The Effect of Warming on Phenology, Physiology, and Leaf Nitrogen in Six Deciduous Tree Species Over the Growing Season

by

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Kathleen Donohue

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environment in the Graduate School of Duke University

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ABSTRACT

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Abstract

There is no consensus on climatic warming’s effect on phenology, photosynthesis, and leaf nitrogen content in temperate deciduous tree species. A major question is whether or how these trees will utilize a longer growing season. Data on leaf photosynthetic rates, leaf nitrogen content, and leaf phenological status were collected weekly or biweekly in 2012 for *Acer rubrum, Liquidambar styraciflua, Liriodendron tulipifera, Quercus alba, Quercus rubra, and Fraxinus americana* at Duke Forest in Orange County, North Carolina. Seedlings were grown in open-topped chambers established in 2009 that were maintained at either ambient or 5 degrees Celsius above ambient temperatures. Half the chambers were shaded, half were under gap conditions.

Four of six species had advanced spring phenology with warming treatment, and four of six species delayed leaf senescence with warming treatment. Shade delayed spring phenology later more than earlier in the season, and gap conditions delayed fall phenology. Warming had an inconsistent effect on photosynthetic rate. Two species increased photosynthetic rate with chambered +5C conditions, another decreased photosynthetic rate in unchambered plots. Half of the species studied had no significant correlation between temperature and photosynthetic rate. The timing of photosynthetic
decline was simultaneous within species across warming treatments, even in species which delayed visible senescence with warming.

There were major differences between years in terms of the effect of warming on nitrogen content and resorption. In 2011, percent leaf nitrogen was lower in warmed chambers, and nitrogen resorption efficiency was not correlated with warming treatment. In 2012, warmed chambers had higher nitrogen concentration and a wider range of nitrogen resorption values than ambient, and resorption efficiency was positively correlated with warming in 2012. It is possible that these differences between years are driven by differences in availability of soil moisture. 2011 was much drier during the senescent period than 2012. The direction and magnitude of warming on nitrogen was consistent within years, but varied by year.

Warming often causes visible change in the timing of phenology, but the timing of photosynthetic decline was unaffected and changes in nitrogen content and resorption were interannually variable. The benefits of delaying visible senescence are unclear. The effect of interactions between warming and soil moisture on leaf processes must be explored further. It is possible that the delay in visible phenology without a concurrent delay in leaf decline is due to a mismatch in environmental cues under a warmer climate. Because of the lack of obvious benefit to plant carbon uptake with warming, it is possible that there is no true extension of the growing season in fall.
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1. Introduction

Trees are sessile, long lived organisms that experience a range of environmental conditions over their lifetimes. In order to cope with this variability, they express phenotypic plasticity in their phenology and physiology. While some have argued that trees have a high potential for rapid local adaptation due to their extensive gene flow over long distances and intensive selection on juveniles (Kremer et al. 2012), ultimately their slow recruitment rate and inadequate migration (Zhu et al. 2012) suggest that phenotypic plasticity will determine which trees survive the bottleneck imposed by climate change.

1.1 Phenological Plasticity

Many species have changed their phenology with the advent of climate change (Parmesan 2007). Phenological plasticity is one way plants can respond to their environment across life stages despite being unable to change location. By adjusting the timing of life events earlier or later in the year, trees can exert control over the conditions faced at different stages (Donohue 2005) and maximize their growth period. Adjusting phenology in response to climate can allow plants to maximize their growing season length in favorable conditions and avoid cold damage in unfavorable conditions. The most important cues for tree phenology are temperature and photoperiod.

Warming is assumed to extend the photosynthetically active period because it often advances the start of the season (Vitasse et al. 2009, Menzel et al. 2005), and it
sometimes delays the end of the growing season (Rosenthal and Camm 1997, Vitasse et al. 2009, Norby et al. 2003). However, the end-of-season effect is not as predictable (Kramer 1995, Vitasse et al. 2010, Piao et al. 2006). Responses differ by species and local environmental conditions.

It is possible that changes in the start of the growing season respond to different drivers than end of season processes. Date of leaf flushing can be predicted with reasonable accuracy by a model including temperature and chilling units (Vitasse et al. 2011, Morin et al. 2009, Vitasse et al. 2010). Growing Degree Days (GDD) after March 1 are also a fairly reliable predictor of bud burst (BB) (Gunderson et al. 2012). Most of the lengthening of the growing season witnessed occurred through the advancement of the spring phenological stages, when light conditions were more advantageous for growth (Vitasse et al. 2009, Augspurger and Bartlett 2003) and water was not limiting. For these reasons, one hypothesis of this study is that warming will advance the start of the growing season. If spring phenology is not advanced with warming, then it is likely that the seedlings are responding to other cues, such as photoperiod.

As mentioned above, the timing of the end of the growing season is less well understood. About half of the phenological variation in senescence can be explained by models incorporating photoperiod and temperature (Vitasse et al. 2009), paying special attention to the air temperature in August and September (Gunderson et al. 2012). Temperature predicts more of the change in the timing of visible senescence than
photoperiod, and appears to be a more important cue in temperate regions (Vitasse et al. 2011, Gunderson et al. 2012, Kramer 1995). Vitasse et al. performed an insightful experiment that tested and ultimately supported this hypothesis (2011) using a reciprocal transplant common garden along an elevational gradient. With elevation as a proxy for temperature variation, and photoperiod invariant among the test plots, senescence reliably declined with increasing altitude. That is, temperature drove the timing of senescence when photoperiod was constant (2011). For this reason, it is hypothesized that warming will delay senescence, but the response may be less consistent than the start-of-season effect. If senescence is not delayed, then warming is not a major cue for fall phenology in that species.

Drought is emerging as another important determinant of the timing of the end of the growing season. With drought, leaves may senesce earlier in gaps than in the understory (Augspurger and Bartlett 2003). In Mediterranean climates, drought has always exerted control on the length of the growing season, but it is becoming a factor in traditionally well-watered areas. In the Mediterranean fir (Abies pinsapo), Lenares et al. observed that growing season extension with warming is greatest at the highest elevations due to the favorable water balance (2012). The delay in the start of the growing season attributed to lower spring temperatures at higher elevation is more than offset by the delay in growth cessation with water draw down (Lenares et al. 2012). This pattern could become more widespread, as hotter summers increase the speed of the
depletion of soil water content. This effect was witnessed in an elevational study of temperate trees, where beech senesced earlier at lower elevations due to drought (Vitasse et al. 2010). In another study, late summer drought caused premature leaf senescence in four species (Gunderson et al. 2012). It is also well known that *Liriodendron tulipifera* can experience drought induced senescence (Burns and Honkala 1990). If warmed seedlings senesce earlier than ambient seedlings, it is possible that they are suffering from drought conditions.

It has been hypothesized that the difference in phenological stage between canopy and understory trees is caused by different temperature sums correlating with height (Augspurger and Bartlett 2003). Temperature sums were greater lower to the ground due to increased radiant heating. In understory seedlings, early emergence added 2 months of favorable light conditions and accounted for ~70% of annual biomass gain (Seiwa 1999). Differences in responsiveness based on height and age are worth noting, because they suggest that trends observed in seedlings, a common study subject due to the relative ease of data collection, may not be relevant to adult trees. However, ultimately recruitment is driven by the success or failure of seedlings, so these studies are still important. Consequences of failing to respond phenologically to the advanced growing season can include declines in abundance (Willis et al. 2008).
1.2 Physiological Plasticity

While shifts in phenology can help trees tailor their life stage-specific needs and vulnerabilities to local environmental conditions, physiological responses are also plastic. Photosynthetic rates acclimate and increase in high light environments (Koike et al. 2001, Herrick and Thomas 2003, Harley et al. 1996). In an experiment both Acer rubrum and Liriodendron tulipifera acclimated to an increase in light by increasing their photosynthetic rate and changing their leaf anatomy (Wallace and Dunn 1980). Interestingly, L. tulipifera, an obligate sun plant, was less able to adjust its morphology than the more versatile A. rubrum.

Photosynthetic rate varies with temperature. Warming has been observed to decrease (Wertin et al. 2011, Way and Sage 2008), increase (Hikosaka et al. 2006, Niu et al. 2008) or have no effect on (Gunderson et al. 2010, Weih and Karlsson 2002) photosynthetic rate. Other experiments have provided conflicting results, where photosynthetic rate increased with temperature in lab conditions but the reverse trend was observed in the field (Gunderson et al. 2000). If there is a negative correlation between photosynthetic rate and warming, then the species are inhibited by warmer temperatures. If there is no effect of warming, then temperature is not the limiting factor of photosynthetic rate in that individual. If warming increases photosynthetic rate, then the individual is growing in conditions less than its temperature optima.
Wertin et al. concluded that photosynthetic decline with warming is triggered by an inability to acclimate respiration to higher temperatures. This disability causes the individual to burn up more resources and experience lower net photosynthesis despite a longer growing season (2011). A failure to control dark respiration with increasing temperature was also observed by Dreyer et al. in 2001.

Cold tolerant plants have been found to be more plastic than cold intolerant plants in their ability to shift their temperature optimum in response to warming or cooling (Yamori et al. 2010). Conflicting results have also been found, where there was no difference between northern and southern populations of *Acer saccharum*’s temperature acclimation potential in terms of photosynthetic rate, but trees from warmer provenance were better able to rein in their dark respiration than their northern conspecifics (Gunderson et al. 2000). All pertinent studies included in this literature review showed trees adjusting their optimum temperature to some degree. There was greater evidence of widespread plasticity than of local adaptation (Gunderson et al. 2010, Lee et al. 2005).

In addition to responding to acute environmental cues, photosynthetic rate follows diurnal and seasonal patterns. Over the course of a day, photosynthetic rates reach their peak and plateau in magnitude between approximately 10 am and 2 pm (Thomas and Hill 1937). This relationship changes under drought conditions. Instead of plateauing, a plant may rapidly decline after reaching peak photosynthesis in mid-
morning (Yin et al. 2006). If seedlings are drought stressed, then their photosynthetic rate will be incongruent with expected diurnual patterns.

Annual photosynthetic rates display seasonality, and they also peak and plateau before experiencing a rapid decline (Jurik 1986). Photosynthetic rates peak in June around the summer solstice, after full leaf expansion, and are relatively stable with a slow decline from June through mid-September. In mid-September photosynthetic rates rapidly diminish (Jurik 1986, Bauerle et al. 2012, Morecroft et al. 2003, Gill et al. 1998, Hikosaka et al. 2007).

Interestingly, even when visible leaf senescence is delayed by warming, not all end of season processes are delayed to the same degree. In the Gunderson et al. experiment, chlorophyll was retained 7 days longer with warming of 4 degrees Celsius, while leaf abscission was delayed an average of 13 days (2012). These extra 6 days without chlorophyll prior to abscission are of questionable utility to the leaf’s carbon balance. This example has implications for other studies which have also found leaf function to cease well before the visible signatures of senescence manifest (Gill et al. 1998, Bauerle et al. 2012). Bauerle et al. (2012) believe that, because photosynthetic decline precedes leaf senescence even, photosynthetic rate is strongly controlled by photoperiod (2012). Bauerle et al. were able to post-pone severe photosynthetic decline by simulating an extended photoperiod (2012).
If plants face impaired functioning late in the growing season, an extended autumn due to warming may delay leaf abscission without adding greatly to carbon gain (Bauerle et al. 2012, Morecroft et al. 2003, Gill et al. 1998). Carbon gain occurs primarily through photosynthesis, and is essential to plant success and survival. Carbon serves as their primary energy storage unit (Mooney 1972). In addition to supplying overall maintenance, a good store of carbon reserves is essential for a competitive advantage following the start of the growing season. Deciduous trees often rely on carbon gains from previous years to jump-start growth of new leaves. Leaves only break even in terms of carbon cost/carbon gain when they are half grown, and before that point are reliant on carbon reserves (Mooney 1972). For this reason, poor performance in terms of carbon uptake can cause a lasting legacy that impacts future success.

Photosynthetic rate can also vary inter-annually dependent upon drought stress and leaf ontogeny (Grassi et al. 2005). Drastic declines in photosynthetic rate that are out of synch with the diurnal and annual pattern, observed either early in the morning (Bassow and Bazzaz 1998, Hinckley et al. 1979) or before the summer solstice (Gunderson et al. 2000), are suggestive of drought stress.

Plants have a variety of plastic responses to environmental cues. This plasticity can include changes in the timing of life events and photosynthetic rate. It has been demonstrated that fitness correlates with the ability of a plant to respond to warming, so
quantifying and relating phenotypic plasticity in trees has applications for predicting success or failure of species and future forest structure.

1.3 Leaf Nitrogen

Leaf nitrogen content can correlate quite closely with maximum photosynthetic rate (Reich et al. 1998, Bauerle et al. 2012, Gunderson et al. 2010). The driver of this relationship is that many of the important mechanisms in the photosynthetic process contain nitrogen (Evans 1989). Specifically, the RuBP proteins of the Calvin cycle (dark reactions of the photosynthetic cycle) and the thylakoids (critical for the light processes) are partially constructed of nitrogen (Evans 1989). Under ideal conditions, it is believed that the positive relationship between photosynthetic capacity and leaf nitrogen content persists across the growing season (Grassi et al. 2005, Reich et al. 1991), with nitrogen concentration peaking in late May/early June and declining rapidly in September (Hikosaka et al. 2007, Niinemets and Tamm 2005).

The positive relationship between photosynthetic rate and leaf nitrogen content can be confounded by drought (Wilson et al. 2000, Grassi et al. 2005, Damour et al. 2008). Damour et al. found that drought was associated with increased nitrogen content per unit area but not per unit mass, and they believed that this effect, combined with changes in photosynthetic parameters, is indicative of nitrogen reallocation within the leaf (2008). If leaf nitrogen content correlates with photosynthetic rate, then the leaves are not drought stressed.
A decline in photosynthetic activity can pre-date decline in leaf green-ness and nitrogen content at the end of the growing season (Bauerle et al. 2012). The end of the growing season is especially important for leaf nitrogen in deciduous perennial species because, prior to abscission, some of the nitrogen is reclaimed from the leaves in the process of resorption. Initial leaf nitrogen content on a mass basis is around 2 percent (Reich et al. 1991, Del Arco et al. 1991, Lee et al. 2005), and the plant can generally translocate about half of that amount (Reich et al. 1991, Del Arco et al. 1991, Kobe et al. 2005). However, resorption rates can range from approximately 25 to 70 percent (Del Arco et al. 1991). Resorption efficiency is important because foliage can contain up to half of a deciduous tree's total plant nitrogen (Mooney 1972).

Most studies find that leaves that senesced earlier were less able to reclaim nitrogen. Early senescing leaves had a higher rate of resorption, but a lower overall percent reclamation (Niinemets and Tamm 2005). Leaf litter nitrogen content was proportional to initial leaf nitrogen content (Norby et al. 2000, Kobe et al. 2005), and trees with higher initial leaf nitrogen content had more nitrogen in their leaf litter (Kobe et al. 2005, Niinemets and Tamm 2005). Another study found that the length of the abscission period can influence the amount of nitrogen reclaimed, especially if the plant has drought-triggered senescence (Del Arco et al. 1991). Under xeric conditions, a more prolonged senescent period with a slower rate of senescence is less efficient at reclaiming nitrogen than a faster, later season resorption (Del Arco et al. 1991).
However, gradually losing leaves and decreasing transpiration are more adaptive than improved nitrogen recovery in xeric environments (Del Arco et al. 1991). Niinemets and Tamm believed the findings of Del Arco’s paper were solely the result of drought conditions, as their own research did not support its conclusions (2005). Leaves that senesce later should have higher rates of resorption.

Previous studies have found that warming can decrease (Norby et al. 2000, Weih and Karlsson 2002, Reich and Oleksyn 2004, Lee et al. 2005, Tjoelker et al. 1999) or increase leaf nitrogen content (Butler et al. 2011). Increased leaf nitrogen in cooler climates is a physiological adaptation because nitrogen use efficiency decreases with lower temperatures (Weih and Karlsson 2002). Dark respiration has a linear relationship with leaf nitrogen (Tjoelker et al. 1999). Warming should decrease nitrogen content in leaves.

Leaf nitrogen content generally tracks photosynthetic capacity (Reich et al. 1998, Bauerle et al. 2012, Gunderson et al. 2010, Hikosaka et al. 2007, Grassi et al. 2005), but this relationship is muddied at the end of the growing season (Bauerle et al. 2012) and under drought conditions (Wilson et al. 2000, Grassi et al. 2005, Damour et al. 2008). Leaf resorption efficiency is often related to initial green-leaf nitrogen content (Kobe et al. 2005, Niinemets and Tamm 2005), while factors such as speed and timing of senescence may also be important (Del Arco et al. 1991, Niinemets and Tamm 2005). Low light and low temperatures increase leaf nitrogen concentration (Norby et al. 2000, Weih and

1.4 Hypotheses

The objective of this study is to clarify the relationships between warming and maximum photosynthetic rate, temperature, and leaf nitrogen and how these relationships develop in conjunction with phenological status across the growing season. These interactions are changing with our changing climate, so identifying and exploring different species responses should provide insight into which populations are likely to face success or failure in our future forests. The uniting theme of this study is that warming may change some leaf processes without changing others, which could reduce the overall impact of warming on effective growing season length.

From this literature review, multiple hypotheses were developed. The first predicted effect of warming is that it will advance spring phenology to a greater and more universal extent than it will delay fall phenology, but fall phenology may also be delayed. If spring phenology is not advanced with warming, then it is likely that the seedlings are responding to other cues, such as photoperiod. If senescence is not delayed, then warming is not the most important cue for fall phenology in that species.
If warmed seedlings senesce earlier than ambient seedlings, it is possible that they are suffering from drought.

If photosynthetic rate decreases with warming, then the species are inhibited by warmer temperatures. If there is no effect of warming, then temperature is not the limiting factor of photosynthetic rate in that individual. If warming increases photosynthetic rate, then the individual is growing in conditions less than its temperature optima. If seedlings are drought stressed, then their photosynthetic rate will be incongruent with expected annual patterns. Photosynthetic rate may decline well before senescence, especially in species with warming-induced delays in fall phenology.

Leaf nitrogen may lose its clear relationship with photosynthetic rate under drought conditions and at the end of the growing season. Trees that senesce later may reclaim a higher percentage of their green-leaf nitrogen content. Warming may decrease leaf nitrogen concentration.
2. Materials and Methods

Seedlings were grown in unchambered control conditions (C) with deer exclosure fencing and no other infrastructure, open top chambered control conditions at ambient temperatures (0) with the full chamber infrastructure, and at open top chambered conditions +5° C (5) above ambient temperatures. Unchambered control plots were approximately 3° C cooler than chambered control plots, and chambered control plots were 5° C cooler than chambered +5 plots. Chambers were replicated under both gap and shade light treatments. The chambers were warmed by pumping air in using plastic ducts hung lengthwise across the middle of the chamber (see figure 1). Chambers measured 3 by 5 meters, and were first established in 2009.
Figure 1: Open-top warming chamber in Duke Forest

In the gap light treatment, this study follows the phenological progress of *Acer rubrum* (common name: red maple, abbrev.: ACRU), *Fraxinus americana* (white ash, FRAM), *Liriodendron tulipifera* (tulip poplar, LITU), *Liquidambar styraciflua* (sweet gum, LIST), *Quercus alba* (white oak, QUAL), and *Quercus rubra* (red oak, QURU). Due to low survival of shade intolerant species in shade treatment, only ACRU, QUAL, and QURU were tracked in shaded chambers.

Phenological measurements were collected weekly in spring and fall, and less frequently in late summer. Technicians observed each seedling, recorded and tracked its phenological status in a database matching observation to species, chamber, and unique ID. Seedlings were given a ranking of 1 to 6 for spring phenology. In non-oak species, stage 1 corresponded to cotyledon emergence; in stage 2 new tissue was visible growing between the cotyledons; in stage 3 very small, folded leaves were visible, in trees older than one year this stage represents bud break; in stage 4 leaves unfolded; in stage 5 leaves expanded; and in stage 6 leaves hardened. In oaks, stage 1 corresponded to no shoot emergence; stage 2 to stem elongation with very tightly folded leaf primordia; stage 3 was defined by distinguishable leaf primordia; stage 4 was marked by leaf elongation without stem growth; and stage 5 and 6 were the same as in the other study species (adapted from Clark lab protocol, Duke University). In the fall, seedlings were ranked from 0 to 2. Stage 0 designated mature leaves with less than 1/3 senesced
material, stage 1 leaves were between 1/3 and 2/3 senesced, while stage 2 leaves were more than 2/3 senesced.

Measurements of photosynthetic rate of these seedlings were also collected across the growing season for all of the previously identified species and treatments. Early in the growing season, the same leaf on the same plant was measured over the course of a day to verify that the daily period during which measurements occurred was capturing photosynthetic rate at its daily maximum. Data collection occurred between 10:00 and 14:00 hours to ensure that measurements were taken during the leaf’s daily midday photosynthetic plateau.

Photosynthetic rate was measured over the 2012 growing season using the LI-COR 6400 XT (LI-6400 XT, LI-COR Biosciences, Lincoln, NE, USA) portable photosynthetic meter (see figure 2). PPFD was fixed at 1500 w/m², flow rate was maintained at 300 μm s⁻¹, CO₂ concentration was set to 380 ppm, and relative humidity was maintained between 40-80%. These settings ensured leaf stomatal closure was not triggered by dry air and that the leaves were light saturated. Photosynthetic rate was measured as μmolCO₂ m⁻² s⁻¹. In shaded chambers, light response curves were performed to test for photo-inhibition at high light levels. Photo-inhibition was not observed at PPFD of 1500, though the shaded seedlings had reached saturated light levels well before that point.
Replication necessary to ensure the relevance of the experimentally measured photosynthetic rate was determined to be 5 measurements each for 3 individuals per species per treatment per sample date. The 5 point measurements of photosynthetic rate were taken per leaf and averaged to obtain an accurate estimate of maximum photosynthetic rate for each individual. Due to time constraints limiting the optimal photosynthetic window for measurements, two days per week were allocated to photosynthetic measurements: one day was given to gap measurements and another to shade measurements. In a given sampling, each study species was measured 45 times if they were present in gap conditions exclusively, and 90 times if they were present in both gap and shade conditions. Depending on species availability, up to 405 photosynthetic measurements were collected per week.
Leaf samples were collected in gap chambers for nitrogen analysis at the height of the growing season (after they had attained peak nitrogen concentration) through late November. Samples were collected in both 2011 and 2012. Two samples were collected per species per treatment on each sample date. Leaf samples were placed in a drying oven set to 60° Celsius for a minimum of 48 hours. After being thoroughly dried, the samples were ground to a fine powder in a ball-mill. The powder was then submitted to a lab for CN analysis. CN analysis produced data on carbon and nitrogen percent by mass. Nitrogen resorption was calculated by subtracting the current nitrogen concentration from the initial concentration, dividing by the initial concentration, and then multiplying the result by 100 to get a percent value.

Most of the factors included in this study respond to both temperature and water availability, so it was necessary to include data on soil moisture to understand the drivers of the observed effects. Soil moisture data were collected biweekly by technicians using a Time-domain Reflectometer (TDR); which produced readings of soil moisture content (%). Five readings were collected per chamber per soil moisture sampling date. These readings were then averaged across chamber to characterize the soil moisture conditions within that particular treatment.

Statistical analyses and graphing were performed in R version 2.12.1 (R Development Core Team, 2010). Exploratory data analysis indicated significant species differences in response to the manipulated variables, so each species response was
modeled separately. ANOVAs were performed to create statistical linear models of the correlations between the fixed factors of chamber treatment, light treatment, and year with the dependent variables of photosynthetic rate, leaf nitrogen content, and leaf nitrogen resorption. The models are defined below.

1. \( f(\text{photosynthetic rate for each species}) = \text{warming} + \text{light} + \text{warming*light} \)

2. \( f(\text{nitrogen concentration for each species}) = \text{warming} + \text{year} + \text{year*warming interaction} \)

3. \( f(\text{nitrogen resorption for each species}) = \text{warming} + \text{year} + \text{year*warming interaction} \)

The statistical models produced coefficients, which were informative as to the direction of the effect with treatment. Positive coefficients indicated positive correlations, while negative coefficients indicated a negative correlation. P-values provided information on the significance of the correlations. Insignificant correlations were marked as ”NS”, marginal correlations were labeled ”.”. Significant results were designated with ”***”, ”**” or ”*” depending on the degree of significance (\( x <0.001 <0.01 <0.05 \), respectively).
3. Results

3.1 Soil Moisture

Soil moisture is an environmental variable that influences all the other factors included in this study, so monitoring soil moisture was important to ensure that we were attributing the response of the independent variables to the correct manipulated variables. Soil moisture content followed a seasonal pattern of peaking in winter and decreasing from the start of the growing season through summer. After reaching its lowest level at approximately day 230, soil moisture concentrations steadily rose as the year progressed. Gap plots had more soil water than shade sites, and chambered +5C plots had less soil water than chambered ambient or unchambered plots. Gap plots also had a wider spread of soil moisture values than shade chambers, which is to say that warming made a bigger difference in terms of soil moisture in gap plots. Chambered +5C conditions had a smaller, though still noticeable, impact on soil moisture content in shade plots. The difference between warmed +5C and ambient chambered plots was more marked under gap conditions than in shade conditions (figures 3 and 4). 2012 was wetter than 2011, as can be seen from figure 5. In summary, soil moisture conditions were highest in gap unchambered plots in 2012 during the fall, and were lowest in shade chambered +5C plots in the summer of 2011.
Figure 3: Soil moisture content (%) in gap plots. For Julian Date, 1 = January 1, 2012. Bars represent standard error.

Figure 4: Soil moisture content (%), shade plots. For Julian Date, 1 = January 1, 2012. Bars represent standard error.
Figure 5: Soil moisture content (%), both years, all light and warming treatments pooled by year. Solid line represents mean, dotted lines represent spread.

### 3.2 Phenology

More than half of all species seemed to advance spring phenology with warming. *L. styraciflua, L. tulipifera, Q. alba,* and *Q. rubra* (figures 8, 9, 10, and 11, respectively) all showed advanced phenology with warming treatment of +5°C above ambient conditions. No species delayed spring phenology with warming treatment.

The species studied in both gap and shade conditions (*A. rubrum, Q. alba* and *Q. rubra*) advanced spring phenology under gap conditions in late spring (see figure 12). In early spring, there was no effect of light treatment. In the fall, gap conditions delayed phenology to a small extent. Direction of the effect of warming remained constant.
across light treatments, though magnitude of effect may have changed. For example, *Q. alba* had a stronger signature of advanced phenology with chambered+5C conditions in shade plots. Conversely, *Q. rubra* did not have as many representatives in shade chambers so there was a wider range in standard error for this plot (figure 11).

In our study more species had a strong visual signature of delayed fall senescence than advanced spring phenology. All species except *L. styraciflua* and *L. tulipifera* delayed the onset of fall phenology with chambered +5C conditions, and the number of days delayed was larger in the fall than in the spring.
Figure 6: Phenology for *Acer rubrum* over the growing season (2012). Julian date is on the x-axis, and phenological status is on the y-axis. Left side graphs are spring phenology, right graphs are fall phenology. Bars represent standard error.
Figure 7: Phenology for *Fraxinus americana* over the growing season in gaps (2012). Julian date is on the x-axis, and phenological status is on the y-axis. Left graph is spring phenology, right graph is fall phenology. Bars represent standard error.

Figure 8: Phenology for *Liquidambar styraciflua* over the growing season in gaps (2012). Julian date is on the x-axis, and phenological status is on the y-axis. Left graph is spring phenology, right graph is fall phenology. Bars represent standard error.
Figure 9: Phenology for *Liriodendron tulipifera* over the growing season in gaps (2012). Julian date is on the x-axis, and phenological status is on the y-axis. Left graph is spring phenology, right graph is fall phenology. Bars represent standard error.
Figure 10: Phenology for *Quercus alba* over the growing season (2012). Julian date is on the x-axis, and phenological status is on the y-axis. Left graph is spring phenology, right graph is fall phenology. Bars represent standard error.
Figure 11: Phenology for *Quercus rubra* over the growing season (2012). Julian date is on the x-axis, and phenological status is on the y-axis. Left graph is spring phenology, right graph is fall phenology. Bars represent standard error.
Figure 12: Phenology for *A. rubrum*, *Q. alba*, *Q. rubra* for both spring and fall by light treatment. Julian date is on the x-axis, and phenological status is on the y-axis. Top graphs are spring phenology, bottom graphs are fall phenology. Bars represent standard error.
3.3 Physiology

There were noticeable species differences in average photosynthetic rate (see table 3). In declining order, species rank in terms of maximum photosynthetic rate from highest to lowest was *L. styraciflua*, *L. tulipifera*, *A. rubrum*, *F. americana*, *Q. rubra*, and *Q. alba* (see figure 13). *Q. alba* and *Q. rubra* increased photosynthetic rate in chambered +5°C plots to a statistically significant degree, while *A. rubrum* decreased its photosynthetic rate in the unchambered plots. Otherwise, the species included in this study seemed photosynthetically impervious to our warming treatments. Light treatment had the largest impact on photosynthetic rate, with shade conditions decreasing rate of photosynthesis in all species considered in the shade plots.

You can also see in figure 14 that photosynthetic decline occurred before the end of the growing season. The growing season was defined as the period between bud-break and leaf senescence greater than 1/3 of the leaf area. *Q. rubra* under gap conditions was used as an example to illustrate this case (figure 14), but the pattern is visible for every species that delayed senescence with fall. The period of photosynthetic activity was roughly the same regardless of warming treatment, but phenology was delayed. Photosynthetic rate of the different warming treatments track each other over the year, but at an offset dependent upon treatment. They declined at the same time, regardless of visible leaf condition.
Graphs of the data reveal other patterns not detected in our statistical analyses (see figure 12). In all species, one can see the signature of a heat wave near Julian Date 183, where photosynthesis was shut down due to excessively high temperatures. During that 3-5 day period, daily highs exceeded 43 degrees Celsius within the chambers.

**Figure 13:** Photosynthetic rate in gap and shade chambers, 2012. Warmed treatment measurements are shown in red, unwarmed treatment measurements are in blue. Lower red and blue bars represent shade chambers, higher red and blue bars represent gap chambers.
Figure 14: Photosynthetic rate and growing season length in *Q. rubra* gap chambers, 2012. Chambered +5 treatment is in red, chambered ambient is in black, and unchambered is in green.
Table 1: ANOVA of warming and light's effect on photosynthetic rate
Model = f(photosynthetic rate of species) = warming + light + light*warming interaction. Estimates indicate direction of response. Positive indicates positive response, negative indicates negative response. Insignificant correlations were marked as "NS", marginal correlations were labeled ".". Significant results were designated with "***", "**" or "*" depending on significance (x less than 0.001, 0.01, and 0.05, respectively). Intercept estimate is average value of case used for comparison.

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3.4 Leaf Nitrogen

The big surprises of this study were the major inter-annual differences in the relationships between nitrogen concentration, nitrogen resorption, and warming treatment. These differing trends are dramatically apparent in our graphs of nitrogen concentration (figure 15), where the relationship between warming and nitrogen content reverse for L. styraciflua and L. tulipifera from 2011 to 2012. A. rubrum did not have a strong relationship between year and leaf nitrogen content. A. rubrum, L. styraciflua, and L. tulipifera were the three species studied in both 2011 and 2012.

In 2011, cooler chambers had higher leaf nitrogen content than warmer chambers. There was no relationship between resorption and warming. In 2012, warmer chambers had higher leaf nitrogen content than cooler chambers (see table 2). L. styraciflua, L. tulipifera, and Q. alba all significantly increased their leaf nitrogen content in chambered +5C plots. F. americana and Q. rubra also increased leaf nitrogen, but their correlations were of marginal significance. Resorption was lower in chambered +5C for Q. alba and Q. rubra, and resorption was higher in unchambered ambient for L. styraciflua (see table 3). In terms of between year differences in resorption, L. styraciflua was the only species with a significant effect. L. styraciflua resorbed less nitrogen in 2012 than in 2011. There was higher overall leaf nitrogen content in 2012 than in 2011.

L. tulipifera had the highest average resorption rate across the growing season.

Resorption rate varied with species and year. Q. alba and Q. rubra decreased nitrogen
resorption in chambered +5 conditions, and *L. styraciflua* increased nitrogen resorption in unchambered plots. *L. styraciflua* also resorbed less in 2012 than in 2011. Much less nitrogen was reclaimed by late November in 2012 compared with late November 2011. In summary, warm treatment leaves had less nitrogen than cool treatment leaves in 2011, and the opposite relationship was observed in 2012. In 2011 there was no relationship between warming treatment and total resorption, and overall resorption was lower in 2012. Nitrogen concentration in 2012 was higher than in 2011. Species differences in nitrogen content and resorption were observed.

At the start of both the 2011 and 2012 senescence periods, initial nitrogen concentration was highest in *Liriodendron tulipifera* in both warm and ambient chambers. Initial nitrogen content in ambient leaves ranged from 1.8-2.0% in 2011 and 1.5-2% in 2012 (see figure 13). In +5Celsius leaves, this concentration ranged from 1.0-1.3% in 2011 and 1.5-2.5% in 2012. In 2011, all species were able to resorb approximately 60% of their initial leaf nitrogen by late November. In 2012, resorption by the end of November ranged from 20-80%.

*L. tulipifera* had the highest resorption percent, while *L. styraciflua* had the lowest. *L. styraciflua* appears not to have started nitrogen resorption by the end of the 2012 study period. In fact, the negative resorption values in 2012 (data not shown) indicate that nitrogen concentration had increased since the initial measurements.
Figure 15: Nitrogen content (%) in gap chambers, 2012 and 2011. Warmed treatment measurements are shown in red, unwarmed treatment measurements are in blue. Julian date is calculated as days since January 1, 2011.
Table 2: ANOVA of warming and year's effect on nitrogen content.
Model = f(nitrogen concentration by species) = warming + year + year*warming interaction.
See table 1 for table legend and definitions.

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### Table 3: ANOVA of warming and year's effect on nitrogen resorption.

Model = \(f(\text{nitrogen resorption by species}) = \text{warming} + \text{year} + \text{year*warming interaction}\). See table 1 for table legend and definitions.

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4. Discussion and Conclusion

4.1 Phenology

The general advancement of spring phenology with warming agreed with the results expected from the literature review (Vitasse et al. 2009, Menzel et al. 2005). This effect was consistent across light conditions for the species represented in both gaps and the understory. This finding suggests two things: first, climate models emphasizing the importance of warming to emergence time in tree species seem to align with observed field results. Second, either none of the species in our study have a chilling requirement (Vitasse et al. 2011, Morin et al. 2009, Vitasse et al. 2010), or winters are not yet warm enough to fail to achieve the chilling requirement, and thus emergence times are not being penalized by warm winters. For species which had a large spring effect, it seems that warming may be the factor which exerts the most control over the timing of emergence.

It is interesting to observe that, when +5C chambered conditions advanced spring phenology, the advancement took place at both the beginning and end of the spring leaf-out period. The differences in spring phenology between gap and shade conditions are most apparent towards the end of the leaf-out period. It is possible that early in the season, prior to canopy closure, the advantages of being a seedling in the gap conditions are minimized. Later in the season, after the overstory has experienced
leaf expansion, differences in both light and temperature between gap and shade plots would be exaggerated.

Our findings contrast with previous studies that found ring porous species typically emerge later than diffuse porous species due to their cold-embolism avoidance strategy (Seiwa 1999). In this study, two of three ring porous species studied advanced their spring phenology with warming. It is possible that their response was of smaller magnitude than the response of diffuse porous species, but it was statistically significant. The possible link between relative responsiveness of phenology at the start of the season and porosity is an area that could be explored further in future studies incorporating more species and a greater temporal period.

Fall phenology also showed a strong relationship with warming. Four of six species delayed the end of the growing season under chambered +5C conditions. This finding fails to support the idea that fall processes may be more cued by photoperiod (Körner and Basler 2010, Bauerle et al. 2012). Our findings bolster the claim that warming frequently delays fall senescence (Rosenthal and Camm 1997, Vitasse et al. 2009, Norby et al. 2003). This study’s results were far less mixed than those typically reported in the literature.

There was no difference in the direction of phenological response to warming between gap and shade chambers. It appears that the importance of various cues does not change dependent upon light environment. The later senescence of gap seedlings
compared to shade seedlings could be a result of higher temperatures in the gaps, increased light availability promoting growth, or the general pattern of lower soil moisture in shade chambers. If drought stress hastens senescence (Augspurger and Bartlett 2003), then it is possible that the combination of lower temperatures and lower soil moisture both contributed to the early leaf senescence in the shade.

It is generally understood that gaps are more biodiverse than the understory, so it can be challenging to avoid the confounding factor of differences in species composition when examining gap-shade differences. One way to get at the truth of effects driven by light conditions would be to examine species found in both gap and shade conditions only. However, this approach has its own limitations. Only studying species with broad physiological tolerances is also problematic.

Another source of error in phenological models is the difficulty in relating individual observations of phenological status with the continuous variable "day of the year" (Clark et al. unpublished). Transitions between stages mean different things, and it is possible that warming impacts the progress of some phenological stages but not others. These relationships are lost in a general linear model. One proposed solution to this issue is to integrate these two variables by using a state-based model (Clark et al. unpublished). This approach could represent a future direction of phenological research.
4.2 Physiology

As expected, the maximum photosynthetic rate was higher in gap chambers than in shade chambers (Koike et al. 2001, Herrick and Thomas 2003, Harley et al. 1996). Additionally, our maximum photosynthetic rates were in-line with reported values (Bassow and Bazzaz 1998). This agreement with the literature supports the validity of our data.

Two species (Q. alba, Q. rubra) in chambered +5 plots increased photosynthetic rate in comparison with ambient temperatures, while one species (A. rubrum) decreased its photosynthetic rate in unchambered plots. It is possible that local climatic conditions are cooler than A. rubrum, Q. alba, and Q. rubra’s preferred temperature regime. Warming had no effect on photosynthetic rate in half of the species considered, so it is possible that photosynthetic rate in these species is limited by factors other than temperature, perhaps soil moisture or light availability. However, the species that showed significant correlations with temperature were represented in both gap and shade conditions, so they were sampled twice as frequently as species found exclusively in the gap. This difference effectively doubled their sample size relative to the other half of the species which showed no relationship between photosynthetic rate and warming treatment. Future studies should measure at least six individuals per treatment per sample day to clarify relationships between photosynthetic rate and warming treatment. Pooling measurements across healthy leaves during the growing
season to enhance sample size should be approached with caution. One of the main points of this study is that leaf appearance is not always a reliable signal of leaf function.

The inconsistent relationship between treatment and photosynthetic rate reflect the current literature on the subject, which is divided on whether warming increases (Hikosaka et al. 2007), decreases (Wertin et al. 2011, Way and Sage 2008), or has no effect (Gunderson et al. 2010, Weih and Karlsson 2002) on photosynthetic rate. Part of this confusion could stem from differences in the responsiveness of warming to temperature over the growing season.

As manifested in the similar slopes of the photosynthetic rate over the growing season, the timing and rate of photosynthetic decline was the same across temperature treatments. This suggests that the timing of photosynthetic decline is similar regardless of phenology or warming, lending support to Bauerle et al.'s hypothesis that photosynthetic decline can precede the visible end of the growing season (2012).

Our findings are further complicated by the differential response of trees depending on light conditions. Warming explained less of the variation in photosynthetic rate in shade chambers. This reduced effect could be attributed to the overall lower maximum photosynthetic rate in shade leaves. Shade leaves are generally considered to be limited by light conditions, so environmental effects that ameliorate other factors may produce limited results. For this reason, it seems that warming had less effect on shade seedlings than gap seedlings.
If one views figure 14, there may be evidence of a shift in the relationship between *Acer rubrum*’s maximum photosynthetic rate and temperature treatment over the growing season. One explanation for red maple’s inconsistent relationship between warming and photosynthesis could be found in last summer’s epidemic of anthracnose fungus. It is possible that red maple seedlings in shade chambers displayed inhibited photosynthesis because they were fighting infection. Anthracnose seemed to be more virulent in shade chambers (personal observation).

All seedlings displayed the expected temporal relationships between photosynthetic rate and warming, which suggests they did not experience severe drought stress the summer of 2012 (Yin et al. 2006). In addition to having higher overall photosynthetic rates, photosynthetic rates declined later in gap seedlings than in shade seedlings, so the benefits to the carbon balance of a seedling growing in gap conditions are two-fold.

### 4.3 Leaf Nitrogen

According to the literature, warm, sunny leaves have lower nitrogen content than cooler, shaded leaves (Norby et al. 2000, Weih and Karlsson 2002, Reich and Oleksyn 2004, Lee *et al.* 2005, Tjoelker *et al.* 1999, Niinemets 1998, Ellsworth and Reich 1992, Koike *et al.* 2001). The leaf with the highest nitrogen content should be a cold, shaded leaf. This relationship held true in 2011, but in 2012 warmed leaves had higher
nitrogen content (see figure 13). The 2012 data disagree with the bulk of existing studies, but they matched the findings of Butler et al. (2011).

The reversal in trends between nitrogen and warming treatments is a tantalizing puzzle because light regime and warming treatment were kept constant between 2011 and 2012. Because the manipulated factors were held constant both years, the cause of this reversal in effect could be attributable to another important, fluctuating environmental factor not controlled for in this study.

The major difference between years was in soil moisture content. Soil moisture content was higher in late summer and fall in 2012 than it was over the same period in 2011. It has recently been demonstrated empirically that increased leaf nitrogen content can be part of drought acclimation in willows in central Sweden (Weih et al. 2011), so it is possible that warmer leaves in 2012 were exposed to enough drought stress to reverse that standard relationship between temperature and nitrogen concentration. However, 2011 was drier than 2012. Perhaps the increase in nitrogen in leaves in 2012 was triggered by a lag effect lingering from the drought of the preceding growing season. Alternatively, 2011 may have been too hot and dry for the soil microbial activity necessary to render nitrogen available for plant uptake. 2012’s soil moisture content could have been within an acceptable range for microbial nitrogen fixation, increasing the availability of nitrogen within the soil.
In both years our leaf nitrogen concentrations were fairly close to reported findings (Wallace and Dunn 1980, Reich et al. 1991, Lee et al. 2005, Kobe et al. 2005). Small differences in concentration are likely due to differences between site conditions between our study and theirs.

Nitrogen content also varied as predicted between species, in that species with typically higher photosynthetic rates had higher nitrogen concentrations than those with lower photosynthetic rates (Reich et al. 1998, Bauerle et al. 2012, Gunderson et al. 2010). The top two species for photosynthetic rate were also the top two species for nitrogen content.

There were inter-annual and treatment differences in nitrogen resorption. More nitrogen was resorbed by late November in 2011 than in 2012, which could support either the hypothesis that the initiation of leaf nitrogen resorption started later in 2012, or that the resorption process was impacted by the same environmental variable that changed the relationship between warming and leaf nitrogen concentration, which in this case is probably soil water content.

Our resorption efficiency matched reported values for both years (Herrick and Thomas 2003), but was closer to median values in 2011 (Del Arco et al. 1991). One potential explanation for the similarity in resorption values for both warmed and ambient chambers in 2011 is that warming does not affect resorption efficiency when soil moisture content is low. There were no significant differences in overall resorption at
the end of the growing season in 2011 by species or warming treatment. Instead of benefiting from the prolonged senescent period with warming by increasing nitrogen reclamation, warmed and ambient seedlings reclaimed proportionally equivalent amounts. This result agrees with previous studies, which observed decreased resorption under drought conditions (Niinemets and Tamm 2005, Del Arco et al. 1991).

2012 showed a wider variation in resorption, more closely approximating Del Arco et al.’s findings of a range between 20-70% (1991). Seedlings from chambers warmed to +5 Celsius above ambient had a larger range of resorption values than those in chambers kept at ambient temperatures in 2012. It is possible that these differences are due to differences in soil moisture content between years.

Drought promoted senescence in 2011 as an explanation for differences from 2012 in resorption efficiency could tie to Niinemets and Tamm’s conclusion that early senescing leaves had a lower overall percent reclamation (2005). If the leaves in 2011 were drought stressed, then that might explain why they had all completed nitrogen resorption by late November, but had a smaller range of resorption percentages than the 2012 leaves.

The wider range of resorption values could also be explained by seedlings manifesting two strategies: either they used the extra time to resorb more nitrogen, or they used the extra time to continue carbon uptake, and so had not yet pulled nitrogen from the leaves. It is likely that not all of the 2012 seedlings had finished senescence by
the end of November. *L. styraciflua* was especially notable for this, as it maintained its nitrogen content until the end of the study with no appreciable decline. In fact, if one refers back to the phenology and leaf nitrogen graphs of *L. styraciflua* (figures 8 and 15), one sees that this species was still transitioning between the "1/3 senesced" and "2/3 senesced" stages at the end of the nitrogen study period.

In terms of rate of resorption, again the data from 2012 differed from 2011. All species resorption progressed at similar relative rates, with the exception of *L. styraciflua*, which had yet to start resorption by late November, 2012. There were significant differences in resorption efficiency between treatments, and there were also significant species differences. The general trend is that the 2012 data exhibited more spread, perhaps suggesting the differences in strategy between species and years. It is possible that the ameliorated soil moisture conditions of 2012 allowed for species differences in strategy to become more visible. 2011’s soil moisture was lowest during the fall senescence period.

All trees’ nitrogen concentrations responded in the same direction within warming treatments, but in opposite directions dependent upon year. Because it appears that three species in 2012 showed negative correlations between temperature and resorption, it is possible that the delay in fall phenology with warming, and thus extended period of leaf greenness in the fall, is tied to the delay in nitrogen resorption. In this study we did witness some degree of an extended growing season in terms of
delayed nitrogen resorption, but not photosynthetic rate, and only in 2012 when soil
moisture was high.

It is possible that sampling methods hid resorption in *L. styraciflua* in 2012,
explaining its minimal resorption by the end of November. *L. styraciflua* is an
indeterminant species (Lechowicz 1984), so it is likely that it was growing new leaves for
the duration of the growing season. Leaf sampling is destructive, so by removing the
old leaves, by design only newer leaves were available to sample at future dates. Leaf
nitrogen content declines with age (Herrick and Thomas 2003), so newer leaves have
higher nitrogen content and have not started the resorption process. Counter-arguments
include that no such detection problem was observed in 2011, and *L. tulipifera* is also an
indeterminant species, but its nitrogen data in 2012 was in keeping with the general
trend. Arguments in favor of destructive sampling introducing error into the data on
indeterminant species are the greater spread of values for *L. styraciflua*, and its apparent
continuation of nitrogen accumulation well towards the end of the growing season, after
the other species and already entered the resorption phase.

**4.5 Conclusions**

A general conclusion to be drawn is that warming alone does not determine
growing season length. It can extend the period of green-ness, often at both the
beginning and end of the growing season, and it may affect photosynthetic rate, but it
does not necessarily extend the photosynthetically active period relative to ambient
conditions. The direction of the effect of warming on photosynthetic rate was more variable and showed species differences. Warming treatment of +5°C did seem to delay nitrogen resorption in some species, so it is possible that the extended period of leaf greenness is tied to the continued presence of nitrogen within the leaf at the end of the growing season, despite the initiation of decline in the photosynthetic mechanisms.

As seen in the nitrogen data, observations can vary widely from year to year dependent upon interactions with other environmental factors. It was interesting to observe such variation in response to temperature within the same annual photoperiod regime. In many cases, drought may be as important if not more so than temperature in determining growing season length.

In this particular study, it appears that our seedlings thrived with warming when it was matched with abundant soil moisture. Conversely, during dry conditions the year before, warming inhibited rather than promoted seedling function. This finding suggests that predicting seedling response may be as linked to soil moisture as it is to temperature. To add another level of complexity, it is quite possible that drought conditions in previous growing seasons may have lingering effects on tree physiology.

Predicting a species’ response to warming is important because it can allow us to make educated guesses about their carbon balance. Increased carbon gain both helps an individual to maintain its own health, and also to out-compete others in the early growing season when trees are most dependent upon their carbon reserves to initiate
growth. However, while temperature matters; different soil moisture conditions can
change the magnitude and direction of a phenological or physiological response.
Instead of one all-inclusive verdict on what warming does to tree function, the answer
seems to be "it depends".
5. References

Augspurger, C., & Bartlett, E. (2003). Differences in leaf phenology between juvenile and adult trees in a temperate deciduous forest. Tree Physiology, 23(8), 517-25.


