Tree Seedling Establishment in Response to Warming and Nitrogen Addition in a Temperate Old Field

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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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TREE SEEDLING ESTABLISHMENT IN RESPONSE TO WARMING AND NITROGEN ADDITION IN A TEMPERATE OLD FIELD

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by

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Graduate Program in Biology

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Abstract

Climate change and increased atmospheric nitrogen deposition over the next century may alter the ability of woody species to germinate and compete with grasses and forbs in temperate old fields. To investigate the responses of seed germination and seedling growth to warming and nitrogen, I transplanted seeds and seedlings into plots of a field experiment and conducted a greenhouse experiment. The combination of warming and nitrogen allowed seeds to germinate earlier, although there was no effect on final germination. In the greenhouse nitrogen increased seedling growth, and warming had little effect. However, in the field, warming significantly decreased the growth and survival of *M. coronaria* seedlings. Overall, my results suggest that while warming and nitrogen may have direct effects on germination and establishment of seedlings, these effects may be outweighed by indirect effects via interactions with drought and herbivory.

Keywords

Establishment, germination, growth, nitrogen, old field, seed, seedling, survival, warming
Contribution and Co-Authorship Statement

Dr. Hugh Henry will be co-author on any published manuscript(s) that arise from the contents of this thesis.

For experiments conducted at the Agriculture Canada and Agri-Food Southern Crop Protection and Food Research Centre, seeds and seedlings were planted into a pre-existing warming and nitrogen addition experiment established in 2006. The plots of this experiment were designed, maintained, and monitored by Dr. Hugh Henry.
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<tbody>
<tr>
<td>ABA</td>
<td>abscisic acid</td>
</tr>
<tr>
<td>ITEX</td>
<td>International Tundra Experiment</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NDVI</td>
<td>normalized difference vegetation index</td>
</tr>
<tr>
<td>NIR</td>
<td>near infrared</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinyl chloride</td>
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<tr>
<td>RED</td>
<td>visible red</td>
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<td>RO</td>
<td>reverse osmosis</td>
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Chapter 1

1 Introduction

1.1 Succession in Old Fields

Old field habitats are areas previously used for production of crops or rangeland that have been abandoned (i.e. no longer managed) for a number of years. Colonization of these areas by vegetation in temperate regions follows a fairly predictable pattern. Fields are first colonized by weedy annual plants, followed by perennial grasses, then shrubs and trees, and these successional changes are based on the ability of each species to tolerate increased shading and competition (Monk and Gabrielson 1985). With time and lack of disturbance, later successional trees and shrubs begin to close the canopy (Baeten et al. 2010) and may return the area back into a secondary forest community. The ability of trees and shrubs to invade old fields depends on a large number of variables including previous land use of the area (Benjamin et al. 2005), size of the soil seed bank (Baeten et al. 2010), proximity to forest edges and seed dispersing adults (Gardescu and Marks 2004), herbivory of seeds and seedlings (Östfeld et al. 1997, Mazia et al. 2013) as well as the ability of woody plants to compete with previously established vegetation.

The mechanisms by which plants interact with each other and their environment to change community composition throughout stages of succession have been studied extensively (Connell and Slater 1977, Tilman 1985, Tilman 1987, Thompson 1987, Gleeson and Tilman 1990). Connell and Slater (1977) grouped the mechanisms of succession in disturbed sites into three main groups, facilitation, tolerance, and inhibition, based on plant-plant interactions. Facilitation refers to the process by which early successional species or individuals alter the surrounding environment in a way that favours the establishment of later successional individuals. Evidence for facilitation has been observed often in old fields, where tall pioneer trees or shrubs decrease competing grass biomass and increase shade and nitrogen beneath their canopy, increasing the
survival of seedlings (Weltzin and McPherson 1999, Siemann and Rogers 2003, Rodriguez-Garcia et al. 2011). Facilitation is most often cited in cases where environmental conditions are unfavorable for growth, indicating there may be an advantage to neighbouring plants during stressful periods (Brooker 2006). Tolerance describes the situation where an early successional species does not favour or inhibit the growth of later successional species with its presence. When an early successional species is disturbed or dies, it is replaced in that spot by a seedling, with the species of the latter determined by the relative proportions of seeds or propagules in the area. In cases of inhibition or competition, early successional species modify their environment in a way that prevents the establishment of later successional individuals. For example, non-native grasses commonly repress the colonization of woody species (Flory and Clay 2010), sometimes for exceptionally long periods (Inouye et al. 1987). The end result of plant-plant interactions depends on the ability of the species involved to outcompete their neighbors within their given environment. The availabilities of water, light, and soil nutrients in the surrounding environment play a large role in plant competitive interactions and may help shape plant communities and long term successional patterns (Grime 1979, Tilman 1985, Thompson 1987). Therefore, the impact of plant interactions on community structure and function may be altered by external forces of climate change and nutrient availability (Brooker 2006), possibly changing the direction or speed of succession in temperate old fields.

1.2 Seedling Establishment in Early Successional Communities
1.2.1 Dispersal, Dormancy and Germination

Establishment of woody species in early successional communities is dependent on the availability of tree and shrub seeds within the soil seed bank. Depending on the method of dispersal, deposition of seed into the soil seed bank follows different patterns. Wind dispersed seeds show a slow decline in seed abundance with distance from parent tree, whereas animal dispersed seeds show a sharp decline in seed abundance with increased
distance from the parent, with most falling directly under or around the canopy (Debussche and Lepart 1992, Clark et al. 1999). Gardescu and Marks (2004) found wind dispersed seeds were present at much higher densities in the middle of an old field than seeds of vertebrate dispersed seeds, which may lead to slower encroachment of vertebrate dispersed species within old field communities (Pinder et al. 1995). Seed dispersal may not only affect distance travelled by seeds, but also the likelihood of the seed surviving and germinating. For example, Kollmann and Schill (1996) found both mice and birds cached nuts during the fall. However, mice tended to bury seeds under old field vegetation and shrub cover, whereas birds were more likely to store seeds on top of the soil in bare or open areas increasing the likelihood of seed desiccation.

Seeds of early successional species can persist in the soil seed bank for long periods of time, and therefore large numbers of seeds can germinate if conditions are right for seedling growth and survival (Bazzaz 1979). To ensure seeds do not germinate during temporarily favourable conditions in an unfavourable winter season, most temperate species have some form of seed dormancy (Bewley 1997). The most common forms of dormancy for temperate woody species are non-deep physiological dormancy, which requires stratification for a given period of time at either a low (spring germinating seeds) or high (autumn germinating seeds) temperature, and physical dormancy, in which the seed coat is impermeable and must be broken down for the seed to imbibe water (Baskin and Baskin 2004). In seeds with physiological dormancy, the level of dormancy generally corresponds with the concentration of absisic acid (ABA) present in seed tissues at maturation, with higher concentrations associated with deeper dormancy (Graeber et al. 2012). Stratification induces the production of gibberellins which inhibit the action of ABA, breaking seed dormancy and increasing the responsiveness of seeds to environmental conditions (Finkelstein et al. 2008).

Once the stratification needs of seeds have been met, non dormant seeds begin germinating when external conditions such as light, oxygen, water, and soil temperature are favourable for seedling growth (Obroucheva 2012). Often germination of early successional species is triggered by an increase in the ratio of red/far red light reaching the seed, which is associated with decreased shading by canopy plants and the appearance
of a gap (Fenner 1985). For example, some early successional trees such as pin cherry exhibit mass germination of buried seeds in response to dieback or removal of adults as light becomes available (Marks 1974). Similar to shading by adult trees, thick layers of grass litter or high biomass of dense turf grasses can decrease total percent germination and increase time to germinate (Facelli 1994, Laborde and Thompson 2013). However, in a study by Gill and Marks (1991), total seedling emergence was lower on bare ground than in the presence of annual or perennial plants, suggesting intermediate amounts of litter may be beneficial to seeds by protecting them from desiccation (Donath and Eckstein 2010, Loydi et al. 2013).

### 1.2.2 Seedling Growth and Survival

Seedling growth in old fields depends largely on the ability of newly germinated seedlings to compete with established vegetation. Shading by perennial herbs, particularly grasses, decreases seedling survival and growth (Mazia et al. 2013, Gill and Marks 1991, Desteven 1991b) by limiting photosynthesis and lowering water use efficiency of seedlings (Burton and Bazzaz 1995, Pantera and Papanastasis 2012). The degree to which competition from neighbouring above ground biomass decreases seedling survival depends on species specific differences in shade tolerance and the ability to maintain adequate rates of photosynthesis even at very low light intensities (Kollmann and Reiner 1996, Grubb et al. 1996). Berkowitz et al. (1995) found seedling growth across a variety of old field communities was low due to trade-offs between site quality and competition. In poor quality sites, growth was primarily limited by physiological tolerance of the seedlings to environmental conditions, while in good quality sites, growth was limited by neighbouring old field vegetation, suggesting the intensity of aboveground competition increases with increasing site quality (Putz and Canham 1992). Seedling establishment is also high in gaps where old field vegetation has died or been disturbed by fire or grazing, because competition for resources decreases (Mazia et al. 2013, Jurena and Archer 2003). However, larger gaps can lead to drier soils, which may increase seedling desiccation (Vandenberghe et al. 2006).
1.3 Global Climate Change

Human activity, mainly the combustion of fossil fuels for energy, transportation and industrial activities, has increased global emissions of carbon dioxide, methane and nitrogen dioxide, which all contribute to warming the Earth through the greenhouse effect. While fluctuations in global temperature and carbon dioxide concentrations in the atmosphere have occurred naturally throughout Earth’s geologic timeline, the concentration of greenhouse gasses in the atmosphere as of 2005 was the highest on record over the last 650,000 years (IPCC 2007). As a result, average global surface temperatures have risen and are anticipated to continue rising by 1.1 to 6.4°C by the year 2100, depending on the particular climatic model applied. Warming will be comparatively higher over land masses than oceans, and will be most intense at high latitudes (IPCC 2007). In southern Ontario, increases in average air temperatures of 3-4°C are expected to occur (Christensen et al. 2007). Across North America, the number of cold nights and nights with frost has decreased, while the number of hot days and nights has increased (Brown et al. 2010). There have been decreases in snowfall in the Northern Hemisphere, and an earlier start to spring (Ahas 1999, Schwartz and Reiter 2000).

Changes to climate across the globe may have long term effects on plant communities, because temperature plays an important role in regulating ecosystem processes, such as the activity of soil microbes (Rustad et al. 2001), nutrient availability through N mineralization (Hobbie 1996, Reich et al. 1997) and trace gas emissions (Cantarel et al. 2012), as well as root growth and turnover (Bai et al. 2010). The extent to which increased temperatures will affect ecosystems may depend on water availability; however, long-term predictions about precipitation are difficult to make, because rainfall is expected to increase in some areas and decrease in others (IPCC 2007). Furthermore, water availability may be affected by warmer temperatures regardless of precipitation due to decreases in snow thickness, increased evaporation, and higher use by plants.
1.4 Responses to Climate Change

1.4.1 Phenology

Climate change is expected to alter global patterns in the timing of plant life cycle events, because temperature plays an important role in the development of plants, such as cues of germination and bud burst in the spring (Walck et al. 2011). Changing temperature regimes may also decrease snow cover and snowpack depth, which can influence the timing of plant growth (Brown and Mote 2009). In temperate ecosystems, milder winters and warmer spring temperatures are associated with earlier leaf out and flowering of herbaceous vegetation (Root et al. 2003). Miller-Rushing and Primack (2008) found patterns in advancement of flowering were strongly related to the average temperatures one or two months prior to flowering, with warmer mean temperatures leading to earlier onset of flowers. While warming may lead to earlier leaf out and flowering, responses to warmer temperatures are often species specific (Primack et al. 2009). In both trees and non-trees species that leaf out and flower earliest in the spring are the most responsive to increases in temperature (Fitter and Fitter 2002, Morin et al. 2009). Native habitat may also play a role, because species with large geographical ranges respond most strongly to temperature cues (Morin et al. 2009). Another factor affecting temperature response can be altitude (Vitasse et al. 2009). For example, Richardson et al. (2006) found that the onset of spring temperatures, and subsequent response in tree phenology, was delayed by about 2.7 days for every hundred meters of elevation. Although increases in spring temperatures generally favour earlier and earlier leaf out of trees in northern latitudes (greater than 40 degrees north), the opposite may be true further south, with the switch point occurring around 35 degrees north latitude (Zhang et al. 2007). In this region, winter buds may not reach low enough temperatures for long enough periods to meet chilling requirements for buds, increasing developmental time and delaying phenological leaf out and flowering responses (Morin et al. 2009, Zhang et al. 2007, Thompson and Clark 2008). Deepened snow cover in some areas due to altered precipitation regimes may also delay tree responses to spring temperatures, because the soil stays cool for a long period of time, despite warm air temperatures (Shutova et al. 2006).
Photoperiod constraints on budburst may play a role in the response of temperate tree species to warming. For example, Heide (1993) found long days reduced the time to bud burst in a large number of northern temperate deciduous trees regardless of the ambient temperature, suggesting that day length may be an important cue for initiating leaf out in the spring. Strong photoperiod control has often been found in later successional forest species, whereas earlier successional species have been found to rely primarily on spring temperatures for bud burst cues (Caffarra and Donnelly 2011, Basler and Koerner 2012). This may constrain the response of later successional trees to long term climate changes (Basler and Koerner 2012).

Climate warming has increased the length of the growing season in temperate ecosystems by about 2.1-4.2 days per decade over the last several decades (Jeong et al. 2011, Richardson et al. 2013). Not only is leaf out happening earlier in spring (Thompson and Clark 2008), but autumn colour and plant senescence are occurring later in the year (Estrella and Menzel 2006). Remote sensing data for North America monitoring seasonal patterns in normalized difference vegetation index (NDVI), a measure of greenness, indicate fall dieback may be delayed by up to 9 days during years with a very hot summer (June – September) and autumn (Jeong et al. 2011, Doi and Takahashi 2008).

### 1.4.2 Productivity

Long term climatic changes can increase plant productivity due to increases in the length of the growing season (Rustad et al. 2001). Because warming is expected to be most intense at high latitudes, effects of temperature on productivity and soil nutrient cycling are expected to be greatest in arctic and tundra systems. These areas have been studied extensively using a variety of passive warming and nutrient addition techniques (Chapin et al. 1995, Chapin and Shaver 1996, Henry and Molau 1997, Robinson et al. 1998, Wahren et al. 2005, Post and Pedersen 2008, Zamin and Grogan 2012). Increases in photosynthetic activity and NDVI indicate plant biomass is increasing in response to earlier snowmelt and a longer growing season, as well as an increase in decomposition and mineralization of N by soil microbes (Myneni et al. 1997, Press et al. 1998, Jonasson et al. 1999, Walker et al. 2006, Sistla et al. 2013). Arft et al. (1999) conducted a meta-analysis investigating the combined results of the international tundra experiment (ITEX)
and found warming significantly increased plant biomass across the Arctic, and that herbaceous species, especially graminoids, were more responsive to warming than woody species. However, a more recent analysis has found that long term warming has increased the dominance of low growing woody shrubs across the tundra (Sistla et al. 2013), indicating community responses to warming may change over time.

In contrast to the consistent increases in productivity observed in arctic systems, studies investigating effects of warming in temperate ecosystems and grasslands have reported mixed results, with warming treatments increasing (Engel et al. 2009, Hutchison and Henry 2010, Kardol et al. 2010), decreasing (Klein et al. 2007, Cantarel et al. 2013), or having very little effect on plant biomass (Dukes et al. 2005, Flanagan et al. 2013).

Unlike arctic plants, in which photosynthesis is limited by temperature most of the year, plants at lower latitudes are closer to their photosynthetic optima and are unlikely to show increased rates of photosynthesis in response to increased temperature (Penuelas et al. 2004). However, warming in spring may allow growth of grasses earlier in the season and accelerate the timing of flower production (Hutchison and Henry 2010, Alward et al. 1999, Cleland et al. 2006). Due to their flexible morphology (Arft et al. 1999) and ability to take up and store nutrients (Shaver and Chapin 1995), grasses that begin growing earlier could dominate the plant canopy and shade out competitors (Dunnett and Grime 1999). Warming is expected to increase soil N mineralization rates and litter turnover (Rustad et al. 2001), and higher nutrient availability may increase productivity. Nevertheless, responses to warming may be limited by water stress, because warming increases evaporation and leads to drier soils (Harte and Shaw 1995, De Boeck et al. 2008, Sherry et al. 2008).

### 1.4.3 Range Shifts

In addition to altering productivity and the timing of plant growth, long term changes to climate may alter species range or distribution across the landscape. Distributions of plants will gradually move pole-ward (or upwards in elevation in the case of alpine systems) and some species are currently being observed in communities they have never previously been a part of (Parmesan and Yohe 2003, Leithead et al. 2012, Telwala et al. 2013). For example, Woodall et al. (2009) found seedling recruitment and establishment
of some temperate trees was higher at the northern part of their range than further south. With increased seedling recruitment concentrated to one side of the range, they predicted the species distribution would move 100 km per century due to cycling between death of older trees and regeneration of new ones. However, not all species may respond to warming with changes in distribution (Zhu et al. 2012). Species that live on complex terrain such as mountain tops or cliff sides may not be able to change their distributions and would be more likely to decrease their range (Heusser 2000). Furthermore, some species may be limited in their distribution by mutualistic relationships with pollinators or mycorrhizal associations (Liu et al. 2010).

1.4.4 Germination and Seedling Establishment

As described above, warming has significant effects on plant productivity and phenology. Increases in annual temperature may alter the ability of early successional woody plants to compete with herbaceous vegetation in old fields by affecting the germination of seeds and establishment of newly germinated seedlings. Many woody species have some form of seed dormancy involving hard seed coats or internal physiological cues that must be broken before germination (Obroucheva 2012). Similar to bud burst, many seeds with physiological dormancy require chilling at a low temperature for a certain period of time (Obroucheva 2012, Walck et al. 2011, Zhang et al. 2007) Those with requirements of long stratification at a cold temperature or very narrow optimum temperature ranges may experience declines in germination percentages or delays in germination if those needs are not met in a warming climate, whereas those with broad ranges or short stratification times would benefit from warmer spring temperatures by germinating earlier, increasing the length of their growing season (Walck et al. 2011, Walck et al. 1997). Increases in ambient temperatures are also associated with increased soil temperature and a decrease in soil moisture content (Classen et al. 2010). Small seedlings may be at a disadvantage when soil moisture is low, because their taproots must compete in the same soil plane as the highly branched and fibrous roots of grasses for water. High temperatures can also decrease seedling competitive ability and increase mortality, particularly during dry periods (Davis et al. 1999; (Mazia et al. 2013).
1.5 Ecosystems and Nitrogen

Nitrogen is essential to plant growth and metabolism and is incorporated into chlorophyll, DNA, amino acids, and other cellular components. However, although 78 percent of the atmosphere is nitrogen, very little is available in forms plants are able to take up and utilize. Converting $N_2$ to reactive nitrogen species ammonium ($NH_3$) or nitrate ($NO_3^-$) usable by plants involves breaking a triple covalent bond and is energetically costly. Creation of reactive nitrogen species happens naturally by lightning and fixation by certain species of bacteria and archea or can be done industrially through the Haber-Bosch process (Galloway 2005).

Nitrogen deposition is the movement of reactive nitrogen species from the atmosphere to the earth’s surface. It can occur through dry deposition of dust and fine particulate matter, or through wet deposition within rain or snow. Nitrogen pollution into the atmosphere and subsequent deposition has increased over the last century in response to the increase in the global population and the corresponding demand for food, energy, and transportation (Galloway 2005, Vitousek et al. 1997). Since the early 1900s, when rates of N deposition were only a fraction of the amount of N fixed by natural means, the nitrogen deposition rate in southern Ontario has climbed to 10-20 kg ha$^{-1}$ y$^{-1}$, and the rate of deposition is expected to continue increasing over the next 40 years, reaching levels as high as 20-50 kg ha$^{-1}$ y$^{-1}$ by the year 2050 (Galloway 2005). Ammonium and nitrate are the two predominant reactive nitrogen species in the atmosphere and they are deposited in different ratios depending on location, with ammonium occurring primarily near agricultural areas and nitrate greater near urban and highly industrialized areas (Galloway 2005).

1.6 Responses to Increased Atmospheric Nitrogen Deposition

1.6.1 Plant Productivity

Increasing nitrogen through atmospheric deposition alters nutrient cycling through terrestrial ecosystems and increases plant biomass. High N availability is associated with increased competition aboveground for light and space (Wilson and Tilman 1991, Wilson
and Tilman 1993), and tends to favour fast growing species that are able to quickly increase their biomass and shade out their neighbours (Lee and Caporn 1998, Aerts 1999, van den Berg et al. 2005) or reduce water available for other species (Zavaleta et al. 2003). Grasses in particular are highly responsive to nitrogen due to their flexible morphology and ability to take up and store nutrients (Shaver and Chapin 1995, Aerts 1999). Similarly, in forested systems, increased nitrogen deposition is associated with a widening and flattening of the upper canopy cover and higher nitrogen content in leaf tissues (McNeil et al. 2007). Increased shading by mature trees decreases seedling recruitment and survival in the forest understory (Catovsky and Bazzaz 2002). Although small nitrogen inputs can increase productivity, chronic nitrogen addition may increase nitrogen leaching (Magill et al. 2000) and deplete the soil of calcium, sodium, potassium, and magnesium (Horswill et al. 2008). Resistance of natural communities to acidification from chronic nitrogen addition differs depending on community structure, environmental conditions, and soil type (Tipping et al. 2013).

While aboveground productivity often increases in response to N addition, studies investigating fine root production have demonstrated little to no increase (Nadelhoffe 2000). However, roots of fast growing species show increased morphological plasticity and ability to absorb nutrients (Crick and Grime 1987), which indicates plants may increase production of cellular components, such as proton pumps and other proteins, that aid in the capture of resources (Jackson et al. 1990). In addition to increasing the productivity of individual plants, greater nutrient capture may deplete soil N before slower growing species are able to absorb it (Aerts 1999). Nitrogen can also affect the availability of soil nutrients by slowing the breakdown of litter by the microbial community (Zak et al. 2008). Furthermore, N addition may decrease the diversity of mycorrhizal fungi and limit the number of successful associations that form (Wollecke et al. 1999, Jarvis et al. 2013).

### 1.6.2 Species Diversity

Small inputs of nitrogen increase productivity in terrestrial ecosystems in the short term by increasing nitrogen mineralization and resource capture (Aber et al. 1998). However, additions of reactive nitrogen over long periods significantly decrease species diversity
and increase the biomass of fast growing, nitrogen demanding species (De Schrijver et al. 2011). This pattern has been observed repeatedly in grasslands (Dupre et al. 2010, Maskell et al. 2010, Araya et al. 2013), and is presumably driven by a loss of forb species richness, particularly those that are adapted to nitrogen limiting conditions (Zavaleta et al. 2003, Stevens et al. 2004). Effects of species declines appear to be long lasting, and chronic nitrogen addition plots show little improvement in species richness for years after nutrient addition ceases (Edmondson et al. 2013, Isbell et al. 2013).

1.6.3 Germination and Seedling Establishment

As described above, nitrogen deposition may have long term effects on plant community structure and function, which can affect the establishment of woody species, especially when combined with the effects of warming. Both warming and nitrogen can increase grass productivity (Hutchison and Henry 2010), leading to a thicker litter layer over the winter. While thicker litter benefits larger seeded species by protecting them from desiccation (Donath and Eckstein 2010), it may be detrimental to smaller seeded species that have fewer carbohydrate reserves, because it lengthens the distance they need to sprout to reach sunlight (Facelli 1994). Interactions between soil moisture and nitrogen may also affect the survival of seedlings once they have emerged. Decreases in soil moisture may alter the ability of soil microbes to mineralize nitrogen and make it available to plants (Araya et al. 2013), which could change the competitive balance between grasses and tree seedlings. Changes to nitrogen availability can also decrease species diversity in grasslands (Dupre et al. 2010). Wright et al. (2013) found lower diversity of herbaceous grassland species decreased the long-term survival of planted oak seedlings. However, those that did survive in the low diversity, grass-dominated plots exhibited increased growth over a three year period, suggesting duration of competition may be important, and that once the hardier tree seedlings clear the top of the grass canopy, they may be released from intense levels of competition (Berkowitz et al. 1995).
1.7 Objectives and Hypotheses

My objective was to examine how climate warming and increased atmospheric nitrogen deposition would affect the germination, growth, and survival of early successional woody species in a temperate old field. I hypothesized that germination of seeds and establishment of seedlings in old fields is a function of both direct and indirect effects of warming and nitrogen. I predicted that germination of woody species would be highest and would occur earliest in the spring in warmed plots due to decreased snow cover and warmer temperatures, but germination would be lowest in nitrogen addition plots as a result of increased grass litter. Based on the assumption that the treatments would increase the growth of neighbouring grasses, I predicted seedling survival would decrease with warming and nitrogen addition in the field, and that surviving seedlings in the treatment plots would have decreased growth, leaf area and biomass. However, I predicted that seedling growth and productivity would increase in response to warming and nitrogen addition treatments in the absence of neighbours in the greenhouse.
Chapter 2

2  Materials and Methods

2.1 Study Site Description

My research was conducted at the Agriculture Canada and Agri-Food Southern Crop Protection and Food Research Centre (43° 01’ 46” N, 81° 12’ 52” W) in London, Ontario, Canada. The site was a former agricultural field that was previously planted with rotations of wheat, corn and soybean; however, it has not been plowed, fertilized, or mowed for at least 30 years. The vegetation at the site was dominated by the perennial grasses *Poa pratensis* L. (Kentucky blue grass) and *Bromus inermis* Leyss. (smooth brome) with patchy distribution of the forb *Cirsium arvense* L. (Canada thistle) and the legume *Lotus corniculatus* L. (bird’s-foot trefoil). The forbs *Asclepias syriaca* L. (common milkweed), *Aster ericodes* L. var. *scabra* Torr. & A. Gray (tall goldenrod), and the vine *Vitis riparia* Michx. (riverbank grape) were also present at lower density. The soil was classified as a well to imperfect drained silt loam glacial till (Hagerty and Kingston 1992), and had an average pH of 7.5 (Turner and Henry 2009).

2.2 Design of Warming and Nitrogen Addition Experiment

The field manipulations consisted of three warming treatments (warming all-year, winter warming only, and no warming) crossed with two N treatments (added N and ambient) with the plots organized in a randomized block design (n=10) (Figure 2.1). Each of the 60 plots had a diameter of 113 cm and extended an additional 10 cm outwards into a buffer zone that received the same experimental treatments. Plots were warmed by 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA), suspended 50 cm above the centre of each plot. These heaters simulated solar heating without giving
Figure 2.1 Schematic of one of the experimental blocks. Plots received either warming through ceramic infrared heaters (solid circles) or no warming through dummy heaters (open circles). Plots within each block were randomly assigned to treatments of nitrogen deposition (N), ambient field conditions (C), or reserve plots (R—not used in this study). Redrawn from Turner and Henry (2009).
off photosynthetically active radiation that can alter plant growth. The heaters were raised by up to 25 cm from mid-June to late-August to avoid scorching grass inflorescences in the plots. In the fertilized treatments, nitrogen was added to the plots by two separate methods: a pulse of aqueous ammonium nitrate in late March at a rate of 2 g m\(^{-2}\) y\(^{-1}\) to simulate a pulse of N at snowmelt, and additional ammonium nitrate in late-May in the form of slow release pellets (Florikan ESA, Sarasota, FL, USA) at a rate of 4 g m\(^{-2}\) y\(^{-1}\) to simulate wet and dry deposition during the rest of the year. These addition rates were designed to approximate predicted deposition rates for this region by the year 2050 (Galloway et al. 2005). The plots received warming and nitrogen treatments starting in 2006. Soil temperature and moisture were monitored at the plot level and recorded year round on an hourly basis. Soil temperatures were monitored using 107-BAM-L temperature probes placed at 1 and 5 cm, while soil moisture was measured at depths of 0-15 cm and 0-30 cm using CS616-L time domain reflectometry probes (Campbell Scientific Corp., Edmonton Alberta, Canada). The 2012 summer growing season was only 1.5°C higher on average than historic climate normals for the area, but was drier than an average year, particularly within the heated plots (Table 2.1). To increase the survival of the newly-transplanted seedlings, I watered each seedling with 40mL of reverse osmosis (RO) water twice a week during periods with no rainfall, for 8 weeks following planting.
**Table 2.1** Mean temperature and total precipitation over the plant growing season (May 1- October 31) and winter (November 1- April 30) for the experimental site compared to the climate normals recorded 1971-2000. (Environment Canada, National Climate Data and Information Archive)

<table>
<thead>
<tr>
<th></th>
<th>1971-2000</th>
<th>2012-2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Temperature (°C)</td>
<td>15.8</td>
<td>17.3</td>
</tr>
<tr>
<td>Growing Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Precipitation (mm)</td>
<td>509.8</td>
<td>411.8</td>
</tr>
<tr>
<td>Growing Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Winter Temperature (°C)</td>
<td>-0.9</td>
<td>-0.5</td>
</tr>
<tr>
<td>Total Winter Precipitation (mm)</td>
<td>474.5</td>
<td>388.8</td>
</tr>
</tbody>
</table>
2.3 Seed Germination Experiment

I investigated the effects of warming and nitrogen addition on the germination of five tree and shrub species (*Elaeagnus umbellata* Thunb. (autumn olive), *Rhamnus cathartica* L. (European buckthorn), *Cornus raemosa* Lam. (grey dogwood), *Rhus typhina* L. (staghorn sumac), and *Fraxinus Americana* L. (white ash)) during the winter of 2012-2013 using the field plots described above. Winter warming-only plots were not used. Each of the 4 remaining treatment combinations was replicated 10 times. Each replicate contained 5 individual seed bags filled with one hundred seeds each of a single species, with the exception of white ash, which contained 20 seeds per bag as a result of seed collection limitations (4 treatments × 10 replicates × 4 species × 100 seeds + 4 treatments × 10 replicates × 1 species × 20 seeds = 16800 seeds total).

I collected seeds of each species as close to London, Ontario as possible (Table 2.2) to minimize ecotypic variation and to reflect the growth environment of individuals commonly found in the area. Seeds growing inside fruit were removed and rinsed with RO water. I placed the seeds inside 10 cm × 7.5 cm bags made of screen (mesh size 1 mm) fastened with hot glue. The screen material allowed access to air and water and permitted the collection of all seeds in the spring, yet it provided little thermal insulation from plot conditions. I placed the bags within the litter layer to approximate the natural deposition of seed. In response to extensive seed predation during the winter, I surrounded the seed bags with small hardware mesh cages (mesh size approximately 1 cm) in early spring.

At the beginning of May, when seeds would naturally be germinating in the field, I collected the seed bags from the field. The number of seeds that had germinated in each bag was recorded and those seeds were removed and discarded. I placed the remaining seeds in a germination cabinet for three weeks to compare germination in the field to the number of truly viable seeds. The germination cabinets were set to alternating day/night temperatures of 20 and 15 ºC, with 80 percent relative humidity and a 14/10 h photoperiod. I split each seed bag between 2 petri dishes lined with filter paper, and moistened with 10 mL of RO water. I covered the *Rhus typhina* (staghorn sumac) petri dishes with lightweight, dark plastic to block the light and allow them to germinate
Table 2.2 Seed collection locations by species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collected From</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fraxinus americana</em> L.</td>
<td>University of Western Ontario Arboretum, London Ontario</td>
<td>43.009139N, 81.272974W</td>
</tr>
<tr>
<td><em>Malus coronaria</em> L.</td>
<td>Roadside, Thorndale Ontario</td>
<td>43.102885N, 81.139104W</td>
</tr>
<tr>
<td><em>Rhus typhina</em> L.</td>
<td>Sarnia road, London Ontario</td>
<td>42.998271N, 81.290483W</td>
</tr>
<tr>
<td><em>Elaeagnus umbellata</em> Thunb.</td>
<td>Joanie’s Woods, Sylvan Ontario</td>
<td>43.168813N, 81.793864W</td>
</tr>
<tr>
<td><em>Cornus racemosa</em> Lam.</td>
<td>Sarnia road, London Ontario</td>
<td>42.990667N, 81.316214W</td>
</tr>
<tr>
<td><em>Rhamnus cathartica</em> L.</td>
<td>Brescia Hill, London Ontario</td>
<td>43.003082N, 81.284919W</td>
</tr>
<tr>
<td><em>Prunus virginiana</em> L.</td>
<td>Purchased from St. Williams Nursery and Ecology Centre, St. Williams Ontario</td>
<td>42.69575N, 80.446384W</td>
</tr>
</tbody>
</table>
(personal communication with Mary Gartshore, St. Williams Nursery and Ecology Centre). Additional water was added as needed to prevent drying of the filter paper. I recorded germination every 2 days and seeds were removed from the petri dish if they had a radicle greater than 1 mm long. Seeds that had turned mouldy or folded in response to a light pressure applied with forceps were noted and discarded.

2.4 Seedling Preparation

I collected seeds of 4 early successional woody species (Fraxinus Americana (white ash), Malus coronaria L. (crab apple), Elaeagnus umbellata (autumn olive), and Rhus typhina (staghorn sumac)) in early October. I removed all seeds grown inside fruit, rinsed them with deionized water and allowed them to air dry for 24 hours. All seeds were stored in plastic Ziploc® containers with damp pine shavings in the dark at 4 ºC for the suggested stratification time (Table 2.3). In late March, seeds of each species were planted in the greenhouse in 3.8 cm diameter × 14 cm deep cone shaped planting containers (Stuewe & Sons, Inc., Oregon, USA) to allow roots to expand downwards without becoming pot bound. Containers were filled with soil retrieved from the top 15 cm of the soil horizon from the field that had been passed through a 2.36 mm sieve, topped with 1 cm of Promix™ soil to prevent cracking of the soil and to minimize seed exposure during germination. After 6 weeks of growth in the greenhouse, once the threat of frost had passed, seedlings from the greenhouse were transplanted into the field plots along with another species, Prunus virginiana L. (choke cherry), that I had purchased from St. Williams Garden Ecology Centre (Figure 2.2). All seedlings were between 3 and 10 cm in height at the time of planting, similar in size to those that had germinated naturally outside. Bulk soil around the roots of each seedling was shaken off before planting to maximize contact of the roots with the treated soil in the plots. Each seedling was surrounded aboveground by a 4 cm long piece of 2.5 cm diameter white PVC pipe with a 1 cm wide strip of copper tape surrounding the top to help protect the stems from herbivory. Initial height of each seedling was recorded from the soil surface to the apical bud.
<table>
<thead>
<tr>
<th>Species</th>
<th>Pre-treatment</th>
<th>Warm Stratification</th>
<th>Cold Stratification</th>
<th>Seed Sowing Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fraxinus americana</em></td>
<td>White Ash</td>
<td>-</td>
<td>90 days at 2-4°C</td>
<td>Insert key into soil seed side down until seed is covered</td>
</tr>
<tr>
<td><em>Malus corinaria</em></td>
<td>Crab Apple</td>
<td>Soak seeds 24 h in room temp water</td>
<td>-</td>
<td>130 days at 2-4°C</td>
</tr>
<tr>
<td><em>Rhus typhinia</em></td>
<td>Staghorn Sumac</td>
<td>Remove seed coat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Cornus racemosa</em></td>
<td>Gray Dogwood</td>
<td>Soak seeds 24 h in room temp water</td>
<td>60 days at 30°C</td>
<td>90 days at 2-4°C</td>
</tr>
<tr>
<td><em>Elaeagnus umbellata</em> *</td>
<td>Autumn Olive</td>
<td>Remove seed coat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Rhamnus cathartica</em> *</td>
<td>European Buckthorn</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 2.2 Placement of tree seedlings and sampling rings within each plot.
2.5 Vegetation Sampling

During the growing season, measures of seedling condition were taken every 2 weeks using a 4 point scale, with 0 indicating the seedling was dead, 1 indicating the seedling was alive, but the shoots and leaves were in poor condition, 2 indicating the seedling was in moderate condition, and 3 indicating the seedling was in good condition. Leaf number was recorded at three points in the growing season: May, July, and September. Measures of percent cover were estimated visually from above the plot in a 5 cm radius around each seedling to determine the degree of shading by the surrounding herbaceous species. Aboveground biomass of grasses was also estimated non-destructively at the subplot level in May, July, and September using leaf height to mass allometry, as described by Hutchison and Henry (2010). The latter estimates were taken from 113 cm$^2$ plastic sampling rings that I had anchored in each of the 60 subplots in early March.

Soil cores were taken in June to estimate the belowground standing crop of the herbaceous species. A 15 cm deep × 2 cm diameter core was removed from the northeast quadrant of each plot approximately 15 cm inside of the plot boundary. Cores were weighed and immersed in RO water to break apart the soil and free the roots. Any rocks, leaf litter or live aboveground biomass were removed and discarded. Roots were collected with forceps and a 500 µm sieve, and dried for 2 days at 65 ºC before being weighed. Biomass measurements were extrapolated to the plot level based on soil surface area.

2.6 Seedling Sampling

Surviving seedlings were destructively harvested in late September at the end of the growing season and final condition scores were recorded. Final height of each seedling was taken, measuring from soil surface to the top of the apical bud. Leaves were counted then removed at the base of the petiole and measured using a Li-3100 leaf area meter to determine total leaf area per seedling. Roots were washed with RO water and gently
blotted dry to remove any soil. All samples were dried at 65 ºC for 2 days before final biomass measurements were taken.

### 2.7 Design of Greenhouse Experiment

The experimental design consisted of two warming treatments (ambient temperature and warmed) crossed with two nitrogen addition treatments (ambient and nitrogen addition) in a randomized design containing 5 blocks (Figure 2.3). The warming was applied at the plot level by 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA) suspended 40 cm above the soil surface supported by a 210 cm long by 90 cm wide by 90 cm tall angle iron framework. Seeds of 4 early successional field species (*Elaeagnus umbellata* (autumn olive), *Rhamnus cathartica* (European buckthorn), *Rhus typhina* (staghorn sumac), and *Fraxinus Americana* (white ash)) were planted individually in 15 cm diameter plastic pots. Each pot was filled with a 3:1 mixture of Promix™ and sieved soil from the field site. Once the seeds had germinated, the pots were placed in a circular array around the centre of each heater, with each array containing 2 pots of each species. Individual pots were randomized within an array every week to help control for positional effects. One pot of each species within each array was assigned a nitrogen addition treatment and a total of 6 g m⁻² was added in the form of ammonium nitrate over the course of the experiment in increments of 2 g m⁻².
**Figure 2.3** Depiction of greenhouse plots. Plots received either warming through ceramic infrared heaters ($W^+$) or no warming ($W^-$). One pot of each species within each plot was assigned to nitrogen addition ($N^+$).
2.8 Greenhouse Seedling Measurements

During the course of the experiment, temperatures in 3 randomly selected plots of each warming treatment were recorded every hour using Log Tag Analyzer temperature loggers, (MicroDAQ Ltd.). I took measurements of seedling height and leaf number at the beginning of the experimental period to provide a baseline measurement of growth for each individual. After 6 weeks, I destructively harvested the seedlings and processed them in the same manner as the seedlings from the field experiment. I used a handheld leaf clip spectroradiometer (Field Spec Handheld Plant Probe, Analytical Spectral Devices, Boulder, CO, U.S.A.) to obtain a measure of the normalized vegetation index (NDVI) for each seedling to quantify differences in leaf greenness between treatments. I took spectral measures from the third leaf from the apical bud of each seedling. Each spectral measurement was the average of 10 spectra taken through the leaf clip. I calculated NDVI using the equation \( \text{NDVI} = \frac{\text{NIR} - \text{RED}}{\text{NIR} + \text{RED}} \), where NIR (near infrared) is the average reflectance from 720-740 nm and RED (visible red) is the average reflectance from 660-680 nm (Figure 2.4).

2.9 Statistical Analyses

For analyzing the treatment effects on seed germination and seedling growth and survival, I used a randomized block ANOVA that included both warming and nitrogen as fixed between-plot factors as well as interaction term for the effects of their treatment combination. For measuring growth of grasses during three points of the growing season, I used the same analysis but with date as an additional fixed factor and plot as a random factor to account for repeated measures of the plots throughout the growing season. Biomass data were transformed by square root to satisfy the assumptions of normality of the ANOVA test. To examine effects of treatment on number of seed bags eaten and seedling mortality, I used a contingency analysis with mortality corrected by treatment. For effects of grass biomass on seedling condition, I used a logistic regression with seedling condition score (on an ordinal scale of 0 to 3) and percent cover of surrounding
grass biomass as factors. I ran all analyses using the Fit y by x and Fit Model platforms in JMP 10.0 (SAS Institute Inc., Cary, NC, USA).
**Figure 2.4** Sample data of reflectance spectra by treatment of *Fraxinus Americana* used to calculate NDVI values. Dotted lines indicate visible red (RED) wavelengths (average reflectance from 660-680 nm) and near infrared (NIR) wavelengths (average reflectance from 720-740 nm).
Chapter 3

3  Results

3.1 Seed Germination

Prior to the installation of the wire exclosures in the spring, almost half of the seed bags were predated. With respect to seed bag predation, there was a significant interaction between warming and N addition pooled over all species (P=0.005), with warming almost doubling the predation of bags in plots with no added N, and intermediate amounts of predation occurring in all N addition plots (Fig. 3.1). Taking into account both predation and lack of germination, for the percentage of seed bags with >0% germination in the field as of 1 May, there was a significant interaction between warming and N addition pooled across species (P=0.043), with N addition promoting increased germination, but only in the warmed plots (Fig. 3.2). When mean percent cumulative germination (including germination in the field) was assessed for seeds subsequently germinated in growth chambers, there were no significant effects on a per species basis (Table 3.2), although percent germination of seeds from warmed N addition plots was consistently high (Figure 3.3). Seeds of Cornus racemosa did not germinate either in the field or the incubator and were therefore excluded from the per species analyses.
Figure 3.1 Percentage of seeds bags predated per treatment, pooled across all species (n=10 for each species). Associated significance test shown in Table 3.1.
Figure 3.2 Percentage of seed bags with >0% germination in the field as of 1 May, taking into account both predation and lack of germination. Data are pooled across all species (n=10 for each species), with the associated significance test shown in Table 3.1.
Figure 3.3 Percent cumulative germination in the growth chamber by treatment for each species: (A) *E. umbellata* (B) *F. americana* (C) *R. cathartica* (D) *R. typhina*. Points show mean germination percentages of seeds per bag (+/- standard error bars). All seeds were incubated at 20 °C/15 °C, with a 16 h photoperiod and 80% RH.
Table 3.1 Summary of contingency test P-values for effect of treatment on number of seed bags predated over the winter and the percentage of seed bags with >0% germination in the field as of 1 May. Analyses were pooled over all species.

<table>
<thead>
<tr>
<th></th>
<th>Bags predated</th>
<th>Bags with germination &gt;0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>&lt;0.001***</td>
<td>0.999</td>
</tr>
<tr>
<td>N</td>
<td>0.194</td>
<td>0.265</td>
</tr>
<tr>
<td>W×N</td>
<td>0.017*</td>
<td>0.043*</td>
</tr>
</tbody>
</table>

W- warming; N- nitrogen. Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
Table 3.2 Summary of ANOVA P-values for effect of treatment on percent germination in the field and total percent cumulative germination (including germination in the field) after 21 days of incubation by species.

<table>
<thead>
<tr>
<th>Effect</th>
<th>E. umbellata</th>
<th>F. americana</th>
<th>R. cathartica</th>
<th>R. typhina</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Field</td>
<td>Total</td>
<td>In Field</td>
<td>Total</td>
<td>In Field</td>
</tr>
<tr>
<td>W</td>
<td>0.790</td>
<td>0.189</td>
<td>0.553</td>
<td>0.218</td>
</tr>
<tr>
<td>N</td>
<td>0.331</td>
<td>0.994</td>
<td>0.682</td>
<td>0.758</td>
</tr>
<tr>
<td>W×N</td>
<td>0.767</td>
<td>0.919</td>
<td>0.131</td>
<td>0.090</td>
</tr>
</tbody>
</table>

W- warming; N- nitrogen. Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
3.2 Greenhouse Experiment

The average daily temperature 2 cm above the soil surface for the non-warmed greenhouse pots was 26.9°C, and the average warming effect was 1.8 °C (Figure 3.4). Seedlings of *E. umbellata, F. americana,* and *R. typhina* had significant increases in growth, total leaf area and aboveground biomass, and changes in leaf number, in response to N addition (Table 3.3; Figs. 3.5 and 3.6), but there were no significant effects on *R. cathartica*. Both *R. cathartica* and *R. typhina* seedlings exhibited significant increases in NDVI (indicating greener tissues) in response to N addition (P=0.0118 and P=0.0407, respectively; Figure 3.7). Nitrogen addition significantly increased the root biomass of *E. umbellata* and *R. typhina* (P=0.026 and P= 0.002, respectively; Table 3.3) Warming had no significant effects on growth, leaf number, total leaf area, biomass or NDVI of *E. umbellata, F. americana,* or *R. cathartica* (Table 3.3). However, for *R. typhina* there was a significant interaction between warming and N addition (P=0.041), whereby N addition increased NDVI, but only in warmed plots (Table 3.3, Figure, 3.7).
Figure 3.4 Average daily temperatures taken 2 cm above the soil surface of non-warmed (dotted line) and warmed (solid line) greenhouse pots (n=3).
Table 3.3 Summary of ANOVA P-values for effect of treatment within the greenhouse on total growth (final height – initial height), change in leaf number (final leaf number-initial leaf number), leaf area, aboveground biomass, root biomass, and normalized difference vegetation index (NDVI), by species.

<table>
<thead>
<tr>
<th>Effect</th>
<th>E. umbellata</th>
<th>F. americana</th>
<th>R. cathartica</th>
<th>R. typhina</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.433</td>
<td>0.345</td>
<td>0.525</td>
<td>0.082</td>
</tr>
<tr>
<td>N</td>
<td>0.031*</td>
<td>0.003**</td>
<td>0.109</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>W×N</td>
<td>0.086</td>
<td>0.184</td>
<td>0.430</td>
<td>0.029*</td>
</tr>
<tr>
<td><strong>Leaf Number</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.772</td>
<td>0.683</td>
<td>0.937</td>
<td>0.814</td>
</tr>
<tr>
<td>N</td>
<td>0.024*</td>
<td>&lt;0.001***</td>
<td>0.166</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>W×N</td>
<td>0.563</td>
<td>0.288</td>
<td>0.899</td>
<td>0.555</td>
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<tr>
<td><strong>Leaf Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.771</td>
<td>0.976</td>
<td>0.780</td>
<td>0.707</td>
</tr>
<tr>
<td>N</td>
<td>0.003**</td>
<td>&lt;0.001**</td>
<td>0.210</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>W×N</td>
<td>0.170</td>
<td>0.930</td>
<td>0.456</td>
<td>0.536</td>
</tr>
<tr>
<td><strong>Aboveground</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.961</td>
<td>0.806</td>
<td>0.380</td>
<td>0.517</td>
</tr>
<tr>
<td>N</td>
<td>0.005*</td>
<td>0.002**</td>
<td>0.513</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>W×N</td>
<td>0.172</td>
<td>0.790</td>
<td>0.735</td>
<td>0.402</td>
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<tr>
<td><strong>Root Biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.544</td>
<td>0.483</td>
<td>0.609</td>
<td>0.295</td>
</tr>
<tr>
<td>N</td>
<td>0.026*</td>
<td>0.106</td>
<td>0.984</td>
<td>0.002**</td>
</tr>
<tr>
<td>W×N</td>
<td>0.188</td>
<td>0.497</td>
<td>0.898</td>
<td>0.373</td>
</tr>
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</table>
### NDVI

<table>
<thead>
<tr>
<th></th>
<th>Value1</th>
<th>Value2</th>
<th>Value3</th>
<th>Value4</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>0.994</td>
<td>0.745</td>
<td>0.971</td>
<td>0.047*</td>
</tr>
<tr>
<td>N</td>
<td>0.311</td>
<td>0.203</td>
<td>0.011*</td>
<td>0.002**</td>
</tr>
<tr>
<td>WxN</td>
<td>0.679</td>
<td>0.247</td>
<td>0.359</td>
<td>0.040*</td>
</tr>
</tbody>
</table>

W- warming; N- nitrogen. Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
Figure 3.5 Mean growth (final height – initial height) (cm) of A) *E. umbellata*, B) *F. americana*, C) *R. cathartica*, D) *R. typhina* after 6 weeks of growth in the greenhouse. Error bars represent standard error (n=6). Associated significance values are listed in Table 3.3.
Figure 3.6 Mean change in leaf number of A) *E. umbellata*, B) *F. americana*, C) *R. cathartica*, D) *R. typhina* seedlings after 6 weeks of growth in the greenhouse. Error bars represent standard error (n=6). Associated significance values are listed in Table 3.3.
Figure 3.7 Mean NDVI (normalized difference vegetation index) of A) *E. umbellata*, B) *F. americana*, C) *R. cathartica*, D) *R. typhina* seedlings after 6 weeks of growth in the greenhouse. Error bars represent standard error (n=6). Associated significance values are listed in Table 3.3.
3.3 Seedling Transplantation into Warmed and N Fertilized Plots in the Field

In addition to experiencing very early snow melt, the field site was subject to drought conditions over much of the experimental period. Consequently, soil volumetric water content was very low during the period in which the seedlings were in the field (May 1-September 30), reaching a minimum value of 0.099 vol/vol in late July. Plots warmed by infrared heaters were significantly drier than ambient plots (P= <0.0001), although even ambient plots were very dry (Fig. 3.8). Warming significantly decreased survival of *M. coronaria* seedlings by approximately one half (chi-squared test; P=0.044), but there were no significant treatment effects on the survival of other species (Table 3.4; Figure 3.9). *M. coronaria* seedlings in warmed plots also produced 33% less biomass and had 57% less total leaf area than those in non-warmed plots (P=0.010 and P=0.009, respectively; Figures 3.10 and 3.11). For seedling height, there was a significant interaction between warming and N addition for *M. coronaria* (P=0.033), with N addition decreasing seedling height in warmed plots (Figure 3.12). Ninety-seven percent of *Prunus virginiana* seedlings were predated, so the species was excluded from further analyses. There were no significant differences in seedling biomass, total leaf area or seedling height for the other species (Table 3.5).
Figure 3.8 Average daily soil volumetric water content of non-warmed plots (dashed line), warmed plots (solid line), and the 2007-2011 site average (dash-dotted line) from 1 May to 1 October 2012 in the field experiment (warmed plots n=20, non-warmed plots n=40).
Figure 3.9 Percent survival of (A) *M. coronaria*, (B) *F. americana*, (C) *E. umbellata* and (D) *R. typhina* seedlings by treatment recorded on September 25, 2012 (n=10, data pooled for ambient and winter warming-only plots; *F. americana* seedlings not planted in winter warming-only plots).
Table 3.4 Summary of Chi-squared P-values for survival of seedlings by species planted across different treatment groups.

<table>
<thead>
<tr>
<th>Species</th>
<th>Survival between treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Elaeagnus umbellata</em></td>
<td>0.300</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>0.273</td>
</tr>
<tr>
<td><em>Malus coronaraia</em></td>
<td>0.044*</td>
</tr>
<tr>
<td><em>Rhus typhina</em></td>
<td>0.179</td>
</tr>
</tbody>
</table>

Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
Table 3.5 Summary of ANOVA P-values for effect of treatment on total biomass, leaf area, and final height by species.

<table>
<thead>
<tr>
<th>Effect</th>
<th>E. umbellata</th>
<th>F. americana</th>
<th>M. coronaria</th>
<th>R. typhina</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.829</td>
<td>0.674</td>
<td>0.010*</td>
<td>0.521</td>
</tr>
<tr>
<td>N</td>
<td>0.900</td>
<td>0.452</td>
<td>0.3382</td>
<td>0.166</td>
</tr>
<tr>
<td>W×N</td>
<td>0.5902</td>
<td>0.410</td>
<td>0.090</td>
<td>0.339</td>
</tr>
<tr>
<td><strong>Leaf Area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.795</td>
<td>0.126</td>
<td>0.009**</td>
<td>0.676</td>
</tr>
<tr>
<td>N</td>
<td>0.888</td>
<td>0.162</td>
<td>0.460</td>
<td>0.483</td>
</tr>
<tr>
<td>W×N</td>
<td>0.581</td>
<td>0.660</td>
<td>0.708</td>
<td>0.551</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.750</td>
<td>0.310</td>
<td>0.619</td>
<td>0.381</td>
</tr>
<tr>
<td>N</td>
<td>0.986</td>
<td>0.281</td>
<td>0.045*</td>
<td>0.129</td>
</tr>
<tr>
<td>W×N</td>
<td>0.339</td>
<td>0.0814</td>
<td>0.033*</td>
<td>0.779</td>
</tr>
</tbody>
</table>

W- warming; N- nitrogen. Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
Figure 3.10 Mean total biomass of (A) *M. coronaria*, (B) *F. americana*, (C) *E. umbellata* and (D) *R. typhina* seedlings by treatment at the end of the summer growing season (n=10, data pooled for ambient and winter warming-only plots; *F. americana* seedlings not planted in winter warming-only plots).
Figure 3.11 Mean total leaf area (cm$^2$) of (A) *M. coronaria*, (B) *F. americana*, (C) *E. umbellata* and (D) *R. typhina* seedlings by treatment at the end of the summer growing season (n=10, data pooled for ambient and winter warming-only plots; *F. americana* seedlings not planted in winter warming-only plots).
Figure 3.12 Mean height (mm) of (A) *M. coronaria*, (B) *F. americana*, (C) *E. umbellata* and (D) *R. typhina* seedlings by treatment at the end of the summer growing season (n=10, data pooled for ambient and winter warming-only plots; *F. americana* seedlings not planted in winter warming-only plots).
The biomass of the two grass species present in the plots were measured to determine the degree of shading seedlings faced in each treatment. Total grass biomass (including both Poa pratensis and Bromus inermis) increased significantly in N addition plots (P=0.036; Fig. 3.13a). This effect was explained by increases in the aboveground biomass of P. pratensis in response to N addition (P=0.025), and there was also a marginally significant increase in P. pratensis biomass with warming (P=0.070; Fig. 3.10b). For B. inermis, there was a significant interaction between N addition and sampling date (P=0.047), which was explained by significant increases in biomass in response to N addition in May, but attenuation of this effect later in the summer when the tillers senesced. For roots (pooled over all species), there was a marginally significant trend of increased biomass in N addition plots (P=0.0516; Table 3.6), but no significant effect of warming (P=0.8806).

When a logistic regression was performed to investigate the relationship between grass cover (in a 5 cm radius around each seedling) and tree seedling condition, increased grass cover significantly increased the probability that the seedling was scored in good condition in May, shortly after the seedlings were planted (E. umbellata - P=0.038; F. americana - P =<0.0001; M. coronaria - P =0.0010; Table 3.6). This effect remained significant for F. americana throughout June and July (P=<0.0001 and P=0.0126, respectively). The condition of R. typhina (planted in late May) was not significantly affected by percent cover early in the growing season, but became significant in July and August (P=0.0450 and P=0.0273, respectively).
Table 3.6 Summary of ANOVA P-values for effect of treatment and date on above and below ground productivity of grasses.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Aboveground Productivity</th>
<th>Root Mass</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>B. inermis</td>
<td>P. pratensis</td>
<td>(All Species)</td>
</tr>
<tr>
<td>W</td>
<td>0.589</td>
<td>0.339</td>
<td>0.070</td>
<td>0.880</td>
</tr>
<tr>
<td>N</td>
<td>0.036*</td>
<td>0.515</td>
<td>0.025*</td>
<td>0.051</td>
</tr>
<tr>
<td>D</td>
<td>&lt;0.001***</td>
<td>&lt;0.001***</td>
<td>0.061</td>
<td>-</td>
</tr>
<tr>
<td>W×N</td>
<td>0.657</td>
<td>0.338</td>
<td>0.153</td>
<td>0.309</td>
</tr>
<tr>
<td>W×D</td>
<td>0.574</td>
<td>0.966</td>
<td>0.676</td>
<td>-</td>
</tr>
<tr>
<td>N×D</td>
<td>0.169</td>
<td>0.046*</td>
<td>0.211</td>
<td>-</td>
</tr>
<tr>
<td>W×N×D</td>
<td>0.996</td>
<td>0.515</td>
<td>0.390</td>
<td>-</td>
</tr>
</tbody>
</table>

W- warming; N- nitrogen; D- date. Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
Figure 3.13 Aboveground biomass A) Total grass biomass, B) *Poa pratensis*, and C) *Bromus inermis* for three sampling dates over the 2012 growing season. Data were pooled over ambient and winter-warmed plots (n=20), vs. all-year warmed plots (n=10). Error bars denote standard errors. Associated significance tests are shown in Table 3.6.
Figure 3.14 Estimated root biomass (all species) in treatment plots. Data were pooled over ambient and winter-only warmed plots (n=20), vs. all-year warmed plots (n=10). Error bars denote standard errors. Associated significance tests are shown in Table 3.6.
Table 3.7 Summary of logistic regression P-values for effect of percent grass cover on seedling condition score over the growing season, by species.

<table>
<thead>
<tr>
<th></th>
<th>E. umbellata</th>
<th>F. americana</th>
<th>M. coronaria</th>
<th>R. typhina</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>0.038*</td>
<td>&lt;0.001***</td>
<td>0.001**</td>
<td>-</td>
</tr>
<tr>
<td>June</td>
<td>0.240</td>
<td>&lt;0.001***</td>
<td>0.059</td>
<td>0.819</td>
</tr>
<tr>
<td>July</td>
<td>0.972</td>
<td>0.012*</td>
<td>0.194</td>
<td>0.045*</td>
</tr>
<tr>
<td>August</td>
<td>0.558</td>
<td>0.099</td>
<td>0.869</td>
<td>0.027*</td>
</tr>
</tbody>
</table>

Asterisks denote significance (* 0.05-0.01, ** 0.01-0.001, *** <0.001)
Chapter 4

4 Discussion

4.1 Woody Species Establishment: Overall Responses to Warming and Nitrogen

My study tested the hypothesis that climate warming and increases in atmospheric nitrogen (N) deposition will affect the germination, emergence and survival of early successional woody species in temperate old fields. The three experiments I conducted were designed to isolate the effects of warming and N on seed germination from their effects on the growth of newly emerged seedlings, both in the presence of neighbours (in the field), and the absence of neighbours (in the greenhouse). For seeds, although the combination of warming and N addition increased average percent germination in the field, contrary to my prediction, there were no significant effects of warming alone or N addition alone on percent germination. In addition, it was clear that treatment effects on seed predation played an important role in determining germination success. As for seedling growth, under controlled conditions in the greenhouse, seedlings grew significantly taller and had greater number of leaves and total leaf area under nitrogen addition, but responded very little to increases in soil surface temperature. However, in the field, warming played a large role in the survival and growth of Malus coronaria seedlings, with seedlings in warmed plots having lower survival, biomass and leaf area than those in ambient plots. Therefore, while the seeds and seedlings of the temperate woody species I examined have the potential to respond directly to warming and nitrogen addition through increased germination and growth responses, the most influential effects on seedling establishment appeared to occur via the indirect effects of warming and nitrogen addition on other factors such as competitor biomass, herbivore activity and soil water content.
4.2 Interaction of Warming and Nitrogen Increases Germination in the Field

As discussed previously, only the combination of warming and N addition increased average percent germination in the field, whereas there were no significant effects of warming alone or N addition alone on percent germination. In warmed plots, earlier snow melt would have exposed the seeds to increased water, sunlight, and air temperature fluctuations earlier in the season than the ambient temperature plots, which could initiate signaling pathways within seeds to break dormancy and begin germination (Fenner 1985; Obroucheva 2012). There can also be direct effects of N addition on seed germination; for example, the priming of seeds with solutions of KNO$_3$ prior to incubation increased germination percentage and decreased total germination time in chestnut-leaved oak (*Quercus castaneifolia*), and the forb California poppy (*Eschscholzia californica*) (Hadinezhad et al. 2013, Lee et al. 2010). In addition to the direct effects of N, increased seed germination can also be caused in part by N addition effects on litter layer thickness. A dense litter cover is associated with decreased light penetration, high humidity, and a reduction in temperature fluctuations (Donath and Eckstein 2010), which may be advantageous for preventing herbivory and desiccation, especially for large seeded species (Donath and Eckstein 2010, Loydi et al. 2013, Burton and Bazzaz 1991). Due to the significant increase in *Poa pratensis* biomass during the growing season in response to N addition, there was a large amount of grass litter that accumulated in the N addition plots. Nevertheless, while increases in litter layer thickness have increased tree and shrub germination in some studies (Gill and Marks 1991, Desteven 1991a), the opposite effect has been observed in others (Facelli 1994), which suggests these responses may be species specific. Since litter acts as a buffer against changing environmental conditions (Donath and Eckstein 2008) it may have helped maintain seed viability throughout the winter in warmed plots where there was less insulation by snow cover (Shimano and Masuzawa 1998), but is unlikely to have directly caused increased germination in the spring.

Although it was not the intention of the field germination trials to examine seed predation, from mid-November until April, seeds from 42% of the bags were eaten,
averaged across all species. Seed predation was highest in warmed plots, intermediate in N addition plots and lowest in ambient temperature plots with no added N. Unlike the ambient temperature plots, the warmed plots lacked continuous snow cover over much of the winter. The meadow vole (*Microtus pennsylvanicus*) was a highly abundant predator at the field site (Moise and Henry 2012), and the increased visibility and exposure of seeds in warmed plots may have increased seed detection in the snow-free plots. Similarly, Shimano and Masuzawa (1998) found predation of beech seeds was 70% higher in sites with no snow cover than in snow-covered sites, because the presence of snow concealed the sight and smell of seeds from rodents. In the N addition plots, increased grass litter may have increased seed predation, which is consistent with the observation that meadow voles preferentially choose habitat with abundant herbaceous cover (Pusenius and Ostfeld 2002). Deeper herbaceous cover may promote a sense of safety for voles (Koivunen et al. 1998), and increased use of densely covered areas increases the chances of seeds being encountered and eaten in these areas (Gill and Marks 1991, Ostfeld and Canham 1993). The percentage of seed bags predated provided an estimate of the degree of predation seeds might experience in patches of decreased snow cover or higher biomass in old fields under future conditions, which may be useful for predicting patterns of succession. However, predation during the winter also decreased the number of seed bags available for germination trials. Therefore, while significant results were obtained when germination was examined in results pooled over all species, potentially significant effects at the species level may have been obscured by reduced statistical power.

In addition to the significant effects of treatment on seed predation, there were also significant differences in seed predation among tree species. *Elaeagnus umbellata* was most frequently eaten, followed by intermediate predation on *Fraxinus americana*, *Rhus typhina* and *Cornus racemosa* seeds. *R. cathartica* was the least favoured, with 40% fewer bags predated than *E. umbellata*. Several other studies of *R. cathartica* have noted low consumption of seeds by small mammals (Gill and Marks 1991, Shahid et al. 2009). Bark, leaves, unripe fruits and seeds of *R. cathartica* contain the chemical emodin, which may affect the health and digestion of small mammals (Sherburne 1972). Differential selection by seed predators may alter the dispersal and establishment of woody species in
old fields (Ostfeld et al. 1997). Higher consumption of native species over invasive species such as *R. cathartica* may also increase the invasion of non-native species in old field habitats (Maron et al. 2012), although such statements cannot be supported conclusively by my study, given the limited number of species used, along with the lack of control for phylogeny (i.e. the intention of my study was to not explicitly compare the responses of native versus invasive species).

Overall, the successful emergence of seeds of early successional woody species in a future environment will likely be influenced by the balance between changes in the timing of germination and predation risk brought about by increasing temperature and N deposition. Earlier germination in the spring may provide an advantage for seedlings by allowing them to emerge when the soil is moist and competing herbaceous biomass is still relatively low (Classen et al. 2010). However, selective predation of palatable species by rodents in patches with decreased snow cover or increased litter may alter species abundance in old fields and lead to a patchy distribution of surviving seedlings (Ostfeld and Canham 1993).

4.3 Seedling Responses to Warming and Nitrogen in the Absence of Neighbours

My greenhouse experiment tested the hypothesis that climate warming and increased atmospheric N deposition will have direct effects on the growth of early successional woody seedlings. As expected, N addition significantly increased the height, leaf number, and leaf area of almost all species without competition, with the exception of *R. cathartica* (Bloor et al. 2008). Given that I used a mixture of field-collected soil and Promix in this experiment, it is possible that *R. cathartica* formed associations with arbuscular mychorrhizal fungi found naturally in the soil, and these associations can enhance N acquisition (Knight et al. 2007), allowing *R. cathartica* to overcome N limitation.
In contrast to the influential effects of N addition, warming did not significantly affect the growth and NDVI of most of the species. Such a result could be explained by the relatively favourable average temperatures of 26 °C experienced by the seedlings in the ambient temperature pots, whereas warming might have moved the seedlings closer to their optimum growth temperatures if ambient temperatures had been cooler. In the case of *R. typhina* seedlings, which exhibited decreased growth and NDVI values in response to warming, it is possible that the warming pushed the seedlings past their growth optima. Although warming can increase desiccation stress, *R. typhina* creates a dense, spreading mat of fine roots (Kock et al. 2008; Voss & Reznicek 2012), that allows it to grow in dry, gravely soils and makes it an ideal early successional species, and in my study *R. typhina* seedlings had 80 percent greater root mass than the other species. Also, pots were watered daily, so it is unlikely that decreased growth by *R. typhina* was caused by desiccation stress.

4.4 Seedling Growth and Survival in Response to Warming and Nitrogen in the Field

In contrast to seedlings grown in the greenhouse, survival of seedlings in the field was low across all species and treatment groups, and seedling height also increased very little over the summer. Other studies investigating seedling establishment in old fields have reported similar results (Gardescu and Marks 2004, Gill and Marks 1991, Berkowitz et al. 1995). Slow growth may be attributed to competition, both above and below ground, with herbaceous plants; Bloor and colleagues (2008) reported that competition with grasses reduced seedling biomass by almost 80 percent under N addition treatments. The type of herbaceous vegetation may also be important in determining the degree of competition experienced by seedlings. For example, in a study by Davis et al. (2005), half as many seedlings survived when grown in the presence of cool season (C<sub>3</sub>) grasses, than in the presence of warm season (C<sub>4</sub>) grasses. The high mortality rates in the case of the former were attributed to changes in soil chemistry and water availability, especially under water limiting periods (Davis et al. 2005). Similarly, the two main grass species at my site were *P. pratensis* and *B. inermis*, which are non-native C<sub>3</sub> grasses, and the 2012 growing
season was unusual in that there was an early snowmelt, combined with drought conditions over a large part of the growing season. Although the grasses responded significantly to N addition, the total aboveground productivity of the grasses was about 45% less than a typical year, and similar to that observed at the site in 2007, another drought year (Hutchison and Henry 2010). The combination of low precipitation and high water demand by the grasses decreased soil moisture to very low levels, which likely stressed the tree seedlings.

There was also variation among species in responses to warming and N addition. *Malus coronaria* seedlings behaved as predicted, with decreased survival, leaf area, and biomass in warmed plots. However, rather than being direct responses to the treatments, these effects seem to have been brought about indirectly due to changes in vegetation composition and soil moisture. *Malus coronaria* is commonly found in open sites along riverbanks and fences and flourishes in moist to well drained soils (Brown 1938, Voss and Reznicek 2012). Therefore, decreased survival and establishment of this species may be a result of a high moisture requirement and inability to tolerate shading (Classen et al. 2010).

Contrary to my predictions, survival of the three remaining species was higher in either the N added (*E. umbellata*) or warmed (*F. americana* and *R. typhina*) plots. Although these trends were not statistically significant, other studies have shown similar seedling responses to cover by herbaceous vegetation under drought conditions (Desteven 1991b, Berkowitz et al. 1995). Although, as described above, herbaceous cover generally decreases seedling survival as a result of competition for light, water or nutrients (Burton and Bazzaz 1995, Davis et al. 1999), shading can promote the establishment of seedlings under some circumstances, particularly when exposure to full sunlight is unfavourable (Gill and Marks 1991, Berkowitz et al. 1995, Dickie et al. 2007). This response is similar to the nurse plant phenomenon in desert ecosystems (Franco and Nobel 1989, Lopez and Valdivia 2007, Masrahi et al. 2012), or facilitation of seedlings by mature trees (Weltzin and McPherson 1999, Rodriguez-Garcia et al. 2011, Dickie et al. 2007, Kellman and Kading 1992), in that intermediate levels of shading by surrounding grasses can benefit seedlings by striking a balance between light levels that are too high for seedlings to
maintain adequate rates of photosynthesis and levels that are too low to support survival and growth (Burton and Bazzaz 1995, Laliberte et al. 2008). Since abundant herbaceous cover is associated with decreased photosynthesis, this interaction would be most likely to benefit species whose seedlings are shade tolerant (Berkowitz et al. 1995).

Facilitation of seedling survival by grasses may also explain why the relationship between percent cover around each seedling and seedling condition scores in my study was pronounced earlier in the growing season, but less so near the end of the growing season. When seedlings were planted in May, soil water was low and the seedlings may have experienced transplant shock from root disturbance during planting and differences in light intensity between the greenhouse and the field. Over the growing season the strength of the relationship between seedling survival and grass cover decreased for some species, coinciding with the gradual senescence of grasses throughout June and July (Hutchison and Henry 2010), as well as the increase in rainfall near the end of July (Fig 3.8).

4.5 Conclusions

An integrated view of my results provides insight into the overall establishment success of seedlings in old fields in the context of climate warming and increased atmospheric N addition. Seeds in warmed, N added plots germinated earlier than those in other plots, which may provide the advantage of an early start to the growing season. However, seeds that were more visible to predators through decreased snow cover in warmed plots or increased biomass in N added plots were at higher risk of predation over the winter. The differences in seedling growth and survival between the conditions in the greenhouse and the field site demonstrated that N and warming may have significant impacts on seedling survival and productivity throughout the first year. However, the critical impacts may be those of the indirect effects of N and warming on the surrounding vegetation, soil moisture and herbivory, rather than direct effects on seedling condition or growth.
4.6 Directions for Future Research

In the current study, I chose to transplant seedlings into the plots in order to maintain a uniform number and initial size of seedlings in the plots. However, in future experiments it would also be useful to sow seeds of early successional woody species directly inside the plots receiving warming and N addition treatments, in order to assess how treatment effects on the timing of germination influence seedling growth and survival, and also to avoid the effects of transplant shock. Likewise, it would be informative to leave the seedlings in the plots for multiple years, so that responses to treatment effects on winter conditions (e.g. reductions in snow cover and increased soil freezing) could be assessed (Drescher and Thomas 2013).

Future studies could also examine the responses of a wider range of early successional woody species, focusing on how differences in the timing of seed dispersal might affect treatment responses. For example, all of the species used in my experiment had fruit that matured and dispersed in the fall, with seeds that remained dormant over winter and germinated in the spring and early summer (Kock et al. 2008; Voss & Reznicek 2012). However, as observed by Classen et al. (2010), species such as silver maple (*Acer saccharinum*), in which keys mature, disperse, and germinate in the spring, may respond differently to changes in temperature and water availability than those that disperse in the fall. Not only is timing of germination important, seed size may also affect the responses of plants to future conditions. Larger seeds are prone to desiccation stress may and be more visible to predators (Donath and Eckstein 2010, Loydi et al. 2013), but smaller seeds may not successfully germinate through dense litter cover due to smaller reserves (Facelli 1994, Laborde and Thompson 2013). Overall, including more species to compare a wider range of germination times and strategies, functional groups or native vs. exotics may provide a better idea of how changing environmental conditions may impact the establishment by woody species in early successional habitats as a whole.

An important component of future environmental change not included in my study was that of elevated atmospheric carbon dioxide, which is linked with increases in global temperatures. In experiments featuring increased carbon dioxide applied at the ground level through free air CO$_2$ enrichment (FACE), seedling survival and establishment have
increased as a result of decreased transpiration and water stress since higher CO$_2$ concentrations allow seedlings to close their stomata for longer periods of time (Picon et al. 1996, Polley et al. 1996, Dermody et al. 2007, Davis et al. 2007). Since desiccation played such a critical role in the survival and establishment of seedlings in my study, it would be valuable to explore the extent to which elevated CO$_2$ might ameliorate water stress and interact with climate warming and increased atmospheric N deposition.
LITERATURE CITED


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