) model (Iverson and Prasad 2001) to predict potential changes in eastern habitat suitability from 1999-2099. This analysis was performed on a county level and our study uses predictions for Huntingdon County, where the study site is located. The selected

<table>
<thead>
<tr>
<th>Month</th>
<th>Average precipitation 2008 (mm)</th>
<th>Average precipitation 2009 (mm)</th>
<th>Average precipitation 2010 (mm)</th>
<th>20% irrigated treatment addition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>42.6</td>
<td>82.6</td>
<td>13.83</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>25.6</td>
<td>26.15</td>
<td>12.4</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>46.59</td>
<td>95.1</td>
<td>15.18</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>63.4</td>
<td>34.8</td>
<td>14.98</td>
</tr>
<tr>
<td>5</td>
<td>109.8</td>
<td>48</td>
<td>30</td>
<td>17.58</td>
</tr>
<tr>
<td>6</td>
<td>68.2</td>
<td>120.8</td>
<td>0</td>
<td>19.55</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>81</td>
<td>6.5</td>
<td>18.26</td>
</tr>
<tr>
<td>8</td>
<td>59.4</td>
<td>72.8</td>
<td>0.7</td>
<td>17.34</td>
</tr>
<tr>
<td>9</td>
<td>71.4</td>
<td>76.4</td>
<td>-</td>
<td>15.11</td>
</tr>
<tr>
<td>10</td>
<td>88</td>
<td>157.4</td>
<td>-</td>
<td>14.23</td>
</tr>
<tr>
<td>11</td>
<td>39.6</td>
<td>44.4</td>
<td>-</td>
<td>14.45</td>
</tr>
<tr>
<td>12</td>
<td>115.4</td>
<td>103.6</td>
<td>-</td>
<td>13.21</td>
</tr>
<tr>
<td>total</td>
<td>597.8</td>
<td>882.59</td>
<td>275.85</td>
<td>186.12</td>
</tr>
</tbody>
</table>
species were then assessed for known physiological and morphological characteristics to provide a basis for response to ambient climate conditions, as well as potential responses to the imposed climate treatments (Table 2.2b-c).

Table 2.2a: Selection of 11 species planted in experimental plots. Importance values for current and predicted changes for Huntingdon County are from Iverson and Prasad (2001) model of habitat suitability (DISTRIB). An average of five global climate models was used to determine potential changes across a suite of environmental variables over time (1999-2099).

<table>
<thead>
<tr>
<th>Species name</th>
<th>Common name</th>
<th>Species Abbreviation</th>
<th>Current Importance Value (1999)</th>
<th>Average Predicted Importance Value Change (2099)</th>
<th>Predicted Success or Failure Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Quercus rubra</em></td>
<td>Northern red oak</td>
<td>QURU</td>
<td>12.25</td>
<td>-1.96</td>
<td>Resident Loser</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>White ash</td>
<td>FRAM</td>
<td>7.75</td>
<td>-2.02</td>
<td>Resident Loser</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>Red Maple</td>
<td>ACRU</td>
<td>28.75</td>
<td>-10.20</td>
<td>Resident Loser</td>
</tr>
<tr>
<td><em>Prunus serotina</em></td>
<td>Black cherry</td>
<td>PRSE</td>
<td>14.5</td>
<td>-5.71</td>
<td>Resident Loser</td>
</tr>
<tr>
<td><em>Betula lenta</em></td>
<td>Black birch</td>
<td>BELE</td>
<td>9.25</td>
<td>+1.33</td>
<td>Resident Winner</td>
</tr>
<tr>
<td><em>Quercus alba</em></td>
<td>White oak</td>
<td>QUAL</td>
<td>7.75</td>
<td>+15.63</td>
<td>Resident Winner</td>
</tr>
<tr>
<td><em>Quercus velutina</em></td>
<td>Black oak</td>
<td>QUVE</td>
<td>6.5</td>
<td>+9.23</td>
<td>Resident Winner</td>
</tr>
<tr>
<td><em>Carya glabra</em></td>
<td>Pignut Hickory</td>
<td>CAGL</td>
<td>5.75</td>
<td>+4.34</td>
<td>Resident Winner</td>
</tr>
<tr>
<td><em>Quercus prinus</em></td>
<td>Chestnut oak</td>
<td>QUPR</td>
<td>15.25</td>
<td>+5.48</td>
<td>Resident Winner</td>
</tr>
<tr>
<td><em>Quercus stellata</em></td>
<td>Post oak</td>
<td>QUST</td>
<td>0</td>
<td>+21.27</td>
<td>Predicted Migrant</td>
</tr>
<tr>
<td><em>Liquidambar styraciflua</em></td>
<td>Sweetgum</td>
<td>LIST</td>
<td>0</td>
<td>+1.92</td>
<td>Predicted Migrant</td>
</tr>
</tbody>
</table>
Table 2.2b: A comparison of the drought tolerance, belowground physiological and morphological characteristic of 11 selected study species planted on the FORCE plots. Species are organized within the table based on their predicted success in future forests (Table 2.2a).


<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Drought tolerance</th>
<th>Drought deciduous</th>
<th>Root structure</th>
<th>Mature tree rooting depth minimum (inches)</th>
<th>Mycorrhizal type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus rubra</td>
<td>mesic</td>
<td>medium</td>
<td>no</td>
<td>tap root</td>
<td>36</td>
<td>ectomycorrhiza12</td>
</tr>
<tr>
<td>Fraxinus americana</td>
<td>mesic</td>
<td>low</td>
<td>no</td>
<td>short tap root and long laterals</td>
<td>40</td>
<td>arbuscular12</td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>dry-mesic</td>
<td>low</td>
<td>yes</td>
<td>shallow</td>
<td>30</td>
<td>arbuscular12</td>
</tr>
<tr>
<td>Prunus serotina</td>
<td>mesic</td>
<td>medium</td>
<td>no</td>
<td>shallow</td>
<td>36</td>
<td>arbuscular12</td>
</tr>
<tr>
<td>Betula lenta</td>
<td>mesic</td>
<td>medium</td>
<td>yes</td>
<td>shallow</td>
<td>28</td>
<td>ectomycorrhiza12</td>
</tr>
<tr>
<td>Quercus alba</td>
<td>mesic</td>
<td>medium</td>
<td>no</td>
<td>tap root</td>
<td>48</td>
<td>ectomycorrhiza12</td>
</tr>
<tr>
<td>Quercus velutina</td>
<td>dry-mesic</td>
<td>low</td>
<td>no</td>
<td>tap root</td>
<td>40</td>
<td>ectomycorrhiza18</td>
</tr>
<tr>
<td>Carya glabra</td>
<td>dry-mesic</td>
<td>medium</td>
<td>no</td>
<td>tap root</td>
<td>48</td>
<td>ectomycorrhiza12</td>
</tr>
<tr>
<td>Quercus prinus</td>
<td>xeric</td>
<td>medium</td>
<td>no</td>
<td>tap root</td>
<td>36</td>
<td>ectomycorrhiza17</td>
</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td>dry-mesic</td>
<td>low</td>
<td>yes</td>
<td>shallow</td>
<td>36</td>
<td>arbuscular16</td>
</tr>
<tr>
<td>Quercus stellata</td>
<td>xeric</td>
<td>high</td>
<td>no</td>
<td>tap root</td>
<td>36</td>
<td>ectomycorrhiza19</td>
</tr>
</tbody>
</table>
Table 2.2c: A comparison of the drought tolerance, aboveground physiological and morphological characteristic of 11 selected study species planted on the FORCE plots. Species are organized within the table based on their predicted success in future forests (Table 2.2a). Values for physiological and morphological characteristics are listed in parentheses when available.


<table>
<thead>
<tr>
<th>Species</th>
<th>Growth form</th>
<th>Stomatal activity in drought</th>
<th>Stomatal pore Size (µ)</th>
<th>Stomatal density (#/cm²)</th>
<th>Osmotic adjustment during drought (MPa)</th>
<th>Predawn water potential during drought (AMPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Quercus rubra</em></td>
<td>semi- determinate¹¹</td>
<td>maintained⁴</td>
<td>Small²⁵ (6)</td>
<td>high²⁵ (66477)</td>
<td>low⁶ (+0.02)</td>
<td>high⁸ (-0.93)</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>determinate¹</td>
<td>reduced⁶</td>
<td>moderate-large²⁵ (17)</td>
<td>low²⁵ (12973)</td>
<td>low⁶ (+0.5)</td>
<td>reduced⁶</td>
</tr>
<tr>
<td><em>Acer rubrum</em></td>
<td>indeterminate¹</td>
<td>reduced⁶</td>
<td>large²⁵ (32)</td>
<td>high²⁵ (70455)</td>
<td>low⁶</td>
<td>reduced⁶</td>
</tr>
<tr>
<td><em>Prunus serotina</em></td>
<td>indeterminate¹</td>
<td>reduced⁵</td>
<td>small²⁵ (10)</td>
<td>low²⁵ (18324)</td>
<td>low⁸</td>
<td>reduced⁸</td>
</tr>
<tr>
<td><em>Betula lenta</em></td>
<td>indeterminate¹</td>
<td>reduced⁸</td>
<td>small²⁵ (7)</td>
<td>high²⁵ (53835)</td>
<td>low⁸</td>
<td>reduced⁸</td>
</tr>
<tr>
<td><em>Quercus alba</em></td>
<td>determinate¹²</td>
<td>maintained⁴</td>
<td>small²⁵ (6)</td>
<td>high²⁵ (62405)</td>
<td>high⁶ (-1.55)</td>
<td>high⁸ (-2.30)</td>
</tr>
<tr>
<td><em>Quercus velutina</em></td>
<td>determinate¹⁴</td>
<td>maintained⁴</td>
<td>moderate-large²⁵ (24)</td>
<td>high²⁵ (40530)</td>
<td>high⁶ (-0.17)</td>
<td>high⁶ (-1.03)</td>
</tr>
<tr>
<td><em>Carya glabra</em></td>
<td>determinate¹⁴</td>
<td>reduced²⁰</td>
<td>small²⁵ (9)</td>
<td>high²⁵ (38162)</td>
<td>moderate²⁰</td>
<td>high²⁰</td>
</tr>
<tr>
<td><em>Quercus prinus</em></td>
<td>determinate¹⁴</td>
<td>maintained⁴</td>
<td>moderate²⁵ (11)</td>
<td>low²⁵ (25758)</td>
<td>high⁶ (-0.33)</td>
<td>high⁶ (-0.77)</td>
</tr>
<tr>
<td><em>Liquidambar styraciflua</em></td>
<td>indeterminate¹⁵</td>
<td>reduced¹⁵</td>
<td>moderate²⁵ (11)</td>
<td>low²⁵ (22443)</td>
<td>low¹⁵</td>
<td>reduced¹⁵</td>
</tr>
<tr>
<td><em>Quercus stellata</em></td>
<td>determinate¹⁴</td>
<td>maintained¹⁰</td>
<td>moderate²⁵ (14)</td>
<td>high²⁵ (42424)</td>
<td>high⁶ (-0.33)</td>
<td>high⁶ (-2.00)</td>
</tr>
</tbody>
</table>
Seeds of the selected species were purchased from a native seed source company (Sheffield Seed Company Locke, NY) or were collected locally in central Pennsylvania. When native Pennsylvania seeds were purchased, the collection location for each seed source was identified and each species was selected from a Pennsylvania location. For the two southern species, purchased seeds were selected from the most northern source available through Sheffield Seed Company. In the fall of 2007, randomly generated plot maps were created to plant seeds within each plot using a 100x100 cm grid and spacing the location of each seed 10 cm apart. These maps were used to document the location of each seed and to monitor initial spring germination. Large seeded species such as *Quercus alba* and *Carya glabra* received a single seed in each randomly generated grid point, while *Quercus stellata* was planted with two acorns at each grid point. Smaller seeded species in the general *Betula, Liquidambar, Acer* and *Fraxinus*, received a predetermined amount of seed at each assigned grid point to ensure adequate seed numbers on each plot. In October 2008, half the germinated seedlings were harvested to make room for new seeds to be planted and for biomass measurements of 1-year-old seedlings. In 2008, a 50x100 cm grid was used to plant seeds within the harvested area of each plot utilizing the same techniques from fall 2007. All surviving seedlings were allowed to remain within the treatment plots for the remainder of the study period. In 2009, the presence of established seedlings prevented the use of the 10 cm grid and limited space required an alternative approach for planting. Within each 2x4 m plot, a group four seed locations were assigned for each species and recorded on the existing seed maps. The seed planting rotation produced data for 1, 2 and 3 year old seedlings by the end of the three year study.

Seeds planted in 2007 experienced heavy seed predation by small mammals, and in 2008 and 2009 PVC seed protectors were constructed an used to cover newly planted seeds during the overwintering period (K. Steiner, personal communication). Browsing by small mammals also led to the loss of seedlings within plots and those seedlings were replaced by recently germinated seedlings grown in the greenhouse from the same seed source as the field planted seedlings. The
Experimental field study presented many challenges with the management and maintenance of the infrastructure and the growth of seedlings. Natural interference and localized disturbances acknowledged as artifacts, or unknown effects, could have affected the results of the experimental treatments and how the seedlings responded. These artifacts included: herbivory by small mammals, seed predation, insect damage, insecticide application, elimination of herbaceous competition through weeding, the potential effects that the heaters had on the height growth of seedlings, and the accumulation of trace elements in the water used for irrigation. The effects of natural interference and disturbances were balanced across treatment plots. The impacts of these artifacts were minimized through the use of randomized complete block design and the distribution of plots within each block. Differential germination and seedling survival over the course of the three years of the experiment resulted in an unequal distribution of the number of individuals per species per treatment (Table 2.3).

Table 2.3: Final seedling distribution by age and treatment based on harvest data from August 2010. Three-year-old seedlings germinated in the spring of 2008, two-year-old seedlings germinated in the spring of 2009 and one-year-old seedlings germinated in the spring of 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>ambient 3</th>
<th>2</th>
<th>1</th>
<th>warmed + irrigated 3</th>
<th>2</th>
<th>1</th>
<th>warmed 3</th>
<th>2</th>
<th>1</th>
<th>irrigated 3</th>
<th>2</th>
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<tbody>
<tr>
<td>Quercus rubra</td>
<td>4</td>
<td>13</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>10</td>
<td>8</td>
<td>3</td>
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<td>8</td>
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<tr>
<td>Fraxinus americana</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<td>6</td>
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<td>0</td>
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<tr>
<td>Acer rubrum</td>
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<td>3</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Prunus serotina</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Betula lenta</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Quercus alba</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>3</td>
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<td>1</td>
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</tr>
<tr>
<td>Quercus velutina</td>
<td>4</td>
<td>3</td>
<td>11</td>
<td>6</td>
<td>9</td>
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<td>3</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Carica glabra</td>
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<td>0</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
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<td>5</td>
</tr>
<tr>
<td>Quercus prinus</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Liquidambar strycaflia</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Quercus stellata</td>
<td>6</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
In August 2010 the FORCE experiment was terminated and the above and below ground biomass from the plots was removed. Prior to excavation, five soil samples were taken from each planted plot to estimate below ground fine root biomass of seedling communities. Plots were then harvested by excavation of whole seedlings, including root systems. Additional methods specific to the research questions described in the objectives will be addressed in detail in each chapter.
Chapter 3

Advances in eastern deciduous tree species germination and seedling leaf phenology in response to increased temperature and precipitation treatments.

Introduction

The distribution of plant species is strongly influenced by climate, especially temperature and precipitation regimes (Stephenson 1990, Pigott and Pigott 1993, Chuine and Beaubien 2001). Habitat distribution models predict major changes in species composition and biomass accumulation in response to these climate changes (Solomon 1986, Pastor and Post 1988, Shugart et al. 1992, Iverson and Prasad 2001). Iverson and Prasad (2001) based their predictions of changes in habitat suitability on DISTRIB, a deterministic regression tree analysis model. This model evaluated the relationship of environmental variables to species distributions and then used them to predict potential suitable habitat across the eastern United States under predicted climate change scenarios. Environmental variables include soil, land use/land cover, elevation, landscape pattern, and climate (Iverson and Prasad 2001). The potential impacts of climate change on habitat suitability for northeastern forests are characterized by an expected northward shift in the habitat of most major trees species. For regions that exist at the intersection of forest types, like central Pennsylvania, at the maple-beech-birch and oak-hickory forest boundary, there is potential loss of current species diversity as species composition changes due to climate driven habitat change. Under current prediction models species may be forced to migrate to remain within a suitable habitat or acclimate to cope with climate changes. When comparing current mean latitudes of mature forest biomass with seedling distribution, Woodall et al. (2009) found that northern species had mean latitude of seedlings that was significantly farther north, suggesting that the process of northward tree migration in the eastern forests is currently occurring at approximately 100 km century$^{-1}$. 
Most temperate deciduous tree species require winter chilling followed by an accumulation of growing degree days above a threshold to trigger germination (Alison and Lechowicz 1992, Heide 1993, Myking and Heide 1995, Chuine et al. 1999). Timing of seedling germination can shift depending on environmental conditions, but these shifts carry potential risks and benefits. Emerge too early and the risk of mortality from frost is high, emerge too late and competition from neighboring seedlings impedes chances of establishing. However, early emergence can provide a crucial period of high light availability, which contributes between 33-98% of total light received throughout the growing season for understory individuals (Gill et al. 1998, Augspurger et al. 2005, Augspurger 2008). Species with the capacity to respond to earlier phenological cues for germination and leaf-out may have a competitive advantage over species that are adapted for later season emergence to avoid frost damage in the early spring (Badeck et al. 2004).

Changes in plant phenology, or the emergence of fully expanded leaves or cotyledons, are considered to be a sensitive and observable indicator of plant responses to climate change (Linderholm 2006, Cleland et al. 2007). Satellite image data showing advancing community-level spring “green up” (Schwartz 1998) and traditional on-the-ground observational studies highlight changes in species-based phenology. These observations include adjustments in the timing of germination and bud burst, as well as changes in photosynthetically active periods or timing of biomass allocation to leaves and roots (Price et al. 2001).

Changes in timing of spring phenological events can also impact competitive abilities of individual seedlings. The potential impact of altered growth that results from earlier germination or leaf-out can lead to changes in mortality and survivorship rates of young tree seedlings that are sensitive to changing environmental conditions (King et al. 2005). However research is needed to understand how a warmer climate may affect germination and seedling survival to strengthen predictions of population dynamics and future distributions of deciduous tree species in the Northeast (Iverson and Prasad 1998, Cramer et al. 2001, Higgins et al. 2003, Neilson et al. 2005,
Pearson 2006, Thuiller et al. 2008, Milbau et al. 2009). A field-based experimental study, as well as, a controlled growth chamber study of tree seed germination, was established to improve our understanding of how climate change may affect the spring phenology in northeastern temperate forests. Specifically the following hypotheses were tested: (a) germination success, timing and leaf-out will occur earlier in response to increased temperatures and precipitation (b) Southern species not currently distributed within study area will germinate and leaf-out earlier in responses to warming and increased precipitation when compared to current northern species, which are found within the study area, under the same treatment conditions (c) Similar advances in germination timing in responses to experimental warming and irrigation field treatments will be observed in controlled growth chamber conditions.

Methods

Field Study Design

Germination success and timing of spring emergence from selected eastern trees were monitored in a three year climate change study at the Stone Valley Forest in Huntingdon County, PA. The site was located at the interface between the Northeast and Mid-Atlantic regions, on the boundary of two dominant forest types: maple-beech-birch and oak-hickory. The latitude and longitude of the site is 40N 85’, 77W 83’. Average annual mean temperatures at the site are 9 °C with an average annual maximum of 15 °C and minimum of 4 °C throughout the year, with a range from 27°C in July to -8°C in January. Mean annual precipitation is approximately 1000 mm. The site is situated on a southeast-facing slope with Oxyaquic Fragiudalfs soils in the Clarksburg series. A 4-hectare area was clear-cut and fenced in August 2007 and 16 plots were established in a 2x2 factorial design with 4 treatments (ambient, irrigated (+20%), warmed (+2°C), and warmed+irrigated (+20% and +2°C)).
Climate manipulation treatments

Field warming treatments had a constant target temperature increase of 1°C during the day and 3°C at night when compared to unwarmed plots, with an average temperature increase of 1.8°C (+/- 0.29°C) from May 2008 through August 2010. Predicted changes in precipitation were simulated with weekly watering additions that produced an effective increase of 23%, with monthly precipitation additions ranging from 12 to 43%. The quantity of water (liquid, snow or ice) that was applied weekly to the irrigated plots was determined based on long-term historical averages (Chapter 2: Experimental Methods and Set-up). Applied irrigation amounts were recorded and the percent effective irrigation treatment was calculated by week, month, year, and experiment duration by dividing the irrigated treatment amount by the ambient precipitation.

Species selection and planting

In the fall of 2007 - 2009 seeds from 9 northern and 2 southern deciduous species were randomly planted into each of the 16 treatment plots. These eleven species were selected based on their current or future importance in the central PA forests based on Iverson and Prasad’s habitat suitability model, which is based on current Forest Inventory Analysis data (2001) (Table 2.2). The selected study species were classified based on their current distribution (northern or southern) and the change in future potential habitat suitability (winners, losers and migrants). Species classified as resident winners are species that are currently present in the central PA forests and are predicted to have increased habitat suitability as climate changes. Resident winner species include Betula lenta L., Quercus alba L., Quercus velutina (Lamb.), Carya glabra (Mill.), and Quercus prinus L. Resident loser species include Quercus rubra L., Fraxinus americana L., Acer rubrum L., and Prunus serotina (Ehrh.). These resident loser species are currently present in central PA forests, but are expected to see a decline in habitat suitability and a loss of
importance in future forest composition. The remaining two species, *Liquidambar styraciflua* L. and *Quercus stellata* (Wangen.), are classified as “predicted migrants” or species not currently present in the central PA forests but predicted to gain in importance as climate change extends their suitable habitat northward. Seeds were purchased from Sheffield Seed Company (Locke, NY) or were collected locally in central Pennsylvania whenever they were available. Purchased seeds of southern species were selected from sources at the northern end of their natural distribution. In preparation for the fall plant, a test for seed viability was performed by using a container of water to float test for *Quercus, Cayra* and *Prunus* seed viability. A float test is performed as a quality check on acorns or hulled seeds by placing the seeds in a container of water, discarding the floaters, and keeping the sinkers for planting. Seeds will float if they have incurred insect damaged or have some air space inside the shell as a result of dehydration. Seeds of all species were soaked in water for 24 hours prior to planting in the field, with the exception of *Carya glabra* nuts, which required 72 hours of soaking prior to planting.

In the fall of 2007, randomly generated plot maps were created to plant seeds within each plot using a 100x100 cm grid and spacing the location of each seed 10 cm apart. These maps were used to document the location of each seed and to monitor initial spring germination. In October 2008, half the germinated seedlings were harvested to make room for new seeds to be planted and for biomass measurements of 1-year-old seedlings. In 2008, a 50x100 cm grid was used to plant seeds within the harvested area of each plot utilizing the same techniques from fall 2007. All surviving seedlings were allowed to remain within the treatment plots for the remainder of the study period. In 2009, the presence of established seedlings prevented the use of the 10 cm grid and limited space required an alternative approach for planting. Within each 2x4 m plot, a group four seed locations were assigned for each species and recorded on the existing seed maps. The seed planting rotation produced data for 1, 2 and 3 year old seedlings by the end of the three year study. Additional details of the field planting methods are described in Chapter 2: Experimental Design and Set-up.
In the following spring of each year, germination was monitored on a weekly basis beginning in March and continuing through the last week of June. Day of germination was determined by the emergence of the first fully expanded leaf for each seedling. The location of each seedling was marked with a small flag, assigned a unique number and monitored throughout the duration of each year of the experiment. Seedlings that remained within the treatment plots the following spring were then monitored weekly for leaf-out in 2009 and 2010. Leaf-out was also defined by the emergence of at least one fully expanded leaf. Seedlings were also monitored for defoliation and other natural damages. Plots were weeded weekly to eliminate competition from other species that were not the focus of this study.

**Growth Chamber Study**

Seeds for the growth chamber germination study came from the same sources as seeds planted in the field in the fall of 2009. In preparation for the greenhouse germination study, the same float test for viability was performed by for *Quercus*, *Carya* and *Prunus* seed viability that was used for field planting. All species, including the remaining float-tested seeds, were then placed into airtight bags containing a basic peat mixture to control moisture. The bags were then placed into a refrigerator to begin cold stratification for 120 days before sowing in March 2010.

Four Conviron reach-in growth chambers (Winnipeg, Canada) were used to simulate spring warming trends in a controlled setting. A single chamber was established as the “unwarmed” growth chamber and was programmed to imitate ambient air temperature data that was collected at the field site during the 2009 growing season. A second chamber was set at 2°C above ambient to simulate the heating effects established at the field site (Figure 3.1). Patterns of spring warming were mimicked at an accelerated pace within the growth chamber (Figure 3.2). Initial chamber temperatures were set at 1°C (unwarmed) and 3°C (warmed) and the containers receiving the treatments were placed inside the chambers and allowed to rest at the initial
temperature for one week. The chamber temperature was then set to increase in 2°C increments in weeks 1-4 and 7-12, with 1°C increases occurring in week 5 and 6, until the final chamber temperature of 23°C (ambient and irrigated) and 25°C (warmed and warmed+irrigated) was reached at the end of 12 weeks. Nighttime temperatures were increased from 2-10°C (ambient and irrigated) and 2-12°C (warmed and warmed+irrigated). Photoperiod within the chambers was controlled to mimic day length for early spring (~8 hours in March-April) and was adjusted to match the corresponding changes in temperature to simulate the lengthening photoperiod of the growing season (12 day/12 night hours).

Figure 3.1: Average, maximum and minimum weekly temperature data collected from the Forest Response to Climate Experiment (FORCE) field site during the 2009 growing seasons which was used to establish growth chamber temperature increase patterns. Weekly observations range from 3/9/2010-6/3/2010. Error bars represent the SE of the mean.
Seeds from each of the 11 study species were planted into 68 liter Sterlite containers containing Promix BX (Premier Horticulture, Inc, Red Hill, PA). Randomly generated seed charts were created to establish planting locations within a 40x50cm grid each container. Eight containers were divided into four treatment groups and were divided between two chambers. Chamber 1: ambient, irrigated (+20% precipitation) and Chamber 2: warmed (+2°C), and warmed+irrigated (+20% precipitation and +2°C). All eight containers were watered for 60 seconds each week using a 1.9 liter heavy duty sprayer (Tolco Corporation, Toledo Ohio). Irrigated treatments received ~20% more watering time (12 additional seconds or 72 seconds total watering time) at each weekly watering event. Throughout the period of incubation, the seeds were monitored daily for germination (emergence of the first fully expanded leaves) and the

Figure 3.2: Comparison of the growth chamber and field site daylight air temperatures during the 2009 growing season.
germinated seedlings were recorded and removed to eliminate confusion from resprouting in subsequent weeks.

**Data analysis**

Average day of germination and leaf-out were calculated and compared by treatment, both annually from 2008-2010 and by species. For annual comparisons of germination and leaf-out data for all species were combined at the treatment level for 2008-2010 and 2009-2010, respectively (Appendix Table B1-2). For species comparisons of germination and leaf-out, data was combined for each individual species across all years of observation and averaged for a treatment type (Appendix Table B3-4). Comparisons of germination and leaf-out were represented as a deviation from ambient, which standardized the response of each treatment to the ambient for each block before annual or species-specific averages were calculated. Species distributions across the treatment plots that were used for the analysis in this chapter can be found in Appendix tables A1-5. For germination success, a species-specific approach was used to compare and combine species data across the three years of observation in all four treatment types. To calculate germination success, the number of germinated seedlings was counted in each treatment plot for each species in 2008-2010. The number of seedlings germinated in each treatment plot was then subtracted from the number of seedlings that germinated in the ambient plot, standardizing germination counts as a deviation from ambient for each species to obtain the average “germination success” in each year.

All data analysis was conducted with Statistical Analysis Software (SAS) or Microsoft Excel. Germination success, day of germination and leaf-out data from each of the three treatment plots was standardized to the corresponding average ambient treatment value. An analysis of variance (ANOVA) was used to test treatment effects in the field study on temperature and germination, leaf-out date and germination success. An alpha value of 0.05 was used to
determine significance. For annual comparisons of germination and leaf-out, year was included as a variable in the model, but was not included in the species-specific comparisons of germination. For growth chamber data analysis, standard error was calculated using n=2, with a single tub treated as a replicate. However, due to the placement of tubs within two growth chambers there was no true replication and statistical analysis was not used to compare the results.

**Results**

On average, warming advanced the day of germination 10 to 20 days for all species during the three years of study when compared to unwarmed treatments (warmed: 135 ±4; unwarmed: 149 ± 3) (p=0.001; Appendix Table C1) (Figure 3.3a). In 2010, leaf-out occurred 5-10 days earlier for seedlings growing on warmed treatment plots when compared to seedlings on unwarmed plots (warmed: 131 ±4; unwarmed: 140± 1) (p<0.0001; Appendix Table C1) (Figure 3.3b). Additional plot, treatment and block averages of raw germination data can be found in Appendix Tables B1-5.
Figure 3.3a: Annual day of germination combined for all species, represented as a deviation from ambient, for three treatments during an experimental field study from 2008-2010. Stars indicate significant difference (p<0.05) from unwarmed treatment plots. Error bars represent the SE of the mean.

Figure 3.3b: Annual day of leaf out combined for all species, represented as a deviation from ambient, for three treatments during an experimental field study from 2009-2010. Stars indicate a significant difference (p<0.05) from unwarmed plots. Error bars represent the SE of the mean.
It was hypothesized that earlier germination would occur for southern species under increased warming and irrigation when compared to northern species on the same treatment plots. However, the difference in species germination and leaf-out responses to warming were not the result of current habitat distribution. Two resident loser species had earlier leaf-out in response to warmed treatments (p=0.038; Appendix Table C2), specifically *Quercus rubra* (warmed: 129 ±3; unwarmed: 143 ±5; post hoc species comparison p=0.0005) and *Fraxinus americana* (warmed: 129 ±5; unwarmed: 144 ±5; p=0.0328) (Figure 3.4b). *Quercus stellata*, a predicted migrant species, also leafed-out earlier on warmed plots when compared to the unwarmed plots (warmed: 126 ±2; unwarmed: 147 ±3; p<0.0001). Certain resident winner species demonstrated a very strong response to the warming treatments, such as *Quercus prinus*, which germinated an average of 40 days earlier on warmed only plots when compared to unwarmed plots (warmed: 139 ±8; unwarmed: 179 ±0) (Figure 3.4a). While germination was not consistent in all treatments (Appendix Table A3), germination success differed by species (p<0.0001; Appendix Table C3) (Figure 3.5). Both northern and southern oak species, *Quercus rubra, Quercus velutina* and *Quercus stellata* had greater germination success over the three years of study, regardless of treatment type (*Quercus rubra*: 1.8 ±0.5 seedlings; *Quercus velutina*: 1.2 ±0.3 seedlings; *Quercus stellata*: 1.9±0.4 seedlings). Additional model outputs including degrees of freedom, F-values and P-values are listed in ANOVA tables (Appendix Table C1).
Figure 3.4a: Average day of germination (represented as a deviation from ambient) for study species planted in experimental temperature and precipitation manipulation plots in a field study from 2008-2010. Species that were not well replicated are not represented on the figure. Error bars represent the SE of the mean.
Figure 3.4b: Average day of leaf out (represented as a deviation from ambient) for study species grown in experimental temperature and precipitation manipulation plots in a field study from 2009-2010. Species that were not well replicated are not represented on the figure. Error bars represent the SE of the mean.
The controlled growth chamber study exhibited a similar response in germination, with germination differing by species (Appendix table B5). There was no difference in the time of germination in response to the growth chamber climate treatments (ambient: 156 ±1; warmed+irrigated: 151 ±0.9; warmed: 151 ±0.8; irrigated: 156 ±0.7). *Quercus* species were the most consistent in time of germination in the growth chamber conditions and germinated in all treatment types (*Quercus alba*: 159 ±3; *Quercus prinus*: 157 ±2; *Quercus rubra*: 148 ±1; *Quercus stellata*: 156 ±0.2; *Quercus velutina*: 150 ±0.3) (Figure 3.6). Warm treatments lacked the
consistent effect in the growth chamber that was observed in the field germination responses (Figure 3.4a). The extreme early germination that was observed for *Quercus prinus* in the field was not replicated in the controlled warming in the growth chamber (-40 days versus -14 days respectively). The average germination success for seeds planted in the growth chamber experiment also demonstrated no response to the imposed treatments (*Quercus alba*: 3.6 ±2 seedlings; *Quercus prinus*: 4.5 ±1 seedlings; *Quercus rubra*: 5.4 ±2 seedlings; *Quercus stellata*: 12.6 ±2 seedlings; *Quercus velutina*: 7.3 ±2 seedlings) (Figure 3.7).

![Figure 3.6: Average day of germination (represented as a deviation from Ambient) for a single accelerated germination growth chamber experiment. Species represented are those that successfully germinated in all 4 experimental treatment types. Error bars represent the SE of the mean.](image-url)
Figure 3.7: Germination success in a controlled growth chamber experiment represented as a deviation from ambient. Negative numbers represent greater germination in ambient treatments, while positive numbers indicate greater germination in climate treatments. Error bars represent the SE of the mean.
Discussion

The earlier occurrence of spring phenological events observed in single and multifactor climate change experiments and field study observations illustrate the influence of warming, altered precipitation patterns and rising CO₂ on plant responses (Arft et al. 1999, Peñuelas et al. 2004, Cleland et al. 2007). Similar advancements in the timing of leaf-out and germination in response to warming and irrigation in my field study have been identified in large-scale observational studies (Schwartz 1998, Parmesan and Yohe 2003, Chuine 2010), as well as experimental studies of phenological change (Walker et al. 1999, Hovenden et al. 2008, Post et al. 2008). Germination was earlier in warmed plots (warmed and warmed+irrigated) than unwarmed plots (ambient and irrigated). Earlier germination and leaf-out on warmed plots provided an additional 10-20 days for early spring photosynthetic activity. However, it is important to highlight the absence of a significant response to warming or irrigation for established seedlings in 2009. This suggests that seedling age may be contributing to the variability in treatment-level responses and that in 2010; the established 3-year-old seedlings may be driving the earlier leaf-out response on the warmed treatment plots.

The advance of spring phenological events that was observed during my three-year study indicates that temperature plays an important role in explaining the germination and leaf-out responses. First, the earlier germination during the three years of experimental treatments demonstrated a consistent response to warming. In contrast, the imposed irrigation treatment had no significant effect on the germination response of any treatment year. However, this experiment does isolate the effect of increasing temperature on the advancement of both germination and leaf-out, consistent with what has been seen in other observational and experimental studies. Increasing air and soil temperatures are phenological cues for both bud burst and leaf-out in seedlings (Augspurger 2008, Linkosalo et al. 2009). Second, leaf-out for 3-year-old seedlings exhibits a strong response to warming in 2010 and differences in the response
of leaf-out in warmed plots between 2009 and 2010 may be the result of an age difference in the seedlings. Most tree seedlings invest resources in defense against external stressors and for energy storage (Dalling and Hubbell 2002, Stamp 2003) and older seedlings will have greater stored resources than their younger competitors (Seiwa 2007). Earlier leaf-out of young seedlings may provide the opportunity for reduced competition from herbaceous vegetation and larger woody trees and shrubs that are not able to alter the timing of their spring phenology under natural conditions. In the presence of other trees and herbaceous competitors, earlier leaf-out can supplies 33-98% of the total irradiance received during the growing season providing a distinct advantage for seedling survival in understory (Augspurger et al. 2005). Although increases in global temperatures have been observed to advance spring green up for large numbers of species (Schwartz 1998), experimental studies have yet to document the shifts in germination timing for individual species (Donohue et al. 2010).

It was initially hypothesized that the timing of germination and leaf-out would vary based on both habitat distribution (northern or southern distributed species) and the predicted changes in habitat suitability under predicted climate change (winners, losers or migrants). However, species’ responses to increased warming and irrigation were not consistent with the initial expectations of success, based on modeled habitat suitability, and germination and leaf-out responses were not based on a species’ current or future habitat distribution. The timing of leaf-out in previous studies has been attributed to the adaptive characteristics of species and there are costs and benefits associated with altering phenological timing. Earlier spring leaf-out can provide an increase in the amount of time a young seedling spends under photosynthetically active environmental conditions (Gill et al. 1998, Augspurger and Bartlett 2003, Augspurger 2008). However, early leaf emergence in northern forests presents the associated risk of frost damage and potentially increase mortality if a hard frost occurs late in the season (HÄNninen 1991, Kramer 1994, Augspurger and Bartlett 2003). Temperature and precipitation are not the only environmental factors that control for germination timing, variation in species-specific
germination response may also be the result of additional internal chemical signals (Chen et al. 2008) or external environmental cues, such as red:far red light and photoperiod (Hakkinen et al. 1998, Lau and Deng 2010), which can delay germination until the required threshold is crossed. Under the controlled environmental conditions of the growth chamber study, the extreme advancement of germination from the field study was not observed. This suggested that the constant increase in temperature and consistent photoperiod may not have been enough of a climate cue to replicate the field study germination response. As global climate continues to change, increasing spring temperatures and altering the warming cues for spring emergence, species that are capable of capitalizing on these changes may experience a lengthened growing season with additional time for photosynthetic activity and growth.

**Conclusions**

Temperature records show that the average global surface temperature has increased approximately 0.2°C every decade over the last 30 years (Hansen et al. 2006). This global increase in temperature coincides with both satellite and traditional phenological observation records, indicating that the emergence of spring vegetation is occurring approximately 8 days early since 1981 (Schwartz 1998). Results from my climate change experiment isolated temperature as a driving environmental factor in the spring phenological response of seedlings. The 2°C warming experienced by seeds and tree seedlings in my study advanced day of germination and leaf-out by 10-20 days. However, species comparisons of spring phenology were not consistent with modeled predications of changing habitat suitability. The lack of consistent responses in the timing of germination and leaf-out among northern species indicated that there are additional environmental and biological factors that may control seedling emergence. The absence of a phenological response to the increased precipitation treatment in this study instead emphasizes the importance of increasing temperature on the timing of spring
phenological events in temperate systems. Climate change experiments have the potential to isolate the relative influence of range of climate variables on specific aspects of plant communities and ecosystem interactions (Cleland et al. 2007). Additional studies are needed across northeastern forests to quantify the extent of the impact of climate change variables (CO₂, N deposition, drought, photoperiod, etc) on spring phenological events, as well as on trees in different life stages to better understand if these responses are environmental, genetic or due to ontogeny.
Chapter 4

The effects of increased temperature and precipitation on relative growth rate and biomass allocation of eastern deciduous tree seedlings

Introduction

Climate is the primary force shaping major biogeographic regions that consists of distinctive plant life forms (Emanuel et al. 1985, Prentice and Solomon 1991, Prentice et al. 1992). The distribution of these biotic regions is explained primarily by annual precipitation and temperature variation (Prentice 1990). As a result, the habitats created by these regional variations in climate contribute to the distribution pattern of species and species associations (Franklin 1993, Hansen et al. 2001). This relationship between species distribution and climate has caused considerable attention to be directed at predicting the effects of climate change on the distribution of species (Pastor and Post 1988, Melillo et al. 1996, Sykes and Prentice 1996, He et al. 1999, Kirschbaum 2000, Aber et al. 2001, Hansen et al. 2001, Iverson et al. 2004). As climate conditions change and habitat suitability is altered, species’ future distribution can be predicted using measureable growth and ecophysiological responses to altered environmental conditions. (Schulze et al. 1994, Baldocchi and Harley 1995, Malhi et al. 1999, Baldocchi et al. 2002).

Plant carbon gains and biomass allocation are influenced by a variety of factors including climate, disturbance, and species tolerance to environmental conditions. Changes in mean temperature and precipitation can alter the allocation of carbon to roots, shoots, and leaves, particularly in young seedlings that lack the stored reserves to withstand prolonged drought or heat stress (Bassow et al. 1994, Tschaplinski et al. 1995, Aber et al. 2001, Buchmann 2002, Lee et al. 2005). Disturbances such as logging or windthrow can open gaps in the canopy and provide increased access to limiting resources (Canham 1989, Peterson and Pickett 1991, Abrams 1992, Abe et al. 1995, Keddy and Drummond 1996, Scheller and Mladenoff 2005, Caplat et al. 2008,
Leithead et al. 2010). However, these disturbance events can also result in additional pressures for seedlings to alter growth, in the form of increased aboveground biomass and height, as they compete for a place in the developing canopy (Bazzaz 1979, Franklin and Forman 1987, Walters et al. 1993b, Niinemets and Tenhunen 1997, Hansen et al. 2001).

Increases in biomass and height are critical in the growth and development of establishing seedlings and can vary widely with species, ontogeny and environmental conditions. Relative growth rates, or biomass production in a given period of time, provides a method of comparing biomass accumulation across species of different sizes, as it standardizes the growth of an individual to its inherent size over a given period of time (Poorter et al. 1990). Species-specific comparisons of relative growth rates provide an explanation for the biomass production of species under varied environmental factors, such as increased temperature or precipitation. Species with high relative growth rates are capable of occupying space and acquiring resources more rapidly and under optimal conditions this provides a competitive advantage over slower growing species (Grime and Hunt 1975). However, as environmental conditions become less favorable, conservative growth strategies may allow species to persist with a reduction in biomass production. These changes in relative growth rate in response to environmental conditions have been expressed through ratios of plant structures that highlight competitive strategies of biomass production and allocation (Poorter et al. 1990, Shipley 2002). Increases in specific leaf area are strongly correlated to increased relative growth rate in understory plants when light is limiting (Walters et al. 1993b, Poorter and van der Werf 1998, Reich et al. 1998a). Similar shifts in biomass allocation to belowground growth, or increased root weight ratios and decreased leaf weight ratios, have been documented in water-limited systems where root competition is high (Tilman 1984, Maranon and Grubb 1993, Ericsson 1995).

This study focuses on the influence of climate change on the growth and biomass allocation of nine northeastern and two southeastern temperate mid-successional tree species under increased temperature and precipitation regimes. The difference in morphological and biomass
allocation responses has been identified in previous studies of leaf structure and proportional biomass partitioning, demonstrating the increase in specific leaf area with increasing growth rates in response to low light environments (Walters et al. 1993b, a, Reich et al. 1998a, Poorter 1999) and similar comparisons can be made under manipulated climate conditions.

In this chapter the influences of simulated climate change and inherent species differences on the relationships of both absolute growth and relative growth rate (RGR) for seedlings is examined. These relationships were addressed with the following question: How do the increased temperature and precipitation treatments affect the absolute and relative growth responses of the selected study species? Relative growth rate can be broken down into both morphological and physiological components and in this paper I focus on the morphological variability in both absolute and relative growth rate and hypothesize that: 1) increased temperature and irrigation treatments drive increased total seedling biomass as allocation to the root system increases to meet the demands of the greater aboveground biomass.  2) increased biomass production in response to manipulated climate treatments will result in an increase the relative growth rate of species predicted to be successful under a climate-species model of changing habitat suitability.

**Methods**

**Species selection**

In the fall of 2007 - 2009 seeds from nine northeastern and two southeastern mid-successional deciduous species were planted using randomly generated plot maps and a 100x100 cm grid with 10 cm spacing for each of the 16 treatment plots. These eleven species were selected based on their current or future importance in the central PA forests based on Iverson and Prasad’s (2001) habitat suitability model, DISTRIB, which is trained on current Forest Inventory
Analysis data (Experimental Setup – Table 2.2). The selected study species were classified based on their current geographical distribution (northern or southern) and the change in future potential habitat suitability. Species classified as “resident winners” are currently present in the central PA forests and are predicted to increase in importance as climate changes. Resident winner species include *Betula lenta*, *Quercus alba*, *Quercus velutina*, *Carya glabra*, and *Quercus prinus*.

Resident losers or species that are currently present in the central PA forests and are predicted to decrease in importance include *Quercus rubra*, *Fraxinus americana*, *Acer rubrum*, and *Prunus serotina*. These species are currently present in central PA forests, but are expected to see a decline in habitat suitability and a loss of importance in future forest composition. The remaining two species, *Liquidambar styraciflua* and *Quercus stellata*, are classified as “predicted migrants” or species not currently present in the central PA forests but are predicted to gain in importance as climate change extends their suitable habitat northward.

**Field Study Design**

Seedlings from selected eastern tree species were monitored in a three-year climate change study at the Stone Valley Forest in Huntingdon County, PA (40N 85°, 77W 83°). The site is located at the interface between the Northeast and Mid-Atlantic regions, on the boundary of two dominant forest types: maple-beech-birch and oak-hickory. Average annual mean temperatures at the site are 9 °C with an average annual maximum of 15 °C and minimum of 4 °C throughout the year, with a range from 27°C in July to -8°C in January. Mean annual precipitation is approximately 1000 mm. The soils at the site are Oxyaquic Fragiudalfs in the Clarksburg series. The site is situated on a southeast slope. A four-hectare area was clear-cut and fenced in August 2007 and 16 plots were established in a 2x2 factorial design with for treatments (ambient, irrigated, warmed, and warmed+irrigated).
Climate manipulation treatments

Field warming treatments were programmed for a temperature increase of 1°C during the day and 3°C at night when compared to unwarmed plots, which resulted in an average temperature increase of 1.8°C (+/− 0.29°C). Plots were heated continuously from May 2008 through August 2010. The quantity of water (liquid, snow or ice) applied weekly to the irrigated plots was recorded and percent effective irrigation treatment was calculated by week, month, year, and experiment duration by dividing the irrigated treatment amount by the ambient precipitation. The effective precipitation treatment over the course of the experiment was +23%, with monthly precipitation additions ranging from 12 to 43%. Detailed explanations of the warming and precipitation manipulations are discussed in Chapter 2: Experimental Design and Methods.

Biomass Measurements

Above ground seedling biomass was measured following the harvest of all individual trees growing on the treatment plots. The seedling harvest occurred on two separate dates over the course of the field study. In October 2008 the first harvest excavated half the one-year-old seedlings on the 16 study plots. The second and final harvest, in August 2010, occurred when the experimental treatments were terminated and all surviving seedlings were removed. To remove seedlings with as much of the root system in tact as possible, study plots were excavated starting from the outer boundaries and moving towards the center of the plot. Species with taproots were excavated as deeply as possible and a targeted soil core was taken to remove any remaining taproot left behind after the seedling removal. This occurred most commonly for Quercus species, whose taproots could extend beyond 30 cm for older seedlings. These soil cores only occurred in 8% of the total number of all seedlings harvested. Following the field harvest, the
seedlings were brought back to the lab where they were broken down into leaves, stems, and roots. Final height and diameter measurements were taken for aboveground stems of each seedling and fresh weights for each component were recorded. Total fresh root weight, root length, total lateral root width and taproot diameter, when present, were recorded for belowground biomass components. Fresh leaf area for each seedling was determined with a leaf area meter (Li-Cor Inc. Lincoln, NE). Following all fresh measurements, seedling components were bagged and dried in a forced-air oven at 60°C for a minimum of three days and then re-weighed for final dry plant mass values.

**Data Analysis**

Data from each 3-year-old individual seedling was averaged by species for each treatment plot and plot-level values were then used to calculate the final treatment-level averages for each species. Of the 11 original study species only seven were used in the biomass comparisons for this chapter due to the uneven distribution of species across the four treatment types (Appendix Table A6). The species included in this analysis were *Quercus rubra*, *Fraxinus americana*, *Acer rubrum*, *Quercus alba*, *Quercus velutina*, *Liquidambar styraciflua* and *Quercus stellata*. Inconsistent germination and seedling mortality due to stressors such as drought, browsing, and defoliation reduced the number of seedlings harvested for final biomass calculation. Species were included in this analysis if they were present in at least two ambient treatment plots and also distributed across the climate treatment plots. Comparisons of biomass, height and leaf, shoot and root morphology were represented as a deviation from ambient, which standardized the response of each treatment to the ambient for each block.

Plant and root trait data were log$_{10}$ transformed to normalize distribution for parametric statistical analysis and linearize the relationship between variables. This transformation also allowed the data to be plotted for all species and treatments on the same log$_{10}$ x-axis scale. Plot,
treatment and block averages of untransformed data can be found in Appendix Table B6-12. Biomass allocation within individual seedlings was compared using biomass ratios of the fraction of leaf (leaf weight ratio; LWR), root (root weight ratio; RWR) or stem (stem weight ratio; SWR) dry mass to the total dry biomass of a seedling (Table 4.1). However, one drawback of this biomass ratio comparison is that it does not take into account the initial variation in size of seedlings of different species. To assess the relative biomass accumulation of the three-year-old seedlings in the study plots, relative growth rate was calculated based on the average one-year biomass for seedlings of each species and the final biomass of each individual three-year old seedling at the time of harvest. Relative growth rates of species by treatment were calculated with average 1-year seedling mass and three year seedling mass with the following equation: \( \frac{\ln M_{d2} - \ln M_{d1}}{(t_2 - t_1)} \). Where \( M_{d1} \) is the average dry mass at the end of a single year of growth, \( M_{d2} \) is the total dry mass at harvest, \( t_1 \) is one year and \( t_2 \) is three years. The average 1-year values were calculated at the species level for each treatment type. This provided an initial average mass (\( M_{d1} \)) for each species and treatment combination. Then individual seedling relative growth rates were calculated for each three-year-old seedling (\( M_{d2} \)) based on the final dry mass at the conclusion of the experimental period (Hunt 1982).

For the three-year-old seedlings of the seven study species, tests for treatment (species*warming*irrigation), main effects, and interactions on seedling growth and morphology were completed using an analysis of variance (ANOVA) mixed model procedure in SAS (SAS institute, Cary NC). When main effects were significant, means were compared with tests of least squares means. When single factor effects (warming, irrigation or species) were found to be significant, seedling response data was combine for the single factor for further comparison of the resulting responses. Additionally, relationships between morphological traits, such as height, biomass, and allocation ratios, and relative growth rate were examined with a linear regression analysis. Regression analysis was used to compare species-specific response to the four treatment
types and an analysis of covariance was used to compare the slopes of linear regression lines for each species. Alpha=0.05 was used to determine the significance of the statistical tests.

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Table 4.1: Variable abbreviations, full names and units for all growth analysis and morphology comparisons used in this chapter.

<table>
<thead>
<tr>
<th>Variable abbreviation</th>
<th>Variable name</th>
<th>Units</th>
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<tbody>
<tr>
<td>RGR</td>
<td>relative growth rate</td>
<td>mg g⁻¹ day⁻¹</td>
</tr>
<tr>
<td>LWR</td>
<td>leaf weight ratio (foliage mass/plant mass)</td>
<td>g⁻¹ g⁻¹</td>
</tr>
<tr>
<td>SWR</td>
<td>stem weight ratio (stem mass/plant mass)</td>
<td>g⁻¹ g⁻¹</td>
</tr>
<tr>
<td>RWR</td>
<td>root weight ratio (root mass/plant mass)</td>
<td>g⁻¹ g⁻¹</td>
</tr>
<tr>
<td>SLA</td>
<td>specific leaf area (foliage area/foliage mass)</td>
<td>cm⁻² g⁻¹</td>
</tr>
<tr>
<td>RLR</td>
<td>root length ratio (root length/plant mass)</td>
<td>m g⁻¹</td>
</tr>
</tbody>
</table>

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Results

Growth and allocation

After three years of growth under experimental treatments the differences in seedling growth and morphology reflect both the treatment responses and inherent species differences. There was no height response to treatment (ambient: 56.6 ±5 g; warmed+irrigated: 89.6±12 g; warmed: 68.8±5 g; irrigated: 54.7±13 g) (warming: p=0.092; irrigation: p=0.75) (Appendix Table C4). However, seedling heights were different among species (p=0.012). Specifically, Quercus velutina seedlings were shorter when compared to Acer rubrum seedlings across all treatment plots (50.5 ±8 cm and 72.2 ±21 cm, respectively; post hoc comparison of species p=0.0173).

Warming significantly increased total seedling biomass for all species by 242% (warmed: 164.9 ± 34 g; unwarmed: 68.2 ±22 g; p=0.012; Appendix Table C4) and basal area by 218% (warmed:
0.0003 ± 0.00005 m²; unwarmed: 0.0001 ± 0.00003 m²; p=0.015) and a 258% increase in stem biomass (warmed: 68.2 ± 22 g; unwarmed: 22.8 ± 8 g; p=0.0145) (Figure 4.1). Seedlings on warmed plots had 213% higher leaf mass (warmed: 29.7 ± 7 g; unwarmed: 13.9 ± 4 g; p=0.012) and 216% greater leaf area (warmed: 5288 ± 2253 cm²; unwarmed: 2443 ± 832 cm²; p=0.029) when compared to seedlings on unwarmed plots (Figure 4.2) (Appendix Table C4). Warming more than doubled average root mass from 33.06 g (± 10.06 g) on unwarmed plots to 76.26 g (± 13.81 g) on warmed plots for an increase of 230% (p=0.0077), regardless of species. Average root length was not significantly different when seedlings were compared by species (Appendix Table B12) or treatments (ambient: 24.5 ±0.7 cm; warmed+irrigated: 25.1 ±1 cm; warmed: 27.5 ±0.8 cm; irrigated: 18.3 ±2 cm) (p>0.05), indicating that while the total biomass of the root system was increasing, the depth of root penetration did not change with increased temperature or irrigation (Figure 4.3). Additional model outputs including degrees of freedom, F-values and P-values are listed in ANOVA tables (Appendix Table C4).
Figure 4.1: Seedling height, biomass and basal area for 3 year old seedlings at harvest, represented as a deviation from ambient. Error bars represent the SE of the mean.
Figure 4.2: Leaf area (cm²) and leaf mass (g) for 3-year-old seedlings of each species at harvest, represented as a deviation from ambient. Error bars represent the SE of the mean.
Figure 4.3: Average root mass (g) and length (cm) for 3 year old seedlings at harvest, represented as a deviation from ambient. Error bars represent the SE of the mean.
Growth and biomass partitioning

When comparing seedling biomass allocation to total seedling mass, all three allocation components (leaf weight ratio, stem weight ratio and root weight ratio) were significantly correlated with total seedling biomass in the warmed+irrigated treatments. This correlation indicates that the increase in both temperature and precipitation stimulated whole seedling growth and shifted biomass allocation. Stem weight ratio was positively correlated to total biomass in both the warmed ($R^2=0.50$, $p<0.001$) and warmed+irrigated ($R^2=0.49$, $p<0.001$) treatments, although the two treatments were not significantly different from one another ($p=0.39$). However both root weight ratio ($R^2=0.15$, $p=0.002$ warmed+irrigated; $R^2=0.29$, $p<0.0001$ warmed) and leaf weight ratio ($R^2=0.21$, $p=0.001$ warmed+irrigated) were negatively correlated to total biomass, but did not differ significantly in the slope of their relationship ($p=0.98$ and $p=0.82$, respectively) (Figure 4.4).

Overall, seedlings allocated more biomass to stem growth as the relative growth rate increased, while allocating proportionally less biomass to leaves and roots (Figure 4.5), suggesting that as seedling grow faster, the gains in biomass were allocated to the increase in woody aboveground tissue. The positive correlation between stem weight ratio and relative growth rate was significant on the warmed and warmed+irrigated treatment ($R^2=0.53$, $p<0.001$ and $R^2=0.47$, $p<0.001$, respectively). However, there was no significant difference in the slopes between the warmed and warmed+irrigated regression lines ($p=0.24$), indicating that the two treatments did not produce differences in the woody biomass allocation of seedlings as their relative growth rate increased.

The specific leaf area for seedlings grown in a high light environment showed no significant relationship to relative growth rate for any treatment type ($p=0.82$) (Appendix Figure D1). However, when the components of specific leaf area (absolute leaf area and absolute leaf mass) were compared across species and treatments, both were significantly higher in warmed plots.
(leaf area – warmed: 5288 ± 2253 cm²; unwarmed: 2443 ± 832 cm²; p=0.029 and leaf mass –
warmed: 29.7 ± 7 g; unwarmed: 13.9 ± 4 g; p=0.012). Additionally, leaf weight ratio had a
significant negative relationship with relative growth rate on irrigated and warmed+irrigated
treatments (R²=0.16, p=0.01 and R²=0.21, p=0.04), however these regression responses were not
significantly different between the treatment plots (p=0.32).

Figure 4.4: Relationships of total seedling biomass (log₁₀ g) with morphological traits of 3 year old
seedlings. Closed circles (○) are for ambient treatment plots, open circles (●) are for
warmed+irrigated treatment plots, open triangles (▼) are for warmed treatment plots and (▲) are
for irrigated treatment plots. Significant regressions are indicated ** (P<0.001) and * (P<0.05)
within the plot, with the R² value listed for each significant relationship. Abbreviations and units for
variables are explained in Table 1.
Figure 4.5: Relationships of relative growth rate (RGR, g g⁻¹ year⁻¹) with morphological traits of 3 year old seedlings. Closed circles (○) are for ambient treatment plots, open circles (●) are for warmed+irrigated treatment plots, open triangles (▼) are for warmed treatment plots and (▽) are for irrigated treatment plots. Significant regressions are indicated ** (P<0.001) and * (P<0.05) within the plot, with the R² value listed for each significant relationship. Abbreviations and units for variables are explained in Table 1.
Similar negative correlations were significant for warmed and warmed+irrigated treatment plots between root weight ratio and relative growth rate (\(R^2=0.34, p=0.001\) and \(R^2=0.14, p=0.05\), respectively), although these regression responses were not significantly different between the treatment types. Root length ratio also had a moderate negative correlation to relative growth rate (Pearson’s correlation, \(p=0.05\)) (Table 4.2), and seedlings on all four treatment types had significant decreases in relative root length as relative growth rate increased. These results indicate that regardless of treatment type, seedlings were producing thicker, shorter roots as the relative growth rate increases (Figure 4.5).

Table 4.2: Correlation matrix for relative growth rate (RGR) and other measured variables across all species and treatments. The upper right half of the matrix shows correlation coefficients for 3 year old seedlings. Correlations significant at \(P<0.05\) are shown in bold. Variable abbreviations and units can be found in Table 4.1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>RGR</th>
<th>LWR</th>
<th>SWR</th>
<th>RWR</th>
<th>SLA</th>
<th>RLR</th>
<th>Biomass</th>
<th>Height</th>
<th>Basal Area</th>
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</thead>
<tbody>
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<td>0.03</td>
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<tr>
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<tr>
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</table>
Species-specific variation in relative growth and biomass allocation

Biomass allocation ratios (stem weight ratio and root weight ratio) were similar among *Quercus* species when compared to the non-*Quercus* species. When species were compared by treatments, the positive relationship between stem weight ratio and relative growth was also observed on the warmed or the warmed+irrigated treatment plots for all *Quercus* species (Figure 4.7). For example, both *Quercus stellata* ($R^2=0.769$, $p=0.014$) and *Quercus rubra* ($R^2=0.735$, $p=0.018$) have significant increases in stem weight ratio with increasing growth rate on warmed-only treatment plots, while *Quercus rubra* ($R^2=0.716$, $p=0.034$), *Quercus alba* ($R^2=0.793$, $p=0.043$) *Quercus velutina* ($R^2=0.722$, $p=0.02$) displayed significant increases in stem weight ratio with increasing growth rate on warmed+irrigated treatment plots (Figure 4.7). *Quercus rubra* and *Quercus stellata* (warmed only) and *Quercus velutina* (warmed+irrigated) had inverse relationships between relative growth rate and stem weight ratio (positive) and root weight ratio (negative), indicating that these species were allocating more to stem growth as the relative growth rate increased (Figure 4.6).

In contrast to the trade-off between stem and root allocation in the *Quercus* species, *Liquidambar styraciflua* was the only species with a positive relationship between root weight ratio and relative growth rate on the ambient treatment plots ($R^2=0.99$, $p=0.05$) (Figure 4.6). This high explained variance can be attributed to the low number of individuals sampled and the similarity of the growth response for the seedlings sampled. However, *Fraxinus americana* did not have significant relationships between root weight ratio or shoot weight ratio and relative growth rate in any treatment type, which suggests that these morphological ratios are not adequately explaining changes in absolute growth that was observed in this species in response to increased temperature or precipitation treatments.
Figure 4.6: Relationship of relative growth rate (RGR, g g⁻¹ year⁻¹) with root weight ratio (RWR, g g⁻¹) for 3 year old seedlings. Closed circles (○) are for ambient treatment plots, open circles (●) are for warmed+irrigated treatment plots, open triangles (▼) are for warmed treatment plots and (▲) are for irrigated treatment plots. Significant regressions are indicated by * (P<0.05) within the plot, with the R² value listed for each significant relationship. Abbreviations and units for variables are explained in Table 1. Only significant correlations are shown with a regression line.
Figure 4.7: Relationship of relative growth rate (RGR, g g$^{-1}$ year$^{-1}$) with stem weight ratio (SWR, g g$^{-1}$) for 3 year old seedlings. Closed circles (○) are for ambient treatment plots, open circles (●) are for warmed+irrigated treatment plots, open triangles (▼) are for warmed treatment plots and (▲) are for irrigated treatment plots. Significant regressions are indicated ** (P<0.001) and * (P<0.05) within the plot, with the R$^2$ value listed for each significant relationship. Abbreviations and units for variables are explained in Table 1. Only significant correlations are shown with a regression line.
I expected to see changes in the overall height response for seedlings grown under the increased temperature and precipitation treatments. Instead, I found that there was no difference in the absolute height for seedlings in response to any treatment type (p>0.05) (Appendix Table B7). However, when relative growth rate was compared to height, I found positive relationships between height and relative growth for most species (Figure 4.8). Warming treatments (warmed and warmed+irrigated) have a significant positive correlation between height and relative growth rate, supporting the absolute growth result that warming also stimulated overall biomass increases and produced taller seedlings more quickly. Smaller seeded species (Acer rubrum, Fraxinus americana and Liquidambar styraciflua) had significant relationships between height and relative growth rate in response to warming (Figure 4.8), although no treatment specific differences were found when the slopes of the regression lines were compared for each species.
Figure 4.8: Relationship of relative growth rate (RGR, g g⁻¹ year⁻¹) with height (log₁₀ cm) for 3 year old seedlings. Closed circles (○) are for ambient treatment plots, open circles (●) are for warmed+irrigated treatment plots, open triangles (▼) are for warmed treatment plots and (▲) are for irrigated treatment plots. Significant regressions are indicated ** (P<0.001) and * (P<0.05) within the plot, with the R² value listed for each significant relationship. Abbreviations and units for variables are explained in Table 1. Only significant correlations are shown with a regression line.
Discussion

After three years of growth under the experimental climate treatments, warming resulted in a significant increase in total seedling biomass regardless of species type or regional origin. Additionally, there was no significant warming by irrigation interaction found for any variable of morphology, refuting the hypothesis that the combination of both increased temperature and precipitation would facilitate increased seedling growth. However, the presence of warming, regardless of the level of irrigation, significantly increased seedling basal area, leaf area, leaf mass and root mass when compared to seedlings on unwarmed treatment plots. While seedlings grown on warmed plots increased in size, patterns between relative growth and biomass allocation were not consistent among species or treatment type. As seedlings compete for space in the developing canopy, there may be increased competitive pressure to grow taller faster, which further supported the increase in height growth with increased relative growth rate.

Growth and seedling traits under manipulated climate treatments

Most previous studies of seedling relative growth rate and biomass accumulation focus on growth responses under light-limited conditions and have strong positive relationships between specific leaf area and relative growth rate as broader, thinner leaves are produced to increase light capture and photosynthetic gains (Walters et al. 1993b, a, Reich et al. 1995a, Reich et al. 1998a, Sack and Grubb 2002). The lack of a significant relationship between specific leaf area and relative growth rate suggests that for seedlings grown in high light environments, leaf morphology is responding to reduce other sources of environmental stress such as water loss. The observed increases both in leaf area and leaf mass were not associated with reduced relative growth rates; rather, the seedlings appear have the physiological mechanisms to cope with the demands of increased photosynthetically active tissue in the high light environment.
Additionally, the absence of a relationship between specific leaf area and relative growth rate may also be the result of the more complex above-ground canopy structure that begins to develop as seedlings age, increasing self-shading and requiring greater woody structural support for increasing leaf mass (Kohyama and Grubb 1994, Mark et al. 1999, Naumburg et al. 2001). Walters et al. (1993a) found that for seedlings grown in varying light conditions, the area of photosynthetically active tissue increases in relation to the amount of carbon that is allocated as leaves. These growth responses were observed to occur in a manner consistent with theories of balanced growth, or the relationship between resource availability and growth, namely that individuals must allocate more biomass to the part of plant that is acquiring the most limiting resource (Poorter et al. 1990, Shipley and Peters 1990, Lambers and Poorter 1992, Walters et al. 1993a, Reich et al. 1998a).

In early successional environments with high light, seedlings demonstrate a consistent shift away from root and leaf biomass allocation in favor of stem biomass allocation (Walters et al. 1993b, Walters and Reich 1996, Reich et al. 1998a). This shift in growth form supports the observed decrease in leaf weight ratio and increase in stem weight ratio in relation to total biomass for seedlings grown under warmed+irrigated treatments in this study (Figure 4.4). In the absence of water stress, increasing air and soil temperatures are likely to stimulate above ground growth (Norby et al. 1992, Luxmoore et al. 1993, DeLucia et al. 1994, Farnsworth et al. 1995, Cunningham and Read 2003, Yin et al. 2007) and for young seedlings that growth occurs in the form of height. I expected to find both root and leaf biomass to increase proportionally on warmed+irrigated treatments. Instead, both the leaf weight ratio and root weight ratio declined with increasing seedling biomass, indicating that seedlings were allocating a greater proportion of their carbon resources to stem and branch structures, refuting the proportional biomass allocation component of hypothesis 1. Another possibility is that that young seedlings will vary in their ability to respond to changes in resource availability and stress through altered morphological

As seedlings grow, changes in the fraction of biomass allocated to specific plant components may be the result of species-specific growth form, such as growth rate or morphological traits (Latham 1992, Long and Jones 1996), drought tolerance (Abrams and Knapp 1986, Abrams 1990, Kubiske and Abrams 1992, Dickson and Tomlinson 1996) and stem and root architecture (Aiba and Kohyama 1997, Poorter et al. 2003). Although the sample size of *Liquidambar styraciflua* seedlings was limited, under ambient northeastern climate conditions this native southern species allocated greater biomass to its root system to support its total relative growth. When the ratio of average root length was compared to seedling biomass, *Liquidambar* seedlings on warmed+irrigated plots demonstrated the same response as *Quercus* species, with a significant negative relationship between root length ratio and relative growth rate indicating that seedlings produced shorter, thicker roots as relative growth rate increased to support increasing overall biomass and longer, thinner roots when relative growth rate was low (Figure 4.5). The absence of significant differences between the rooting behaviors of the study species is important to consider, given the inherent rooting architecture of the tap-rooting species (*Quercus* species) and the shallower rooting, small seeded species. Not only was the relationship between root length ratio and relative growth rate strongly negative across all treatment types, but when comparing root length as a deviation from ambient there was only a weak relationship to warming treatments (p=0.07) across all species. The combination of these morphological and biomass allocation characteristics indicate that within our study site, rooting was influenced by other environmental factors, such as water or nutrient availability or that the increase in temperature and precipitation was not great enough to limit root growth within the time frame of this study.

For seedlings in high resource environments higher relative growth rates and increased biomass allocation to stems is necessary to emerge from the understory as stands self-thin over time (Bazzaz and Miao 1993, Walters et al. 1993a, Seiwa and Kikuzawa 1996, Beckage and
Clark 2003). Way and Oren (2010) found that for deciduous species, increased height growth was often greater than the corresponding increases in shoot mass; suggesting that stem elongation could be responsive to greater resource availability. This increase was evident across this study, with significant positive relationships between height and relative growth rate, particularly among seedlings on warmed and warmed+irrigated treatment plots. Although there was no significant difference in the slopes of the four treatment types, unwarmed plots had no significant relationships between relative growth rate and height (Figure 4.9). Increases in both height and biomass may also drive a decline in leaf weight ratio as seedlings age, since plants allocate proportionally more biomass to stem growth for support as height increases (Walters et al. 1993b), a result that was observed in this study as well.

Height (Figure 4.6) had stronger, positive correlations to relative growth rate for more species and treatment combinations than any other measured variable of morphological form or structure. While there was no observed difference in absolute height among treatments, the relationship between individual seedling height and the corresponding relative growth rate can be used to better understand how young trees are responding to manipulated climate conditions. Studies have examined intraspecific trade-offs between survival and relative growth rate along resource gradients, such as light (Walters et al. 1993b, Kobe et al. 1995, Reich et al. 1998a, Seiwa 1998, Poorter 1999). In high-light environments, earlier vertical growth is advantageous for seedling establishment because the light availability to the seedling is drastically improved for taller stems (Ross and Harper 1972, Givnish 1982, King 1990, Seiwa 2000, 2007). Quantifying the relationship between height and the rate of seedling growth enables simple field measurements to be used to assess seedling biomass accumulation in developing stands under changing climate conditions.
Conclusion

Studies of relative growth rate and seedling biomass partitioning have highlighted that no single trait can provide basis for fundamental differences in species ecology (Walters et al. 1993a). Predictions of species success and failure in future conditions require the consideration of the ecologically important morphology and physiology traits that both affect and are affected by a suite of environmental characteristics. Results from this study found that increasing temperature has a stimulating effect on the biomass and growth of young, deciduous seedlings. Seedling responses to increasing temperature resulted in an overall increase in total biomass, with the greatest proportional increase found in above ground woody tissue. This increase in stem and branch biomass also drives a strong positive relationship between relative growth rate and seedling height for most of the mid-successional species included in this study. While it was expected that species' morphological responses as young seedlings would reflect species-climate envelope model predictions of success and failure based on habitat suitability of mature forests, the results of this study did not support that initial hypothesis and all seven species increased their biomass accumulation in response to warming. Biomass accumulation is heavily proportioned to stem growth and height was unaffected by treatment conditions, suggesting that the first three years of seedling growth are strongly limited by seedling capacity for total growth in an effort to maintain a place in the developing canopy. Additionally, changes in the total seedling leaf area and leaf mass did not respond as expected, increasing in response to warming, suggesting that additional growth or environmental factors were influencing the development of leaf characteristics and morphology. The mechanisms that drive this increasing biomass in response to increased warming and precipitation are still not clear. Field-based studies focused on determining the tradeoffs in morphological traits and their relationship to physiological responses, such as photosynthesis and water potential, can identify how species are acclimating to changing environmental conditions.
Chapter 5

The effects of seedling morphology and leaf characteristics on the photosynthetic responses of temperate deciduous tree seedling grown under increased temperature and precipitation treatments.

Introduction

Primary productivity, or energy produced through the photosynthetic responses of plants, is strongly influenced by global climate. As climate changes, predicted increases in temperature and altered precipitation patterns will influence growing season conditions and impact species’ morphology and physiology (Reich et al. 1998a, Wright and Westoby 1999, Montgomery 2004). Changes in plant level responses have the ability to alter community and ecosystem-level productivity, which can further impact global climate. Among ecosystems that are sensitive to plant-level responses, forested ecosystems are one of the most important, covering 30% of terrestrial biomes and store approximately 45% of terrestrial carbon, with temperate forests estimated to account for nearly 20% of the global plant biomass (Elser et al. 2010). Temperate forest ecosystems, like those found in the eastern United States, are predicted to experience changes in temperatures ranging from 2-6°C coupled with an increase in precipitation up to 20% (Bonan 2008). Morphological and physiological responses of forest tree species have become a focus of ecological studies in an effort to understand the interactions between climate change and temperate forests. The effects of changes in climate can be seen clearly in the forest community through seedling leaf morphology, due to their small stature and limited reserves (Ollinger et al. 2007).

availability and changes in leaf structure can be used to measure plant responses to changing environmental conditions (Ellsworth and Reich 1992, Reich et al. 1998a). Specific leaf area (SLA; leaf area per unit leaf mass) has been used as a proxy of leaf thickness or density, which has a strong correlative relationship to photosynthetic rates (Abrams and Kubiske 1990, Abrams et al. 1994). Increased irradiance, temperature or limited water availability can cause seedlings to reduce specific leaf area, producing thicker or denser leaves, and greater mass distributed over less surface area. This reduction in surface area can also limit the photosynthetic capacity of the leaves by reducing the surface area of chloroplasts that are exposed to intercellular space, reducing the potential for light interception (Abrams et al. 1994, Poorter and Evans 1998, Reich et al. 1998a).

Specific leaf area and foliar nitrogen are positively related to photosynthesis and both are sensitive to environmental conditions. Species with higher specific leaf area generally have higher mass based nitrogen ($N_{mass}$) and area based photosynthetic capacity ($A_{area}$). Approximately half of leaf nitrogen is invested in the structure of chloroplasts and the primary carboxylation enzyme, Rubisco. There is a strong correlation between a species capacity for photosynthetic activity and the foliar nitrogen concentration of its leaves (Evans and Poorter 2001). Additionally, species with a higher nitrogen concentration tend to have higher photosynthetic N-use efficiency (photosynthetic capacity per unit leaf N) (Field and Mooney 1986, Reich et al. 1995b, Reich et al. 1997, Hikosaka 2004, Montgomery 2004, Osada et al. 2010). Thicker leaf structure may result in lower photosynthetic rates that are a result of variation in leaf nitrogen distribution within the leaf tissue (Hikosaka 2004, Wright et al. 2005), self-shading (Hikosaka 2004), reduced/slow intercellular diffusion of CO$_2$ (Hikosaka and Terashima 1995, Ackerly 1999, Montgomery 2004). The relationships between leaf structure, photosynthesis and nitrogen has important implications in understanding the physiology of leaf function and the ability to predict whole tree level responses to changing environmental conditions (Parkhurst 1994).
While leaf-level physiological and morphological measurements are a common method used to assess seedling responses to environmental changes, they do not provide a complete representation of whole-plant physiology. Stand-level measures of gas exchange and net ecosystem exchange have been measured using eddy covariance (Reich et al. 1998b) and modeled by scaling leaf and plant level measurements to whole stand estimations (Goulden et al. 1996, Curtis et al. 2002, Baldocchi 2003). When leaf-level assimilation is scaled to whole plant leaf area there is a tighter correlation with whole plant carbon gain when compared to leaf-level photosynthesis alone (Friend et al. 1993, Eamus 1996, Lavigne et al. 1997, Sievänen et al. 2000). A species may have low investment of resources in a single leaf, but may produce an increased number of leaves allowing it to keep pace with species that may invest high levels of C and N into a few leaves. However, it is important to consider the limitations of whole seedling estimations, namely that seedlings are not homogenous in their leaf development and self shading, and that nutrient partitioning among leaves can lead to over-estimations as seedling architecture becomes more complex (Montgomery 1999).

This study focuses on the influence of climate change on leaf morphology and physiology of early successional tree species grown under increased temperature and precipitation conditions. I expected that seedlings would respond to increased temperature and precipitation treatments after 3 growing seasons by altering their leaf morphology and physiology to maintain photosynthetic activity. This expectation was addressed with the following questions: How do the functional relationships between leaf physiological and morphological variables change when seedlings are grown under increased temperature and precipitation treatments? Do leaf-based physiological traits change when scaled to the photosynthetic and growth responses of the whole seedling? I hypothesize that: 1) Leaf-level photosynthetic rates will be maintained under warming and irrigation treatments through increased leaf mass, area and physiology. 2) Whole-seedling photosynthetic rates will increase with warming and irrigation treatments through increased seedling growth and foliar N. The potential implications of these physiological and
morphological responses to increasing temperature and precipitation treatments provide a more in depth understanding of the range of photosynthetic carbon gains in broadly distributed temperate tree species as regional climates change.

**Methods**

**Field Study Design**

Seedlings from selected eastern trees were monitored in a three-year climate change study at the Stone Valley Forest in Huntingdon County, PA (40N 85', 77W 83'). The site was located at the interface between the Northeast and Mid-Atlantic regions, on the boundary of two dominant forest types: maple-beech-birch and oak-hickory. Average annual mean temperatures at the site are 9 °C with an average annual maximum of 15 °C and minimum of 4 °C throughout the year, with a range from 27°C in July to -8°C in January. Mean annual precipitation is approximately 1000 mm. The soils at the site are Oxyaquic Fragiudalfs in the Clarksburg series. The site is situated on a southeast-facing slope. A 4-hectare area was clear-cut and fenced in August 2007 and 16 plots were established in a 2x2 factorial design with four treatments (ambient, irrigated, warmed, and warmed+irrigated).

Field warming treatments had a constant target temperature increase of 1°C during the day and 3°C at night when compared to unwarmed plots, which produced an average temperature increase of 1.8°C (+/- 0.29°C). Plots were heated continuously from May 2008 through August 2010. Predicted changes in precipitation were simulated by irrigating treatment plots weekly through the course of the experiment. The quantity of water (liquid, snow or ice) applied weekly was recorded and percent effective irrigation treatment was calculated by week, month, year, and experiment duration by dividing the irrigated treatment amount by the ambient precipitation. This produced an effective irrigation treatment of 23%, with monthly irrigation treatments ranging
from 12 to 43% above ambient. Detailed explanations of the warming and precipitation manipulations are discussed in Chapter 2: Experimental Design and Methods.

**Gas Exchange**

Within the 16 study plots, seedlings of each tree species present were selected for gas exchange measurements during the 2010 growing season (June-July) (Appendix table A7a and b). Gas exchange measurements were repeated monthly during the growing season. Measurements of photosynthetic CO$_2$ assimilation and leaf-level conductance were made on fully expanded leaves under ambient conditions with a portable leaf chamber and infrared gas analyzer (LiCor 6400, Li-Cor Inc. Lincoln, NE) under full sunlight from 0900-1500, local time. These measurements were made under similar conditions (full sunlight during the same daylight hours) during the growing season. For each sampling date, three leaves were measured per seedling, and these measurements were averaged for a seedling value to be used in subsequent analyses. Selected seedlings were re-sampled each year, as long as they persisted in the plot. When a seedling was lost due to mortality, it was replaced with another seedling of the same species from the treatment plot if one was present. This sampling resulted in 73 three-year-old seedlings being sampled during the 2010 growing season (Appendix table A7a and b).

**Leaf Nitrogen**

During the 2010 growing season, leaves were collected following July pre-dawn and mid-day water potential measurements (described in Chapter 6). Sampled leaves were brought back to the lab, placed in labeled paper bags and dried at 60°C for three days. After dry weight was recorded, each leaf was ground to a fine powder to homogenize the sample. Ground samples were weighed and rolled into 2.500-3.500 mg subsample tins and then run through a CHNSO
Seedling Biomass

In August 2010 all above- and below-ground seedling biomass was removed from the study plot for detailed post-harvest measurements. Harvested seedlings were brought back to the lab, where they were rendered into leaves, stems, and roots. Final height and basal stem diameter were measured for aboveground stems of each seedling and fresh weights for each component (i.e. stems, leaves, roots) were recorded. Fresh leaf mass was weighed for each seedling and leaf area was measured using a portable leaf area meter (Li-Cor Inc. Lincoln, NE). Following all fresh measurements, seedling components were bagged and dried in a forced-air oven at 60°C for a minimum of three days and then re-weighed for final dry plant mass values. Specific leaf area (SLA) for each seedling was determined by dividing its leaf oven dry mass by leaf area (cm² g⁻¹).

Relative growth rates of species by treatment were calculated with average 1-year seedling mass and three year seedling mass with the following equation: (ln M_{d2} - ln M_{d1})/(t_2-t_1). Where M_{d1} is the average dry mass at the end of a single year of growth, M_{d2} is the total dry mass at harvest, t_1 is one year and t_2 is three years. The average 1-year values were calculated at the species level for each treatment type. This provided an initial average mass (M_{d1}) for each species and treatment combination. Then individual seedling relative growth rates were calculated for each three-year-old seedling (M_{d2}) based on the final dry mass at the conclusion of the experimental period (Montgomery 2004).
Data Analysis

For species-specific comparisons of photosynthesis and leaf-level and whole seedling biomass and area, data from each 3-year-old individual seedling was averaged by species for each treatment plot and plot-level values were then used to calculate the final treatment-level averages for each species. Species were included in this analysis if there were seedlings present in a minimum of two ambient plots as well as being distributed across the climate treatment plots. Of the 11 original study species only seven were used in the biomass and photosynthesis comparisons for this chapter due to the uneven distribution of species across the four treatment types. The species included in this analysis are *Quercus rubra*, *Fraxinus americana*, *Acer rubrum*, *Quercus alba*, *Quercus velutina*, *Liquidambar styraciflua* and *Quercus stellata*. Species distributions in the plots for the analysis in this chapter can be found in Appendix table A7 and A8. Inconsistent germination and seedling mortality due to stressors such as drought, browsing, and defoliation reduced the number of seedlings present during the third year of photosynthesis measurements, therefore not all species could be included in final analyses. Comparisons of both whole-seedling and leaf-level photosynthesis, leaf biomass and area and foliar chemistry were represented as a deviation from ambient, which standardized the response of each treatment to the control for each block before treatment or species-specific averages were calculated.

Leaf structure (i.e. specific leaf area and leaf area ratio), leaf-level and whole seedling photosynthesis and foliar chemistry measurements were analyzed using a mixed model analysis of variance (ANOVA) for a randomized complete-block, split-plot design. For the three-year-old seedlings of the seven study species, tests for treatment (species*warming*irrigation), main effects, and interactions on seedling growth and morphology were completed using an analysis of variance (ANOVA) mixed model procedure in SAS (SAS institute, Cary NC). When main effects were significant, means were compared with tests of least squares means. When single factor effects (warming, irrigation or species) were found to be significant, seedling response data were
combine for the single factor for further comparison of the resulting responses. The interrelationship among leaf structure, foliar nitrogen, gas exchange and the warming treatment on photosynthesis was analyzed using linear regression analysis and analysis of covariance (ANCOVA) to test for similarity of slope. All statistical comparisons were considered significant at $\alpha=0.05$.

**Results**

**Leaf-level photosynthesis and leaf biomass and leaf area of all seedlings**

While gas exchange measurements were collected in both June and July of 2010, only photosynthesis measurements from the July 2010 sampling were used for comparisons of gas exchange and foliar chemistry. When all seedling leaf-level photosynthetic rates were compared by treatment, warming increased photosynthesis (warmed: $8.8\pm0.3$ $\mu$mol m$^{-2}$ s$^{-1}$; unwarmed: $6.6\pm0.8$ $\mu$mol m$^{-2}$ s$^{-1}$; $p=0.024$; Appendix Table C5) (Figure 5.1). However, species leaf-level photosynthesis rates were different regardless of treatment. *Acer rubrum* seedlings had significantly lower photosynthetic rates ($4.8\pm0.5$ $\mu$mol m$^{-2}$ s$^{-1}$) when compared to *Fraxinus americana* ($8.1\pm0.4$ $\mu$mol m$^{-2}$ s$^{-1}$; post hoc comparison of species $p=0.0423$), *Quercus alba* ($8.8\pm0.3$ $\mu$mol m$^{-2}$ s$^{-1}$; $p=0.0026$) and *Quercus stellata* ($9.9\pm1.3$ $\mu$mol m$^{-2}$ s$^{-1}$; post hoc comparison of species $p=0.0001$).
Warming significantly increased the total leaf area and leaf mass of seedlings by over 200% (Biomass chapter Figure 4.2). However, leaf area ratio (total leaf area/total seedling biomass; LAR) decreased by an average of 7% under warmed conditions (warmed: $31.5 \pm 5$ cm$^2$ g$^{-1}$; unwarmed: $33.7 \pm 2$ cm$^2$ g$^{-1}$; p=0.0078; Appendix C5), confirming results from the previous chapter which suggests that seedlings allocated less biomass to leaf growth than to stem or root growth (Figure 5.2). While the ratio of leaf biomass per seedling (leaf weight ratio; LWR) may have decreased, the morphology of the leaves (specific leaf area; SLA) had a significant interaction between warming and species (p=0.0354; Appendix C5). *Quercus rubra* seedlings had greater specific leaf area on warmed plots when compared to unwarmed plots (warmed: $38.2 \pm 10$ cm$^2$ g$^{-1}$; unwarmed: $32.5 \pm 6$ cm$^2$ g$^{-1}$; post hoc comparison of species p=0.0064), indicating that the seedlings were producing thinner leaves with greater surface area as growing under warmer temperatures (Figure 5.2). In contrast, *Fraxinus americana, Quercus alba* and *Quercus stellata* had lower average specific leaf area measurements on warmed plots when compared to
unwarmed plots, indicating that these species were producing thicker, denser leaves with less surface area on warmed plots (Fraxinus americana warmed: 20.9±1 cm² g⁻¹; unwarmed: 26.9±2 cm² g⁻¹; p=0.33; Quercus alba warmed: 25.9±3 cm² g⁻¹; unwarmed: 32.1±7 cm² g⁻¹; p=0.09; and Quercus stellata warmed: 22.5±2 cm² g⁻¹; unwarmed: 29.8±2 cm² g⁻¹; p=0.6). Additional plot, treatment and block averages of raw data can be found in Appendix Table B13 and B14.

Figure 5.2: Comparison of 3 year old seedling leaf morphology. Specific leaf area and leaf area ratio are shown on a log₁₀ scale for comparison on a similar scale and are represented as a deviation from ambient. Negative values indicate a response that is lower than that of ambient grown seedlings. Error bars represent SE of the mean.
The concentration of foliar carbon and nitrogen did not respond to warming and irrigation treatments (foliar N: ambient: 12.8 ± 0.3 mg; warmed+irrigated: 13.8 ± 2 mg; warmed: 17.7 ± 2 mg; irrigated: 19.7 ± 2 mg; warmed: p=0.54; irrigated: p=0.90; foliar C: ambient: 286.9 ± 15 mg; warmed+irrigated: 317.0 ± 59 mg; warmed: 438.0 ± 8 mg; irrigated: 371.0 ± 16 mg; warmed: p=0.53; irrigated: p=0.39; Appendix Table C5). Foliar nitrogen was different among species (p=0.003; Appendix Table C5). *Acer rubrum* had significantly less foliar nitrogen (7.9±1 mg) than both *Quercus velutina* (24.7±2 mg; p=0.014) and *Quercus rubra* (23.5±3 mg; p=0.011). Similarly, *Acer rubrum* also had significantly less foliar carbon (180±29 mg) when compared to *Quercus rubra* (487.4±27 mg; p=0.002), *Quercus velutina* (531±52 mg; p=0.005) and *Fraxinus americana* (487.9±151 mg p=0.008) (Figure 5.3). The model outputs including degrees of freedom, F-values and P-values are listed in ANOVA tables (Appendix table C5).
Leaf-level morphology and physiology

For this study, the relationship between leaf-level photosynthesis and foliar N was not consistent for species and treatment combinations. *Quercus stellata* ($R^2:0.10$, $p=0.48$) and *Liquidambar styraciflua* ($R^2:0.75$, $p=0.07$) were the only two species that displayed a positive, linear relationship between foliar N and photosynthesis, although these relationships were not displayed in the figure due to the absence of a significant regressions between photosynthesis and
foliar N. For both of these southern species, warming treatments raised the measured rate of photosynthesis when foliar N values were similar when compared to unwarmed treatments (Figure 5.4). *Quercus stellata* leaf nitrogen and photosynthetic capacity were not significantly different when compared between warmed and unwarmed plots (p=0.356). In contrast, on warmed plots *Quercus rubra* (R²:0.25, p=0.31) and *Acer rubrum* (R²:0.42, p=0.11) have higher photosynthetic rates at low levels of foliar N. *Acer rubrum* also displayed limited range in both leaf-level photosynthesis rates and foliar N content on both warmed and unwarmed plots (Figure 5.4).

![Figure 5.4: Comparison of leaf-level photosynthesis for 3-year-old seedlings and foliar N content. Black circles (●) represent seedlings are grouped by warmed (warmed and warmed+irrigated) and open circles (○) represent unwarmed (ambient and irrigated). Regression lines are only present when significant. R² values for each regression are indicated next to the treatment type for each species. Significance for each regression line is indicated next to R² as not significant (ns, p>0.05) or significant (*, p<0.05 or **, p<0.001).](image)
For most of the study species, specific leaf area fell within a similar range of values (100-200 cm² g⁻¹). However, for all species, except *Liquidambar styraciflua* ($R^2$:0.14, $p=0.85$) and *Quercus velutina* ($R^2$:0.05, $p=0.63$), photosynthetic rates were higher with increasing specific leaf area on warmed plots, which suggests that the seedlings are producing thinner leaves, with greater surface area for light interception on warmed plots (Figure 5.5). It is important to note that the $R^2$ values for the majority of these relationships is less than 0.2, suggesting that for these seedlings, specific leaf area does not serve as a strong predictor variable for leaf-level photosynthesis.

![Figure 5.5: Comparison of leaf-level photosynthesis for 3 year old seedlings and specific leaf area (SLA; cm²/g). Black circles (●) represent seedlings are grouped by warmed (warmed and warmed+irrigated) and open circles (○) represent unwarmed (ambient and irrigated). $R^2$ values for each regression line are indicated next to the treatment type for each species. Significance for each regression line is indicated next to $R^2$ as not significant (ns, $p>0.05$) or significant (*, $p<0.05$ or **, $p<0.001$).](image-url)
Physiological responses to differences in biomass allocation

Estimates of total seedling foliar nutrients showed a significant increase in foliar C and N on warmed treatment plots, when leaf-level values were scaled up using total seedling leaf mass (seedling N warmed: 724.2 ±229 mg; unwarmed: 264 ±52; p=0.009; seedling C warmed: 16860 ±4850 mg; unwarmed: 5266 ±709 mg p=0.006) (Figure 5.6). This increase is primarily driven by the 213% increase in leaf biomass that occurred in seedlings grown under warmed treatment plots (Figure 4.2). This change in foliar C and N should result in subsequent changes in the whole seedling photosynthetic responses. In fact, estimates of whole seedling photosynthesis had the expected positive, linear relationship for all species, except Quercus alba on unwarmed plots when compared to whole seedling foliar N (Figure 5.7). Acer rubrum was had the strongest positive relationship between increasing foliar N and whole seedling photosynthetic rates on unwarmed plots (R^2=0.97, p=0.001) and a similar positive increase on warmed plots, although the relationship was not significant (R^2=0.50, p=0.07). When seedling relative growth rate was compared to whole seedling photosynthetic rates, it was found that warming increased photosynthesis and growth for the study species (Figure 5.8). Three of the seven study species had significant positive increases in photosynthetic rates with increased relative growth rate on warmed treatment plots, including Quercus rubra (R^2=0.78 p=0.047), Acer rubrum (R^2=0.85 p=0.003) and Quercus stellata (R^2=0.76 p=0.011).
Figure 5.6: Estimation of whole seedling foliar N and C content based on total leaf biomass. All figures are represented as a deviation from ambient. Negative values indicate that the treatment value is lower than the average ambient value. Error bars represent SE of the mean.
Figure 5.7: Comparison of estimated whole seedling photosynthesis for 3-year-old seedlings and total foliar N content. Black circles (●) represent seedlings are grouped by warmed (warmed and warmed+irrigated) and open circles (○) represent unwarmed (ambient and irrigated). $R^2$ values for each regression line are indicated next to the treatment type for each species. Significance for each regression line is indicated next to $R^2$ as not significant (ns, p>0.05) or significant (*, p<0.05 or **, p<0.001).
Figure 5.8: Comparison of estimated whole seedling photosynthesis for 3-year-old seedlings and relative growth rate. Black circles (●) represent seedlings are grouped by warmed (warmed and warmed+irrigated) and open circles (○) represent unwarmed (ambient and irrigated). R^2 values for each regression line are indicated next to the treatment type for each species. Significance for each regression line is indicated next to R^2 as not significant (ns, p>0.05) or significant (*, p<0.05 or ***, p<0.001).
Discussion

Leaf-level responses of physiology and growth

In this study, it was expected that increased temperature and irrigation treatments would alter the development of seedling leaf morphology and physiology to maintain leaf-level photosynthetic rates under manipulated climate conditions. While warming treatments increased the average leaf-level photosynthetic rates of seedlings, species responses varied. *Acer rubrum* seedlings displayed little variation across the treatments, with almost no difference in photosynthetic rates under increased temperature or precipitation. *Acer rubrum* had significantly lower leaf-level photosynthetic rates when compared to *Fraxinus americana*, *Quercus alba* and *Quercus stellata*, a result that is supported by other studies of early successional species in high light (Way and Oren 2010).

Leaf morphological features, such as specific leaf area and leaf biomass, have been attributed to leaf-level photosynthetic function (Field and Mooney 1986, Abrams and Kubiske 1990, Reich et al. 1991, Reich et al. 1998a, Montgomery 2004). Warming significantly increased the total amount of leaf mass and leaf area across all study species, however the ratio of leaf mass to total biomass on warmed plots was lower when compared to unwarmed plots. This suggests that increased temperatures are driving seedlings to allocate a greater proportion of biomass to stem or root growth to support the resource demands of photosynthetically active tissues (Jurik 1986, Reich et al. 1990, Kloeppele et al. 1993, Kubiske and Abrams 1994, Abrams 1998). While the ratio of leaf biomass per seedling decreased with warming, leaf morphology differed by species. This change in specific leaf area provides one potential mechanism for the increases in photosynthetic responses to warming that were observed among study species. Both *Quercus*...
rubra and Quercus velutina had greater specific leaf areas on warmed plots, which suggest that increased temperatures may be driving these two species to produce thinner leaves with greater surface area to increase the photosynthetically active surface area. In contrast, Fraxinus americana, Quercus alba and Quercus stellata, the three species that differed significantly from Acer rubrum in their photosynthetic rates, also had lower specific leaf area on warmed plots indicating that the higher leaf-level photosynthetic rates were occurring in thicker, denser leaves with a lower surface area to mass ratio. Understory seedlings exhibit higher photosynthetic rates with increasing specific leaf area, as surface area increases to maximize light interception in low light environments (Jurik 1986, Reich et al. 1991, Abrams et al. 1994, Abrams 1998). However, in this study, seedlings were not limited by overstory shading and had a limited range of specific leaf area measurements. This absence of a significant correlation between specific leaf area and photosynthesis could be attributed to species differences in foliar nitrogen content, which plays a critical role in photosynthetic capacity, particularly for open-grown seedlings (Jurik 1986, Olszyk et al. 1998, Hawkins et al. 1999, Niinemets 1999, Slaton and Smith 2002, Montgomery 2004).

High foliar nitrogen content is often strongly related to increased photosynthetic rates because nitrogen is an important component of the two central biochemical determinants of photosynthetic capacity: chlorophyll, the primary receptor of solar energy and Rubisco, the primary carboxylation enzyme (Jurik 1986, Reich et al. 1991, Walters et al. 1993b, Abrams et al. 1994, Abrams 1998, Reich et al. 1998a, Poorter 1999). Foliar nitrogen did not show any significant response to warming or irrigation treatments, suggesting that the seedlings did not alter the allocation of structural components in leaf tissues when grown under warming or irrigation. Additionally, when photosynthetic rates were compared to foliar nitrogen values, this study did not result in consistent positive relationship across all study species. The two southern species, Quercus stellata and Liquidambar styraciflua, were the only species with increasing leaf-level photosynthesis corresponding to increasing foliar nitrogen. Although Quercus stellata had higher photosynthetic responses for a given foliar nitrogen level on the warmed treatment plots,
the relationship between the responses on warmed and unwarmed plots were significantly different. This suggests that *Quercus stellata* seedlings are capable of increasing their photosynthetic capacity at given foliar nitrogen levels as temperatures on the warmed treatment plots increased (Reich et al. 1990, Abrams and Mostoller 1995, Niinemets 1997, Abrams 1998, Reich et al. 1998b). Since increased temperatures can increase the efficiency of photosynthetic responses through increased enzymatic activity, it is expected that a species with low foliar nitrogen content would not experience the same level of photosynthetic response that species with higher nitrogen content display. However, the ability of young seedlings to utilize physiological and morphological tradeoffs to maintain photosynthetic capacity at the leaf-level also grants them the ability to respond to increased temperature and precipitation at the whole seedling scale.

**Physiological responses to differences in biomass allocation**

While leaf-level measurements provide an easily accessible means to determine basic plant responses, it is important to consider the structure and function of the entire seedling when working to determine their overall response of increasing temperature and precipitation. Warming treatments increased whole-plant estimates of photosynthesis, foliar carbon and nitrogen. This result was not unexpected given the 213% increase in leaf biomass for seedlings grown on warmed treatment plots, which would lead to these corresponding increases as leaf-level values were scaled up to whole seedling biomass. Montgomery (2004) found that leaf-level assimilation scaled by whole plant leaf area is more tightly correlated with whole plant carbon gain rather than with leaf-level photosynthesis alone. When considering whole plant traits, such as leaf area and branching architecture, it is expected that the relationship between leaf and whole plant photosynthesis may differ, even if seedlings do not vary in their leaf-level photosynthesis rates, foliar nitrogen content or leaf area, as a result of self-shading which reduces light penetration to lower leaves reducing overall photosynthetic responses (Montgomery 1999).
Whole-seedling estimates for *Quercus rubra* were used to compare photosynthetic capacity and foliar nitrogen; a change in the slope of the leaf-level relationship suggests that the increase in leaf biomass and leaf area allowed *Quercus rubra* seedlings to increase the total photosynthetic rates through increased leaf tissues, rather than increased foliar nitrogen concentrations. Similar results have suggested that in response to increased light, species that are more architecturally responsive in branch number, leaf number or total leaf area will be able to increase their total photosynthetic capacity (Kolb et al. 1990). Whole seedling responses that can be scaled up using total leaf area or leaf biomass can be used to evaluate seedling and species level responses to increased temperature and precipitation.

For seedlings in this study there was a positive relationship between increasing relative growth rate and increased whole seedling photosynthetic capacity ($A_{\text{area}}$). The overall increase in relative growth rate under warmed treatment conditions suggests that seedlings may be capable of achieving greater photosynthetic capacities as a result of morphological and physiological adjustments that enable greater carbon gains and corresponding increases in biomass allocation that further enable them to maximize growth rates. There is evidence to support the importance of morphological and physiological traits, such as photosynthesis and net assimilation rates, in explaining seedling growth responses (Sipe and Bazzaz 1994, Kubiske et al. 1997, Muraoka et al. 2003, Montgomery 2004). As temperature increases, photosynthetic capacity also increases as a result of the increased efficiency of the enzymes responsible for carbon fixation (Sipe and Bazzaz 1994, 1995, Bassow and Bazzaz 1997, Abrams 1998, Beaudet et al. 2000, Muth and Bazzaz 2003). Additionally, seedlings grown in high light environments maintain a high photosynthetic capacity and increased maximum assimilation rate to meet the high energy demands of building and maintaining the morphological structures required to support the increased above and below ground growth.
**Acer rubrum: leaf traits and physiological responses to climate treatments**

Abrams (1998) defined *Acer rubrum* as a “super-generalist” with low resource requirements and the ability to function reasonably well under a range of environmental conditions. While *Acer rubrum* has the ability to tolerate environmental stress and maintain physiological activity, there is not clear evidence for a single trait that enables this species to be successful under such wide ranging environmental conditions. The result of this study support that idea that *Acer rubrum* invests fewer resources to the construction of individual leaves and provides one possible explanation for the lack of variation in leaf-level photosynthetic measurements in response to the increased warming and irrigation treatments. This reduced resource investment was seen in the difference in foliar carbon content coupled with the observed differences in specific leaf area (Figure 5.3) suggests that leaf mass and density influence the amount of carbon that seedlings invest in their photosynthetically active tissues. Both foliar carbon and nitrogen content varied little, which also coincided with the low photosynthetic rates measured among the study seedlings across treatments. Abrams (1998) attributed the low photosynthetic rates of *Acer rubrum* across a range of site conditions to be the result of modest leaf morphological characteristics and low nutrient levels, which is consistent with the measured responses in this study across both warmed and irrigated treatments. This low photosynthetic rate was also observed on warmed treatment plots in this study. The decreasing leaf-level photosynthetic rate found with increasing foliar nitrogen were consistent with the leaf structural and physiological responses observed in this study and suggests that there are other factors influencing the physiological response of this species.

*Acer rubrum* had the strongest positive relationship between whole seedling photosynthesis and total foliar nitrogen on warmed plots and although the relationship on the unwarmed plots was not significant. This species appears to overcome modest leaf-level carbon gains through whole seedling investments. This change in the photosynthetic response for this species is
supported by field observations and measurements that demonstrate that *Acer rubrum* can survive and thrive on a variety of sites and exhibits a suite of morphological and ecophysiological characteristics that make it difficult to use a single leaf-level trait as a measure of success or failure under manipulated climate change treatments (Abrams 1998). The results of this field study provide evidence that *Acer rubrum* can maintain competitive whole seedling levels of photosynthetic activity in response to increasing temperatures, which may enable this species to maintain its current importance in central Pennsylvania forests as climate continues to change.

**Conclusion**

Overall, the morphology and physiology of whole seedlings responded to warming as total biomass on those plots also increased. Increased temperatures resulted in an increase in photosynthesis although it was not the result of corresponding increases in foliar N. However, when the leaf-level responses were scaled up to incorporate the increased foliar biomass, seedlings grown under all treatment types displayed the expected positive relationship between whole-seedling photosynthetic estimates and whole-seedling foliar N, emphasizing the importance of leaf-level resource allocation and the role it plays in controlling whole-seedling growth and physiological responses. *Acer rubrum* is an example of how the leaf-level photosynthetic and foliar N responses fail to reflect the species’ ability to maintain competitive whole-seedling levels of photosynthesis through modest resource investments across a increasing foliar biomass regardless of the implemented climate treatments further emphasizing the ability of broadly distributed temperate species to tolerate moderate climate changes. Warming also showed strong positive correlations between relative growth rate and photosynthetic capacity, suggesting that the increase in temperature facilitated an increase in assimilation and growth for the study species. Although single leaf-level and whole seedling morphological and physiological variables did provide some explanation for the change in total seedling growth and biomass, leaf-
level traits as they are related to photosynthetic response do not provide a complete explanation of the observed patterns under manipulated climate change treatments.
Chapter 6

Water relations, gas exchange and morphology of deciduous tree seedlings after three years of increased temperature and precipitation manipulations.

Introduction

As climate changes, seedlings must be able to exhibit plasticity in growth, photosynthetic capacity and leaf water relations to compete in changing habitat conditions (Reich et al. 1990, Abrams and Mostoller 1995, Abrams 1998). Photosynthetic activity is influenced by stomatal control, regulating the rate of CO\(_2\) influx and water lost through transpiration, and can be affected by both environmental factors and internal signaling (Bazzaz and Sipe 1987). In general, stomata open as light availability increases, which allows for an increase in gas exchange for photosynthesis. However, under high light conditions, increasing leaf temperatures and evapotranspirative demands may result in stomatal closure and increase the risk of heat or water stress photoinhibition (Farquhar and Cowan 1974, Raschke 1975, Jarvis and Davies 1998, Jones 1998). This tradeoff to maintain high photosynthetic carbon gains required for rapid growth while balancing leaf water loss through transpiration is necessary for plants to avoid developing stressful internal water deficits (Ludlow and Björkman 1984, Smith et al. 1989). Stomatal conductance and photosynthesis may vary with morphological characteristics of seedling growth, such as leaf thickness or stomatal size and number, which can impact gas exchange rates during stressful conditions such as high temperatures or altered precipitation patterns (Cowan 1978, Tilman 1988, Loreto and Sharkey 1990, Chaves 1991, Ort et al. 1994, Cornic and Massacci 1996, Flexas and Medrano 2002).

Tradeoffs in biomass allocation are often linked to the ability of a plant to tolerate or exploit changing environmental conditions through altered growth and photosynthetic responses (Kubiske and Abrams 1992, Chapin et al. 1993, Kloeppe1 et al. 1993, Abrams et al. 1994,
Dickson and Tomlinson 1996). Increased growth and changes in the allocation of biomass to roots, stems and leaves can alter the demands for photosynthetic carbon gains and water uptake. Increased carbon allocation to root systems of seedlings in open, high light environments may have the capacity to improve the overall plant water status by increasing the below-ground surface area for water absorption (Tilman 1988). An increase in aboveground biomass allocation, particularly in leaves, can result in a greater potential for carbon gain through increased photosynthetic capacity. However, if this increase in leaf mass does not result in a corresponding increase in root biomass, it may result in a limitation for the capacity of water uptake and increase the potential of seedling water stress. Schwinning and Ehleringer (2001) found that plant water conductance does not limit whole plant carbon gain when root:shoot ratios are large, but small root:shoot ratios decrease plant water potential, leaf conductance and ultimately, whole-plant growth, despite the greater leaf area. While changes in seedling morphology and biomass allocation can influence the ability of seedlings to acquire resources necessary for growth, understanding the mechanisms that drive these changes in growth are crucial as changing environmental conditions alter the responses of developing seedlings.

The ability of seedlings to cope with changing environmental conditions, like increasing temperature or changes in precipitation regimes, through changes in gas exchange and tissue water relations is critically important in mediating environmental stress (Schwinning and Ehleringer 2001). Gas exchange responses to leaf water potential are well documented, with changes in leaf water potential driving stomatal closure in a relatively large number of species subjected to declining soil moisture (Abrams et al. 1990, Kozlowski et al. 1991, Ni and Pallardy 1992, Pallardy and Rhoads 1993, Abrams 1994, Givnish 1995, Dickson and Tomlinson 1996, Hanson and Weltzin 2000). Predawn water status, or soil-to-leaf hydraulic conductance, and stomatal response are strongly influenced by soil and root water status (Reich and Hinckley 1989, Ellsworth and Reich 1992, Abrams et al. 1994, Schwinning and Ehleringer 2001). Plant water potential is strongly correlated to gas exchange, particularly predawn water status, since water
stressed individuals are less likely to achieve high photosynthetic rates as soil water availability declines over the course of the day (Reich and Hinckley 1989). As soil water availability decreases or evapotranspiration demands increase, differences in plant water relations can be used to identify species variability in gas exchange through osmotic adjustment and stomatal conductance (Ni and Pallardy 1991, Kleiner et al. 1992, Abrams et al. 1994). Comparisons of species-specific gas exchange response to predawn water status can also be used to draw connections between physiological process, like photosynthesis, and growth, which are crucial in determining successful establishment in developing forest stands.

Previous chapters already have demonstrated that warming results in greater total seedling biomass and photosynthetic rates. I wanted to determine if physiological responses and biomass gains were influenced by changing water relations under increased temperature and/or precipitation. The objective of this chapter was to investigate the relationship among biomass allocation, leaf gas exchange and water relations in seven early successional species grown under increased temperature and precipitation treatments designed to simulate predicted climate change conditions in central Pennsylvania. I hypothesized that seedlings grown under warmer and wetter conditions would alter their morphology by increasing root growth and maintaining gas exchange activities, while regulating water loss through stomatal conductance. Root growth and biomass allocation were previously discussed in Chapter 4 and it was found that warming increased the total belowground biomass of the study seedlings, but did not alter the root length or proportion of total seedling biomass allocated to the coarse root system. The current hypothesis will be tested by comparing plot-level fine root biomass, belowground allocation responses, and root: shoot biomass with seedling water relations and leaf-level gas exchange measurements.
Methods

Field Study Design

Seedlings from selected eastern trees were monitored in a three-year climate change study at the Stone Valley Forest in Huntingdon County, PA (40N 85’, 77W 83’). The site was located at the interface between the Northeast and Mid-Atlantic regions, on the boundary of two dominant forest types: maple-beech-birch and oak-hickory. Average annual mean temperatures at the site are 9 °C with an average annual maximum of 15 °C and minimum of 4 °C throughout the year, with a range from 27°C in July to -8°C in January. Mean annual precipitation is approximately 1000 mm. The soils at the site are Oxyaquic Fragiaudalfs in the Clarksburg series. The site is situated on a southeast-facing slope. A four-hectare area was clear-cut and fenced in August 2007 and 16 plots were established in a 2x2 factorial design with four treatments (ambient, irrigated, warmed, and warmed+irrigated).

Field warming treatments had a constant target temperature increase of 1°C during the day and 3°C at night when compared to unwarmed plots, resulting in an average temperature increase of 1.8°C (+/- 0.29°C) from May 2008 through August 2010. Predicted changes in precipitation were simulated by adding +23% precipitation above unwatered plots over the course of the experiment, with monthly precipitation applications ranging from 12 to 43%. The quantity of water (liquid, snow or ice) that was applied weekly to the irrigated plots was determined based on long-term historical averages (Chapter 2: Experimental Methods and Set-up). The irrigation amount was recorded and the percent effective irrigation treatment was calculated by week, month, year, and experiment duration by dividing the irrigated treatment amount by the ambient precipitation.

Soil volumetric water content measurements were taken every two weeks during the early months of the 2010 growing season (Julian day 65-170, or March - June) using a Theta Probe –
type ML2x (Delta-T Devices, Cambridge, England) on each plot. At each plot three measurements were taken within the inner 25 cm boarder and the mean volumetric water content value was calculated (Figure 6.1).

**Water potential**

Differences in soil water availability were assessed using plant water potential measurements for three-year-old seedlings across four manipulated temperature and precipitation treatments. Predawn (Ψpd) and midday (Ψmd) leaf water potential were measured on single sampling dates in May, June and July 2010. Leaf water potentials were measured at predawn between 0300 and 0600 on a single leaf from selected three-year-old seedlings in each treatment type using a pressure chamber (PMS Instrument Co., Corvallis, OR, USA) in the week following gas exchange measurements. During the predawn sampling a second leaf from each seedling was prepared for midday sampling by covering it in aluminum foil and sealing in a plastic bag while still attached to the seedling. Midday water potential was measured between 1200 and 1500 solar time using the previously prepared leaf from each seedling. All leaves were severed at the petiole near the point of branch attachment using a razor blade, before being placed into the pressure chamber.

**Seedling biomass**

In August 2010 all above- and below-ground biomass was removed from the study plot and brought back to the lab where they were deconstructed into leaves, stems, and roots. Final height and basal diameter measurements were taken for aboveground stems of each seedling and fresh weights for each component were recorded. Fresh leaf mass was obtained for each seedling and
leaf area was measured using a portable leaf area meter (Li-Cor Inc. Lincoln, NE). Following all fresh measurements, seedling components were dried in a forced-air oven at 60°C for a minimum of three days and then re-weighed for final dry plant mass values. Specific leaf area was determined for each individual seedling by dividing the oven dry mass by area (cm² g⁻¹).

Plot-level fine root biomass was measured at the time of harvest using 5 soil cores taken with an AMS hammer (AMS Inc. American Falls, ID), prior to the excavation of the seedlings. Cores were taken 0.5 meters in from each corner. Cores were 5 cm in diameter and taken to a depth of 15 cm. Samples were not taken from warmed+irrigated or irrigated plots in Block 1 because soils were heavily disturbed by seedling harvest in these plots before it was decided to collect soil cores. Soil cores were homogenized in the field and brought back to the laboratory and allowed to air dry. Soil samples were sieved to remove soil and separate root by diameter class. The remaining roots were washed and allowed to air dry before being oven dried at 60°C for a minimum of three days and re-weighed.

Loss on ignition (LOI) for the calculation of percent organic matter was performed on root samples. Oven dried fine root samples were placed into aluminum tins and oven dried at 60°C and allowed to cool in a desiccator before being weighed for initial mass. Cooled sampled were then placed into a muffle furnace set at 450°C and allowed to remain in the oven for 16 hours. Samples were then cooled to 200°C before being removed and allowed to cool to room temperature in a desiccator. Samples were re-weighed for final mass. Loss on ignition percentage was calculated using the following formula ((initial mass-final mass)/initial mass)*100. This percentage was then used to calculate the dry mass of the fine root biomass to determine the amount of below ground carbon by treatment in the absence of soil aggregates.
Gas Exchange

Within the 16 study plots, seedlings of each species present were selected for gas exchange measurements during the 2010 growing seasons. Gas exchange measurements were repeated every four weeks during June and July. Measurements of photosynthetic CO₂ assimilation and leaf-level conductance were made on fully expanded leaves with a portable leaf chamber and infrared gas analyzer (LiCor 6400, Li-Cor Inc. Lincoln, NE) under full sunlight from 0900-1500, local time. These measurements were made under similar conditions (full sunlight during the same daylight hours) during each of the sample dates. For each sampling date, three leaves were measured per seedling, and these measurements were averaged for a seedling value to be used in subsequent analyses. Selected seedlings were re-sampled each month, or as long as they were present on the plot. When a seedling was lost due to mortality, it was replaced with another seedling of the same species from the treatment plot if one was present, resulting in 73 three-old seedlings from the seven study species being sampled during the 2010 growing season (Appendix Table A9).

Data Analysis

For species comparisons of pre-dawn and mid-day water potential and root:shoot ratios, data from each individual seedling was averaged by species for each treatment plot and plot-level values were then used to calculate the final treatment-level averages for each species. Species were included in this analysis if there were seedlings present in a minimum of two ambient plots as well as being distributed across the climate treatment plots. The species included in this analysis are Quercus rubra, Fraxinus americana, Acer rubrum, Quercus alba, Quercus velutina, Liquidambar styraciflua and Quercus stellata. Species distributions across the treatment types for the analysis in this chapter can be found in Appendix table A9. Inconsistent germination and
seedling mortality due to stressors such as drought, browsing, and defoliation reduced the number of seedlings present during the third year of gas exchange and water status measurements, therefore not all species could be included in final analyses. Comparisons of both treatment-level fine root biomass and species-specific root:shoot ratios were represented as a deviation from ambient, which standardized the response of each treatment to the control for each block before treatment or species-specific averages were calculated.

Treatment level fine root biomass and species-specific root:shoot ratios were analyzed using a mixed model analysis of variance (ANOVA) for a randomized complete-block, split-plot design. For the three-year-old seedlings of the seven study species, tests for treatment (species*warming*irrigation), main effects, and interactions on seedling growth and morphology were completed using an analysis of variance (ANOVA) mixed model procedure in SAS (SAS institute, Cary NC). When main effects were significant, means were compared with tests of least squares means. For the seasonal measurements of pre-dawn and mid-day plant water status a repeated-measures ANOVA was used to determine the effects of treatment, species and month of sampling on seedling water status. When single factor effects (warming, irrigation or species) were found to be significant, seedling response data was combine across the single factor for further comparison of the resulting responses. The interrelationship among pre-dawn plant water status, gas exchange and warming treatment (warmed versus unwarmed plots) on photosynthesis was analyzed using linear regression analysis and analysis of covariance (ANCOVA) to test for similarity of slope. All statistical comparisons were considered significant at $\alpha=0.05$. 
Results

Seedling Water Status

On average, lower predawn water potential ($\Psi_{pd}$) measurements were recorded for all seedlings on warmed treatments plots ($p=0.0083$; Appendix Table C6) over three months of the growing season (May: -0.28±0.05 MPa, June: -0.48±0.08 MPa, July: -0.66±0.08 MPa) when compared to unwarmed plots in the same period (May: -0.03±0.01 MPa, June: -0.46±0.2 MPa, July: -0.37±0.07 MPa) (Figure 6.2). Differences in midday water potential ($\Psi_{md}$) were similarly affected by the warming treatments (May: -0.48±0.05 MPa, June: -1.2±0.1 MPa, July: -1.5±0.2 MPa) when compared to the unwarmed treatment plots (May: -0.11±0.05 MPa, June: -0.79±0.1 MPa, July: -1.0±0.1 MPa), decreasing over the three month sampling period ($p=0.002$; Appendix Table C6) (Figure 6.3). While there was no significant interaction between species and warming for midday water potentials ($p=0.55$; Appendix Table C6), moderate to severe water deficits occurred on warmed plots. Moderate midday water deficits, defined when $\Psi < -1.0$ MPa, were observed for most species on warmed plots.

![Figure 6.1: Volumetric water content for March 2010 – June 2010 based on hand probe measurements from planted plots. Error bars represent ± SE of the mean. Filled circles (●) represent warmed treatment plots, filled squares (■) represent warmed+irrigated treatment plots, open diamonds (◇) represent ambient treatment plots and open triangles (△) represent irrigated treatment plots.](image-url)
Figure 6.2: Seasonal patterns of pre-dawn water potential ($\Psi_{pd}$) for the 7 study species for the 2010 growing season. Error bars represent ± SE of the mean. Filled circles (●) represent warmed treatment plots, filled squares (■) represent warmed+irrigated treatment plots, open diamonds (◇) represent ambient treatment plots and open triangles (△) represent irrigated treatment plots.
Figure 6.3: Seasonal patterns for mid-day water potential ($\Psi_{mad}$) for the 7 study species for the 2010 growing season. Error bars represent SE of the mean. Filled circles (●) represent warmed treatment plots, filled squares (■) represent warmed+irrigated treatment plots, open diamonds (◇) represent ambient treatment plots and open triangles (△) represent irrigated treatment plots.
Pre-dawn water potentials decreased significantly over the course of the three sampling dates and differed by species (p<0.0001; Appendix Table C6). In general, non-oak species (*Acer rubrum*, *Fraxinus americana* and *Liquidambar styraciflua*) displayed a range of average pre-dawn water potential levels (May: -0.15± 0.05 MPa; June: -0.4 ±0.1 MPa; July: -0.5 ±0.1 MPa) over the three sampled dates in the 2010 growing season. Species changes in midday water potential were significantly different regardless of treatment (p<0.0001; Appendix Table C6), with lower water potentials being recorded at the end of the 2010 sampling period. *Quercus stellata* experienced mid-day values ranging on average, from -0.2 to -1.6 MPa over the three sampling dates (May: -0.2± 0.08 MPa; June: -0.9± 0.2 MPa; July: -1.6± 0.3 MPa). Severe July midday water deficits, defined when Ψ < -2.0 MPa, occurred in both *Quercus velutina* (warmed, Ψ$_{\text{md}}$= -2.0 ±0.32 MPa) and *Quercus stellata* (warmed+irrigated, Ψ$_{\text{md}}$= -2.1±0.33 MPa). Additional plot, treatment and block averages of raw monthly data can be found by species in Appendix Table B15-20.

**Root and Shoot Morphology**

Increase in root:shoot ratio on warmed plots was not found (Figure 6.4) and can be attributed to the greater above ground growth of woody stem tissue, which significantly increased with total seedling biomass increases on warmed plots (warmed: 164.9 ± 34 g; unwarmed: 68.2 ±22 g; p=0.012) (Figure 4.1). There was no significant difference in the average root length of the study species on warmed or unwarmed plots (warmed: 26.2 ±1 cm; p =0.064 and unwarmed: 21.4 ±2 cm; p=0.243, respectively) (Figure 4.3). One notable change in the root development of the seedlings was the change in the relationship between relative root length, or the ratio of average root length in relation to seedling relative growth rates, with seedlings producing shorter, thicker roots as growth increased to support increasing overall biomass and longer, thinner roots when seedlings were small (Figure 4.5). Plot-level fine root biomass (<2 mm diameter)
displayed no significant difference (p>0.05) among the four treatment types (Figure 6.5). When
the fine root samples were combusted for loss on ignition to determine carbon content, there were
also no significant differences in the fine root biomass remaining following loss on ignition
(p=0.259 irrigated and p=0.571 warmed) (Figure 6.5). The model outputs including degrees of
freedom, F-values and P-values are listed in ANOVA tables (Appendix table C6).

Figure 6.4: Root:Shoot represented as a deviation from ambient for seedlings used in
gas exchange and water potential sampling. Error bars represent ± SE of the mean.

Figure 6.5: Comparison of total dry weight of fine root samples following loss on
ignition. No significant differences were found between treatment types (irrigated:
p=0.2593; warmed: p=0.5710 and warmed+irrigated: p=0.3712. Error bars represent ±
SE of the mean.
Gas Exchange and Plant Water Potential

Results from previous chapters showed that warming significantly increased the photosynthetic rates for all seedlings (p=0.024) (Figure 5.1). However, there were not strong correlations between photosynthetic rates and predawn $\Psi_{pd}$ despite the significant differences between warmed and unwarmed treatment plots for July predawn water potential and photosynthetic rates when compared as independent responses (Figure 6.6). Only *Quercus stellata* had a significant correlation between pre-dawn water potential and photosynthetic rate ($R^2=0.57$, $p=0.045$) in warmed plots, indicating that this drought tolerant species had a higher photosynthetic rates at lower predawn water potentials ($\Psi_{pd}$). The correlation between predawn water potential and photosynthetic rates was not observed for *Quercus stellata* seedlings on the unwarmed plots ($R^2=0.03$, $p=0.73$), where predawn values ranged from 0 to -0.4 MPa and photosynthesis rates increased as water potential values were closer to 0 MPa. Although there was a lack of significant correlations between predawn water potential and photosynthetic rates, for most species photosynthetic rates were consistent across a limited range of predawn water potentials. This was particularly true for both *Acer rubrum* and *Quercus alba*, which maintained consistent rates of photosynthesis across a range of soil water availabilities in both warmed ($R^2=0.02$ $p=0.82$; $R^2=0.00$ $p=0.70$ respectively), and unwarmed treatment plots ($R^2=0.01$ $p=0.77$; $R^2=0.01$ $p=0.97$ respectively).
Figure 6.6: Correlation of leaf-level photosynthetic rates (A) measured during full sunlight hours (0900-1500) to predawn water potential (-Ψpd) to for species on warmed (warmed and warmed+irrigated) treatment plots and unwarmed (ambient and irrigated) treatment plots. Black circles (●) represent seedlings are grouped by warmed (warmed and warmed+irrigated) and open circles (○) represent unwarmed (ambient and irrigated). R² values for each regression line are indicated next to the treatment type for each species. Significance for each regression line is indicated next to R² as not significant (ns, p>0.05) or significant (*, p<0.05 or **, p<0.001).
Daily stomatal conductance (g) was expected to decrease as soil water availability decreased, particularly for less drought tolerant species such as *Acer rubrum*, *Fraxinus americana* and *Liquidambar styraciflua*. The more drought tolerant *Quercus* species were expected to maintain stomatal conductance under drying soil conditions. Instead stomatal conductance was not significantly correlated with predawn water potential ($\Psi_{pd}$) for any species on warmed or unwarmed treatments (Figure 6.7). The response of stomatal conductance to decreasing water potentials for *Acer rubrum* were similar to the photosynthetic response of the species, maintaining a consistent level of conductance regardless of the soil moisture availability (unwarmed: $R^2=0.01$, $p=0.90$; warmed: $R^2=0.05$, $p=0.62$). This suggests that the photosynthetic response may not have been tightly controlled by the predawn soil water availability when $\Psi_{pd}$ is above -1.2 MPa. Instead, there was an absence of clear relationships between stomatal conductance and predawn soil water availability, which suggests that the significant increase in photosynthetic rates on the warmed plots was not limited by soil water availability and morphological development on the response of gas exchange.
Figure 6.7: Correlation of conductance to water vapor (g) obtained during gas exchange measurements taken during full sunlight hours (0900-1500) to predawn water potential (-Ψpd) for species on warmed (warmed and warmed+irrigated) treatment plots and unwarmed (ambient and irrigated) treatment plots. Black circles (●) represent seedlings grouped by warmed (warmed and warmed+irrigated) and open circles (○) represent unwarmed (ambient and irrigated). R² values for each regression line are indicated next to the treatment type for each species. Significance for each regression line is indicated next to R² as not significant (ns, p>0.05) or significant (*, p<0.05 or **, p<0.001).
Discussion

Increases in growing season temperature and precipitation were expected to alter the plant-soil water relations to provide a mechanistic explanation for increases in gas exchange and growth that were observed in the study seedlings on the warmed plots. During the 2010 growing season, soils became drier as the growing season progressed from the time of the spring thaw in early April through the peak of seedling leaf-out in mid-June. This seasonal depression in measured soil water content often coincides with increasing plant photosynthetic activity (Ellsworth and Reich 1992). As a result of the decreasing soil water availability it was expected that plant tissue water potentials would reflect the seasonal decrease in available soil moisture.

Lower predawn water potentials, measured under increasing temperatures, may serve as an indicator of plant water stress and can be the result of differences in soil-water conditions, night-time temperatures or variability in water extraction capabilities of the study species (Kubiske and Abrams 1992, Nielsen and Jørgensen 2003, Baldocchi et al. 2004). When plant water relations were compared across treatments for seven study species, similar patterns of decreasing predawn water potentials were observed, particularly for the warmed treatment plots as soil moisture availability declined. In contrast, most *Quercus* species were not found to vary significantly in their predawn water potentials between warmed and unwarmed treatment plots as the growing season progressed, which suggests that these more drought tolerant species may have greater recovery capabilities that allow for increased soil water extraction in the predawn hours (Ellsworth and Reich 1992, Sellin 1999, Leuzinger et al. 2005).

While declines in predawn water status suggested reduced ability for plant water status to recover under declining soil water availability, similar decreases in the midday water potentials were also observed among the seedlings grown under increased temperatures. The significant difference between July midday water potentials for most species suggests that warming increased the demands for plant water use. However, it is important to note that declining midday
leaf water potentials do not always indicate plant water stress. Low midday water potentials can occur as a result of either rapidly transpiring, well-watered individuals or through a water stressed individual with partially closed stomata (Reich and Hinckley 1989, Abrams 1990, Bréda et al. 1995, Caspersen and Kobe 2001). *Quercus stellata* had similar deficits on both the warmed and unwarmed treatment plots suggesting that these seedlings maintained physiological activity at lower water potentials, regardless of the treatment conditions. As mature trees, *Quercus* species are considered to be drought tolerant, maintaining photosynthetic activities at low soil water availability, through physiological and morphological adaptations, such as deep roots and osmotic adjustments (Reich and Hinckley 1989, Abrams and Mostoller 1995, Bond and Kavanagh 1999, Williams and Araujo 2002).

**Root and Shoot Morphology**

As temperature and precipitation increased, seedlings were expected to alter the ratio in of biomass allocation to roots to compensate for the lower soil water availability. In general, decreasing soil water availability is strongly correlated to shifts in biomass allocation as plants increase root biomass and decrease leaf production to limit water loss (Kubiske and Abrams 1992, Chapin et al. 1993, Kloeppe1 et al. 1993, Abrams et al. 1994, Dickson and Tomlinson 1996, Hanson and Weltzin 2000, Thomas and Gausling 2000). However, in this study seedlings reduced root:shoot ratios with decreasing water availability through an increases in aboveground woody stem tissue. Increases in total root biomass occurred for warm treatment seedlings, although there was no significant increase in the root length for any species or treatment, suggesting that changes in biomass allocation was not being driven by changes in soil water availability alone.

Rooting length often increases on water limited sites, as plants increase the surface area available for water absorption to maintain physiological function (Caldwell and Richards 1986, Bongarten and Teskey 1987, Nilsson and Albrektson 1993, Pallardy and Rhoads 1993, Joslin et
al. 2000). It was expected that seedlings with obligate taproot formations, such as *Quercus* species, would have greater root length and root length per unit biomass when compared to non-taproot forming species. However, I found no difference in the root length or root length ratio, or root length to seedling biomass ratio, values among species. This generalized increase in growth in response to moderate warming, coupled with minimal species variability, could be attributed to the physical limitations of seasonal biomass accumulation in young trees (Eissenstat 1991, Pallardy and Rhoads 1993, van Hees 1997, Joslin et al. 2000). Although there were differences in total seedling root biomass in warming treatments, when treatment-level fine root biomass was compared across the four treatment types, no significant difference was observed. Joslin *et al.* (Sipe and Bazzaz 1994, Canham et al. 1999, Caspersen et al. 2000) observed similar constant fine root mass over the course of a five year precipitation manipulation study and attributed this to the resilience of the temperate forest ecosystem to maintain fine root production even during periods of water limitation. The ability of seedlings to maintain biomass production, even as soil water availability declined over the growing season, suggests that the study species possess plasticity in their physiological responses that allow them to maintain gas exchange activities required for carbon fixation and growth (Joslin et al. 2000).

**Gas Exchange and Plant Water Potential**

Gas exchange is particularly sensitive to soil moisture availability and the ability of species to maintain physiological activity under increasing soil water deficits has been attributed to their degree of drought-tolerance (Abrams 1988, Canham et al. 1996, Sack and Grubb 2002, Sack 2004) (Ni and Pallardy 1991, Ellsworth and Reich 1992). While the expectations of changes in root length, fine root biomass and root:shoot ratio responses to the manipulated climate treatment were not observed, there were significant gains in total seedling biomass on warmed plots that were the result, in part, of increasing photosynthetic rates (Figure 5.1).
Predawn water status is often highly correlated to the photosynthetic rate and stomatal conductance of plants (Gebre et al. 1998, Aranda et al. 2005). It was hypothesized that photosynthetic rates would decline with increasingly negative predawn water potentials as seedlings limited photosynthetic activity to preserve plant water status. Instead it was found that there was no significant correlation between leaf-level photosynthetic rates and predawn water potential for most species and treatments. While predawn water potential declined in July for most species, it did not coincide with a reduction in the leaf-level photosynthetic rates on warmed treatment plots. This suggests that soil water deficits that were observed to be significantly lower on warmed plots were not low enough to impair photosynthesis in the study seedlings, regardless of species drought tolerance. One exception was the response of *Quercus stellata*, a highly drought tolerant species, which had increasing photosynthetic rates as predawn soil water deficits increased under warmed conditions. Drought tolerant *Quercus* species are capable of osmotic adjustment, or a decrease in osmotic potential in response to water deficits, which has been associated with the capacity for sustained stomatal conductance, photosynthesis and growth during periods of water stress (Rambal 1984, Ellsworth and Reich 1992, Gallego et al. 1994). In contrast to this drought adapted response, *Acer rubrum* displayed a relatively consistent photosynthetic response, regardless of the measured values of predawn water potential. This result continues to support previous findings that this species is highly flexible across the imposed climate treatments and is able to maintain relatively constant photosynthetic activity across a range of environmental conditions (Wilson et al. 1980, Turner 1986, Parker and Pallardy 1988, Abrams 1990, Kubiske and Abrams 1992, Pallardy and Rhoads 1993).

Similar declines in conductance, with increased soil water deficits, were expected on warmed treatment plots if seedlings were experiencing water limitations that impaired their physiological activities. Instead, it was found that there were no significant relationships between predawn plant water potential and conductance, which often occur under increasing temperature or decreasing soil water availability (Abrams and Kubiske 1990, Houle 1994, Abrams and
which had high photosynthetic rates on warmed plots, did not show significant changes in stomatal conductance in response to declining predawn water potentials. This is further supported by the increased photosynthetic rates of seedlings on warmed plots (Figure 5.1), which indicates that the decreasing plant water potential could, in fact, be the result of active gas exchange activity and transpirational water loss, as opposed to water stress. The responsiveness of seedling stomatal conductance does not seem to be strongly influenced by predawn water status, regardless of warming or irrigation treatment.

Conclusions

Plant water relations emphasize the balance of resource availability and physiological responsiveness that seedlings require to cope with changing environmental conditions. After three years of growth under warmer temperatures, seedling photosynthesis and growth increased, regardless of soil water status, for all seven study species. While predawn water potential can be used to highlight changes in plant water stress, if a system is not strongly water limited the relationship between these variables becomes less correlated, which was the observed response in this study. It was expected that declining soil moisture would result in changes in seedling morphology and physiology, but the seedlings grown on the treatment plots displayed few significant changes in their responses to the increased temperature or precipitation manipulations. Warming was decreased seedling water potentials, however these decreases did not correspond to declines in biomass allocation (root:shoot), photosynthetic rates or stomatal conductance responses. Instead, it was found that early seedling growth was not limited by changes in plant water potential and the increased rates of photosynthesis are potential cause for the decreases in predawn water potentials observed over the course of the 2010 growing season. It is important to consider that these study species are broadly distributed and can often be found in high resource
environments, like a post-harvest forest, which are generally associated with higher plasticity in the range of responses to climate change (Ellsworth and Reich 1992, Thomas and Eamus 1999).
Chapter 7

Synthesis

As global climate continues to change, field-based studies of the seedling-stage of forest development are crucial for understanding the relationship between climate change and future forest dynamics. Increases in temperature stimulate tree growth through interaction with soil water and nutrient availability (Meinzer 2002, Adams et al. 2009). In natural environments, the allocation of resources and partitioning of biomass among seedlings is often a response that balances available resources with the demands of biotic and abiotic stressors (Tilman 1993). Understanding what factors control and influence growth under the spatial and temporal heterogeneity of natural field studies is a complex problem that has been a focus of ecological studies ranging from genetic characteristics of traits (Brand 1990, Lopushinsky and Max 1990, Graves and Aiello 1997, Tjoelker et al. 1998, Saxe et al. 2001, Gunderson et al. 2010), to phenotypical expression and plasticity of morphological characteristics and physiological responses (Walters et al. 1993b) and ecosystem-scale community responses (Bradshaw-Jr and Stettler 1995, Dickson et al. 1998, Aitken et al. 2008). Quantifying these factors is a necessary step in developing effective statistical models that will enable more accurate predictions of species’ responses to regional and global climate change (Valladares et al. 2000, Vitasse et al. 2010). Small-scale field studies provide a first step in determining how changes in morphology and physiology may drive changes in seedling growth rates under a range of manipulated climate conditions (O’Neill et al. 2008, Sagnard et al. 2010).

Climate change experiments have the potential to isolate the relative influence of a range of manipulated climate variables on the phenology, morphology and physiology of both individual species and community interactions (O’Neill et al. 2008, Wang et al. 2010, Chmura et al. 2011). Predictions of species success and failure in future habitat conditions require the consideration of ecologically important morphological and physiological traits that are affected by environmental
conditions. Moderate increases in temperatures can enhance photosynthetic activity, as long as there is soil water available to maintain evapotranspirative losses. However, if air temperatures cross a species’ metabolic optimum for carbon assimilation, photoinhibition can occur and if this is coupled with water limitations, this can result in carbon starvation and eventually, mortality. In this study moderate increases in temperature had a stimulating effect on the phenology, growth and physiology of young, eastern deciduous seedlings.

Changes in the timing of phenological events and the corresponding morphological and physiological responses associated with establishment and growth can alter the competitive abilities of seedlings. These changes can include adjustments in the timing of germination and leaf-out, as well as changes in photosynthetically active periods, or timing of biomass allocation to leaves and roots (Reich et al. 1998a, Beier et al. 2004, Bader et al. 2009, Gunderson et al. 2009, Kardol et al. 2010). The moderate warming experienced by seeds and tree seedlings in my study advanced day of germination and leaf-out by 10-20 days, which is well within the reasonable expectation of seedling responses observed under natural global temperature increases (Cleland et al. 2007). This two week advancement to the start of the spring growing season has the potential to provide individual seedlings with additional access to light and soil resources if they were capable of altering their phenological responses to emerge under earlier spring warming. It is possible that the interaction of increased growing season length and increased photosynthetic activity may have contributed to the greater total biomass of seedlings on the warmed treatment plots when compared to the unwarmed plots.

While it was expected that seedling morphological and physiological responses would reflect the conceptual model predictions of species’ success and failure, the results of this study did not support those predictions. Biomass accumulation was heavily proportioned to stem growth, although height was unaffected by treatment conditions. The demand on seedlings to maintain convergent height within crowded, high-light stands may have also contributed to the lack of a height response among seedlings, while the increases in stem biomass maintained structural
stability as seedlings grew (Price et al. 2001). Warming treatments also resulted in strong positive
correlations between relative growth rate and photosynthetic capacity, indicating that the increase
in temperature was facilitating an increase in assimilation and growth for all the study species.
However, the mechanisms that drive this increasing biomass accumulation and photosynthetic
rate in response to increased warming are still not clear. Identifying tradeoffs in traits related to
physiological variables, such as leaf morphology and water potential versus seedlings
establishment and persistence was a focus of this study.

After three years of growth under moderate temperature increases, there was an increase in
photosynthesis response and growth for seedlings of seven species. Seedlings on warmed
treatment plots displayed lower leaf area ratios and altered leaf morphology, while climate
treatments did not appear to alter leaf-level measures of foliar carbon and nitrogen. At the leaf-
level, the expected relationships between photosynthesis, foliar nitrogen and specific leaf area
were not observed and suggested that, for small seedlings, whole-tree estimates may provide a
more complete understanding of the relationships between photosynthesis and potential
explanatory mechanisms under increased warming. Species, such as Acer rubrum, which
invested minimal amounts of foliar carbon and nitrogen to each leaf, may still have the ability to
achieve high whole seedling photosynthesis rates through an investment in a greater total amount
of photosynthetically active tissue distributed over the entire seedling. However, to support higher
whole-seedling levels of photosynthetic activity observed under the warming treatments,
seedlings needed to develop the belowground biomass to have access to adequate water
resources.

Plant water relations were used to understand the balance of resource availability and
physiological responsiveness to changing environmental conditions. Although leaf-level and
whole seedling morphological and physiological variables did demonstrate a change in total
seedling growth and biomass, the leaf-level traits and their relationship to photosynthetic
responses did not provide a complete explanation of the patterns observed under manipulated
climate change treatments. Predawn water status can be used to highlight changes in plant water stress under changing environmental conditions. However, if a system is not water limited, the relationship between these variables becomes less correlated, which was the observed response in this study. Warming decreased seedling water potentials, however these decreases did not correspond to declines in root:shoot ratios, photosynthetic rates or stomatal conductance responses. Instead, it was found that seedling growth was not limited by changes in plant water status and the increased rates of photosynthesis are potential cause for the decreases observed over the course of the 2010 growing season. It is important to consider that these study species can often be found in high resource environments across a broad habitat distribution, both of which generally result in greater plasticity in the range of trait responses to climate change (Augsburger 2008). Understanding how species may respond to moderate climate change conditions during the highly sensitive seedling phase of development can be used to identify changes in species distribution as habitat suitability is altered with increasing temperature and precipitation.

Although the scope of this research project was limited to plots within a single stand, the implications of the results suggests that, under moderate climate change, temperate forests systems may have a greater capacity for resilience at the seedling stage than previously expected. Increasing the temperature and precipitation experienced by developing seedlings in this study showed that the responses of individuals and species may be limited by the developmental constraints of small tree morphology and physiology. The results of this study suggest that, if resources are plentiful, three-year-old deciduous seedlings are still limited by the biological constraints of carbon gain and biomass construction that they are capable of achieving within a single growing season. Seedling success was defined by the ability of an individual to grow larger, or accumulate more biomass, throughout the course of the study. I found that regardless of species type or native habitat range, the growth and biomass accumulation was stimulated by the moderate increase in temperature, with no significant detectable effect of increasing
precipitation. However, in order for a seedling to grow and attain canopy position, it must first be present as a seed to establish when space becomes available. Although individual trees may be limited by their physiological capacity to acquire carbon and grow, the ability of a parent tree to send out propagules that successfully germinate and establish, may actually prove to be a major contributing factor in the development of regenerating forest stands under changing global climate. Once the initial forest canopy has developed, then natural forest successional characteristics, such as shade tolerance, will drive stand development and species replacement. It is also important to consider the range of genetic variability that individual species with broad geographic ranges may posses, enabling them to withstand moderate changes in regional climate and produce offspring with a greater genetic plasticity, increasing the resilience of developing forest stands to changing climate conditions.

This research has highlighted the importance of additional observational and experimental work that is needed to identify the role of genetic adaptation and phenotypic plasticity of both mature trees and their future offspring. The field-based manipulation study that was done at the FORCE site enabled me to examine the effects of multi-factor climate manipulation under natural environmental fluctuations and disturbances, such as insect outbreaks and climate variability. A future direction for continuing this work includes using comparisons of mature individuals which occur at the margins of species’ distribution ranges. Natural climate gradients exist within the north to south distribution of all 11 species that were selected for this study and understanding the physiological and morphological responses of these stands to natural seasonal climate variation can provide insight into the range of responses that established natural stands may have to changing climate. Collecting the propagules of individuals at the edges of species’ ranges also enables an additional opportunity for further study of ecotypic adaptation and the plasticity of seedlings to climate change. The next step in understanding the growth responses that were observed in the field will be to move into controlled greenhouse experimentation, with tightly controlled environmental conditions and greater seedling replication. This will enable
tests for both short and long term photosynthesis and respiration responses to a wider range of climate manipulations to compare baseline responses recorded under natural fluctuations. Additional climate variables can be introduced and replicated in greenhouse and growth chamber studies to simulate a variety of predicted climate scenarios, including increased atmospheric gases, such as CO$_2$ and O$_3$, as well as greater increases in temperature and controlled fluctuations in the intensity and frequency of precipitation events. If common garden experiments and controlled experimental greenhouse studies verify that seedling-level responses are consistent across a species’ range, then that has implications for the types of environmental stress that a species may be able to tolerate as climate changes. Ultimately, the goal of this type of comparative growth response study is to quantify how climate may effect forest development and enable researchers to incorporate physiological parameters into species distribution and habitat suitability models.
References


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Appendix A

Table A1: Count of annual seed germination from 2008-2010 combined for all species. Germination counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented annually and plot-level totals are represented across all three years (2008-2010).

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<th>irrigated</th>
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<td>9 15 4 11 39</td>
<td>6 5 9 14 34 4</td>
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<td>2009</td>
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<td>20 18 3 16 57</td>
<td>5 14 6 13 38</td>
<td>14 6 11 6 37</td>
</tr>
<tr>
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<td>10 12 10 5 37</td>
<td>11 8 9 7 35</td>
<td>11 6 4 7 28</td>
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<td>22 27 24 34 107</td>
<td>29 16 19 22 86</td>
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Table A2: Count of annual seedling leaf-out from 2009-2010 combined for all species. Leaf-out counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented annually and plot-level totals are represented across all three years (2009-2010).

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<th>warmed</th>
<th>irrigated</th>
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Table A3: Count of species-specific germination combined for all years of experimental treatments (2008-2010). Germination counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold.

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Table A4: Count of species-specific leaf-out combined for all years of experimental treatments (2009-2010). Leaf-out counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold.

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Table A5: Count of species-specific growth chamber germination. Germination counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with replicate-level totals represented by container (1, 2). Treatment-level totals (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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<td>4 10</td>
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Table A6: Count of three-year-old seedlings species-specific final biomass and growth used in data analysis for seedling biomass, seedling height, basal area, leaf area, leaf mass, root mass, and root length. Biomass counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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Table A7a: Count of three-year-old seedlings species-specific June leaf-level photosynthesis and used in data analysis for photosynthesis, leaf area ratio, specific leaf area. Biomass counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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Table A7b: Count of three-year-old for seedlings species-specific July leaf-level photosynthesis and used in data analysis for photosynthesis, leaf area ratio, specific leaf area. Biomass counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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Table A8: Count of three-year-old seedlings species-specific leaf-level foliar chemistry used in data analysis for leaf-level foliar nitrogen and carbon and whole seedling foliar nitrogen and carbon estimates. Biomass counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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Table A9: Count of three-year-old seedlings species-specific plant-water status used in the data analysis for pre-dawn and mid-day water potentials and leaf-level gas exchange. Biomass counts are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level totals (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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Appendix B

Table B1: Mean day of seed germination from 2008-2010 combined for all species. Germination means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors (blocks 1-4) are represented annually in bold. When no recorded data (nd) is present the label is indicated in light gray.

| Treatment and block | Year | ambient | | warmed+irrigated | | warmed | | irrigated | | mean | se | mean | se | mean | se | mean | se |
|-------------------|------|---------|------|-----------------|------|-----------|------|-----------|------|-----------|------|-----------|------|-----------|------|-----------|
|                   | 2008 | 145     | 139  | 166  | 143 | 148 | 6.1 | 130 | 136 | 135 | 148 | 137 | 3.9 | 136 | 126 | 144 | 139 | 157 | 3.8 | 131 | 163 | 141 | 171 | **152** | 9.3 |
|                   | 2009 | 155     | 149  | nd   | 160 | 155 | 3.1 | 142 | 141 | 157 | 146 | 146 | 3.7 | 137 | 137 | 138 | 133 | 136 | 1 | 154 | 141 | 155 | 154 | **151** | 3.3 |
|                   | 2010 | 138     | 132  | 152  | 152 | 143 | 5.2 | 116 | 126 | 144 | 118 | 126 | 6.4 | 125 | 125 | 131 | 135 | 129 | 2.5 | 132 | 149 | 154 | 146 | **145** | 4.8 |

Table B2: Mean day of seedling leaf-out from 2009-2010 combined for all species. Leaf-out means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors (blocks 1-4) are represented annually in bold. When no recorded data (nd) is present the label is indicated in light gray.

| Treatment and block | Year | ambient | | warmed+irrigated | | warmed | | irrigated | | mean | se | mean | se | mean | se | mean | se |
|-------------------|------|---------|------|-----------------|------|-----------|------|-----------|------|-----------|------|-----------|------|-----------|------|-----------|
|                   | 2009 | 143     | 139  | 137  | 137 | 138 | 1.9 | 144 | 132 | 135 | 135 | 136 | 2.6 | 143 | 132 | 132 | 126 | 133 | 3.6 | 142 | 134 | 141 | 142 | **140** | 2 |
|                   | 2010 | 143     | 139  | 143  | 143 | 141 | 1.2 | 126 | 121 | 125 | 124 | 124 | 1 | 133 | 125 | 133 | 126 | **129** | 2.1 | 145 | 137 | 146 | 141 | **142** | 2 |
Table B3: Means of species-specific day of germination combined for all years of experimental treatments (2008-2010). Germination means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level means represented by block (1, 2, 3, 4). Treatment-level means and standard errors (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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<th>warmed+Irrigated</th>
<th>1 2 3 4 mean se</th>
<th>warmed</th>
<th>1 2 3 4 mean se</th>
<th>irrigated</th>
<th>1 2 3 4 mean se</th>
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</thead>
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<td>138 138 134 129</td>
<td>135 2.2</td>
<td>132 133 129</td>
<td>132 1.16</td>
<td>130 157 134 142</td>
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</tr>
<tr>
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<td>179 nd</td>
<td>179 nd</td>
<td>149 164 15</td>
<td>nd nd</td>
<td>157 8</td>
<td>141 183 nd nd</td>
<td>162 20.8</td>
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<tr>
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<td>106 122 149 nd</td>
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<td>nd nd</td>
<td>165 nd</td>
<td>168 nd 179 no</td>
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<tr>
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<td>119 132 113 125</td>
<td>122 4.09</td>
<td>nd nd</td>
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<td>179 148 nd nd</td>
<td>164 15.5</td>
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<tr>
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<td>143 130 150 138</td>
<td>140 4.26</td>
<td>nd 131 133 130</td>
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<td>140 12.4</td>
<td>nd nd 179 nd</td>
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<td>138 4.06</td>
<td>120 122 137</td>
<td>160 135 9.15</td>
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<td>126 3.53</td>
<td>133 150 142 178</td>
<td>151 9.62</td>
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</tr>
</tbody>
</table>

Table B4: Means of species-specific day of leaf-out combined for all years of experimental treatments (2009-2010). Leaf-out means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot-level means represented by block (1, 2, 3, 4). Treatment-level means and standard errors (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

<table>
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<th>Species</th>
<th>Treatment and block</th>
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<th>1 2 3 4 mean se</th>
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<th>1 2 3 4 mean se</th>
<th>warmed</th>
<th>1 2 3 4 mean se</th>
<th>irrigated</th>
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<td>135 2.83</td>
<td>140 117 125 131</td>
<td>128 4.71</td>
<td>132 125 144 117</td>
<td>129 5.65</td>
<td>144 150 146 159</td>
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<td>97 102 113 102</td>
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<td>135 143 132 132</td>
<td>135 2.62</td>
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<td>137 1.57</td>
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Table B5: Means of species-specific day of germination for a growth chamber study. Germination means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with replicate-level means represented by tub (1, 2). Treatment-level means and standard errors (blocks 1-4) are represented in bold. When no recorded data (nd) is present the label is indicated in light gray.

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<td>152 nd</td>
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<td>152 0</td>
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<td>152 nd</td>
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</tr>
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<td>Betula lenta</td>
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<td>171 nd</td>
<td>171 nd</td>
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<td>nd nd</td>
<td>143 nd</td>
<td>143 nd</td>
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<td>154 nd</td>
<td>nd nd</td>
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<td>171 nd</td>
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<td>123 6.3</td>
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<td>nd nd</td>
<td>nd nd</td>
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<td>164 7.2</td>
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<td>147 143</td>
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<td>145 1.9</td>
<td>170 154</td>
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<td>152 150</td>
<td>151 0.8</td>
<td>145 145</td>
<td>145 0.4</td>
<td>152 155</td>
<td>153 1.3</td>
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</tr>
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</table>
Table B6: Means of species-specific final biomass (g) of three-year-old seedlings. Biomass mean are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
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<th>Species</th>
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<th>irrigated</th>
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<td>179</td>
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<td>nd</td>
<td>15.2</td>
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<td>nd</td>
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Table B7: Means of species-specific final height of three-year-old seedlings (cm). Height means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
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<th>Species</th>
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<th>irrigated</th>
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<td>4</td>
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<td>114</td>
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<td>44</td>
<td>30</td>
<td>24</td>
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<td>nd</td>
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Table B8: Means of species-specific final diameter (cm) of three-year-old seedlings. Diameter means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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<th>warmed</th>
<th>irrigated</th>
</tr>
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<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
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Table B9: Means of species-specific final leaf area (cm²) of three-year-old seedlings. Leaf area means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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<th>warmed</th>
<th>irrigated</th>
</tr>
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<td>1 2 3 4</td>
<td>1 2 3 4</td>
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<td>1940 336</td>
<td>1940 336</td>
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Table B10: Means of species-specific final leaf mass (g) of three-year-old seedlings. Leaf mass means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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Table B11: Means of species-specific final root mass (g) of three-year-old seedlings. Root mass means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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Table B12: Means of species-specific final root length (cm) of three-year-old seedlings. Root length means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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Table B13a: Means of species-specific June leaf-level photosynthetic rates (μmol m⁻² s⁻¹) of three-year-old seedlings. Leaf-level photosynthetic rates means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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Table B13b: Means of species-specific July leaf-level photosynthetic rates (μmol m$^{-2}$ s$^{-1}$) of three-year-old seedlings. Leaf-level photosynthetic rates means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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Table B14: Means of species-specific leaf-level percent foliar nitrogen (%) of three-year-old seedlings. Percent foliar nitrogen means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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Table B15: Means of species-specific May pre-dawn water potential (MPa) of three-year-old seedlings. May pre-dawn water potential means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

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</tr>
<tr>
<td>Quercus alba</td>
<td>nd nd nd nd nd nd Nd nd</td>
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<td>-0.3 -0.3 -0.3 -0.3 0</td>
<td></td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>nd nd nd nd nd nd Nd nd</td>
<td>nd -0.3 -0.3 -0.3 -0.3 0</td>
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<tr>
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<td>nd -0.3 -0.3 -0.3 -0.3 0</td>
<td>-0.3 -0.3 -0.3 -0.3 0</td>
<td>-0.3 -0.3 -0.3 -0.3 0</td>
<td></td>
</tr>
<tr>
<td>Quercus velutina</td>
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<td>nd -0.3 -0.3 -0.3 -0.3 0</td>
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</table>

Table B16: Means of species-specific June pre-dawn water potential (MPa) of three-year-old seedlings. June pre-dawn water potential means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment and block</th>
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<th>warmed</th>
<th>irrigated</th>
</tr>
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<tbody>
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<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td></td>
</tr>
<tr>
<td>Acer rubrum</td>
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<td>nd -0.4 -0.4 -0.4 -0.4 0.4</td>
<td>0.4 -0.4 -0.4 -0.4 0.4</td>
<td>0.4 -0.4 -0.4 -0.4 0.4</td>
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</tr>
<tr>
<td>Fraxinus americana</td>
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<td>nd -0.7 -0.7 -0.7 -0.7 0</td>
<td>-0.7 -0.7 -0.7 -0.7 0</td>
<td>-0.7 -0.7 -0.7 -0.7 0</td>
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</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td>nd -0.1 -0.1 nd -0.1 0</td>
<td>nd -0.7 -0.7 -0.7 -0.7 0</td>
<td>-0.7 -0.7 -0.7 -0.7 0</td>
<td>-0.7 -0.7 -0.7 -0.7 0</td>
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</tr>
<tr>
<td>Quercus alba</td>
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<tr>
<td>Quercus rubra</td>
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<td>nd -0.5 -0.5 -0.5 -0.5 0</td>
<td>-0.5 -0.5 -0.5 -0.5 0</td>
<td>-0.5 -0.5 -0.5 -0.5 0</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Quercus velutina</td>
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<td>nd -0.5 -0.5 -0.5 -0.5 0</td>
<td>-0.5 -0.5 -0.5 -0.5 0</td>
<td>-0.5 -0.5 -0.5 -0.5 0</td>
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</tr>
</tbody>
</table>
Table B17: Means of species-specific July pre-dawn water potential (MPa) of three-year-old seedlings. July pre-dawn water potential means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment and block</th>
<th>ambient</th>
<th>warmed+irrigated</th>
<th>warmed</th>
<th>irrigated</th>
</tr>
</thead>
<tbody>
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<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>nd -0.3 -0.6 -1</td>
<td>-0.6 0.2</td>
<td>nd -1 -0.8 0.6</td>
<td>-0.8 0.1</td>
<td>-0.4 -0.1 -0.8 -0.2</td>
</tr>
<tr>
<td>Fraxinus americana</td>
<td>nd 0 -0.6</td>
<td>-0.3 0.3</td>
<td>nd -1 -1.1 -0.8</td>
<td>-1.1 0.1</td>
<td>-0.8 -0.6 -1.2 -0.8</td>
</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td>nd -0.5 -0.2</td>
<td>-0.4 0.2</td>
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<td>-0.2 -0.4</td>
<td>nd -0.9 nd nd -0.9</td>
</tr>
<tr>
<td>Quercus alba</td>
<td>nd -0.2 -0.5 -0.3</td>
<td>-0.3 0.1</td>
<td>nd -1.1 -1.3 -0.4</td>
<td>-0.9 0.3</td>
<td>nd nd -0.6 -0.5</td>
</tr>
<tr>
<td>Quercus rubra</td>
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<td>-0.5 nd</td>
<td>nd -0.6 -0.8 -0.7</td>
<td>-0.7 0.1</td>
<td>nd nd -1.2 -0.8</td>
</tr>
<tr>
<td>Quercus stellata</td>
<td>nd -0.3 -0.2 -0.1</td>
<td>-0.2 0.1</td>
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<td>-0.5 0.0</td>
<td>nd nd -0.6 -0.7</td>
</tr>
<tr>
<td>Quercus velutina</td>
<td>nd nd -0.6 -0.2</td>
<td>-0.4 0.2</td>
<td>nd -0.8 -0.7 nd nd</td>
<td>-0.7 0.0</td>
<td>nd nd -0.5 -0.4</td>
</tr>
</tbody>
</table>

Table B18: Means of species-specific May mid-day water potential (MPa) of three-year-old seedlings. May mid-day water potential means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment and block</th>
<th>ambient</th>
<th>warmed+irrigated</th>
<th>warmed</th>
<th>irrigated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
</tr>
<tr>
<td>Acer rubrum</td>
<td>nd -0.3 -0.5 -0.9</td>
<td>-0.5 0.2</td>
<td>nd -0.5 -0.8 -0.4</td>
<td>-0.2 0.2</td>
<td>nd -0.4 -0.8 -0.7</td>
</tr>
<tr>
<td>Fraxinus americana</td>
<td>nd nd nd nd</td>
<td>nd -0.6 -1 -0.8</td>
<td>nd -0.8 0.3</td>
<td>nd -0.6 -0.7 nd</td>
<td>-0.6 nd nd -0.5</td>
</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td>nd nd nd nd</td>
<td>nd -0.5 -0.6 -0.2</td>
<td>nd -0.3 0.1</td>
<td>nd -0.7 nd nd nd</td>
<td>-0.7 nd nd nd nd</td>
</tr>
<tr>
<td>Quercus alba</td>
<td>nd nd nd nd</td>
<td>nd nd -0.5 -0.4</td>
<td>nd -0.5 -0.5</td>
<td>nd nd -0.6 -0.7</td>
<td>-0.5 0.0 0.0 0.38</td>
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<td>Quercus rubra</td>
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<td>nd nd -0.4 -0.3</td>
<td>nd -0.5 -0.4</td>
<td>nd nd -0.7 -0.5</td>
<td>-0.5 0.16 nd nd nd nd</td>
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<tr>
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<td>nd nd -0.3 0.3</td>
<td>nd -0.6 -0.5 -0.5</td>
<td>nd nd nd nd nd</td>
</tr>
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</table>
Table B19: Means of species-specific June mid-day water potential (MPa) of three-year-old seedlings. June mid-day water potential means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment and block</th>
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<th>warmed+irrigated</th>
<th>warmed</th>
<th>irrigated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
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</tr>
<tr>
<td>Acer rubrum</td>
<td>nd -1 -1.2 -1.3 0.1</td>
<td>-1 -1.5 -1.1 -1.2 0.2</td>
<td>-1 -1.9 -1.8 -2.1 -1.7 0.2</td>
<td>0 -0.9 -1.1 -0.7 0.3</td>
<td></td>
</tr>
<tr>
<td>Fraxinus americana</td>
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<td>-1 -1.6 0 nd -0.9 0.5</td>
<td>-1.4 -0.8 -1.8 nd -1.3 0.28</td>
<td>-1 0 -1 nd -0.7 0.3</td>
<td></td>
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<td>Liquidambar styraciflua</td>
<td>nd -0.5 -0.3 nd -0.4 0.1</td>
<td>-1.1 -1.3 -0.5 -0.3 -0.8 0.2</td>
<td>nd -0.1 nd nd -0.1 nd</td>
<td>nd -0.1 nd nd -0.1 nd</td>
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</tr>
<tr>
<td>Quercus alba</td>
<td>nd -1 -0.9 -1.2 0.1</td>
<td>-1.6 -1 -1.3 -1.3 0.2</td>
<td>nd nd nd -1.8 -1.2 -1.5 0.3</td>
<td>nd nd -0.8 nd -0.9 0.1</td>
<td></td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>nd nd -0.7 -0.8 -0.7 0</td>
<td>-1.1 nd -1.5 nd -1.2 -1.2 0.1</td>
<td>nd nd nd -1.7 -1.4 -1.5 0.18</td>
<td>nd nd -1 -1.6 -1.3 0.3</td>
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<tr>
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<td>-0.8 -1.5 -1.3 -1.2 0.2</td>
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<td>nd nd -0.6 nd nd -0.3 0.3</td>
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</tr>
<tr>
<td>Quercus velutina</td>
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<td>-1.5 -1.5 nd -1.5 0</td>
<td>-1.3 -1 -1.6 -1.1 -1.2 0.12</td>
<td>-1 -0.8 -0.7 -1.6 -1 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Table B20: Means of species-specific July mid-day water potential (MPa) of three-year-old seedlings. July mid-day water potential means are represented for each treatment (ambient, warmed+irrigated, warmed and irrigated) with plot level totals represented by block (1, 2, 3, 4). Treatment-level means and standard errors are represented in bold (blocks 1-4). When no recorded data (nd) is present the label is indicated in light gray.

<table>
<thead>
<tr>
<th>Species</th>
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<th>warmed+irrigated</th>
<th>warmed</th>
<th>irrigated</th>
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<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
<td>1 2 3 4 mean se</td>
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</tr>
<tr>
<td>Acer rubrum</td>
<td>nd -1.2 -1.4 -1.3 0.1</td>
<td>-1.3 -1.7 -1 0.5</td>
<td>-2.2 -1.2 -2.5 -1.2 -1.7 0.37</td>
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</tr>
<tr>
<td>Fraxinus americana</td>
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<td>-1.6 -2.1 -2.2 nd -2 0.2</td>
<td>-1.4 -1.6 -2.6 nd -1.9 0.37</td>
<td>-1.1 0 -1.5 nd -0.9 0.4</td>
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</tr>
<tr>
<td>Liquidambar styraciflua</td>
<td>nd -0.4 -0.4 nd -0.4 0</td>
<td>-0.5 -0.5 -1.2 -0.6 -0.7 0.2</td>
<td>nd nd nd -0.3 nd nd -0.3</td>
<td>nd nd -0.3 nd nd -0.3 nd</td>
<td></td>
</tr>
<tr>
<td>Quercus alba</td>
<td>nd -1.2 -1.4 -1.2 0</td>
<td>-1.6 -2.3 -1.9 -1.4 0.7</td>
<td>nd nd -2.1 -1.5 -1.8 0.33</td>
<td>-0.8 nd -1.2 nd -1 0.2</td>
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<tr>
<td>Quercus rubra</td>
<td>nd nd -1 nd -1 nd</td>
<td>-1.3 nd -2.5 -1.6 -1.8 0.4</td>
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<td>nd nd -1.2 -0.8 -1 0.2</td>
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<tr>
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<td>-1.6 -1.9 nd nd -1.7 0.2</td>
<td>-1.5 -2.4 -2.7 -1.4 -2 0.32</td>
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</table>
Appendix C

Table C1: ANOVA for leaf-out and germination means for each year between 2008-2010 with all species values averaged into ambient, warmed, irrigated and warmed+irrigated treatments. Block was considered a random effect. Significant effects are indicated in bold text.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Leaf Out</th>
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<th>Germination</th>
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</thead>
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<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Irrigated</td>
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<td>0.02</td>
<td>0.8851</td>
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<td>57.51</td>
<td>&lt;0.0001</td>
<td>1</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>0.17</td>
<td>0.6818</td>
<td>2</td>
</tr>
<tr>
<td>Warmed*Irrigated</td>
<td>1</td>
<td>0.15</td>
<td>0.7056</td>
<td>1</td>
</tr>
<tr>
<td>Year*Warmed</td>
<td>1</td>
<td>18.53</td>
<td>&lt;0.0001</td>
<td>2</td>
</tr>
<tr>
<td>Year*Irrigated</td>
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<td>3.36</td>
<td>0.0675</td>
<td>2</td>
</tr>
<tr>
<td>Year<em>Warmed</em>Irrigated</td>
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<td>-</td>
<td>-</td>
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</tbody>
</table>

Table C2: ANOVA for leaf-out and germination means for each species values recorded across the years of study (2008-2010) averaged into ambient, warmed, irrigated and warmed+irrigated treatments. Block was considered a random effect. Significant effects are indicated in bold text.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Leaf Out</th>
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<th>Germination</th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Irrigated</td>
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<td>0.12</td>
<td>0.7380</td>
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<tr>
<td>Warmed</td>
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<td>&lt;0.0001</td>
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<td>8</td>
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<tr>
<td>Irrigated*Warmed</td>
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<td>0.3627</td>
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</tr>
<tr>
<td>Species*Irrigated</td>
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<td>0.68</td>
<td>0.6861</td>
<td></td>
</tr>
<tr>
<td>Species*Warmed</td>
<td>7</td>
<td>2.15</td>
<td><strong>0.0381</strong></td>
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Table C3: ANOVA for germination success means for each species values recorded across the years of study (2008-2010) averaged into ambient, warmed, irrigated and warmed+irrigated treatments. Block was considered a random effect. Significant effects are indicated in bold text.

<table>
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<tr>
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</tr>
<tr>
<td>Warmed</td>
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</tr>
<tr>
<td>Species</td>
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</table>

Table C4: ANOVA for seedling biomass, seedling height, basal area, leaf area, leaf mass, root mass and root length means for each species values recorded for three-year-old seedlings averaged into ambient, warmed, irrigated and warmed+irrigated treatments. Block was considered a random effect. Significant effects are indicated in bold text.

<table>
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<th>Seedling Height</th>
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<tr>
<td>Irrigated</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>Species* Irrigated</td>
<td>6</td>
<td>1.01</td>
</tr>
<tr>
<td>Species* Warmed</td>
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<td>0.9</td>
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Table C5: ANOVA for seedling photosynthesis, leaf area ratio, specific leaf area, leaf-level nitrogen, leaf-level carbon, total seedling nitrogen and total seedling carbon means for each species values recorded for three-year-old seedlings averaged into ambient, warmed, irrigated and warmed+irrigated treatments. Block was considered a random effect. Significant effects are indicated in bold text.

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Table C6: ANOVA for seedling pre-dawn water potential, mid-day water potential, root:shoot ratio and fine root biomass means for each species values recorded for three-year-old seedlings averaged into ambient, warmed, irrigated and warmed+irrigated treatments. Block was considered a random effect. Significant effects are indicated in bold text.

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Appendix D

Table D1: A record of field samples taken during the 2008-2010 study period. Months during which samples were taken are indicated by an X under the corresponding column. Biometry measurements include height (cm) and diameter (mm) of each seedling. Photosynthesis measurements were taken for up to three leaves/seedling during full sunlight hours. Water potential measurements include pre-dawn and mid-day samples and the leaves were taken back to the lab for foliar chemistry analysis.

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Figure D1: Relationships of relative growth rate (RGR, g g⁻¹ year⁻¹) with total seedling mass (log10 g), specific leaf area (cm² g⁻¹) and seedling height (cm) and stem weight ratio (g g⁻¹) of 3 year old seedlings. Closed circles (○) are for ambient treatment plots, open circles (●) are for warmed+irrigated treatment plots, open triangles (▼) are for warmed treatment plots and (▲) are for irrigated treatment plots. Significant regressions are indicated ** (P<0.001) and * (P<0.05) within the plot. Abbreviations and units for variables are explained in Table 1.
Rebekah J. Wagner - Vita

**Education**

2007-2011 Pennsylvania State University University Park, PA
- PhD in Ecology with Dr. Margot Kaye.

2005 – 2007 Pennsylvania State University University Park, PA
- MS in Ecology with Dr. Marc Abrams.

2000 – 2005 University of California, Davis Davis, CA
- BS in Evolution and Ecology.

**Publications**

- **Tree ring growth and wood chemistry response to interannual and manipulated precipitation variation for two temperate Quercus species.** Rebekah Wagner, Marc Abrams, Margot Kaye, Paul Hanson, Madhavi Martin. Accepted with revisions to *Tree Ring Research* January 2012.
- **Impacts of imposed climate manipulations on the ecological thresholds of a recently harvested central Pennsylvania forest.** Marshall McDaniels, Rebekah Wagner, Christy Rollinson, Margot Kaye, Jason Kaye, Bruce Kimball, Marc Abrams. Submitted to *Forest and Agricultural Meteorology* 2010.
- **Seedling morphology and leaf characteristics on the photosynthetic responses of temperate deciduous tree seedlings grown under increased temperature and precipitation.** Rebekah Wagner, Margot Kaye, Jason Kaye, Marc Abrams. In preparation for submission 2012

**Presentations and Posters**

- **Presentation: Phenological and morphological responses of seven deciduous tree seedlings to three years of increased temperature and precipitation treatments.** Rebekah Wagner, Margot Kaye, Jason Kaye, Marc Abrams. Ecological Society of America Conference. Organized Oral Session Presentation, Austin, TX. August 2011


- **Presentation: Physiological and morphological responses of temperate deciduous tree seedlings to increased temperature and precipitation regimes in the northeastern United States.** Rebekah Wagner, Margot Kaye, Jason Kaye, Marc Abrams. International Union of Forest Research Organizations Conference. Antalya, Turkey March 2010.

**University committees and activities**

- Xi Sigma Pi – Natural Resource Honors Fraternity 2006 - Present
- Curriculum Chairperson for the Ecology Graduate Student Organization 2008- 2009
- Concepts in Ecology course student coordinator 2008- 2009