

Phenological responses of herbaceous plants, shrubs, and tree seedlings to  
experimental climate change conditions in northern Minnesota

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## **Dedication**

This thesis is dedicated to my partner, Aaron S. David.

## **Abstract**

Changing climate, specifically warmer temperatures, has been linked to changes in phenology, the timing of biological events such as leaf out and flowering. Despite the growing use of phenology as a tool to assess the degree of climate change or understand potential changes to species distributions, among others, phenological changes of herbaceous plants and shrubs remain less studied and thus less understood. In northern temperate and boreal forests, the understory and groundlayer composes the majority of plant biodiversity, is a major component of nutrient cycling, and is a filter for tree regeneration, among other important ecosystem contributions. Understanding the potential phenological changes of the forest groundlayer to predicted changes in climate, such as warming and altered precipitation, can inform how these processes and contributions may change. In this thesis I examine how experimental warming and warming coupled with decreased rainfall impact several phenological stages of herbaceous plants, shrubs, and tree seedlings over multiple years. This study takes place within the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) project in northern Minnesota.

First, to assess the phenological response of 13 herbaceous plants and 3 shrubs to warming I analyzed data collected over five years (2009-2013) at two sites, two canopy covers, and in three warming treatments: ambient temperature, +1.7°C and +3.4°C. Overall, warming extended the growing season, primarily through earlier leaf unfolding in the spring. Notably warmer, earlier springs advanced leaf unfolding more than cooler springs. Compared to advance of budburst of tree seedlings, the groundlayer advances more in warming, possibly allowing for more carbon gain in the spring. Flowering

advanced under warming, though more so for fall blooming species than for spring blooming species. This advance may be attributed to aforementioned carbon gain from earlier leaf unfolding, as the two events appear correlated. Warming did not alter senescence for most species, though several species did delay senescence with warming. Finally, the length of the growing season differed between canopies for several species, primarily through delayed senescence in the closed canopy habitat compared to the clearcut.

Second, I compared the phenological responses of the understory community and tree seedling community under manipulated rainfall and temperature treatments. For both communities I analyzed data collected in 2012-2014 at two sites and one habitat, open canopy. Experimental treatments included the three warming treatments crossed with two rainfall treatments (ambient and 40% reduction). Combining the two sites, there are six replicate plots of each warming treatment (ambient temperature, +1.7°C and +3.4°C) crossed with each rainfall treatment, for a total of 36 plots. Overall, community level phenological responses of the groundlayer and tree seedlings were not altered by rainfall treatments. Thus the larger springtime advance of the groundlayer relative to the tree seedling community in warming (reviewed in Chapter 1) is maintained even when soil moisture is lower.

Finally, I examined the difference in flowering responses of spring and fall blooming herbaceous species to warming and rainfall treatments. Only fall blooming species altered flowering phenology to rainfall manipulation. Two species (*Eurybia macrophylla* and *Solidago spp.*) flowered at the same time in ambient rainfall and

ambient warming conditions as well as ambient rainfall and warmed conditions, but diverged in flowering time in the warmest, driest treatment. This divergence led to a month long gap between when each flowered

These results highlight potentially sensitive species or altered competitive interactions under future climate change conditions. Such changes may have consequences for increased fitness, changes in species persistence and community composition.

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# **Chapter 1 Response of boreal forest herbaceous and shrub layer phenology to experimental warming**

## **Introduction**

Plant species are potentially sensitive to changing climate conditions, given their sessile nature and inability to rapidly migrate (Walther et al. 2002). The timing of phenological events such as flowering and leaf out are already changing in response to warming temperatures (Walther et al. 2002; Parmesan, 2006; Schwartz et al. 2006; Cleland et al. 2007). Though they make up a small portion of forest biomass, groundlayer plants provide major contributions to the ecosystem as a whole. For instance, herbaceous plant communities influence both nutrient flux (Muller 2014) and tree regeneration (Gilliam, 2007). Shrubs provide forage for animals (Stormer and Bauer, 1980) and influence chemical composition and nutrient cycling (Tappeiner and John, 1973; Nilsson and Wardle, 2005). Thus changes to life cycle stages of these plants can have impacts beyond the fitness of the individual plant itself.

Changes in phenology could have complex consequences for different aspects of plant fitness. Earlier leaf out may put a plant at risk during spring frosts (Inouye, 2008; Chuine, 2010). However, these same advances in growing season may increase reproductive output (Jacques et al. 2015) and enhance the ability to capture light (Farnsworth et al. 1995) and early season nutrients (Muller et al. 2014), thus increasing overall growth and fitness (De Frenne et al. 2010; Cleland et al. 2012); or change the 'match' of leaf to herbivore phenology, with potentially negative or positive consequences for the plant (Schwartzberg et al. 2014, Uelman et al. 2016). An extension or reduction in the growing season could alter the carbon balance with more primary production or more decomposition (Gu et al. 2003; Piao et al. 2007) or increase soil moisture by initiating earlier senescence (Zavaleta et al. 2003a).

Potential climate change related shifts in the phenology of the groundlayer plant species of northern forests have been little studied. Herbaceous phenology experiments to date have focused on grasslands (Cleland et al. 2006; Sherry et al. 2007), the arctic (Arft

et al. 1999), or other temperature limited systems at higher altitudes (Inouye, 2008). In the few forest understory warming experiments conducted on herbaceous plants, warming induced earlier emergence and earlier senescence (Farnsworth et al., 1995). For example, De Frenne et al. (2011) observed earlier emergence in *Anemone nemorosa* in warmed plots in a temperate deciduous forest in Belgium. In a greenhouse study, *Panax quinquefolius* exhibited earlier leaf senescence and decreased photosynthesis when warmed +5°C (Jochum et al. 2007) .

Here, we examine the phenological responses of 16 groundlayer plant species at the ecotone between the temperate and boreal forests, a region for which we currently have little knowledge of phenological responses. Specifically, we studied the effects of increased temperature on the timing of vegetative and reproductive events for 12 forbs, 3 shrubs and 1 fern (Table 1-1). We tracked these plants for five years in two different light environments, a clearcut (open canopy) and an intact forest setting (closed canopy). This study took place in the Boreal Forest Warming of an Ecotone in Danger (B4WarmED) field experiment located in the temperate-boreal forest ecotone in northern Minnesota. The B4WarmED project is a free-air and soil warming experiment installed *in situ* (Rich et al. 2015), resulting in the warming of planted tree seedlings and naturally occurring groundlayer vegetation.

Within the context of the B4WarmED project, we examined how warming affected the vegetative phenology and reproductive phenology including overall impacts on the resultant growing season length of 17 groundlayer species (Figure 1-1). Given that the 13 of our 16 study species are herbaceous, we hypothesized that increased warming would cause earlier leaf unfolding and hasten senescence for the community as a whole (Farnsworth et al. 1995; Jochum et al. 2007). Conversely, we could expect woody shrubs to emerge earlier but delay senescence, as observed for tree species (Montgomery et al. submitted). Herbaceous plants typically emerge once soils warm and early enough in the season to take advantage of high light conditions before the overstory canopy closes (Bazzaz and Bliss 1971), thus we expected warmer temperatures to trigger even earlier emergence. Shallow roots systems of herbaceous plants may make them more sensitive to

changes in soil processes (e.g. earlier nutrient cycling, late summer moisture deficits) that correspond to warmer temperatures (Farnsworth et al. 1995).

For similar reasons we hypothesized that flowering and fruiting of groundlayer plants would advance with warming, as has been observed previously (Fitter et al. 1995); Miller-Rushing and Primack 2008; Penuelas et al. 2002). We hypothesized that warmer ambient spring temperatures would further advance the timing of leaf out and flowering when also exposed to warmer temperatures by melting snow and thawing the soil, thus allowing increased water and nutrient availability as well as crossing needed growing degree day accumulation (if required by the plant) earlier in time. Furthermore, species that naturally emerge earlier in the season may be more sensitive to warmer temperatures and better equipped to emerge earlier in the season than later emerging species by increasing developmental rates that are more temperature dependent in the spring but temperature limited in the fall (Sherry et al. 2007) or due to differences in species' resource needs throughout the growing season (Ackerly, 2003), which may lead earlier emerging species to display different phenological responses early in the season than later emerging species.

Growing season length, defined here as the days between leaf unfolding and senescence, might be influenced by warming in one of several ways. Earlier leaf unfolding may shift the timing of the entire growing season without changing its length (Figure 1-1, Scenario 1), if both emergence and senescence advance similarly (Starr, Oberbauer, and Pop, 2000). Alternatively, earlier leaf unfolding could result in a longer growing season, due to a stronger advance in the timing of leaf unfolding than in senescence (Figure 1-1, Scenario 2). Differing habitats may influence responses among species. Within the open canopy, we hypothesized that warming may further advance leaf unfolding compared to the closed canopy given the higher light levels (and warmer temperatures). That same exposure may cause earlier senescence in the open compared to the closed canopy, particularly if warming in the clearcut exacerbates low soil moisture conditions.

## **Methods**

### *Study Site and System*

Phenology data for groundlayer plants were collected in the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) experiment (Rich et al. 2015). The project consists of two upland forested sites approximately 150 km apart. One site was located at the southern edge of the temperate-boreal forest ecotone at the Cloquet Forestry Center in Cloquet, Minnesota (46°31'N, 92°30'W; 4.5° C mean annual temperature, 807 mm mean annual precipitation). The second site was located further north at the Hubachek Wilderness Research Center in Ely, Minnesota (47°55'N, 92°30'W; 3.0° C MAT, 722 mm MAP). There are a total of 72 7.1m<sup>2</sup> plots divided evenly between sites. Each site was divided into six blocks, containing 2 replicates each of the three heat treatments. Heat treatments included unwarmed control plots (ambient) and plots with simultaneous above (via infrared lamps) and belowground (via buried resistance cables) warming that elevated the temperature to two target levels above ambient, + 1.7 °C and + 3.4 °C (Table 1-2 and Table 1-3). Half of the plots in each site were in an open (receiving ~80% of full sunlight) and closed (~5-10% of full sunlight) canopy. The open canopy was a clearcut area, while the closed canopy had a 40-60 year old aspen-birch-fir canopy cover. We planted seedlings of 11 tree species from bare-root stock in random distribution into all of the plots in the spring of 2008, 2011, 2012, and 2013 (Reich et al. 2015). Using planting bars we created the minimum disturbance possible in the plots, allowing the naturally occurring herbaceous and shrub plant community to remain intact. An annual census of every tree seedling planted in the plots revealed that with a 15cm radius around each seedling the herbaceous layer comprised approximately 41% (ambient plots) to 47% (warmed plots) and the shrub layer comprised approximately 21% (ambient plots) and 24% (warmed plots) of available space.

### *Study species*

All of the species in this study are perennials that naturally occur in the temperate-boreal ecotone of northern Minnesota and though ranges vary, most are commonly found throughout much of the United States and Canada (Table 1-1). Under ambient conditions,

all species leaf out in early to mid-May and most are intermediate to very shade tolerant. The majority are herbaceous forbs, except for *Diervilla lonicera*, *Corylus cornuta*, and *Rubus idaeus*, which are shrubs, and *Pteridium aquilinum*, a fern. Most have well-developed rhizomes that allow them to persist for multiple years and expand growth vegetatively. The two *Solidago* species, *Solidago altissima* and *Solidago gigantea*, and two *Hieracium* species, *Hieracium aurantiacum* and *Hieracium pratense*, were combined into one species category due to the limited replication among treatments and similar life history traits between species.

### *Data Collection*

We tracked vegetative and reproductive phenology of the most common, naturally occurring groundlayer plant species in the B4WarmED plots weekly for five growing seasons (i.e. during April – November). Multiple phenophases were defined and data collectors noted the most common phenophases present for each species within each plot on a weekly basis (see Appendix Table 1 for full descriptions of phenophases). Timing of phenophases were recorded as day of year (DOY), with DOY 1 corresponding to January 1. Observations started at time of treatment turn on and ended at treatment turn off. We created a 42-page protocol that included detailed descriptions and photos of each plant in every particular phenophase. Observers were assigned to particular sites and new, seasonal observers worked with long-term staff until they consistently recorded phenophases similarly as long term staff and each other. Observers regularly sent images of phenophases observed to each other via smartphones to maintain consistency and accuracy.

In 2009-2013 we recorded vegetative phenology for 14 species (see Appendix table 2 for replication). Phenophases included ‘plant emerging,’ ‘leaf unfolding,’ ‘leaves fully expanded,’ ‘start of senescence’ and ‘plant shoot dead’. We recorded ‘leaf unfolding’ when the leaves of the plant were starting to unfold and expand away from the stem. We recorded ‘start of senescence’ when leaves began to wither or change color due to end of year senescence or extreme drought stress. The growing season length was calculated as the length of time between ‘leaf unfolding’ and ‘start of senescence.’ We



chose ‘leaves unfolding’ as the start of the season indicator because most carbon capture is likely beginning at this stage (Jacques et al. 2015), and because we could not confidently identify species before this stage. We chose start of senescence as the end of season indicator because discoloration of leaves signals nutrient reallocation within the plant for future storage and an end of photosynthetic carbon gain. Not all species were recorded for start of senescence due to leaves remaining green over winter. Due to technical data collection errors, there are no data for start of senescence in the year 2009.

In 2009-2013 we recorded reproductive phenology as ‘closed flower buds’ and ‘open flowers’ for ten species and ‘immature fruit,’ and ‘ripe fruit’ for seven of the most commonly fruiting species (see Appendix Table 3 for replication). Within each plot, when the species was present, we tracked the first individual displaying these reproductive phenophases, due to many species not flowering or fruiting every year. Farnsworth et al. (1995) saw similar constraints in tracking flowering phenology due to the rarity of sexual reproduction among herbaceous perennials. Many groundlayer perennials can take 4-10 years to first reproduction (Bierzychudek, 1982). We recorded ‘open flowers’ when at least one flower was open with reproductive structures accessible to pollinators. We recorded ‘ripe fruit’ when at least one mature fruit was present on the plant and ready for dispersal. To determine when fruit was mature, our protocol detailed typical size, shape and coloration of ripened fruit for each species using descriptions and pictures.

### *Statistical analysis*

We used mixed effects analysis of variance (ANOVA) models to assess the responses of day of year (DOY) of leaf unfolding, flowering, fruiting, start of plant senescence, and growing season length. Given the unbalanced replication of individual species records among years, canopies and sites, and to effectively understand individual species’ responses, we ran mixed effects ANOVA models first for a subset of species using block nested in site as a random effect with canopy, year, warming treatment, and species, with all 2- and 3- way interactions among variables (Appendix Table 4 and Appendix Table 5). Observing species x warming interactions, we ran a separate model

for each individual species to parse out individual responses. If the species was recorded at both sites we combined data from both sites and used block nested in site as a random effect. Otherwise we used block as a random effect. In individual species models we included canopy (if applicable), year, warming treatment, and all 2- and 3- way interactions among variables. We examined significant results ( $p < 0.05$ ) using Tukey-HSD post hoc tests to evaluate significant differences among treatment variables at  $\alpha = 0.05$ . We used JMP 10 statistical analysis software (JMP 10.0, SAS Institute, Cary, NC) to analyze mixed effects models. We used R Studio (RStudio: Integrated Development Environment for R) to run linear models on change in flowering as a function of change in leaf unfolding in warming compared to ambient for two groups, late and early blooming species. The ggplot2 package from R was used to create figures (Wickham 2009).

## Results

### *Leaf unfolding*

All fourteen species showed a main effect of year on timing of leaf unfolding while eleven also showed a main effect of warming. Additionally, five species showed a year x warming interaction and several showed a main effect of canopy, or warming or year interactions with canopy, on leaf unfolding (Table 1-4 for summary, Appendix Table 6 for model results). Year was the strongest effect for all species. Leaf unfolding occurred earliest in 2010, approximately on May 3, on average, and latest in 2013, approximately on June 1. Warming had the second strongest effect, advancing leaf unfolding for all species except for *Uvularia sessilifolia*, *Rubus idaeus* and *Lathyrus venosus*. Average advance of leaf unfolding across species and among years ranged from 0.8-6.4 days earlier in +1.7°C warming and 1.0-12.7 days in +3.4°C warming. Thus, average rate of change per degree warming ranged similarly from 0.4-4.1 days/°C in +1.7°C and 0.3-3.7 days/°C in +3.4°C warming (Figure 1-2). However, species varied in magnitude of response to different levels of warming. Four species (*Diervilla lonicera*, *Galium triflorum*, *Solidago spp.*, *Pteridium aquilinum*) did not alter leaf unfolding in +1.7°C compared to ambient, but showed an average advance of ~10 days in +3.4°C warming.

Three species (*Aralia nudicaulis*, *Streptopus roseus*, *Eurybia macrophylla*) showed a linear response to warming, advancing 5.5 days in +1.7°C and 10 days in +3.4°C warming. Four species (*Corylus cornuta*, *Maianthemum canadense*, *Rubus pubescens*, *Trientalis borealis*) advanced at similar rates for both +1.7°C and +3.4°C.

We found support in almost half of the study species for our hypotheses that warmer, earlier springs would advance leaf unfolding. We observed a trend for those species with a year x warming interaction: advances to warming were largest in early spring years, 2010 and 2012, compared to advances in other years with cooler average spring temperatures (See Table 1-3 for average temperatures). Species such as *Aralia nudicaulis*, *Eurybia macrophylla*, *Rubus pubescens*, and *Diervilla lonicera* showed this trend. Leaf unfolding of *Maianthemum canadense* showed the most varied response to warming among the years, with earlier leaf unfolding occurring only in +1.7°C warming in 2010, no change in leaf unfolding with warming in 2009 or 2011, and a 16 and 7.5 day advance in +3.4°C and +1.7°C warming in 2012.

Contrary to our hypotheses that exposure differences between canopies would influence timing of leaf unfolding, only *Eurybia macrophylla* and *Diervilla lonicera* showed a warming x canopy interaction while *Maianthemum canadense* showed a main effect of canopy. For these three species leaf unfolding occurred earlier in the closed canopy compared to the open canopy.

#### *Start of senescence*

We observed a main effect of year on start of senescence for eleven of the species, as well as a main effect of warming for five species, a warming x year interaction for four species and a main effect of canopy, or warming or year interactions with canopy for five species (see Table 1-4 for general summary, Appendix Table 7 for model results).

Contrary to our hypotheses, post hoc tests showed five species significantly delayed senescence in +3.4°C warming compared to ambient: *Aralia nudicaulis* (8 days), *Corylus cornuta* (6 days), *Maianthemum canadense* (5 days in +1.7°C with no change in +3.4°C), *Eurybia macrophylla* (12 days), and *Solidago spp* (14 days). Across all species and among four years, delay of senescence ranged from 3.0-9.9 days in +1.7°C and 1.3-13.6

days in +3.4°C warming. Average rate of change across species and among years ranged from 1.7-5.8 days/°C under +1.7°C warming and -0.6-4.0 days/°C under +3.4°C warming (Figure 1-3).

Most species with a significant main effect of year senesced earliest in 2010, corresponding to 2010 having the coldest ambient fall temperatures by several degrees (see Table 1-3), though timing of senescence in ambient plots only varied by approximately 10 days among years. Three species (*Maianthemum canadense*, *Rubus pubescens*, and *Galium triflorum*) showed strongest responses (i.e. delay of senescence) to warming in 2010 compared to other years. Despite delaying senescence in 2010 in warming, senescence of *Maianthemum canadense* occurred 10 days earlier in +3.4°C warming in 2012 and in 2013 senescence of *Galium triflorum* occurred 12 days earlier in +3.4°C warming.

Finally, canopy influenced timing of senescence for a few species. *Diervilla lonicera*, *Rubus pubescens* and *Rubus idaeus* senesced earlier in the open compared to the closed canopy in ambient conditions. *Eurybia macrophylla*, showed a stronger delay of senescence in warming in the closed canopy compared to the open, with a 17 day (5 days/°C) in the closed compared to an 8 day (2.3 days/°C) delay in the warming treatment in the open canopy.

#### *Growing season length*

We observed a main effect of year on growing season length for all species except *Solidago spp* as well as a main effect of warming for 11 species, a warming x year interaction for three species and a main effect of canopy, or interaction with canopy by warming or year for three species (see Table 1-4 for general summary, Appendix Table 8 for model results). Yearly differences in growing season length correspond to trends in leaf unfolding: the earliest year leaf unfolding occurred in ambient conditions, 2010, was the year we recorded the longest growing season (128 days) and the year leaf unfolding occurred the latest, 2013, was the year we recorded the shortest growing season length (106 days). This suggests an extension in accordance with hypothesized scenario 2

(Figure 1-1), though an extension due to earlier leaf unfolding and delayed senescence was apparent for several species.

Post hoc tests show the growing season was extended with warming for all but two species (*Lathyrus venosus* and *Uvularia sessilifolia*). Growing seasons were extended between 11 (*Rubus pubescens*) and 30 days (*Solidago spp.*). On average across four years the extension in growing season compared to ambient ranged from 5.1-15.6 days in +1.7°C and 5.7-22.3 days in +3.4°C warming. Average rate of change for the community as a whole was 3.0-9.2 days/°C in +1.7°C and 1.6-6.5 days/°C in +3.4°C warming (Figure 1-4).

We observed shorter growing seasons in the open canopy than in the closed canopy for *Diervilla lonicera* and *Rubus idaeus* and this trend was most pronounced for *Rubus idaeus* in 2012. Only the growing season of *Eurybia macrophylla* showed a canopy × warming interaction. In the open canopy *Eurybia macrophylla* showed a 16 day extension in both warming treatments and in the closed canopy had a 25 and 29 day extension in both warming treatments.

### *Flowering*

Of nine species, we found a main effect of year on flowering for all but *Solidago spp.*, as well as a main effect of warming for all but *Galium triflorum* and *Lathyrus venosus*, a warming x year interaction for three species and a main effect of canopy and a canopy x year interaction for *Eurybia macrophylla* (see Table 1-4 for general summary, Appendix Table 9 for model results). Our hypothesis that early, warm springs would advance springtime phenological events was supported as most species flowered earliest in warm spring years (2010 and 2012) and latest in 2013.

Early (June) flowering and late (August) flowering species differed markedly in their response to warming, with late flowering species typically showing a larger magnitude of response, especially in early spring years 2010 and 2012. Among early blooming species that responded and among years, flowering advanced with the range of 0.8-7.7 days and 6.9-12.8 days under +1.7°C and +3.4°C warming, with a rate of change of 0.4-5.1 days/°C for +1.7°C warming and 2.0-3.7 days/°C for +3.4°C warming. Late

blooming *Eurybia macrophylla* significantly advanced flowering under warming in the early spring years of 2010 and 2012 with an advance in those years ranging from 19.6-20.8 days in +1.7°C and 32.7-33.9 days in +3.4°C, with modest advances in the other years ranging from approximately 2-4 days. *Solidago spp.* advanced by an average of 11 days in +1.7°C and 20 days in +3.4°C warming across three years.

When the change in timing of flowering under warming was plotted as a function of change in timing of leaf unfolding (for species with data for both phenophases), we observed a strong correlation between the two phenophases for late blooming species but not early blooming species (Figure 1-5). The slope of the line for early blooming species was not significantly different from 0 (intercept=-4.65, slope = 0.15, p=0.25) indicating there was not a strong relationship between advances in leaf unfolding and advances in flowering. The late blooming species showed a relationship that indicates for every one day advance in leaf unfolding we can expect an advance in flowering by a little over half a day (intercept=-8.68, slope = 0.73, p=0.07).

Finally, *Eurybia macrophylla*, the only species that flowered consistently in both canopies, typically flowered earlier in the open canopy. This trend may be driven by the year 2010 when we recorded a 40 day difference between the open and closed canopy flowering times.

### *Fruiting*

Of five species, we observed four species with a main effect of year on timing of fruit maturation, while *Eurybia macrophylla* showed a main effect of warming and a canopy x year interaction and *Galium triflorum* showed a canopy x warming interaction (see Table 1-4 for general summary, Appendix Table 10 for model results). Most species fruited earliest in 2012, though species varied in whether fruiting occurred latest 2011 or 2013.

The two species with warming effects or interactions, *Eurybia macrophylla* and *Galium triflorum*, varied in magnitude and direction of response (Figure 1-6). *Galium triflorum* showed a significant warming x canopy interaction, but post hoc tests show no significant difference between warming treatments within the two canopies. We observed

the strongest response to warming on timing of fruit ripening of *Eurybia macrophylla*. On average across 5 years, *Eurybia macrophylla* fruited approximately 12 days (3.6 days/°C) earlier in +3.4°C warming and 4 days (2.3 days/°C) earlier in +1.7°C. Despite a significant canopy x year interaction, *Eurybia macrophylla* did not differ in timing of fruit maturation between the two canopies.

## **Discussion**

Changes in groundlayer phenology in a warmer climate have important implications for individual species' fitness and the variety of ecosystem functions groundlayer species provide such as carbon and nutrient cycling and enhanced biodiversity. Different responses to warming among species, as we observed in our study, can lead to altered species interactions (e.g., competition, Bradley et al. 1999) or changes in pollinator-plant relationships (Hegland et al. 2009). Overall, species responded to warming with earlier leaf unfolding, earlier flowering, little change in fruit maturation, and an overall extension of the growing season, with little change in start of senescence. However multiple species showed interactions between warming and other factors such as year or habitat, or no change in response to any factors, including warming, indicating potential non-responders. While temperature appears to be the main cue for the majority of the species in this study, other species may rely on other phenological cues, such as daylength. Earlier leafing and flowering due to warmer temperatures could increase productivity and reproductive success, but may also lead to frost damage or mismatches with pollinators.

Earlier leaf unfolding with warming was the strongest, most consistent response of herbaceous plants and shrubs, which supports our hypothesis and results of other studies (Farnsworth et al. 1995). Advances were within the range of previous warming experiments, which predict an advance of approximately 1.4-7 days/C (Ishioka et al. 2013, Rollinson and Kaye 2012, De Frenne et al. 2011, Farnsworth et al. 1995). Responses appeared species-specific with no clear link to life history characteristics as determinants of the magnitude of response, contrary to our hypotheses that early leafing

species would experience larger advances in leaf unfolding. Leaf unfolding of all species is completed within one month, which is perhaps not enough time difference to show a temporal pattern in species' timing of leaf unfolding.

Warm springs coupled with the warming treatment further advanced leaf unfolding of herbaceous plants, a relationship previously observed in tree budburst (Fahey et al. 2016, Montgomery et al. submitted), but fall temperature and the warming treatment had little effect on senescence for most species. One explanation for this difference is the importance of temperature forcing springtime events with photoperiod being a minor cue (Iversen et al. 2009, Cornelius et al. 2013). Conversely, photoperiod or fruit maturation (Bierzzychudek, 1982), which also did not respond to warming, may play stronger roles in cuing senescence than warming. If snowmelt and temperatures above freezing continue to become earlier as predicted (Kling et al. 2003), the groundlayer may be equipped to respond accordingly and extend the growing season into the springtime.

Alternatively, the growing season of several species was extended in both directions through advanced leaf unfolding and delayed senescence, contrary to our hypothesis and to other studies (Farnsworth et al. 1995, Starr et al. 2000, Jochum et al. 2007). These species included late seeding species and a woody shrub. In late seeding species, senescence may be delayed relative to earlier seeding species until after fruit maturation occurs in the late fall (Jacques et al. 2015). Woody shrubs may have more access to belowground resources and more storage reserves with which to buffer stresses that induce senescence in forbs (Farnsworth et al. 1995). Of those species delaying senescence, two species also senesced earlier in warming in several years, indicating conditions may need to be favorable (i.e. more moisture, warmer temperatures) to allow for delayed senescence in some sensitive species. Optimal conditions for delayed senescence may be more prevalent in an intact forest canopy, as we observed a longer growing season and delayed senescence in the closed canopy compared to the open for several species, a response also observed for *Acer* spp. tree seedlings in B4WarmED (Montgomery et al. submitted). The closed canopy may delay senescence more effectively due to overhead trees moderating microclimate by reducing wind speeds and retaining heat (Chen et al. 1995).



Earlier leafing of the groundlayer in the spring can be beneficial for increased carbon gain as photosynthesis rates of herbaceous plants peak before overstory trees leaf out and close the canopy, reducing light availability to the groundlayer (Bazzaz and Bliss 1971, Neufeld and Young 2014). However, earlier leaf unfolding will not be as advantageous if overstory trees respond just as strong, or stronger than groundlayer species to warming. In ambient conditions there is a natural gap of approximately 3 weeks between leaf unfolding of groundlayer species and full canopy expansion of tree seedlings (B4WarmED unpublished data). Some groundlayer species (0.1-13.1 days advanced of leaf unfolding in +3.4°C) have the ability to advance spring phenology more than tree seedlings (2.4-7.2 days advance of budbreak in +3.4°C, Montgomery et al. submitted) in warming. The larger advance of the groundlayer relative to seedlings creates an opportunity for the groundlayer to be exposed to more light early on, capturing more carbon and improving growth (Mahall and Bormann, 1978; Rothstein and Zak, 2001).

As hypothesized and as observed in other studies (Sherry et al. 2007), flowering advanced with warming, though more so for fall blooming species relative to spring blooming species. Fall blooming species may respond to different abiotic cues than spring blooming species, such as spring blooming species being more sensitive to higher temperatures. Alternatively, earlier leaf unfolding may also enhance carbon capture also leading to earlier flowering (Sola and Ehrlén 2007). This explanation is demonstrated in the response of fall blooming species, but no strong trend was observed in spring blooming species (Figure 1-7). In “average” springs years, 2011 and 2013, the advance in leaf unfolding and advance of flowering of fall blooming species nearly follow a 1 to 1 relationship; for every day advance in leaf unfolding followed one day advance in flowering. However, flowering in warmer spring years, 2012 and 2010, deviate from this line with a one day advance in leaf unfolding leading to a two or more day advance in flowering (see again Figure 1-7). One explanation for the lack of relationship between leaf unfolding and flowering for early blooming species is the short window between leaf unfolding (May) and flowering (June). However, this explanation does not explain why fall blooming species, which also unfold leaves in May, advance leaf unfolding at a larger

magnitude than early blooming species. It is possible that leaf unfolding and flowering rely on different cues or a combination of cues. The larger advance in leaf unfolding for late blooming species may allow them to capture more carbon in early spring, translating into more stored carbon to allocate to reproduction (Jacques et al., 2015). One potentially negative outcome of this pronounced response to warming is the risk of frost damage, if leafing or flowering advances more than last freeze dates (Schwartz, Ahas, and Aasa, 2006).

Differing responses among species highlights potentially vulnerable species and enhanced competitors in the face of climate warming (Bradley et al. 1999). For example, *Eurybia macrophylla* was one of the strongest responders to warming in every phenophase, leading to one of the longest growing seasons under warming. The large advances of *Eurybia macrophylla* may provide a competitive advantage. *Eurybia macrophylla* is clonal and can exclude other species by growing in dense mats (Schulz and Adams, 1995) shading out lower growing vegetation (Jacques et al. 2015). Though community composition did not change drastically in response to warming (B4WarmED unpublished data), over time, and with improved fitness of individual species that respond positively to increased temperatures (Zavaleta et al. 2003b; De Frenne et al. 2010) or enhanced early season nutrient capture (Muller, 2014) anticipated with a warmer climate, we may observe shifts in dominance of species like *Eurybia macrophylla* and a decrease in other less responsive species. More long term research into climate change effects on phenology, with corresponding physiological responses, in the groundlayer forest community and the related effects of phenology responses on community composition are needed.

Warming enhanced the growing season of the majority of groundlayer species of the temperate-boreal forest by inducing earlier leaf unfolding with little change in the timing of senescence. Flowering advanced with warming, especially for late blooming species. Changes to phenology may be advantageous to individual species fitness in they increase carbon gain or detrimental if novel changes increase risk of frost damage or mismatches with pollinators or herbivores. Our work contributes to the growing body of knowledge of how phenology may change with rising temperatures and highlights

responses of the groundlayer in particular, an understudied system. Phenological responses of the groundlayer to warming contribute to our understanding of how individual species may fare in a warmer world, which adds to our understanding of how community composition and multiple aspects of forest function may change in the coming decades.

Table 1-1. List of species that were tracked for phenology in descending order from earliest leaf unfolding species to latest leaf unfolding.

| <b>Species</b>               | <b>Family</b>    | <b>Growth habit</b> | <b>Preferred Canopy</b>                    | <b>Range</b>   |
|------------------------------|------------------|---------------------|--|--|
| <i>Fragaria virginiana</i>   | Rosaceae         | Forb                | dry open fields, woodland edges, roadsides | all US, Canada   |
| <i>Galium triflorum</i>      | Rubiaceae        | Forb                | Woods                                      | all US, Canada   |
| <i>Lathyrus venosus</i>      | Fabaceae         | Forb                | Woods and thickets                         | eastern, Midwest US, Alaska, eastern Canada            |
| <i>Hieracium spp.</i>        | Asteraceae       | Forb                | Fields, roadsides, disturbed soils         | Introduced throughout US Canada                        |
| <i>Rubus idaeus</i>          | Rosaceae         | Shrub               | Dry or moist fields, woods or roadsides    | US (exclude far southeast), Canada                     |
| <i>Rubus pubescens</i>       | Rosaceae         | Forb                | Damp woods and bogs                        | Midwest, northern US, Canada                           |
| <i>Diervilla lonicera</i>    | Caprifoliaceae   | Shrub               | Dry or rocky soil                          | Eastern US, Canada                                     |
| <i>Trientalis borealis</i>   | Primulaceae      | Forb                | Rich woods and bogs                        | northwest, Midwest and eastern US Canada               |
| <i>Maianthemum canadense</i> | Liliaceae        | Forb                | Moist woods                                | eastern, Midwest US, Canada                            |
| <i>Eurybia macrophylla</i>   | Asteraceae       | Forb                | Woods                                      | Eastern US, Eastern Canada                             |
| <i>Solidago spp</i>          | Asteraceae       | Forb                | Moist open places                          | All US Canada  |
| <i>Uvularia sessilifolia</i> | Liliaceae        | Forb                | Moist Woods, thickets                      | Eastern, Midwestern US Canada                          |
| <i>Corylus cornuta</i>       | Betulaceae       | Shrub               | Moist woods and thickets                   | southeastern US, Midwest, northern US, southern Canada |
| <i>Aralia nudicaulis</i>     | Araliaceae       | Forb                | Woods                                      | eastern, Midwest, northern US and Canada               |
| <i>Streptopus roseus</i>     | Liliaceae        | Forb                | Rich woods                                 | Eastern US   |
| <i>Pteridium aquilinum</i>   | Dennstaedtiaceae | Fern                | Degraded land                              | all US, southern Canada                                |

Figure 1-1. Hypotheses and generalized results of phenology. Box around timeline indicates length of growing season. In first hypothesized response the growing season shifts in time but does not change in length. In the second hypothesized response the growing season extends mainly driven by larger advances in leaf unfolding

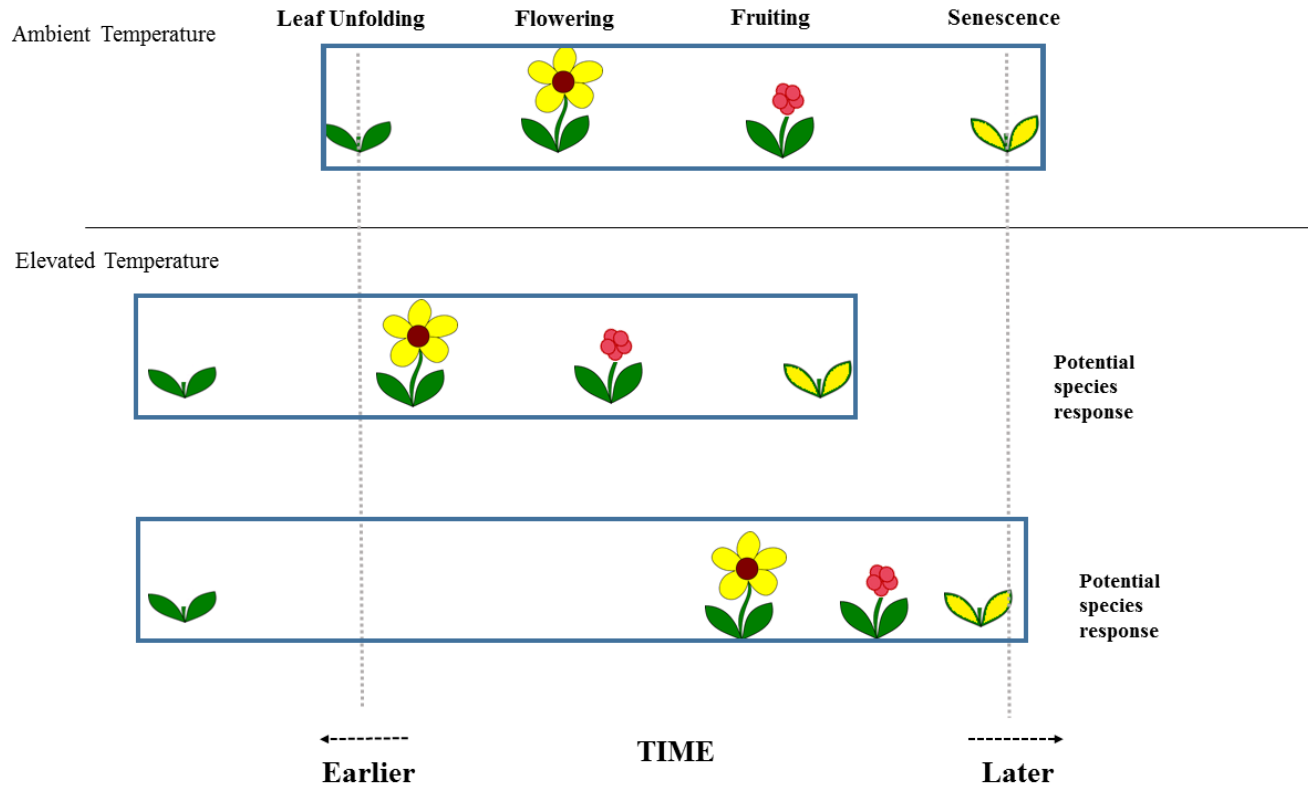


Table 1-2. Average aboveground target temperature with realized delta throughout the growing season (April-November) averaged across site, canopies, and plots.

| <b>Target Temperature</b> | <b>Year</b> | <b>Delta</b> |
|---------------------------|-------------|--------------|
| +1.7°C                    | 2009        | 1.81±0.41    |
| +3.4°C                    | 2009        | 3.45±0.75    |
| +1.7°C                    | 2010        | 1.84±0.26    |
| +3.4°C                    | 2010        | 3.48±0.44    |
| +1.7°C                    | 2011        | 1.40±0.34    |
| +3.4°C                    | 2011        | 2.67±0.63    |
| +1.7°C                    | 2012        | 1.45±0.36    |
| +3.4°C                    | 2012        | 2.92±0.53    |
| +1.7°C                    | 2013        | 1.40±0.57    |
| +3.4°C                    | 2013        | 2.78±1.13    |

Table 1-3. Aboveground temperatures averaged across sites and canopies and plots of each warming treatment for the growing season (April-November), the first week of April (WOY 14) when plants tend to emerge, and the first week of September (WOY 36) when senescence generally begins of each year.

| <b>Treatment</b> | <b>Year</b> | <b>Growing season above ground temp (°C)</b> | <b>WOY 14 above ground temperatures (°C)</b> | <b>WOY 36 above ground temperatures (°C)</b> |
|------------------|-------------|--|--|--|
| Ambient          | 2009        | 10.04 ± 5.92                                 | -0.34 ± 1.69                                 | 15.96 ±0.19                                  |
| Ambient          | 2010        | 11.08 ± 6.77                                 | 7.05 ± 2.36                                  | 10.76±0.05                                   |
| Ambient          | 2011        | 10.54 ± 6.93                                 | -0.19 ± 1.46                                 | 14.84±0.21                                   |
| Ambient          | 2012        | 11.31 ± 7.65                                 | 6.44 ± 1.38                                  | 17.62±0.15                                   |
| Ambient          | 2013        | 9.93 ± 8.05                                  | -1.90 ± 2.60                                 | 16.29±0.07                                   |

Table 1-4. Summarized general response to warming according to post hoc Tukey HSD test of four phenophases and growing season length for individual species in order of timing of leaf unfolding in ambient conditions. Bold response indicates significance at  $p < .05$ , italicized indicates significance at  $p < 0.10$  to main effect of warming. Symbols indicate significant interactions at  $p < 0.05$ : \* = year x warming interaction. † = canopy x warming interaction.  $\times$  = canopy x warming x year interaction, but see text for description of directions of interactions. Blank spaces indicate no data analyzed for response. See Appendix Tables 6-10 for full ANOVA results.

|                              | Leaf Unfolding                       | Flowering          | Fruiting         | Start of senescence | Growing Season Length |
|------------------------------|--------------------------------------|--------------------|------------------|---------------------|-----------------------|
| <i>Fragaria virginiana</i>   |                                      | <b>Advanced *</b>  |                  |                     |                       |
| <i>Galium triflorum</i>      | <b>Advanced</b>                      | No change          | No change †      | No change *         | <i>No change *</i>    |
| <i>Lathyrus venosus</i>      | No change                            | No change          |                  | No change           | No change             |
| <i>Hieracium spp</i>         |                                      | <b>Advanced</b>    |                  |                     |                       |
| <i>Rubus idaeus</i>          | <i>No change</i>                     |                    |                  | No change           | <b>Extended</b>       |
| <i>Rubus pubescens</i>       | <b>Advanced *</b>                    | <b>Advanced *</b>  | No change        | No change *         | <b>Extended *</b>     |
| <i>Diervilla lonicera</i>    | <b>Advanced *†</b>                   |                    |                  | <i>No change</i>    | <b>Extended *</b>     |
| <i>Trientalis borealis</i>   | <b>Advanced</b>                      | <b>Advanced</b>    | <i>No change</i> | No change           | <b>Extended</b>       |
| <i>Maianthemum canadense</i> | <b>Advanced *</b>                    | <b>Advanced</b>    |                  | <b>Delayed *</b>    | <b>Extended *</b>     |
| <i>Eurybia macrophylla</i>   | <b>Advanced *<math>\times</math></b> | <b>Advanced *†</b> | <b>Advanced</b>  | <b>Delayed †</b>    | <b>Extended *†</b>    |
| <i>Solidago spp.</i>         | <b>Advanced</b>                      | <b>Advanced</b>    | No change        | <b>Delayed</b>      | <b>Extended</b>       |
| <i>Uvularia sessilifolia</i> | <i>No change</i>                     |                    |                  | <i>No change</i>    | <i>No change</i>      |
| <i>Corylus cornuta</i>       | <b>Advanced</b>                      |                    |                  | <b>Delayed</b>      | <b>Extended *</b>     |
| <i>Aralia nudicaulis</i>     | <b>Advanced *</b>                    |                    |                  | <b>Delayed</b>      | <b>Extended</b>       |
| <i>Streptopus roseus</i>     | <b>Advanced</b>                      |                    |                  | No change           | <b>Extended</b>       |
| <i>Pteridium aquilinum</i>   | <b>Advanced</b>                      |                    |                  | <i>No change</i>    | <b>Extended *</b>     |

Figure 1-2. Change in leaf unfolding per degree Celsius among five years. Average DOY leaf unfolding is averaged over plots of each treatment. Change was calculated by taking the difference in warming from ambient temperature. Species are in order of earliest leaf unfolding in ambient to latest. Bars indicate  $\pm 1$  SE.

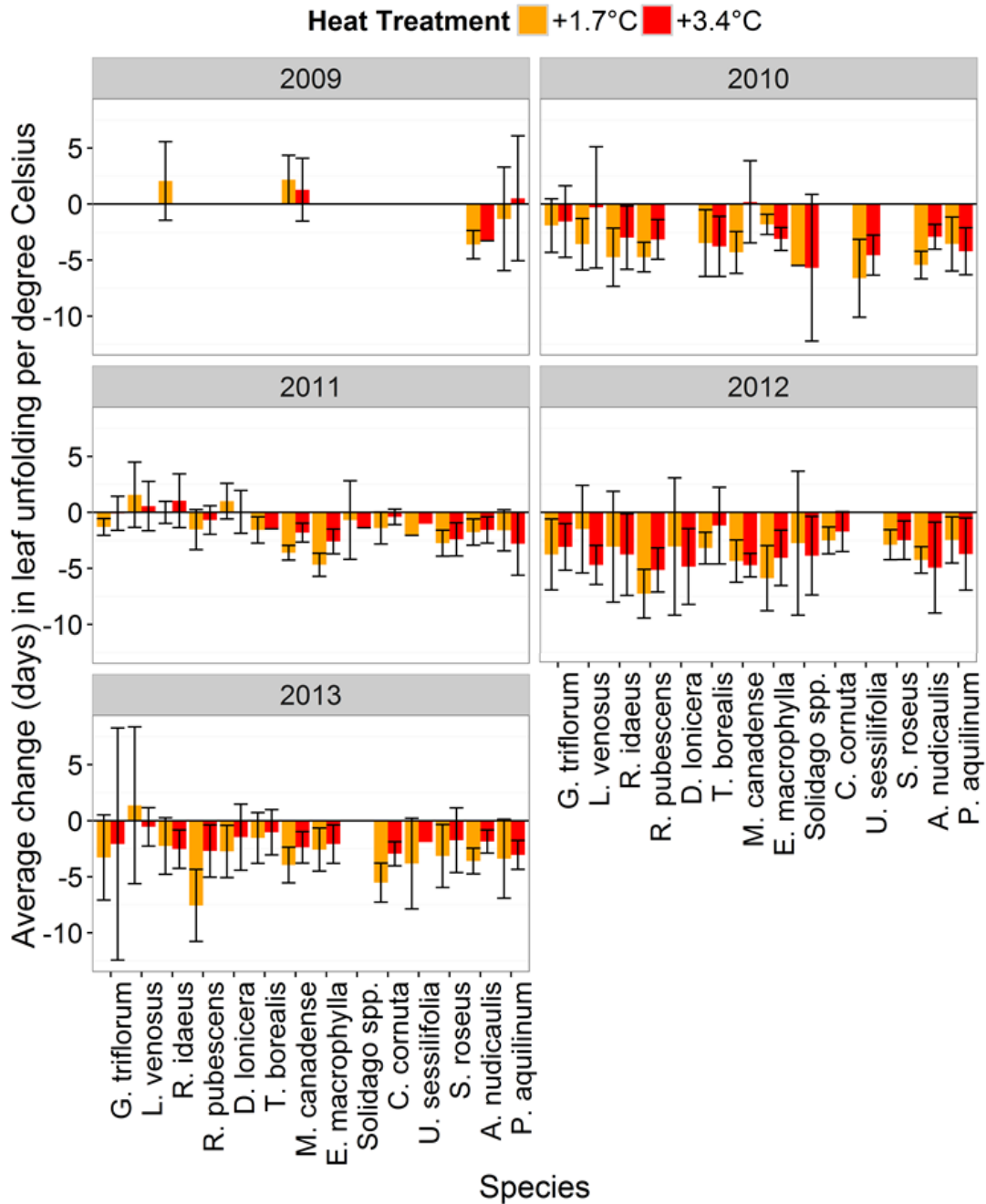




Figure 1-3. Average change in senescence per degree Celsius among four years. Average DOY senescence occurred was averaged between sites and plots of each treatment. Species are in order of timing of senescence in ambient conditions. Bars indicate  $\pm 1$  SE.

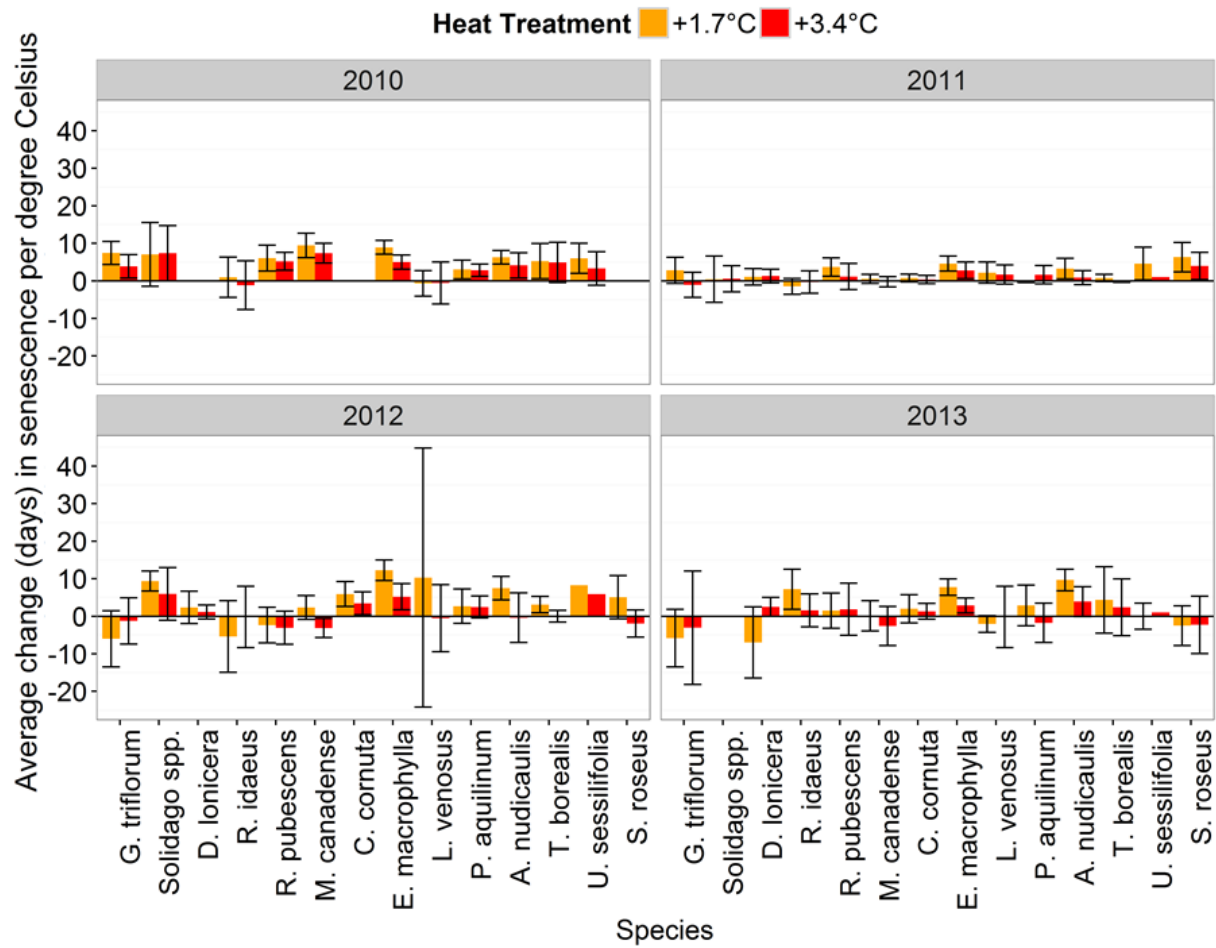


Figure 1-4. Average change in growing season length in warming treatments per change in degree Celsius among four years. Average growing season length was averaged between sites and plots of each treatment. Species are in order of timing of leaf unfolding in ambient conditions. Bars indicate  $\pm 1$  SE.

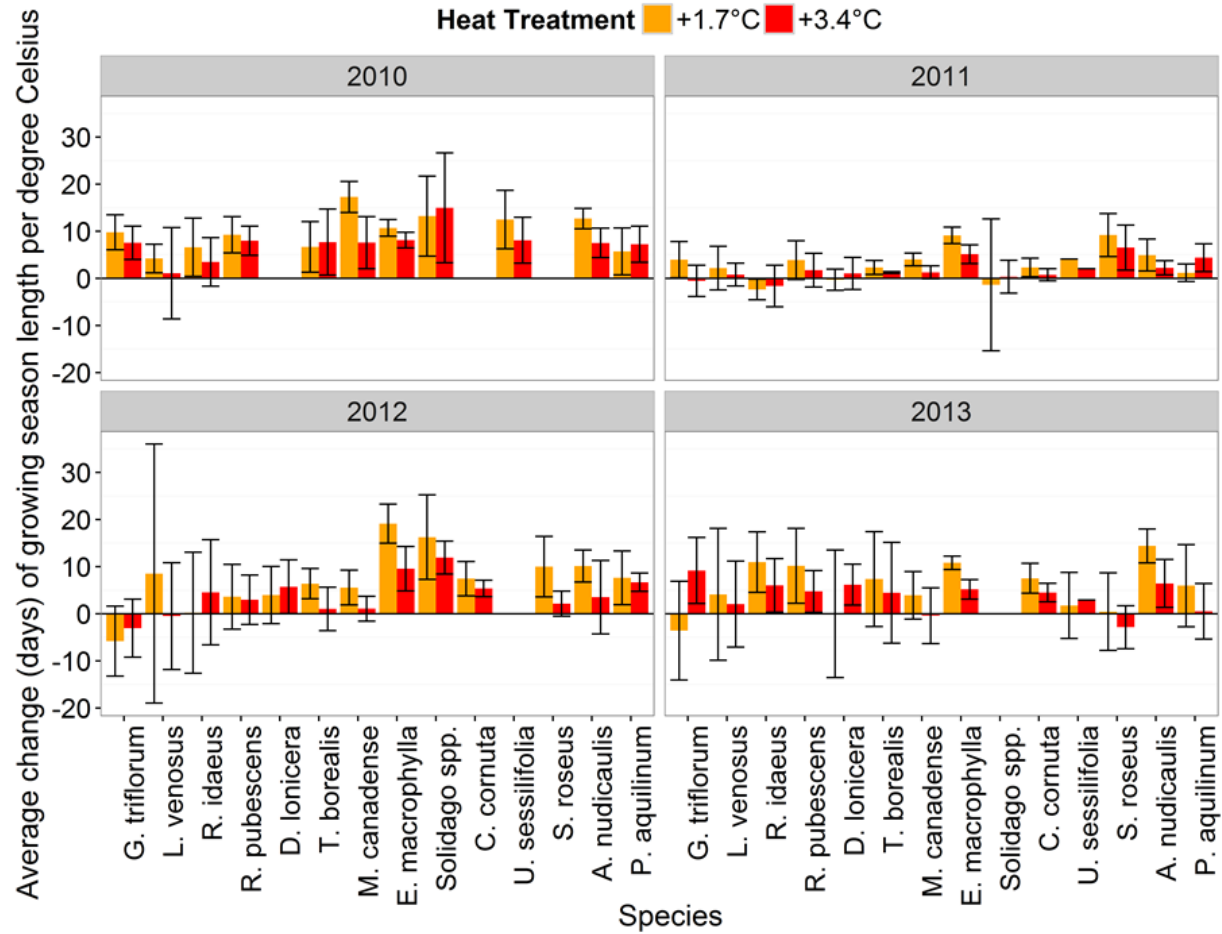


Figure 1-5 Change (delta) in flowering as a function of change (delta) in leaf unfolding in warmed plots compared to ambient are plotted over four years for early and late blooming species. Points represent individual species in each category of bloom time, with error bars indicating  $\pm 1$  SE of response. Delta flowering and delta leaf unfolding are calculated separately for each species by taking the average DOY each phenophase occurred in each warming treatment across two sites and canopies in each year, then taking the difference in the elevated temperature treatments from ambient.

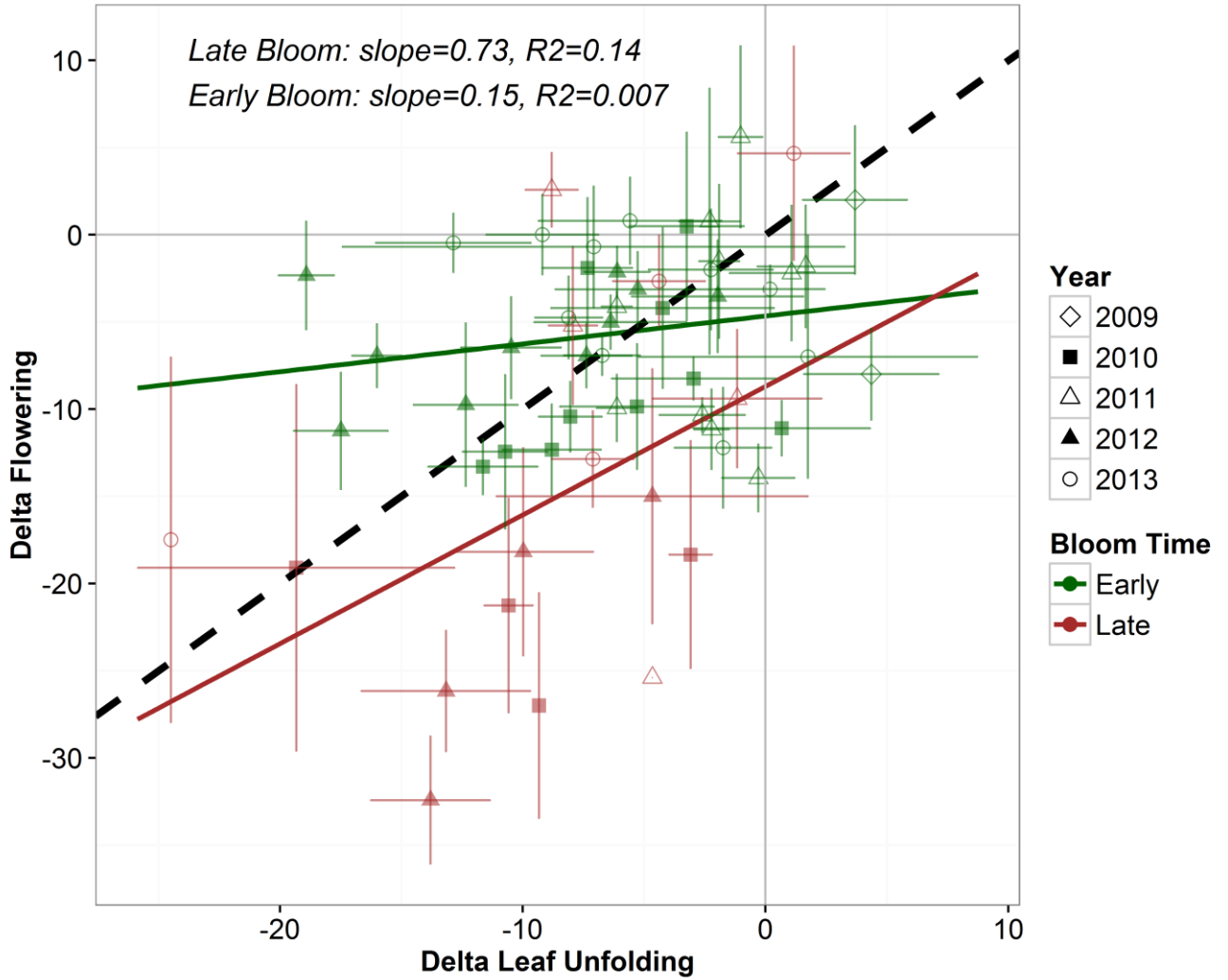
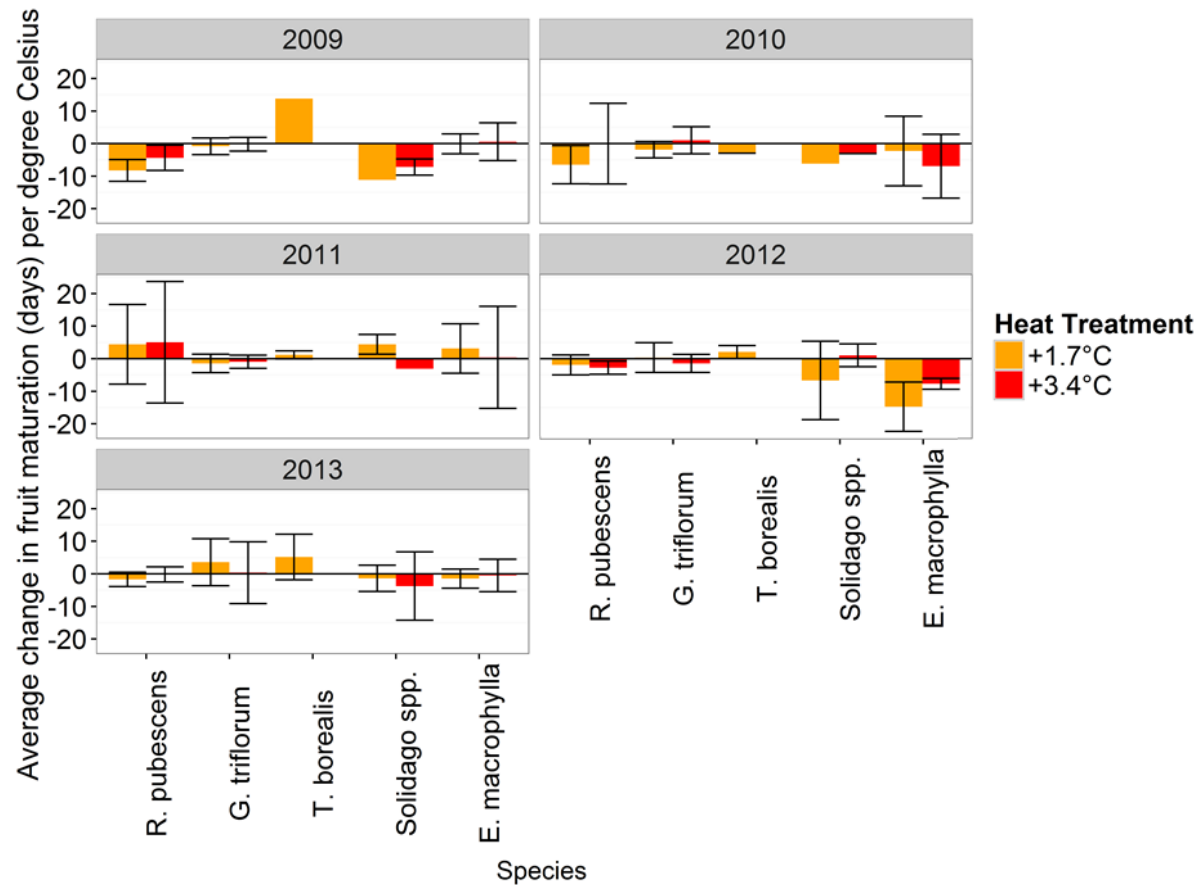


Figure 1-6. Average change in timing of fruit maturation per degree C for five species. Fruiting time is averaged over five years, two sites, and two canopies (when applicable) for each temperature treatment. Change per degree Celsius was calculated by taking the difference in warming from ambient temperature, then dividing the difference by nominal warming treatment (i.e. 3.4). Species in order of timing fruit maturation occurs in ambient temperature. Bars indicate  $\pm 1$  SE over all individuals.



## **Chapter 2 Effects of the interactions of warming and experimental rainfall removal on phenology of forest understory and tree seedling communities.**

### **Introduction**

Temperature is a main driving force for cuing the main life cycle events, or phenology, of plants. Studies examining the effects of warming on phenology are increasingly common (Farnsworth et al. 1995; Inouye, 2008; Fu et al. 2013), while studies examining the effects of drought on phenology are mainly in dry or Mediterranean environments (Penuelas et al. 2004; Bernal et al. 2011). This gap occurs despite large-scale models of drought showing a mixture of increased, unchanged, or shorter growing seasons dependent on the environment (Ivits et al. 2014) and the fact that drought can be one of the most damaging of extreme weather events (Orsenigo et al. 2014). Furthermore, little is known about the interactive or additive effects of drought and temperature on phenology. Expanding the knowledge of the interactive or additive effects of temperature and soil moisture on phenology in a broader range of ecosystems can better inform our understanding of how climate change may influence natural systems (Penuelas et al. 2004; Xie et al. 2015) especially with regards to mutualisms (Hegland et al. 2009), community composition (Wolkovich et al. 2013), biodiversity (Bellard et al. 2012), and vulnerable species identification (Bradley et al. 1999).

While manipulative warming and precipitation experiments of herbaceous and woody plants exist, many are focused on moisture-limited and arid systems (Reynolds et al. 1999; Prieto et al. 2009; Crimmins et al. 2010; Bernal et al. 2011) leaving more mesic systems largely understudied. Furthermore, many experiments focus on the phenological responses of added precipitation (Zavaleta et al. 2003b; Cleland et al. 2006; Sherry et al. 2007; Rollinson and Kaye, 2012), while a number of regions are predicted to see reductions in precipitation and or more extremes. Manipulative experiments combining warming with reduced precipitation add to our current understanding of how plants, and ultimately ecosystems, will respond in a changing world and is a more powerful study

than assessing shifting biological responses to warming alone (Hyvonen et al. 2007; Luo et al. 2008).

Warming has been shown to positively affect phenology by extending the growing season by advancing early season phenology (Farnsworth et al. 1995; Schwartzberg et al. 2014; Jacques et al. 2015; Chapter 1) and delaying senescence (Chapter 1, Montgomery et al. submitted). However, growing season advantages from warming may be negated due to warming effects on other resources, such as soil moisture. Warmer temperatures naturally decrease soil moisture through increased evapotranspiration and evaporative demand, indicating plants are able to utilize resources effectively in warming despite decreased soil moisture. A continued decrease in soil moisture may limit plants' abilities to transport water and nutrients or store carbon, and in turn shorten the growing season. For example, in temperate and boreal tree seedlings enhanced photosynthetic gains due to increased temperatures are negated when soil moisture is reduced (Reich et al. unpublished data). Other studies have shown decreases in bud size from summer drought, which may have implications for the health of primary growth the following year (Montserrat-Marti et al. 2009). Ultimately understanding the combined effects of temperature and water availability in a changing climate can help predict species' ability to persist in changing conditions (Crimmins et al. 2011).

Different functional groups may alter phenological responses to abiotic changes in ways that alter species' competitive niches and resource use (Sherry et al. 2007; Rollinson and Kaye, 2012). An important relationship that may be altered in seasonal deciduous forests is the relationship between the herbaceous and shrub layer (i.e. the groundlayer community) and the tree community. In northern deciduous forests the time between groundlayer emergence and tree canopy closure is a time of high light availability at ground level leading to carbon capture in the groundlayer (Rothstein and Zak, 2001; Bazzaz and Bliss 1971). If groundlayer plants respond more strongly than woody species, as has been shown in +2°C warming (Rollinson and Kaye, 2012), light limited herbaceous plants (Whigham, 2004) may benefit from increased light exposure (Van Calster et al. 2008). Alternatively, in semi-arid ecosystems the herbaceous layer

cover increased in response to tree mortality due to increased resources no longer being utilized by competing woody vegetation (Rich et al. 2008). Given that trees are increasingly threatened by drought and warming, even in ecosystems that are not thought to be water-limited (Allen et al. 2010) we may expect the relationship between the groundlayer and tree community to change even in semi-mesic systems. Understanding the varying responses of both the groundlayer and tree seedling communities alone and jointly can help predict potential community or ecosystem change.

The goal of this study was to enhance knowledge of how phenology may respond to climate change in several ways. First, the phenology of the groundlayer, herbaceous plants and shrubs in particular, is understudied compared to the phenology of trees. Second, the phenology of the temperate and boreal forest groundlayer is less commonly studied than grassland and high altitude systems (Cleland et al. 2006; Sherry et al. 2007; Dunne et al. 2003). Finally, prior manipulative studies either lack interactive effects on phenology of multiple abiotic changes or use treatments that are not applicable to expected changes in other ecosystems, such as northern forests. For instance, a common treatment is adding precipitation in conjunction with warming (e.g. Rollinson and Kaye 2012) while northern forests are expected to receive reduced rainfall with warmer temperatures according to current climate change projections (Handler et al. 2014).

Our study took place in the Boreal Forest Warming of an Ecotone in Danger (B4WarmED) field experiment located in the temperate-boreal forest ecotone in northern Minnesota (Reich et al. 2015). The B4WarmED project uses infra-red heaters and buried soil cables to raise above and below ground temperatures of planted seedlings and the naturally occurring vegetation (Rich et al. 2015). For three field seasons we combined this temperature manipulation with a rainfall manipulation treatment in a clearcut area, excluding rainfall periodically throughout the summer to simulate reduced number and amount of rainfall events.

We examined how decreased rainfall impacted the timing of leaf out, senescence, and growing season length for the tree seedling community and groundlayer community. We asked, does reduced rainfall independently alter phenology or interactively affect

phenological response to warming? We hypothesized that the late timing of the onset of our rainfall exclusion treatment (i.e summer months instead of spring when early season phenophases occur) would have minimal impacts on early season phenophases unless prior year drought in the fall influenced the response in consecutive years by processes such as premature budset (Tinus 1974, Duryea 1984), which may hinder growth the following spring (White 1987, Khan et al. 1996, Breda et al. 2006), or lack of soil moisture recharge after spring thaw, which could delay budburst in less drought tolerant species (Sonesson and Eriksson 2003). We previously observed a combination of no change or a delay of senescence due to warming in B4WarmED herbaceous plants (Chapter 1) and tree seedlings (Montgomery et al. submitted), but hypothesized that warming coupled with decreased rainfall would hasten senescence due to stress associated with soil drying (Marchin et al. 2010; Scholz et al. 2014), thus shifting or decreasing the total growing season length (Figure 2-1).

Furthermore, we asked, do the responses of the understory community (herbs and shrubs) to warming, decreased rainfall, and warming combined with decreased rainfall differ from the response of the tree seedling community (Figure 2-1)? We previously observed that leaf unfolding of herbaceous plants (Chapter 1) advanced more with warming compared to bud burst of tree seedlings (Montgomery et al. submitted) possibly providing more opportunities for the groundlayer to capture light and carbon. We hypothesized that in rainfall exclusion plots the increased sensitivity to soil moisture of shallow rooted species in the groundlayer (Farnsworth et al. 1995) compared to the tree seedlings with more extensive root systems or water storage capacity, may lead to stronger negative effects of rainfall exclusion on the groundlayer compared to little or no change in phenology of the tree seedling community. Thus a change in the overlap of the communities may occur, possibly allowing tree seedlings to leaf out before the groundlayer., However, the combined stress of warmer temperatures with reduced rainfall may shift the growing season of both communities in a similar direction (e.g. delay season start and advance senescence) but again the magnitude of response may be



larger for the groundlayer community due to the increased sensitivity to growing conditions.

## **Methods**

### *Study Site and System*

Data were collected in the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) experiment (Rich et al. 2015). The project has two upland forested sites in Cloquet (46°31'N. 92°30'W) and Ely (47°55'N. 92°30'W), Minnesota; both sites are located within the temperate-boreal forest ecotone. In this current study we used 36 - 7.1m<sup>2</sup> plots divided evenly between sites. Each site contains three blocks in a clearcut area. Within each block are two replicates of the three heat treatments. Heat treatments included unwarmed control plots (ambient) and plots with simultaneous above (via infrared lamps) and below-ground (via buried resistance cables) warming that elevated the temperature to two target levels above ambient, + 1.7 °C and + 3.4 °C. Within each block a rainfall manipulation treatment, simulating drought, was installed in half of the plots, thus each block contains one replicate of each warming treatment with and without rainfall manipulation. Rain removal started June 1 (DOY 153) and ended the last day of September (DOY 274),

The heat treatment is operated in open-air plots using a feedback control system to maintain the target temperature above ambient throughout the growing season (roughly April through November), see Rich et al. (2015) for more details on the heat treatment manipulation. The rainfall manipulation relies on custom-made 20m<sup>2</sup> heavy duty tarps on a furling system to be deployed only during individual rainfall events. The rainfall manipulation treatment removes approximately 40% of the total rainfall from June through September (Figure 2-2). Manual rain gauges above the vegetation within the plots and time-domain reflectometry probes ~20cm below soil surface record rainfall and soil moisture within each plot to estimate efficacy of the rainfall removal.

Seedlings from 10 species, 5 temperate, 5 boreal and one non-native, were planted from bare-root stock in random distribution throughout but in comparable numbers into all of the plots in the spring of 2008, 2011, 2012 and 2013 before initiation of the rainfall removal treatment. Planting bars were used, creating minimal disturbance thus allowing the naturally occurring herbaceous and shrub plant community to remain intact. Spacing between seedlings is 20 cm in all directions, leaving space for the groundlayer species to grow.

### *Data Collection*

Phenology records examining the response of groundlayer leaf unfolding, flowering, start of senescence, and total growing season length to drought coupled with warming were recorded every week in each plot from 2012-2014. Phenophases were recorded as day of year (DOY) starting January 1, though observations started at time of heat treatment turn on and ended at heat treatment turn off. Data collectors were assigned to particular sites and communicated definitions of phenophases through written protocols and electronic communication for data collection consistency.

‘Leaves Unfolding’ was chosen as the start of the season indicator as it was the earliest phase when identification of herbaceous species was most accurate, and was scored when the leaves of the plant were starting to unfold and expand away from the stem. ‘Start of Senescence’ was scored when leaves began to wither or change color due to end of year senescence or extreme drought stress. The growing season length was calculated as the length of time between ‘Leaves Unfolding’ and ‘Start of Senescence.’ For these phenophases, the record for each species in each plot was determined by observing which phenophases was most commonly being displayed. Flowering was defined by the presence of open flowers, such that reproductive structures were available for pollination. Within each plot, when the species was present, we tracked the leading individual’s flowering phenology due to many species and individuals not flowering every year.

Seedling phenology was collected twice weekly in the spring and fall and once a week in mid-June to mid-August. Two individuals, one from the “older” cohort from 2008 and one from the “young” cohort of either 2012 or 2013, of the eleven species planted in each plot were tracked consistently throughout the growing season (see Montgomery *et al* submitted, for more details). We scored the first day of year each seedling first displayed the following phenophases: budbreak and start of senescence (1/3 of leaves were exhibiting underlying pigments from chlorophyll breakdown, see Appendix 1 for full definitions). Growing season length was calculated as the number of days between budbreak and start of senescence. Out of the eleven species tracked, we are including the six native deciduous tree species in our analyses.

### *Statistical analysis*

We used mixed effects analysis of variance (ANOVA) models to assess the responses of average day of year (DOY) of the aforementioned phenophases and average growing season length. To assess if the two communities responded differently to the manipulation conditions, we ran an ANOVA using plant type (i.e. tree seedling or groundlayer plant), year, warming treatment and rainfall treatment as fixed effects, including all 2- and 3- way interactions, with plot nested in block nested in site as the random effect. To test for the effects of excluded rainfall and excluded rainfall combined with warming on the groundlayer community and the tree seedling community in general, we ran separate models for each phenophase and growing season length of each community including the following independent variables: year, warming treatment, drought treatment and all 2- and 3-way interactions among variables with species and block nested in site as random effects. Community analysis of the total growing season excludes conifers, as their timing of senescence is not an adequate indicator of season end. Due to our focus on the interaction of warming and rainfall manipulation, we do not discuss in-depth results of the warming treatment alone, but instead refer the reader to Chapter 1 and Montgomery et al. (submitted) for an in-depth analysis of the effects of warming on individual species of the groundlayer community (Chapter 1) and tree

seedling community (Montgomery et al. submitted) Chapter 1 and Montgomery et al. (submitted) are better equipped to address results pertaining to phenological responses to warming on an individual species level, given the replication of herbaceous and tree seedling phenology over five years in two canopy types with 24 plots of replicate warming treatments (compared to three years, one canopy, and 12 replicates of each warming treatment in this current study). This current study seeks to build on information gained from those two studies.

## **Results**

### *Treatment Manipulation*

Variation in volumetric soil water content (VWC) at week-to-week scale was due to our exclusion of roughly 40% of the total rainfall (Figure 2-2) Thus, drying periods were followed by recharge from proceeding rainfall events, which varied by week and site. The warming treatment also contributed to soil drying, with +1.7°C and +3.4°C warming consistently showing lower VWC than ambient temperatures in both rainfall manipulation set ups, though the excluded rainfall conditions consistently led to lower VWC regardless of warming treatment relative to the paired ambient rainfall plots (Table 2-1).

### *Community comparison*

We observed main effects of year, warming and community type and an interaction of year x community type for all three phenology metrics (Table 2-2). In addition, there were significant interactions for year x warming and warming x community type for leaf unfolding (Table 2-2). There was no effect of rainfall manipulation on any of the phenology metrics. In two of the three years, averaged across all treatments, seedlings broke bud several days earlier than the groundlayer showed leaf unfolding. In ambient conditions, the groundlayer shows leaf unfolding (May 24) an average of 8 days after seedling break bud (May 16). However, with warming the

groundlayer advances leaf unfolding to a larger extent than seedlings advance budbreak (10 day advance vs 4 day advance), resulting in the groundlayer showing leaf unfolding only 2 days after budbreak has occurred for seedlings in +3.4°C warming (Figure 2-3). The groundlayer senesced earlier (September 3) on average than the seedlings (September 18). Senescence was not significantly altered by warming for the groundlayer species. However, the tree seedlings delayed senescence by almost one week when warmed (interaction of warming x community type  $P=0.08$ ). Overall, the groundlayer showed consistently shorter growing season lengths compared to the seedlings. However, both community types advanced the growing season with warming by an average of ~11 days in +3.4°C warming compared to ambient.

#### *Groundlayer phenology: community response*

We found main effects of year, for all groundlayer phenophases, warming for leaf unfolding and growing season length and a year x warming interaction for leaf unfolding. There were no significant effects of rainfall manipulation on the groundlayer community's phenophases (Table 2-3). Ultimately the growing season of the groundlayer was extended by warming, primarily through advances in leaf unfolding and no change in timing of senescence from warming, consistent with previous findings (Chapter 1)

#### *Tree seedling phenology: community response*

We observed significant main effects of year, warming, and a year x warming interaction for all phenophases. In addition, we found a year x rainfall and a year x warming x rainfall interaction on timing of budbreak for deciduous tree seedlings (Table 2-4). Tree seedling budbreak was the only phenophase with an effect of the rainfall manipulation. This response was small: in 2013 we observed earlier budbreak by 2.2 days in the ambient rainfall conditions compared to excluded rainfall, with no differences between rainfall manipulation treatments in other years (Tukey's HSD,  $P<0.05$ ).

## **Discussion**

Expanding our knowledge of how different plant communities may respond to multiple changing drivers of phenology better informs our understanding of how climate change may impact particular functional groups and plant community interactions. We found that phenology of the groundlayer and tree seedling communities responded primarily to warming and did not change in response to summer and fall rainfall reduction treatments. However, both communities responded differently in magnitude to warming which led to increased duration of the high light window prior to canopy closure for groundlayer plants. A longer growing season for the groundlayer community relative to the tree seedling community could alter competitive interactions between the groundlayer and tree seedling communities.

Contrary to our hypotheses that warming coupled with soil drying would decrease or shift the growing season through earlier senescence (Figure 2-1), we did not observe a community level response for the groundlayer or tree seedling phenology. This lack of response may be explained by several mechanisms. First, soil moisture may not be a strong cue for phenology, rather daylength or temperature are stronger cues for phenology (Richardson et al. 2013). Moreover, species may display other responses that did not manifest as a phenological response, such as decreased photosynthetic rates or increased mortality in response to lowered soil moisture (B4WarmED unpublished data). Finally, the timing of the rainfall manipulation treatment, while in accordance with model predictions, may not have spurred the same changes as would have been observed if the reduced rainfall had occurred earlier in the season, such as before budbreak or leaf unfolding. Alternatively, it is possible our rainfall removal treatment may not have been extreme enough to elicit a phenological response (Figure 2-2).

Overall, warming had the strongest impact on altering the start of season overlap between the groundlayer and tree seedling communities. Previous work has shown warming advances leaf unfolding of groundlayer species more relative to budbreak of tree seedlings (Chapter 1, Montgomery et al. submitted) creating a larger opportunity for groundlayer species to receive light and capture carbon. Our current study shows that decreased rainfall does not alter this relationship (Figure 2-3). Furthermore, maximum

leaf unfolding of the groundlayer occurred at nearly twice the rate of crown expansion of the tree seedling layer under warming. Once the tree seedling layer reaches full crown expansion light available to the groundlayer is reduced (Jacques et al. 2015). Increased access to resources early in the season can increase reproduction (Jacques et al. 2015), survival (Augsburger et al. 2008) and fitness (De Frenne et al. 2010).

The possible positive outcomes for the groundlayer due to an earlier growing season could have negative consequences for the tree seedling community. Improved fitness, as previously mentioned, may give the groundlayer a competitive advantage over regenerating tree seedlings (Gilliam, 2007), possibly reducing tree seedling regeneration if groundlayer species can gain more light and nutrients. Earlier herbaceous plant emergence has led to increase nutrient uptake (Muller, 2014), reducing resources available for the tree seedling community. In effect, the groundlayer may then alter the species composition of the future overstory canopy (Gilliam, 2007). However, measuring the physiological and community composition responses of both communities to changes in climate can elucidate unknowns about such potential outcomes. For example, even though warming increased the growing season length with no phenological response to reduced rainfall in our current study, other studies have shown reduced root biomass (Jochum et al. 2007) or crown thinning (Breda et al. 2006) in response to reduced soil moisture and high temperature. In conjunction with changes in the timing of the growing season, these physiological responses may contribute to potential changes in community interactions.

The southern boreal forest ecotone where our study takes place is a relatively mesic system and understudied when examining the response of phenology to manipulated climate change conditions such as drought or increased temperatures. Reduced precipitation had no significant effect on the phenology of the groundlayer or tree seedling community. Our study adds to previous studies that highlight the apparently weak, or lack of, relationship between phenology and soil moisture, which was previously observed in grassland, high elevation and arid systems (Reynolds et al. 1999; Dunne et al. 2003; Sherry et al. 2007; Prieto et al. 2009; Crimmins et al. 2010; Bernal et al.

2011) but largely unexplored in northern forests. Warming altered the overlap in season start between the groundlayer and the tree seedling community by inducing a stronger season start response from the groundlayer, with implications for higher light and carbon gain early in the season for herbaceous plants and shrubs. Future research examining physiological responses of both communities to drought and warming are needed to elaborate on these findings. Our findings suggest that the phenology of the groundlayer community, compared to the tree seedling community, is more sensitive to temperature, which has implications for competition and community composition in northern forests. .





Figure 2-2. Weekly volumetric soil water content records for two rainfall (ambient and 40% excluded rainfall) and three temperature (ambient, +1.7°C, +3.4°C) treatments for 18 weeks of the growing season starting WOY 22 (week of June 1) and ending WOY 39 (last week of September). Three years and two sites are graphed separately. Each point is average of six replicate plots.

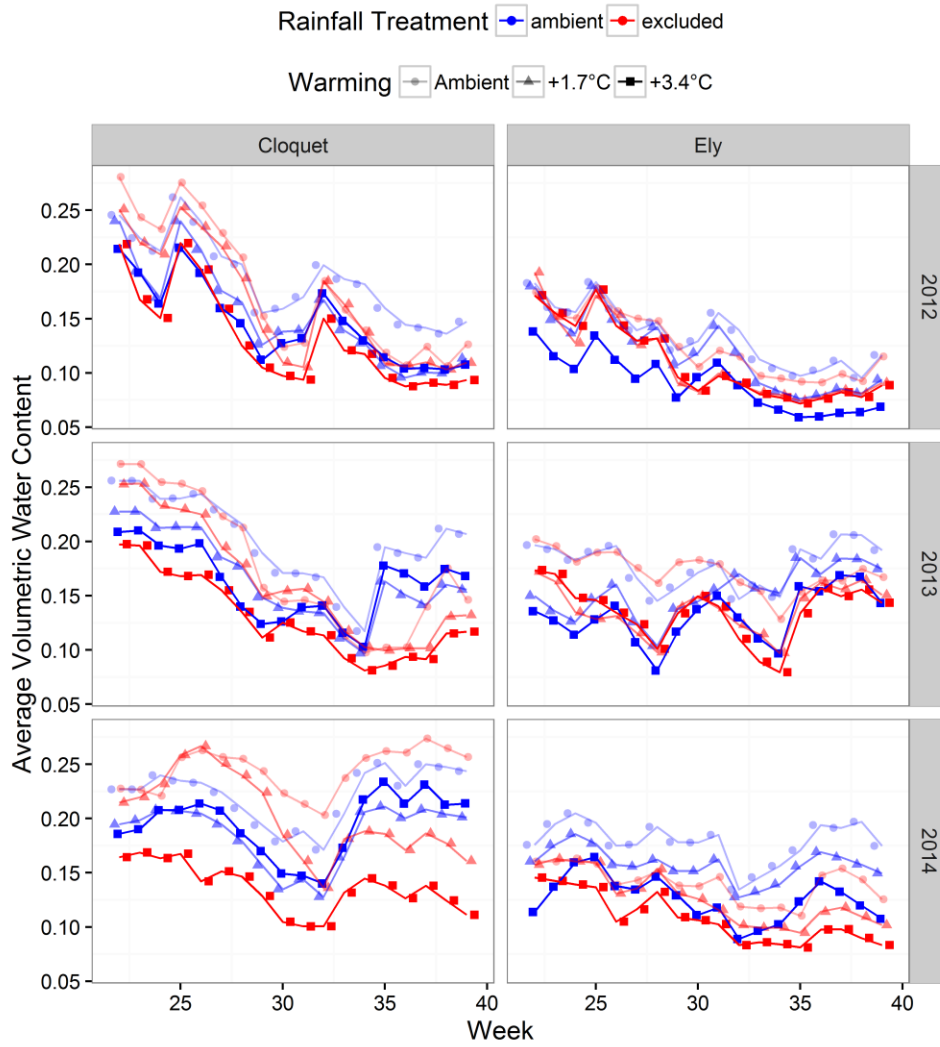


Table 2-1. Average volumetric soil water content (VWC) from June 1 through September 30 over three years and all rainfall and warming treatments. VWC is averaged between two sites and all plots of the same treatments.

| <b>Year</b> | <b>Rainfall</b> | <b>Warming</b> | <b>Mean VWC <math>\pm</math> SE</b> |
|-------------|-----------------|----------------|-------------------------------------|
| 2012        | Ambient         | Ambient        | 0.158 $\pm$ 0.002                   |
| 2012        | Excluded        | Ambient        | 0.148 $\pm$ 0.002                   |
| 2012        | Ambient         | +1.7°C         | 0.133 $\pm$ 0.002                   |
| 2012        | Excluded        | +1.7°C         | 0.133 $\pm$ 0.002                   |
| 2012        | Ambient         | +3.4°C         | 0.116 $\pm$ 0.002                   |
| 2012        | Excluded        | +3.4°C         | 0.117 $\pm$ 0.001                   |
| 2013        | Ambient         | Ambient        | 0.188 $\pm$ 0.001                   |
| 2013        | Excluded        | Ambient        | 0.172 $\pm$ 0.002                   |
| 2013        | Ambient         | +1.7°C         | 0.158 $\pm$ 0.001                   |
| 2013        | Excluded        | +1.7°C         | 0.148 $\pm$ 0.002                   |
| 2013        | Ambient         | +3.4°C         | 0.145 $\pm$ 0.001                   |
| 2013        | Excluded        | +3.4°C         | 0.129 $\pm$ 0.001                   |
| 2014        | Ambient         | Ambient        | 0.201 $\pm$ 0.001                   |
| 2014        | Excluded        | Ambient        | 0.193 $\pm$ 0.002                   |
| 2014        | Ambient         | +1.7°C         | 0.171 $\pm$ 0.001                   |
| 2014        | Excluded        | +1.7°C         | 0.163 $\pm$ 0.001                   |
| 2014        | Ambient         | +3.4°C         | 0.160 $\pm$ 0.002                   |
| 2014        | Excluded        | +3.4°C         | 0.121 $\pm$ 0.001                   |

Table 2-2. Mixed effects ANOVA comparing the ground layer and tree seedling communities for two phenophases and growing season length. Block nested in site and species are random effects.

| Source                              | Leaf unfolding |             |                |                  | Start of senescence |             |                 |                  | Growing season length |             |                 |                  |
|-------------------------------------|----------------|-------------|----------------|------------------|---------------------|-------------|-----------------|------------------|-----------------------|-------------|-----------------|------------------|
|                                     | DF             | DFDen       | F              | P                | DF                  | DFDen       | F               | P                | DF                    | DFDen       | F               | P                |
| Year                                | <b>2</b>       | <b>2333</b> | <b>1028.07</b> | <b>&lt;.0001</b> | <b>2</b>            | <b>2287</b> | <b>237.7881</b> | <b>&lt;.0001</b> | <b>2</b>              | <b>1180</b> | <b>375.2225</b> | <b>&lt;.0001</b> |
| Warming                             | <b>2</b>       | <b>2334</b> | <b>87.01</b>   | <b>&lt;.0001</b> | <b>2</b>            | <b>2286</b> | <b>7.5482</b>   | <b>0.0005</b>    | <b>2</b>              | <b>1180</b> | <b>30.7479</b>  | <b>&lt;.0001</b> |
| Rainfall                            | 1              | 2333        | 0.01           | 0.8976           | 1                   | 2287        | 0.0114          | 0.9148           | 1                     | 1179        | 0.1523          | 0.6964           |
| Community type                      | <b>1</b>       | <b>2334</b> | <b>114.29</b>  | <b>&lt;.0001</b> | <b>1</b>            | <b>2287</b> | <b>297.2997</b> | <b>&lt;.0001</b> | <b>1</b>              | <b>1180</b> | <b>297.1985</b> | <b>&lt;.0001</b> |
| Year x Warming                      | <b>4</b>       | <b>2333</b> | <b>8.90</b>    | <b>&lt;.0001</b> | 4                   | 2286        | 1.5591          | 0.1826           | 4                     | 1179        | 0.3451          | 0.8475           |
| Year x Rainfall                     | 2              | 2333        | 2.00           | 0.1351           | 2                   | 2286        | 2.1701          | 0.1144           | 2                     | 1179        | 1.7129          | 0.1808           |
| Year x Community type               | <b>2</b>       | <b>2333</b> | <b>65.95</b>   | <b>&lt;.0001</b> | <b>2</b>            | <b>2286</b> | <b>156.9146</b> | <b>&lt;.0001</b> | <b>2</b>              | <b>1179</b> | <b>84.3993</b>  | <b>&lt;.0001</b> |
| Warming x Rainfall                  | 2              | 2333        | 0.33           | 0.7181           | 2                   | 2286        | 0.5158          | 0.5971           | 2                     | 1179        | 0.1669          | 0.8463           |
| Warming x Community type            | <b>2</b>       | <b>2333</b> | <b>13.80</b>   | <b>&lt;.0001</b> | 2                   | 2286        | 2.5097          | 0.0815           | 2                     | 1180        | 0.0295          | 0.9710           |
| Rainfall x Community type           | 1              | 2333        | 2.18           | 0.1397           | 1                   | 2287        | 0.0939          | 0.7592           | 1                     | 1179        | 0.0001          | 0.9907           |
| Year x Warming x Rainfall           | 4              | 2333        | 1.64           | 0.1597           | 4                   | 2286        | 1.2176          | 0.3012           | 4                     | 1179        | 0.6081          | 0.6568           |
| Year x Warming x Community type     | 4              | 2333        | 1.06           | 0.3726           | 4                   | 2286        | 1.1683          | 0.3228           | 4                     | 1179        | 1.5505          | 0.1853           |
| Year x Rainfall x Community type    | 2              | 2333        | 0.38           | 0.6825           | 2                   | 2286        | 1.5957          | 0.2030           | 2                     | 1179        | 0.9622          | 0.3824           |
| Warming x Rainfall x Community type | 2              | 2333        | 1.21           | 0.2966           | 2                   | 2286        | 0.5661          | 0.5678           | 2                     | 1180        | 0.4566          | 0.6335           |

Figure 2-3. Phenophases for tree seedlings and groundlayer communities, averaged over species, sites and years. Lines connect phenophases to highlight the length of the time the canopy is closing (faded line between budbreak and crown expansion) and total growing season length. Bars indicate  $\pm 1$  SE.

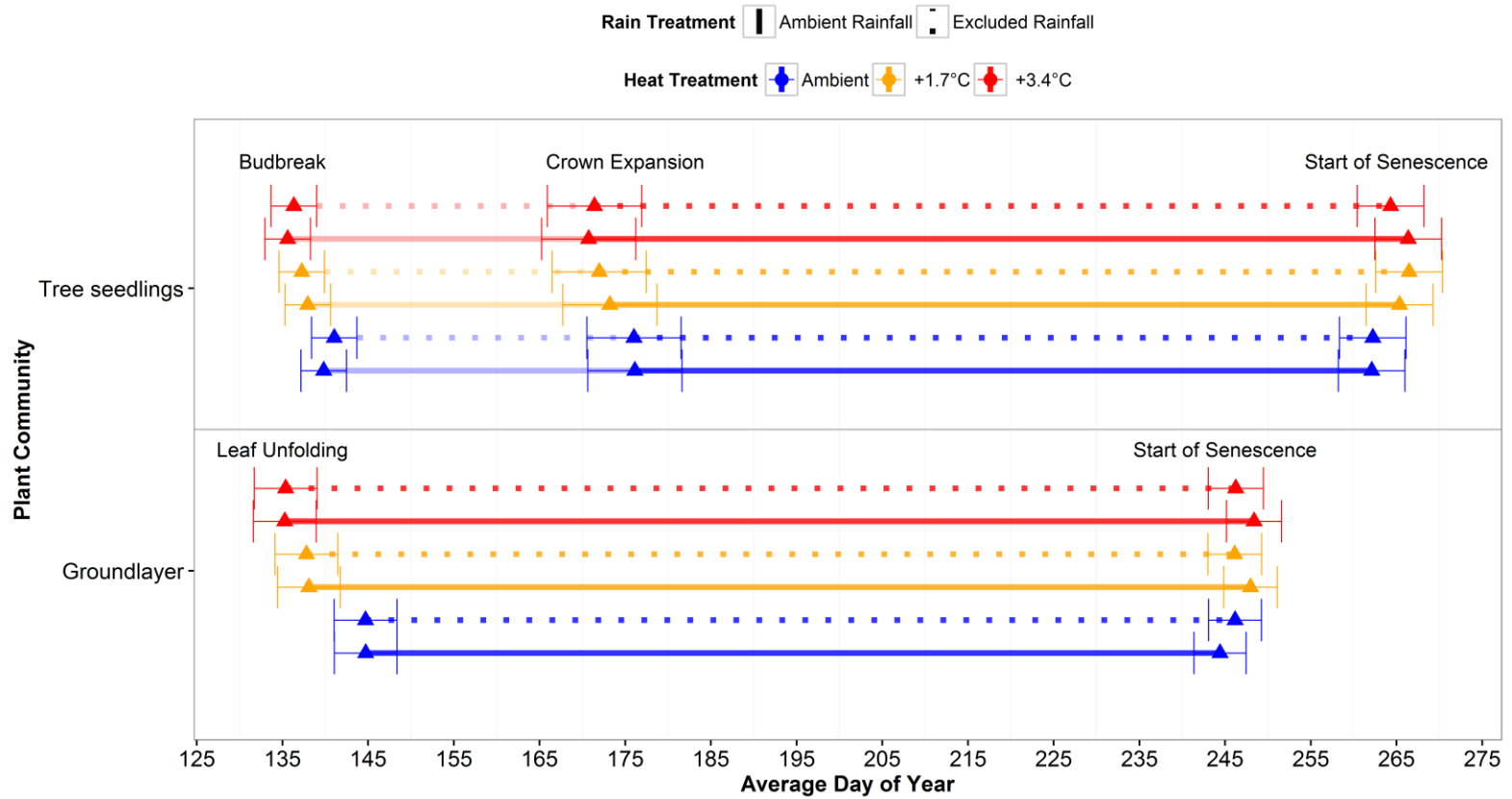


Table 2-3. Mixed effects ANOVA results for three phenophases for groundlayer species: leaf unfolding, start of senescence, and total growing length

| Source of variation       | Leaf unfolding<br>n=473 |       |               |                  | Start of Senescence<br>n=459 |       |               |                  | Growing Season Length<br>n=415 |       |               |                  |
|---------------------------|-------------------------|-------|---------------|------------------|------------------------------|-------|---------------|------------------|--------------------------------|-------|---------------|------------------|
|                           | DF                      | DFD   | F             | P                | DF                           | DFD   | F             | P                | DF                             | DFD   | F             | P                |
| Year                      | 2                       | 445.5 | <b>502.81</b> | <b>&lt;.0001</b> | 2                            | 456.6 | <b>207.78</b> | <b>&lt;.0001</b> | 2                              | 409.9 | <b>284.20</b> | <b>&lt;.0001</b> |
| Warming                   | 2                       | 444.4 | <b>53.06</b>  | <b>&lt;.0001</b> | 2                            | 454.6 | 0.59          | 0.5505           | 2                              | 408.7 | <b>12.77</b>  | <b>&lt;.0001</b> |
| Rainfall                  | 1                       | 444.3 | 0.01          | 0.9299           | 1                            | 454   | 0.14          | 0.7107           | 1                              | 408   | 0.17          | 0.6791           |
| Year x Warming            | 4                       | 444.1 | <b>4.46</b>   | <b>0.0015</b>    | 4                            | 451.6 | 0.14          | 0.9683           | 4                              | 407.6 | 0.69          | 0.6019           |
| Year x Rainfall           | 2                       | 444.3 | 0.03          | 0.9611           | 2                            | 452.4 | 1.94          | 0.1453           | 2                              | 408.2 | 1.33          | 0.2650           |
| Warming x Rainfall        | 2                       | 444.2 | 0.02          | 0.9768           | 2                            | 453.7 | 0.72          | 0.4857           | 2                              | 408.2 | 0.30          | 0.7428           |
| Year x Warming x Rainfall | 4                       | 444   | 0.12          | 0.9769           | 4                            | 451.3 | 0.12          | 0.9765           | 4                              | 407.3 | 0.29          | 0.8852           |

Figure 2-4. Average DOY leaf unfolding occurred (left side) and start of senescence occurred (right side) for groundlayer plants in three years, three warming treatments and two rainfall treatments.

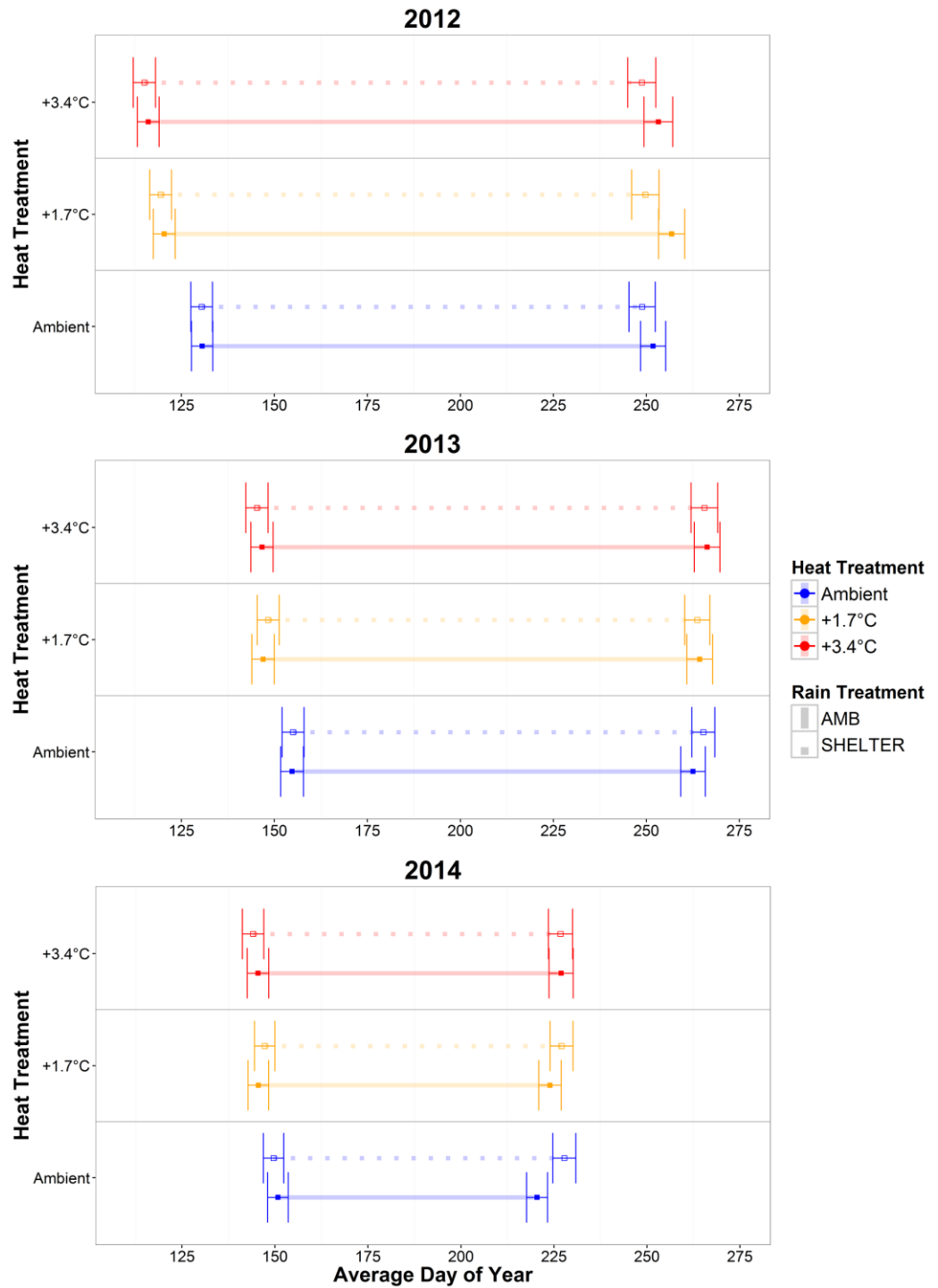
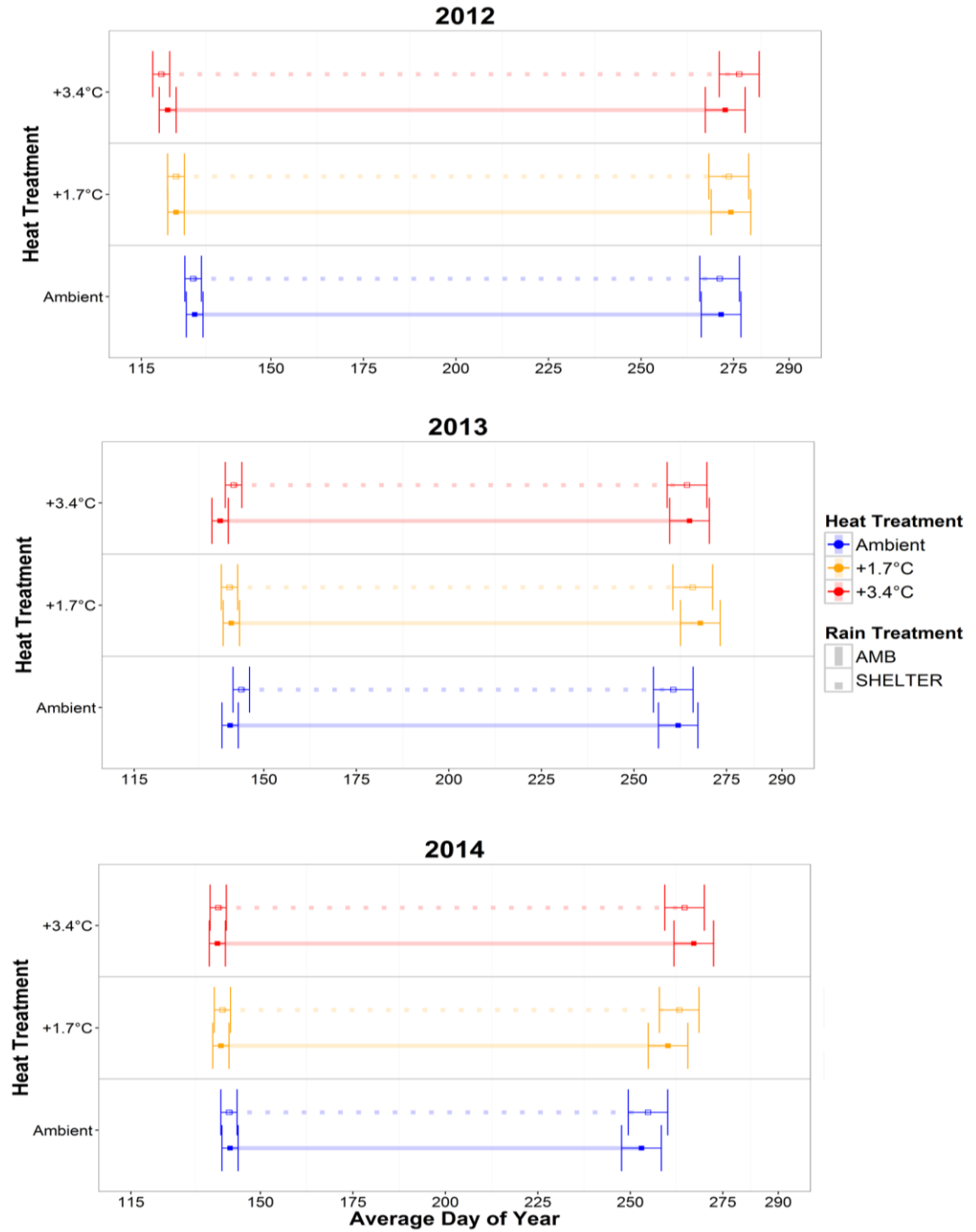


Table 2-4. Mixed effects ANOVA results for three phenophases of 6 deciduous tree seedlings. Plot nested in block nested in site, seedling cohort and species are random effects.

| Source of variation       | Budbreak |              |              |                  | Crown Expansion |              |              |               | Senescence |              |              |                  | Growing season length |              |              |               |
|---------------------------|----------|--------------|--------------|------------------|-----------------|--------------|--------------|---------------|------------|--------------|--------------|------------------|-----------------------|--------------|--------------|---------------|
|                           | DF       | DFDen        | F            | P                | DF              | DFDen        | F            | P             | DF         | DFDen        | F            | P                | DF                    | DFDen        | F            | P             |
| Year                      | <b>2</b> | <b>4.221</b> | <b>58.16</b> | <b>0.0008</b>    | <b>2</b>        | <b>4.674</b> | <b>36.69</b> | <b>0.0014</b> | <b>2</b>   | <b>1077</b>  | <b>50.61</b> | <b>&lt;.0001</b> | <b>2</b>              | <b>4.288</b> | <b>11.45</b> | <b>0.0190</b> |
| Warming                   | <b>2</b> | <b>31.22</b> | <b>45.46</b> | <b>&lt;.0001</b> | <b>2</b>        | <b>30.08</b> | <b>5.94</b>  | <b>0.0067</b> | <b>2</b>   | <b>29.14</b> | <b>5.83</b>  | <b>0.0074</b>    | <b>2</b>              | <b>29.29</b> | <b>11.73</b> | <b>0.0002</b> |
| Rainfall                  | 1        | 31.01        | 1.57         | 0.2184           | 1               | 30           | 0.01         | 0.9673        | 1          | 29.12        | 0.01         | 0.9421           | 1                     | 29.2         | 0.01         | 0.8890        |
| Year x Warming            | <b>4</b> | <b>1846</b>  | <b>10.53</b> | <b>&lt;.0001</b> | <b>4</b>        | <b>1063</b>  | <b>5.71</b>  | <b>0.0001</b> | <b>4</b>   | <b>1792</b>  | <b>6.86</b>  | <b>&lt;.0001</b> | <b>4</b>              | <b>729</b>   | <b>2.59</b>  | <b>0.0355</b> |
| Year x Rainfall           | <b>2</b> | <b>1849</b>  | <b>7.73</b>  | <b>0.0005</b>    | 2               | 1062         | 0.04         | 0.9514        | 2          | 1791         | 1.21         | 0.2961           | 2                     | 728.4        | 1.87         | 0.1545        |
| Warming x Rainfall        | 2        | 30.96        | 0.44         | 0.6444           | 2               | 29.99        | 0.06         | 0.9417        | 2          | 29.1         | 0.01         | 0.9988           | 2                     | 29.26        | 0.13         | 0.8775        |
| Year x Warming x Rainfall | <b>4</b> | <b>1850</b>  | <b>3.44</b>  | <b>0.0082</b>    | 4               | 1062         | 0.58         | 0.6731        | 4          | 1791         | 1.73         | 0.1386           | 4                     | 729          | 1.40         | 0.2302        |



Figure 2-5. Average DOY leaf unfolding occurred (left side) and start of senescence occurred (right side) for tree seedlings in three years, three warming treatments and two rainfall treatments.



## **Chapter 3** Reproductive phenology of herbaceous plants in response to manipulated rainfall and experimental warming

### **Introduction**

Within the next century, temperatures are predicted to continue to rise, as has already been documented (IPCC 2013, Walsh et al. 2014), with other interacting climate variables changing in tandem. Specifically, altered rainfall patterns are expected to coincide with changes in temperature (Karl and Trenberth 2003). Phenology has become a trusted tool for tracking patterns of climate change (Menzel, 2002; Cleland et al. 2007), and is an important component of predicting how future climate change conditions may alter ecological relationships and species persistence. Warming temperatures have been linked to both earlier flowering events (Fitter and Fitter 1995) and delayed flowering depending on time of year and environmental conditions (Sherry et al. 2007). Alterations in timing of flowering have numerous impacts including creating gaps for novel species to invade (Sherry et al. 2007; Wolkovich and Cleland 2014), or causing pollinator-plant mismatches (Hegland et al. 2009). However, despite the expectation that warmer temperatures will be coupled with changes in precipitation, experiments examining phenology in the face of this future scenario are lacking (but see Rollinson and Kaye 2012; Sherry et al. 2007; Cleland et al. 2006).

Flowering phenology is triggered by a combination of temperature, soil moisture and photoperiod (Rathcke and Lacey 1985). Generally, perennials are triggered mainly by temperature accumulation, but some plants, depending on the habitat, have shown either rainy seasons or dry seasons may initiate flowering (Rathcke and Lacey 1985 and sources within). Alpine grasses have responded to snowmelt, possibly as a cue signaling moisture availability and nutrient cycling in the soil (Walker et al. 1995). Yet grassland studies have shown little evidence of a strong phenological response of grassland herbs to added precipitation (Sherry et al. 2007, Rollinson and Kaye 2012, Cleland et al. 2006). To our knowledge, flowering phenology in response to warmer temperatures and reduced

rainfall has not been studied in mesic systems such as temperate or southern boreal forests.

We tested the response of flowering to experimental warming and manipulated rainfall conditions for five herbaceous groundlayer plants. We hypothesized that warming would advance flowering, but the enhanced stress from decreased rainfall would cancel out advances in timing of flowering or delay timing of flowering. Furthermore, few expected to see fall blooming species most impacted by the combined warming and rainfall treatment, given they had the longest exposure to the stressful environment of higher temperatures and limited soil moisture. Our study took place in the Boreal Forest Warming of an Ecotone in Danger (B4WarmED) project, an *in situ* forest experiment in northern Minnesota. In addition to the lack of phenology experiments related to warming combined with reduced rainfall, northern forests are also understudied, making this study unique in its contribution of our understanding of how reproductive phenology of the groundlayer will respond to future climate change conditions.

## **Methods**

### *Study Site and System*

Data were collected in the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) experiment (Rich et al. 2015). The project has two upland forested sites in Cloquet (46°31'N. 92°30'W) and Ely (47°55'N. 92°30'W), Minnesota; both sites are located within the temperate-boreal forest ecotone. In this current study we used 36 - 7.1m<sup>2</sup> plots divided evenly between sites. Each site contains three blocks in a clearcut area. Within each block are two replicates of the three heat treatments. Heat treatments included unwarmed control plots (ambient) and plots with simultaneous above (via infrared lamps) and below-ground (via buried resistance cables) warming that elevated the temperature to two target levels above ambient, + 1.7 °C and + 3.4 °C. Within each block a rainfall manipulation treatment, simulating drought, was installed in half of the plots, thus each block contains one replicate of each warming treatment with and without

rainfall manipulation. Rain removal started June 1 (DOY 153) and ended the last day of September (DOY 274),

The heat treatment is operated in open-air plots using a feedback control system to maintain the target temperature above ambient throughout the growing season (roughly April through November), see Rich et al. (2015) for more details on the heat treatment manipulation. The rainfall manipulation relies on custom-made 20m<sup>2</sup> heavy duty tarps on a furling system to be deployed only during individual rainfall events. The rainfall manipulation treatment removes approximately 40% of the total rainfall from June through September. Manual rain gauges above the vegetation within the plots and time-domain reflectometry probes ~20cm below soil surface record rainfall and soil moisture within each plot to estimate efficacy of the rainfall removal.

#### *Data Collection*

We recorded flowering phenology observations every seven days in each plot from 2012-2014. Phenophases were recorded as day of year (DOY) starting January 1, though observations started at time of heat treatment turn on and ended at heat treatment turn off. Within each plot, when the species was present, we recorded the first DOY we observed flowering in the leading individual of each studied species. Data collectors were assigned to particular sites and communicated definitions of phenophases through written protocols and electronic communication for data collection consistency.

Flowering was defined by the presence of open flowers, such that reproductive structures were available for pollination. All species tracked were herbaceous perennials that were not planted into the plots, rather they regenerated from rhizome systems after disturbance from the initial experimental set up.

#### *Statistical analysis*

We used mixed effects analysis of variance (ANOVA) models to assess the responses of average day of year (DOY) of the reproductive phenophases. To test for the effects of excluded rainfall and excluded rainfall combined with warming on timing of

flowering of the groundlayer community as a whole we ran two separate models, the first combining spring blooming species and the second combining fall blooming species (Table 3-1). Both models included the following independent variables: year, species, warming treatment, rainfall treatment and all 2- and 3-way interactions among variables with block nested in site as the random effect.

To effectively understand individual species' responses, and isolate the effects of excluded rainfall and interactive effects of excluded rainfall combined with warming, we ran mixed effects models for each individual species of the groundlayer community with warming and rainfall manipulation as main effects with a warming x drought interaction (Table 3-2). Year and block nested in site were the random effects. We examined the significant results ( $p < 0.05$ ) of each ANOVA using Tukey-HSD post hoc tests to evaluate significant differences among treatment variables at  $\alpha = 0.05$ .

## Results

Spring blooming species showed a main effect of year, warming, and species, as well as a year x species interaction on timing of flowering (Table 3-1). Species was the strongest fixed effect. Overall, the warming treatment affected the spring blooming species similarly (as shown by no significant warming x species interaction) with warming advancing flowering by an average of 8 days compared to ambient. We observed no significant response of flowering to the rainfall manipulation. This lack of response to the rainfall manipulation was confirmed in the individual species models (Table 3-2). Flowering typically occurred by June 9 in ambient warming and ambient rainfall conditions, several days after the rainfall manipulation was started (June 1).

We observed main effects of warming, year and species on timing of flowering for fall blooming species, as well as several interactions with species including a warming x species x rainfall reduction interaction (Table 3-1). Flowering of the fall blooming species, *Eurybia macrophylla* and *Solidago spp.*, responded to warming and reduced rainfall but *Solidago spp.* was the only species with a warming x rainfall interaction on flowering (Table 3-2). Both species advanced flowering with +3.4°C

warming and ambient rainfall, by 19 and 16 days *Solidago* spp. and *Eurybia macrophylla*, respectively. The response of the two species diverged in the +3.4°C warming coupled with excluded rainfall conditions. Flowering of *Solidago* spp. was delayed by 7 days in +3.4°C warming and excluded rainfall. However, flowering advance of *Eurybia macrophylla* was largest in +3.4°C warming with excluded rainfall, advancing 22 days.

## **Discussion**

Spring blooming groundlayer species did not alter flowering phenology in response to altered rainfall or altered rainfall all combined with warming. This result differed from the two fall blooming species, whose flowering phenology diverged in response to altered rainfall combined with warming. *Solidago* spp. in particular delayed flowering in the warmest, driest treatment while *Eurybia macrophylla* continued to advance flowering in the warmest, driest conditions. Differing responses may indicate future changes to ecological relationships, such as changes in plant-pollinator interactions (Hegland et al. 2009) or opening niches for invasive species to exploit (Sherry et al. 2007).

The moderate advance in spring flowering under warmer temperatures and the lack of response of spring flowering to altered rainfall is not surprising. The lack of response of spring flowering to altered rainfall may be due to the experimental design of the study system: rainfall removal began approximately one week after flowering occurs in ambient conditions. The timing of this manipulation leaves little time for the plants to respond to the stressful conditions, as flower development has already occurred at this time. However, it is unlikely that reduced rainfall events will occur in the spring in northern Minnesota (Walsh et al. 2014), therefore we can expect the conditions imposed by the experimental design and the flowering response to be in accordance with future conditions. The lack of year x rainfall interaction indicates that there is no lag response, i.e. reduced soil moisture the previous year did not impact flowering time the following year. Studies in boreal forests have shown understory species use belowground resources

to initiate growth and then to store resources for the following year to enable flowering (Mooney and Billings 1960, Walker et al. 1995). If decreased soil moisture later in the year impacted translocation ability or resource accumulation on our study species, we may expect to see a delay in flowering the following year after a dry summer or fall. By not observing this lag response, we can infer that phenological cues for flowering override any resource limitations acquired the previous year or that reduced rainfall later in the season did not impact resource allocation at the end of season, as senescence did not appear effected by reduced rainfall (Chapter 2).

Fall (i.e. August) flowering species were the only species showing phenological changes to rainfall exclusion. Generally, *Eurybia macrophylla* and *Solidago spp* flower within days of each other in mid-August, and warmer temperatures with ambient rainfall advance flowering of both species at a similar rate. However, in +3.4°C with rainfall exclusion, the hottest and driest conditions, *Eurybia macrophylla* advanced flowering by three weeks (into the end of July) while *Solidago spp* delayed flowering by almost one week (into the end of August). Thus in +3.4°C rainfall exclusion plots there was a month difference between when *Eurybia macrophylla* flowered compared to *Solidago spp*.

Similarly, (Sherry et al. 2007) observed a divergence of flowering in grassland species, though with no effect of altered precipitation on this timing. The ability of *Eurybia macrophylla* to advance in warm and dry conditions may be related to this species' ability to cope with hot, dry environments (Ahlgren, 1960), as it is one of the first species to rebound in the hot, dry conditions after a fire. It is an aggressively rooted species, perhaps allowing it to derive more moisture from deeper into the soil or over a wider area. Conversely, *Solidago spp* may delay flowering in warm and dry conditions as a means of phenological escape (Sonesson and Eriksson 2003), thus avoiding flowering until soil moisture was restored, or was delayed due to an inability to acquire enough soil moisture until later in the season.

Our observations highlight the importance of examining a range of species found in a habitat and observing responses in the wide range of conditions predicted to change with climate change. Spring and fall blooming species responded differently in

magnitude and direction. Furthermore, examining the responses of fall blooming species in warming alone did not capture the full response of these species. Examining the flowering response in the context of warming combined with altered rainfall provides a fuller picture of responses expected in a changing climate (Hyvonen et al. 2007; Luo et al. 2008). These differences in responses among species, particularly the divergence in flowering times, may lead to other species filling this new temporal niche (Sherry et al. 2007), reduce pollen availability for pollinators that rely on synchronous flowering (Moeller, 2004), or create mismatches in timing of flowering with pollinator availability (Hegland et al. 2009). More studies combining warming with reduced rainfall are needed to enhance our understanding of the phenology shifts which may take place in forested areas of North America, pinpointing sensitive species and elucidating potential changes in ecological relationships. Furthermore, studies of the physiology coupled with phenology are needed to understand the mechanisms behind such responses to increased warming and altered rainfall.



Table 3-1. Flowering response of three spring blooming species (*left: Hieracium spp., Lathyrus venosus, Fragaria virginiana*) and two fall blooming species (*right: Solidago spp., Eurybia macrophylla*), block nested in site is the random effect.

|                              | Flowering: spring blooming species |              |               |                  | Flowering: fall blooming species |              |              |                  |
|------------------------------|------------------------------------|--------------|---------------|------------------|----------------------------------|--------------|--------------|------------------|
| Source of variation          | DF                                 | DFDen        | F             | P                | DF                               | DFDen        | F            | P                |
| Warming                      | <b>2</b>                           | <b>134.2</b> | <b>10.66</b>  | <b>&lt;.0001</b> | <b>2</b>                         | <b>90.97</b> | <b>10.46</b> | <b>&lt;.0001</b> |
| Year                         | <b>2</b>                           | <b>134.6</b> | <b>88.33</b>  | <b>&lt;.0001</b> | <b>2</b>                         | <b>88.95</b> | <b>8.16</b>  | <b>0.0006</b>    |
| Species                      | <b>2</b>                           | <b>136.7</b> | <b>151.46</b> | <b>&lt;.0001</b> | <b>1</b>                         | <b>52.59</b> | <b>9.30</b>  | <b>0.0036</b>    |
| Rainfall                     | 1                                  | 134.8        | 0.25          | 0.6113           | 1                                | 90.12        | 0.01         | 0.8941           |
| Warming x Year               | 4                                  | 134          | 0.36          | 0.8341           | 4                                | 88.88        | 0.99         | 0.4127           |
| Warming x Species            | 4                                  | 135.4        | 1.78          | 0.1349           | 2                                | 91.35        | 2.20         | 0.1156           |
| Warming x Rainfall           | 2                                  | 135.7        | 0.26          | 0.7709           | <b>2</b>                         | <b>90.35</b> | <b>6.46</b>  | <b>0.0024</b>    |
| Year x Species               | <b>4</b>                           | <b>134.5</b> | <b>6.74</b>   | <b>&lt;.0001</b> | 2                                | 89.04        | 0.77         | 0.4642           |
| Year x Rainfall              | 2                                  | 133.9        | 1.87          | 0.1575           | 2                                | 88.33        | 0.04         | 0.9534           |
| Species x Rainfall           | 2                                  | 134.6        | 0.37          | 0.6849           | <b>1</b>                         | <b>90.88</b> | <b>8.91</b>  | <b>0.0036</b>    |
| Warming x Year x Species     | 8                                  | 134.2        | 1.58          | 0.1354           | 4                                | 88.69        | 0.61         | 0.6496           |
| Warming x Year x Rainfall    | 4                                  | 134.1        | 0.99          | 0.4109           | 4                                | 88.53        | 1.13         | 0.3460           |
| Warming x Species x Rainfall | 4                                  | 135.5        | 0.47          | 0.7522           | <b>2</b>                         | <b>91.69</b> | <b>6.92</b>  | <b>0.0016</b>    |
| Year x Species x Rainfall    | 4                                  | 134          | 1.17          | 0.3232           | 2                                | 88.34        | 0.80         | 0.4507           |

Figure 3-1. Average change in days flowering occurred in manipulated conditions compared to ambient rainfall and ambient temperature for three spring blooming herbaceous perennials. Average phenophase timing was calculated by averaging the phenophase onset over three years and all plots of comparable treatments for each species. Average difference was calculated by taking the difference in warming and/or rainfall exclusion from ambient temperature and ambient precipitation. Bars indicate  $\pm 1$  SE. Species in descending order of timing of flowering in ambient conditions.

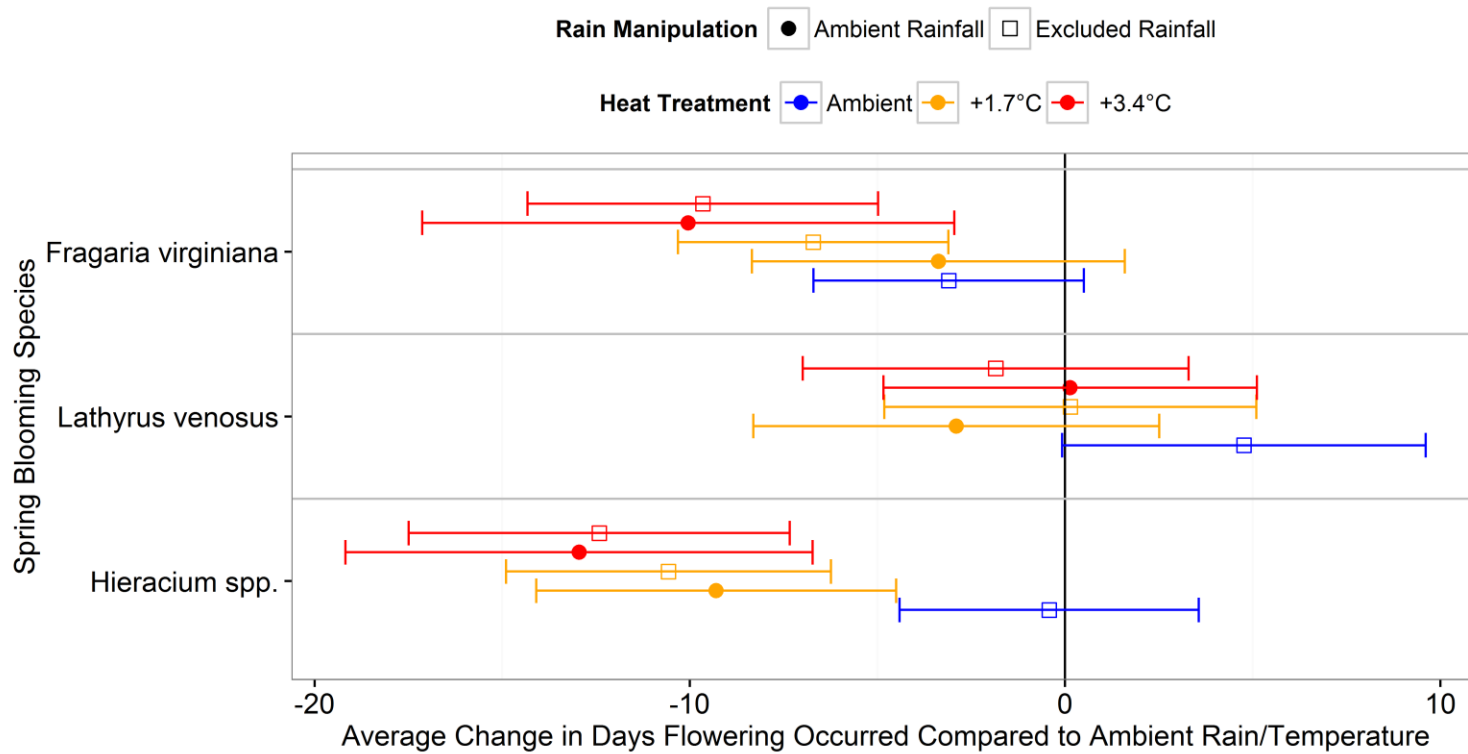


Figure 3-2. Average change in days flowering occurred in manipulated conditions compared to ambient rainfall and ambient temperature for two fall blooming herbaceous perennials. Average phenophase timing was calculated by averaging the phenophase onset over three years and all plots of comparable treatments for each species. Average difference was calculated by taking the difference in warming and/or rainfall exclusion from ambient temperature and ambient precipitation. Bars indicate  $\pm 1$  SE.

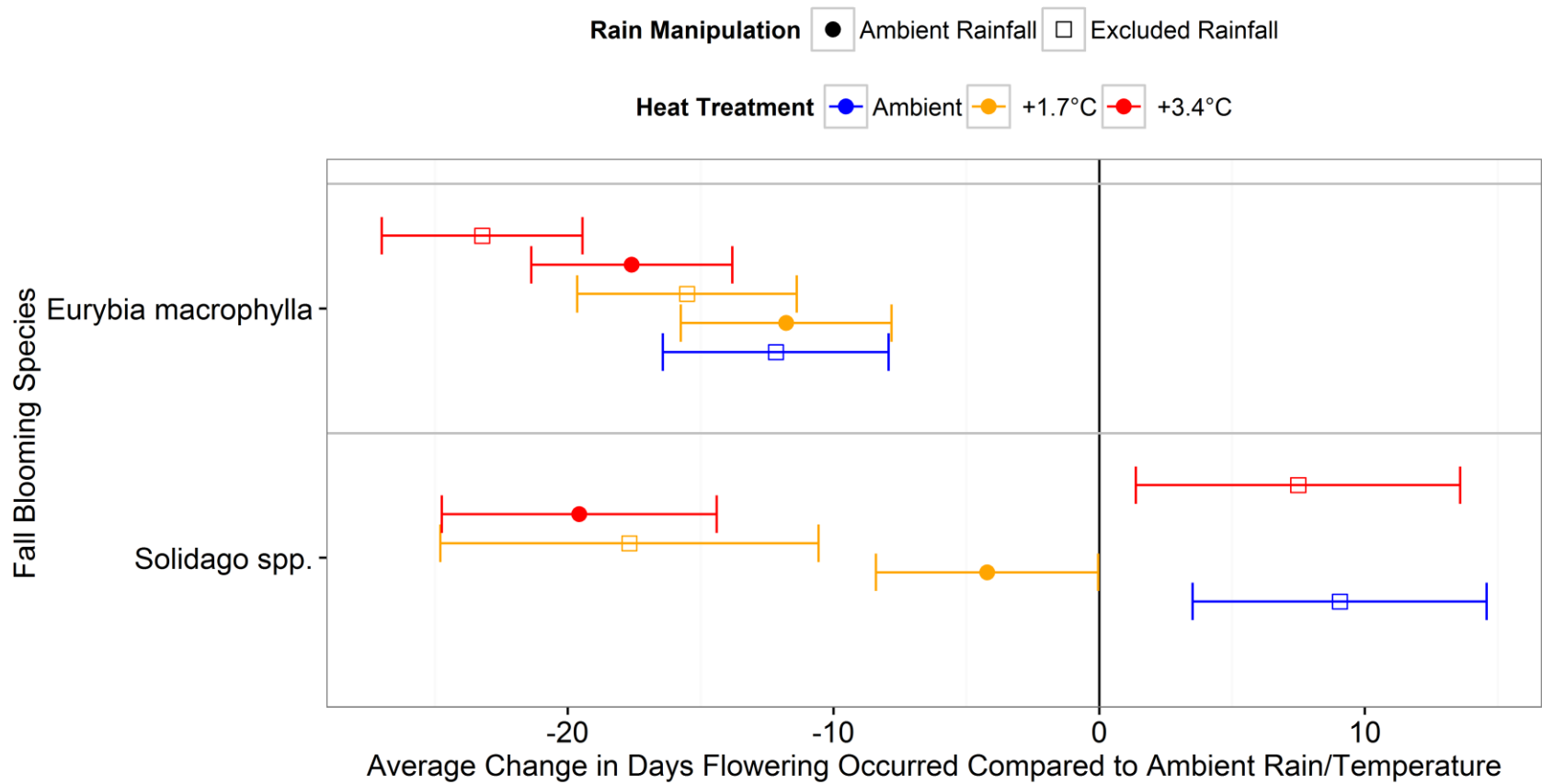


Table 3-2. Summarized responses of main effects of warming and excluded rainfall for flowering response of groundlayer species (when year and block nested in site are random).

| Species                    | n  | Warming     |               | Rainfall    |               | Warming x Rainfall |               |
|----------------------------|----|-------------|---------------|-------------|---------------|--------------------|---------------|
|                            |    | F           | P             | F           | P             | F                  | P             |
| <i>Eurybia macrophylla</i> | 90 | <b>9.24</b> | <b>0.0003</b> | <b>7.84</b> | <b>0.0065</b> | 0.98               | 0.3800        |
| <i>Fragaria virginiana</i> | 78 | <b>4.94</b> | <b>0.0100</b> | 1.75        | 0.1907        | 0.69               | 0.5042        |
| <i>Hieracium spp.</i>      | 52 | <b>6.98</b> | <b>0.0025</b> | 0.02        | 0.8825        | 0.03               | 0.9686        |
| <i>Lathyrus venosus</i>    | 55 | 1.71        | 0.1929        | 0.01        | 0.9251        | 0.58               | 0.5619        |
| <i>Solidago spp.</i>       | 36 | <b>7.43</b> | <b>0.0026</b> | <b>4.63</b> | <b>0.0404</b> | <b>9.25</b>        | <b>0.0009</b> |

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## Appendix Tables and Figures

Appendix Table 1. Definitions of phenophases

| Plant Type              | Phenophase              | Definition   |
|-------------------------|-------------------------|--|
| Groundlayer Phenology   | New Leaves Not Present  | When living growth from the previous year can still be seen such as a woody stem or an old leaf (that is still alive). This stage lasts until buds break or new leaf emerges.  |
|                         | Plant Emerging          | The plant emerges from the ground or at least one bud breaks (the scales of a bud separate revealing underlying leaf tissue) until one or more leaves have begun unfolding.  |
|                         | Leaf Unfolding          | At least one leaf on the individual plant is unfolding. A leaf is considered "unfolding" once it has grown enough so that the two halves of the leaf blade have begun to spread apart like an open book.   |
|                         | Leaf 100%               | At least one leaf on the plant is completely mature (their normal size, flattened and in their normal orientation).  |
|                         | Ready to Flower         | At the first sign of a reproductive structure until the flowers are open (flowers are forming but not open yet).   |
|                         | Flowering               | Plant is flowering (flowers are open), and the reproductive structures are available for pollination.  |
|                         | Fruit Appears           | At least one un-ripened fruit appears (you are able to see a fruit but it is not fully ripe yet). There can be a stage between F and FRA in which nothing will be marked, to keep track of where the plant is reproductively, make a comment in the notes, so you know to keep on looking carefully. |
|                         | Fruit Ripe              | At least one ripe fruit is present on the plant (see description of each species) until it falls off.  |
|                         | Start of Senescence     | The loss of rigidity in the non-woody parts of plants due to lack of water from senescence or drought stress (leaf orientation changes).   |
|                         | Plant Dead              | No green or alive tissue seen on plant.  |
| Tree Seedling Phenology | Budbreak                | Scales on (any) bud parted and the underlying tissue is visible  |
|                         | One Leaf Expanded       | Any leaf is full sized in length/area, and is flattened and in normal orientation.   |
|                         | Crown Expansion         | 95% of leaves are full size in length/area, and are flattened and in normal orientation  |
|                         | Second flush            | New leaves present and expanding   |
|                         | Beginning of Senescence | 1/3 of leaves show breakdown of chlorophyll revealing underlying pigments  |
|                         | Completion of Leaf Drop | 2/3 of leaves abscised or lost color/turned completely brown   |

Appendix Table 2. Sites, years and canopies included in analyses for four vegetative phenology responses of 14 groundlayer species. Dashes indicate no data is available. Sites: C=Cloquet, E=Ely. Canopy: C=Closed, O=Open.

| Species  | Leaf unfolding   |      |             |        | Start of senescence |      |             |        | Growing season length |      |             |        |
|--|------------------|------|-------------|--------|---------------------|------|-------------|--------|-----------------------|------|-------------|--------|
|  | N                | Site | Years       | Canopy | N                   | Site | Years       | Canopy | N                     | Site | Years       | Canopy |
| <i>T. borealis</i>   | 85               | C, E | 2010-2013   | C      | 81                  | C, E | 2010-2013   | C      | 78                    | C, E | 2010-2013   | C      |
| <i>A. nudicaulis</i>   | 105              | C, E | 2009-2013   | C      | 114                 | C, E | 2010-2013   | C      | 101                   | C, E | 2010-2013   | C      |
| <i>C. cornuta</i><br><i>D. lonicera</i><br><i>S. roseus</i>    | 127<br>84<br>91  | C, E | 2011-2013   | O, C   | 132<br>80<br>85     | C, E | 2011-2013   | O, C   | 122<br>72<br>73       | C, E | 2011-2013   | O, C   |
| <i>M. canadense</i><br><i>P. aquilinum</i><br><i>R. idaeus</i> | 227<br>93<br>111 | C, E | 2009-2013   | O, C   | 189<br>110<br>114   | C, E | 2010-2013   | O, C   | 175<br>91<br>103      | C, E | 2010-2013   | O, C   |
| <i>E. macrophylla</i><br><i>R. pubescens</i>                   | 247<br>165       | C, E | 2010-2013   | O, C   | 241<br>162          | C, E | 2010-2013   | O, C   | 238<br>114            | C, E | 2010-2013   | O, C   |
| <i>G. triflorum</i>  | 148              | C, E | 2010-2013   | O, C   | 70                  | C, E | 2010-2013   | C      | 62                    | C, E | 2010-2013   | C      |
| <i>L. venosus</i>  | 90               | C, E | 2010-2013   | O      | 68                  | C, E | 2010-2013   | O      | 60                    | C, E | 2010-2013   | O      |
| <i>Solidago spp</i>  | 25               | C    | 2010-2012   | O      | 37                  | C    | 2010-2013   | O      | 23                    | C    | 2010-2012   | O      |
| <i>U. sessilifolia</i>   | 27               | C    | 2010, 11,13 | C      | 29                  | C    | 2010, 11,13 | C      | 23                    | C    | 2010, 11,13 | C      |

Appendix Table 3 Sites, years and canopies included in analyses for two reproductive phenology responses of 9 groundlayer species. Dashes indicate no data is available. Sites: C=Cloquet, E=Ely.

| Species   | Flowering      |         |           |              | Fruiting |             |           |              |
|---|----------------|---------|-----------|--------------|----------|-------------|-----------|--------------|
|   | n              | Site(s) | Years     | Canopy(s)    | n        | Site(s)     | Years     | Canopy(s)    |
| <i>E. macrophylla</i>   | 224            | C, E    | 2009-2013 | Open, Closed | 90       | C, E        | 2009-2013 | Open, Closed |
| <i>F. virginiana</i><br><i>Hieracium spp</i><br><i>L. venosus</i> | 82<br>53<br>91 | C, E    | 2009-2013 | Open         |          | ---         | ---       | ---          |
| <i>G. triflorum</i>   | 79             | C, E    | 2009-2013 | Open         | 144      | C, E        | 2009-2013 | Open         |
| <i>M. canadense</i>   | 84             | C, E    | 2009-2013 | Closed       |          | ---         | ---       | ---          |
| <i>R. pubescens</i>   | 73             | C, E    | 2009-2013 | Closed       | 115      | C, E        | 2009-2013 | Closed       |
| <i>Solidago spp</i>   | 37             | C       | 2010-2013 | Open         | 30       | C           | 2010-2013 | Open         |
| <i>T. borealis</i>  | 68             | C, E    | 2009-2013 | Closed       | 33       | E (no +3.4) | 2009-2013 | Closed       |

Appendix Table 4. Mixed effects analysis of variance results for response of leaf unfolding, start of senescence, and growing season length for 8 species included in full model (with enough power for all 2 and 3 way interactions) over four years. Block nested in site is random effect.

| Source                     | Leaf unfolding |       |        |        | Start of senescence |       |       |        | Growing season length |       |       |        |
|----------------------------|----------------|-------|--------|--------|---------------------|-------|-------|--------|-----------------------|-------|-------|--------|
|                            | DF             | DFDen | F      | P      | DF                  | DFDen | F     | P      | DF                    | DFDen | F     | P      |
| Species                    | 5              | 869.9 | 117.93 | <.0001 | 4                   | 716   | 29.56 | <.0001 | 4                     | 639.1 | 84.86 | <.0001 |
| Year                       | 3              | 867.3 | 577.61 | <.0001 | 3                   | 711.3 | 8.37  | <.0001 | 3                     | 635.2 | 71.23 | <.0001 |
| Canopy                     | 1              | 10.45 | 0.06   | 0.8079 | 1                   | 10.98 | 8.08  | 0.0160 | 1                     | 12.04 | 5.12  | 0.0429 |
| Warming                    | 2              | 867.8 | 89.92  | <.0001 | 2                   | 711.6 | 12.59 | <.0001 | 2                     | 635.7 | 57.42 | <.0001 |
| Species x Year             | 15             | 867.3 | 6.03   | <.0001 | 12                  | 711   | 5.02  | <.0001 | 12                    | 634.6 | 3.78  | <.0001 |
| Species x Canopy           | 5              | 869.9 | 3.44   | 0.0043 | 4                   | 716   | 1.74  | 0.1387 | 4                     | 639.1 | 2.27  | 0.0594 |
| Species x Warming          | 10             | 867.8 | 2.20   | 0.0159 | 8                   | 711.9 | 3.59  | 0.0004 | 8                     | 635.5 | 3.49  | 0.0006 |
| Year x Canopy              | 3              | 867.3 | 0.96   | 0.4068 | 3                   | 711.2 | 0.91  | 0.4344 | 3                     | 635   | 2.16  | 0.0910 |
| Year x Warming             | 6              | 867.2 | 5.45   | <.0001 | 6                   | 710.4 | 3.76  | 0.0011 | 6                     | 634   | 3.70  | 0.0012 |
| Canopy x Warming           | 2              | 867.8 | 0.44   | 0.6430 | 2                   | 711.6 | 3.47  | 0.0315 | 2                     | 635.6 | 5.36  | 0.0049 |
| Species x Year x Canopy    | 15             | 867.3 | 2.88   | 0.0002 | 12                  | 710.9 | 2.70  | 0.0014 | 12                    | 634.6 | 2.53  | 0.0028 |
| Species x Year x Warming   | 30             | 867.2 | 1.57   | 0.0262 | 24                  | 710.5 | 2.08  | 0.0018 | 24                    | 634   | 1.20  | 0.2273 |
| Species x Canopy x Warming | 10             | 867.7 | 0.98   | 0.4505 | 8                   | 711.8 | 1.36  | 0.2086 | 8                     | 635.4 | 1.18  | 0.3036 |
| Year x Canopy x Warming    | 6              | 867.1 | 0.41   | 0.8708 | 6                   | 710.5 | 0.64  | 0.6945 | 6                     | 633.9 | 0.19  | 0.9770 |

Appendix Table 5. Mixed effects analysis of variance results for response of flowering and fruiting for species included in full model (with enough power for all 2 and 3 way interactions) over five years (2009-2013). Block nested in site is random effect. Models are separated by canopy condition due to limited replication between species among canopies.

| Source                   | Flowering: closed canopy |       |        |        | Flowering: open canopy |       |        |        | Fruiting: closed canopy |       |        |        | Fruiting: open canopy |       |        |        |
|--------------------------|--------------------------|-------|--------|--------|------------------------|-------|--------|--------|-------------------------|-------|--------|--------|-----------------------|-------|--------|--------|
|                          | DF                       | DFDen | F      | P      | DF                     | DFDen | F      | P      | DF                      | DFDen | F      | P      | DF                    | DFDen | F      | P      |
| Warming                  | 2                        | 242   | 17.10  | <.0001 | 2                      | 178.1 | 39.25  | <.0001 | 2                       | 88.17 | 1.65   | 0.1977 | 2                     | 65.61 | 0.77   | 0.4634 |
| Year                     | 4                        | 241   | 35.89  | <.0001 | 4                      | 177.9 | 122.91 | <.0001 | 4                       | 88.12 | 8.63   | <.0001 | 4                     | 65.27 | 11.30  | <.0001 |
| Species                  | 3                        | 243.6 | 136.39 | <.0001 | 2                      | 78.34 | 42.49  | <.0001 | 1                       | 88.67 | 262.32 | <.0001 | 1                     | 67.25 | 370.53 | <.0001 |
| Warming x Year           | 8                        | 241   | 1.19   | 0.3036 | 8                      | 178   | 1.49   | 0.1625 | 8                       | 87.4  | 0.41   | 0.9093 | 8                     | 64.24 | 1.61   | 0.1393 |
| Warming x Species        | 6                        | 242.2 | 1.18   | 0.3152 | 4                      | 178.2 | 2.86   | 0.0248 | 2                       | 87.46 | 1.54   | 0.2190 | 2                     | 65.4  | 0.49   | 0.6102 |
| Year x Species           | 12                       | 241.2 | 4.27   | <.0001 | 8                      | 178.1 | 1.34   | 0.2243 | 4                       | 88.5  | 0.93   | 0.4492 | 4                     | 65.68 | 5.94   | 0.0004 |
| Warming x Year x Species | 24                       | 241   | 1.28   | 0.1761 | 16                     | 178   | 1.24   | 0.2372 | 8                       | 87.35 | 0.85   | 0.5607 | 8                     | 64.68 | 1.75   | 0.1035 |

Appendix Table 6. Response of leaf unfolding. Block nested in site is the random effect. Significant results indicated in bold ( $p < 0.05$ ).

|                              | Year          |                  | Canopy |        | Warming      |                  | Year x Canopy |               | Year x Warming |                  | Canopy x Warming |        | Year x Canopy x Warming |        |
|------------------------------|---------------|------------------|--------|--------|--------------|------------------|---------------|---------------|----------------|------------------|------------------|--------|-------------------------|--------|
|                              | F             | P                | F      | P      | F            | P                | F             | P             | F              | P                | F                | P      | F                       | P      |
| <i>Aralia nudicaulis</i>     | <b>126.11</b> | <b>&lt;.0001</b> | ---    | ---    | <b>45.82</b> | <b>&lt;.0001</b> | ---           | ---           | <b>2.35</b>    | <b>0.0229</b>    | ---              | ---    | ---                     | ---    |
| <i>Corylus cornuta</i>       | <b>135.26</b> | <b>&lt;.0001</b> | 4.39   | 0.0602 | <b>10.67</b> | <b>&lt;.0001</b> | 1.18          | 0.3108        | 1.92           | 0.1130           | 0.07             | 0.9269 | 1.11                    | 0.3535 |
| <i>Diervilla lonicera</i>    | <b>72.30</b>  | <b>&lt;.0001</b> | 0.09   | 0.7720 | <b>7.74</b>  | <b>0.0010</b>    | 0.40          | 0.6691        | <b>4.56</b>    | <b>0.0028</b>    | 3.03             | 0.0554 | 1.26                    | 0.2970 |
| <i>Eurybia macrophylla</i>   | <b>335.07</b> | <b>&lt;.0001</b> | 0.40   | 0.5426 | <b>59.39</b> | <b>&lt;.0001</b> | <b>3.59</b>   | <b>0.0146</b> | <b>2.81</b>    | <b>0.0118</b>    | 1.08             | 0.3418 | 2.01                    | 0.0656 |
| <i>Gallium triflorum</i>     | <b>99.88</b>  | <b>&lt;.0001</b> | 3.32   | 0.0939 | <b>3.46</b>  | <b>0.0346</b>    | <b>3.484</b>  | <b>0.0181</b> | 0.61           | 0.7181           | 0.03             | 0.9746 | 0.05                    | 0.9992 |
| <i>Lathyrus venosus</i>      | <b>40.29</b>  | <b>&lt;.0001</b> | ---    | ---    | 1.36         | 0.2624           | ---           | ---           | 1.60           | 0.1604           | ---              | ---    | ---                     | ---    |
| <i>Maianthemum canadense</i> | <b>117.64</b> | <b>&lt;.0001</b> | 0.01   | 0.9987 | <b>9.72</b>  | <b>&lt;.0001</b> | <b>4.57</b>   | <b>0.0015</b> | <b>5.97</b>    | <b>&lt;.0001</b> | 0.36             | 0.6959 | 0.52                    | 0.8365 |
| <i>Pteridium aquilinum</i>   | <b>27.69</b>  | <b>&lt;.0001</b> | 1.24   | 0.3006 | <b>10.44</b> | <b>&lt;.0001</b> | 0.89          | 0.4689        | 1.33           | 0.2379           | 0.74             | 0.4770 | 0.91                    | 0.5060 |
| <i>Rubus idaeus</i>          | <b>33.33</b>  | <b>&lt;.0001</b> | 0.01   | 0.9923 | 2.85         | 0.0629           | 0.97          | 0.4263        | 0.84           | 0.5690           | 0.19             | 0.8250 | 0.46                    | 0.8776 |
| <i>Rubus pubescens</i>       | <b>129.58</b> | <b>&lt;.0001</b> | 0.01   | 0.9992 | <b>28.71</b> | <b>&lt;.0001</b> | 0.56          | 0.6444        | <b>3.77</b>    | <b>0.0017</b>    | 1.41             | 0.2471 | 1.28                    | 0.2725 |
| <i>Solidago spp.</i>         | <b>14.34</b>  | <b>0.0021</b>    | ---    | ---    | <b>14.5</b>  | <b>0.0148</b>    | ---           | ---           | 1.22           | 0.3413           | ---              | ---    | ---                     | ---    |
| <i>Streptopus roseus</i>     | <b>91.30</b>  | <b>&lt;.0001</b> | 0.19   | 0.6698 | <b>17.53</b> | <b>&lt;.0001</b> | 0.02          | 0.9725        | 0.37           | 0.8263           | 0.69             | 0.5018 | 0.9553                  | 0.4379 |
| <i>Trientalis borealis</i>   | <b>82.69</b>  | <b>&lt;.0001</b> | ---    | ---    | <b>6.49</b>  | <b>0.0026</b>    | ---           | ---           | 1.19           | 0.3243           | ---              | ---    | ---                     | ---    |
| <i>Uvularia sessilifolia</i> | <b>37.93</b>  | <b>0.0001</b>    | ---    | ---    | 3.06         | 0.0910           | ---           | ---           | 0.85           | 0.5127           | ---              | ---    | ---                     | ---    |

Appendix Table 7. Response of start of senescence for individual species. Block nested in site is the random effect. Significant results indicated in bold ( $p < 0.05$ ).

|                              | Year         |                  | Canopy      |               | Warming      |                  | Year x Canopy |               | Year X Warming |                  | Canopy x Warming |               | Year x Canopy x Warming |               |
|------------------------------|--------------|------------------|-------------|---------------|--------------|------------------|---------------|---------------|----------------|------------------|------------------|---------------|-------------------------|---------------|
|                              | F            | P                | F           | P             | F            | P                | F             | P             | F              | P                | F                | P             | F                       | P             |
| <i>Aralia nudicaulis</i>     | <b>16.08</b> | <b>&lt;.0001</b> | ---         | ---           | <b>10.57</b> | <b>&lt;.0001</b> | ---           | ---           | 1.89           | 0.0901           | ---              | ---           | ---                     | ---           |
| <i>Corylus cornuta</i>       | <b>57.19</b> | <b>&lt;.0001</b> | 0.02        | 0.8842        | <b>4.61</b>  | <b>0.0120</b>    | 0.55          | 0.5761        | 1.24           | 0.2979           | 2.01             | 0.1377        | 2.23                    | <i>0.0710</i> |
| <i>Diervilla lonicera</i>    | <b>4.57</b>  | <b>0.0146</b>    | <b>7.96</b> | <b>0.0162</b> | <i>3.12</i>  | <i>0.0519</i>    | <b>3.38</b>   | <b>0.0412</b> | 1.92           | 0.1201           | 1.74             | 0.1856        | 0.35                    | 0.8463        |
| <i>Eurybia macrophylla</i>   | <b>14.15</b> | <b>&lt;.0001</b> | 2.87        | 0.1199        | <b>31.11</b> | <b>&lt;.0001</b> | 1.84          | 0.1407        | 1.11           | 0.3580           | <b>3.83</b>      | <b>0.0232</b> | 0.96                    | 0.4563        |
| <i>Galium triflorum</i>      | 1.582        | 0.2044           | ---         | ---           | 0.21         | 0.8123           | ---           | ---           | <i>2.18</i>    | <i>0.0596</i>    | ---              | ---           | ---                     | ---           |
| <i>Lathyrus venosus</i>      | <b>6.47</b>  | <b>0.0008</b>    | ---         | ---           | 0.33         | 0.7176           | ---           | ---           | 0.39           | 0.8826           | ---              | ---           | ---                     | ---           |
| <i>Maianthemum canadense</i> | <b>13.24</b> | <b>&lt;.0001</b> | 2.29        | 0.1632        | <b>4.16</b>  | <b>0.0174</b>    | 2.09          | 0.1043        | <b>6.95</b>    | <b>&lt;.0001</b> | 0.17             | 0.8473        | 0.58                    | 0.7431        |
| <i>Pteridium aquilinum</i>   | 1.17         | 0.3263           | 0.07        | 0.7937        | 2.38         | <i>0.0990</i>    | 0.68          | 0.5679        | 1.79           | 0.1121           | 1.31             | 0.2755        | 0.22                    | 0.9693        |
| <i>Rubus idaeus</i>          | 2.04         | 0.1141           | <b>7.05</b> | <b>0.0228</b> | 0.45         | 0.6405           | <b>2.75</b>   | <b>0.0473</b> | 0.47           | 0.8323           | 2.11             | 0.1274        | 0.64                    | 0.6948        |
| <i>Rubus pubescens</i>       | <b>5.43</b>  | <b>0.0015</b>    | <i>3.64</i> | <i>0.0887</i> | 0.67         | 0.5128           | 0.68          | 0.5668        | <b>3.66</b>    | <b>0.0022</b>    | 0.19             | 0.8275        | 1.54                    | 0.1691        |
| <i>Solidago spp.</i>         | <b>3.52</b>  | <b>0.0292</b>    | ---         | ---           | <b>3.65</b>  | <b>0.0406</b>    | ---           | ---           | 0.63           | 0.7083           | ---              | ---           | ---                     | ---           |
| <i>Streptopus roseus</i>     | <b>16.17</b> | <b>&lt;.0001</b> | 0.02        | 0.8976        | 1.39         | 0.2564           | 0.85          | 0.4326        | 0.97           | 0.4326           | 0.46             | 0.6326        | 1.73                    | 0.1543        |
| <i>Trientalis borealis</i>   | <b>4.50</b>  | <b>0.0061</b>    | ---         | ---           | 1.46         | 0.2393           | ---           | ---           | 0.61           | 0.7230           | ---              | ---           | ---                     | ---           |
| <i>Uvularia sessilifolia</i> | <b>8.63</b>  | <b>0.0013</b>    | ---         | ---           | <i>3.65</i>  | <i>0.0505</i>    | ---           | ---           | 0.54           | 0.7727           | ---              | ---           | ---                     | ---           |

Appendix Table 8. Mixed effects ANOVA results for response of growing season length. Individual species are analyzed with block nested in site or block when only one site is available are random effects. Significant results indicated in bold ( $p < 0.05$ ).

|                              | Year         |                  | Canopy       |               | Warming      |                  | Year x Canopy |               | Year x Warming |               | Canopy x Warming |               | Year x Canopy x Warming |        |
|------------------------------|--------------|------------------|--------------|---------------|--------------|------------------|---------------|---------------|----------------|---------------|------------------|---------------|-------------------------|--------|
|                              | F            | P                | F            | P             | F            | P                | F             | P             | F              | P             | F                | P             | F                       | P      |
| <i>Aralia nudicaulis</i>     | <b>36.08</b> | <b>&lt;.0001</b> | ---          | ---           | <b>33.35</b> | <b>&lt;.0001</b> | ---           | ---           | 1.89           | 0.0923        | ---              | ---           | ---                     | ---    |
| <i>Corylus cornuta</i>       | <b>13.62</b> | <b>&lt;.0001</b> | 0.91         | 0.3578        | <b>13.39</b> | <b>&lt;.0001</b> | 0.30          | 0.7437        | 2.23           | 0.0712        | 1.73             | 0.1820        | 0.81                    | 0.5214 |
| <i>Diervilla lonicera</i>    | <b>15.39</b> | <b>&lt;.0001</b> | <b>19.22</b> | <b>0.0010</b> | <b>13.68</b> | <b>&lt;.0001</b> | 1.46          | 0.2418        | 2.43           | 0.0596        | 0.93             | 0.3993        | 1.17                    | 0.3335 |
| <i>Eurybia macrophylla</i>   | <b>28.01</b> | <b>&lt;.0001</b> | 0.270        | 0.6142        | <b>61.06</b> | <b>&lt;.0001</b> | 2.48          | 0.0623        | 1.98           | 0.0706        | <b>3.88</b>      | <b>0.0221</b> | 1.26                    | 0.2729 |
| <i>Galium triflorum</i>      | <b>18.25</b> | <b>&lt;.0001</b> | ---          | ---           | 2.79         | 0.0716           | ---           | ---           | 2.30           | 0.0502        | ---              | ---           | ---                     | ---    |
| <i>Lathyrus venosus</i>      | <b>16.08</b> | <b>&lt;.0001</b> | ---          | ---           | 0.53         | 0.5923           | ---           | ---           | 0.11           | 0.9943        | ---              | ---           | ---                     | ---    |
| <i>Maianthemum canadense</i> | <b>43.53</b> | <b>&lt;.0001</b> | 0.37         | 0.5601        | <b>13.38</b> | <b>&lt;.0001</b> | 0.57          | 0.6381        | <b>3.48</b>    | <b>0.0030</b> | 0.50             | 0.6041        | 0.82                    | 0.5532 |
| <i>Pteridium aquilinum</i>   | <b>33.56</b> | <b>&lt;.0001</b> | 1.42         | 0.2774        | <b>17.04</b> | <b>&lt;.0001</b> | 1.79          | 0.1583        | <b>2.43</b>    | <b>0.0359</b> | 1.92             | 0.1538        | 0.69                    | 0.6509 |
| <i>Rubus idaeus</i>          | <b>3.70</b>  | <b>0.0152</b>    | <b>9.07</b>  | <b>0.0111</b> | <b>4.33</b>  | <b>0.0165</b>    | <b>3.11</b>   | <b>0.0315</b> | 0.10           | 0.4337        | 2.24             | 0.1131        | 0.32                    | 0.9216 |
| <i>Rubus pubescens</i>       | <b>25.18</b> | <b>&lt;.0001</b> | 2.41         | 0.1495        | <b>5.77</b>  | <b>0.0043</b>    | 1.55          | 0.2180        | <b>2.61</b>    | <b>0.0405</b> | 1.48             | 0.2310        | 1.35                    | 0.2575 |
| <i>Solidago spp</i>          | 2.6          | 0.116            | ---          | ---           | <b>5.87</b>  | <b>0.0146</b>    | ---           | ---           | 1.23           | 0.3446        | ---              | ---           | ---                     | ---    |
| <i>Streptopus roseus</i>     | <b>23.22</b> | <b>&lt;.0001</b> | 0.01         | 0.9543        | <b>3.46</b>  | <b>0.0391</b>    | 0.71          | 0.4980        | 1.38           | 0.2562        | 0.50             | 0.6041        | 1.64                    | 0.1795 |
| <i>Trientalis borealis</i>   | <b>19.35</b> | <b>&lt;.0001</b> | ---          | ---           | <b>4.33</b>  | <b>0.0171</b>    | ---           | ---           | 0.98           | 0.4480        | ---              | ---           | ---                     | ---    |
| <i>Uvularia sessilifolia</i> | <b>29.30</b> | <b>&lt;.0001</b> | ---          | ---           | 3.68         | 0.0539           | ---           | ---           | 0.99           | 0.4444        | ---              | ---           | ---                     | ---    |



Appendix Table 9. ANOVA results for response of flowering for nine individual species. Block nested in site is the random effect. Significant results indicated in bold (p<0.05).

|                            | <i>E. macrophylla</i> |                  | <i>F. virginiana</i> |                  | <i>G. triflorum</i> |               | <i>Hieracium spp</i> |               | <i>L. venosus</i> |                  | <i>M. canadense</i> |                  | <i>R. pubescens</i> |                  | <i>Solidago spp</i> |               | <i>T. borealis</i> |                  |
|----------------------------|-----------------------|------------------|----------------------|------------------|---------------------|---------------|----------------------|---------------|-------------------|------------------|---------------------|------------------|---------------------|------------------|---------------------|---------------|--------------------|------------------|
| <b>Source of variation</b> | <b>F</b>              | <b>P</b>         | <b>F</b>             | <b>P</b>         | <b>F</b>            | <b>P</b>      | <b>F</b>             | <b>P</b>      | <b>F</b>          | <b>P</b>         | <b>F</b>            | <b>P</b>         | <b>F</b>            | <b>P</b>         | <b>F</b>            | <b>P</b>      | <b>F</b>           | <b>P</b>         |
| Canopy                     | <b>26.25</b>          | <b>0.0001</b>    | ----                 | ----             | ----                | ----          | ----                 | ----          | ----              | ----             | ----                | ----             | ----                | ----             | ----                | ----          | ----               | ----             |
| Warming                    | <b>14.47</b>          | <b>&lt;.0001</b> | <b>10.83</b>         | <b>&lt;.0001</b> | 1.43                | 0.2458        | <b>8.01</b>          | <b>0.0014</b> | 1.50              | 0.2281           | <b>22.51</b>        | <b>&lt;.0001</b> | <b>20.41</b>        | <b>&lt;.0001</b> | <b>6.94</b>         | <b>0.0042</b> | <b>9.04</b>        | <b>0.0004</b>    |
| Year                       | <b>13.49</b>          | <b>&lt;.0001</b> | <b>38.24</b>         | <b>&lt;.0001</b> | <b>4.16</b>         | <b>0.0049</b> | <b>7.06</b>          | <b>0.0002</b> | <b>10.38</b>      | <b>&lt;.0001</b> | <b>92.56</b>        | <b>&lt;.0001</b> | <b>41.25</b>        | <b>&lt;.0001</b> | 1.29                | 0.2990        | <b>29.64</b>       | <b>&lt;.0001</b> |
| Canopy x Warming           | 0.43                  | 0.6492           | ----                 | ----             | ----                | ----          | ----                 | ----          | ----              | ----             | ----                | ----             | ----                | ----             | ----                | ----          | ----               | ----             |
| Canopy x Year              | <b>14.89</b>          | <b>&lt;.0001</b> | ----                 | ----             | ----                | ----          | ----                 | ----          | ----              | ----             | ----                | ----             | ----                | ----             | ----                | ----          | ----               | ----             |
| Warming x Year             | <b>5.85</b>           | <b>&lt;.0001</b> | <b>2.38</b>          | <b>0.0258</b>    | 1.71                | 0.1137        | 0.57                 | 0.7942        | 0.73              | 0.6614           | 1.30                | 0.2554           | <b>2.29</b>         | <b>0.0335</b>    | 0.78                | 0.5897        | 0.59               | 0.7749           |
| Canopy x Warming x Year    | 0.80                  | 0.5956           | ----                 | ----             | ----                | ----          | ----                 | ----          | ----              | ----             | ----                | ----             | ----                | ----             | ----                | ----          | ----               | ----             |

Appendix Table 10. ANOVA results for response of fruit maturation for five individual species. Block nested in site is the random effect. Significant results indicated in bold ( $p < 0.05$ ).

|                         | <i>E. Macrophylla</i> |                  | <i>G. triflorum</i> |                  | <i>R. pubescens</i> |               | <i>Solidago spp</i> |        | <i>T. borealis</i> |                  |
|-------------------------|-----------------------|------------------|---------------------|------------------|---------------------|---------------|---------------------|--------|--------------------|------------------|
| Source of variation     | F                     | P                | F                   | P                | F                   | P             | F                   | P      | F                  | P                |
| Year                    | <b>23.26</b>          | <b>&lt;.0001</b> | <b>11.42</b>        | <b>&lt;.0001</b> | <b>3.67</b>         | <b>0.0084</b> | 2.31                | 0.1093 | <b>10.91</b>       | <b>&lt;.0001</b> |
| Canopy                  | 0.42                  | 0.5377           | 0.001               | 0.9817           | 0.60                | 0.4482        | ----                | ----   | ----               | ----             |
| Warming                 | <b>4.11</b>           | <b>0.0219</b>    | 0.60                | 0.5487           | 0.54                | 0.5808        | 2.06                | 0.1669 | <i>3.18</i>        | <i>0.0877</i>    |
| Canopy x Year           | <b>2.96</b>           | <b>0.0274</b>    | 0.70                | 0.5935           | 0.88                | 0.4783        | ----                | ----   | ----               | ----             |
| Warming x Year          | 1.58                  | 0.1520           | 0.40                | 0.9151           | 0.61                | 0.7621        | 1.01                | 0.4702 | 1.15               | 0.3546           |
| Canopy x Warming        | 1.48                  | 0.2352           | <b>3.10</b>         | <b>0.0491</b>    | 0.36                | 0.6962        | ----                | ----   | ----               | ----             |
| Year x Canopy x Warming | 1.61                  | 0.1420           | 0.75                | 0.6417           | 0.41                | 0.9094        | ----                | ----   | ----               | ----             |