Species-specific flowering phenology responses to experimental warming and drought alter herbaceous plant species overlap in a temperate–boreal forest community

Karen E. Rice^{1,2,*}, Rebecca A. Montgomery¹, Artur Stefanski¹, Roy L. Rich^{1,3} and Peter B. Reich^{1,4}

¹University of Minnesota, Department of Forest Resources, St. Paul, MN 55108 USA, ²University of Florida Extension Education, Fort Lauderdale, FL 33314, USA, ³Smithsonian Environmental Research Center, Edgewater, MD, 21037, USA and ⁴Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia *For correspondence. E-mail kerice@umn.edu

Received: 21 August 2020 Returned for revision: 30 July 2020 Editorial decision: 17 August 2020 Accepted: 24 August 2020 Electronically published: 27 August 2020

• **Background and Aims** Warmer temperatures and altered precipitation patterns are expected to continue to occur as the climate changes. How these changes will impact the flowering phenology of herbaceous perennials in northern forests is poorly understood but could have consequences for forest functioning and species interactions. Here, we examine the flowering phenology responses of five herbaceous perennials to experimental warming and reduced summer rainfall over 3 years.

• Methods This study is part of the B4WarmED experiment located at two sites in northern Minnesota, USA. Three levels of warming (ambient, ± 1.6 °C and ± 3.1 °C) were crossed with two rainfall manipulations (ambient and 27 % reduced growing season rainfall).

• **Key Results** We observed species-specific responses to the experimental treatments. Warming alone advanced flowering for four species. Most notably, the two autumn blooming species showed the strongest advance of flowering to warming. Reduced rainfall alone advanced flowering for one autumn blooming species and delayed flowering for the other, with no significant impact on the three early blooming species. Only one species, *Solidago* spp., showed an interactive response to warming and rainfall manipulation by advancing in +1.6 °C warming (regardless of rainfall manipulation) but not advancing in the warmest, driest treatment. Species-specific responses led to changes in temporal overlap between species. Most notably, the two autumn blooming species diverged significantly in their flowering timing. In ambient conditions, these two species flowered within the same week. In the warmest, driest treatment, flowering occurred over a month apart.

• **Conclusions** Herbaceous species may differ in how they respond to future climate conditions. Changes to phenology may lead to fewer resources for insects or a mismatch between plants and pollinators.

Key words: Flowering, climate change, boreal forest, temperate forest, phenology.

INTRODUCTION

During this century, temperatures are predicted to continue to rise (IPCC, 2014; Walsh *et al.*, 2014), with other interacting climate variables changing in tandem. Specifically, altered rainfall patterns are expected to coincide with rising temperatures (Karl and Trenberth, 2003). Along with these changes in climate, ecological relationships are expected to change.

Phenology, the study of the timing of biological events, 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 ecosystems and species persistence. Warming temperatures are linked to both earlier (Fitter *et al.*, 1995; Rice *et al.*, 2018) and delayed flowering (Sherry *et al.*, 2007) in herbaceous plants, depending on the time of year and environmental conditions. However, despite the expectation that warmer temperatures will be coupled with changes in precipitation, experiments testing the effects of multiple environmental factors on phenology are rare (but see Cleland *et al.*, 2006; Sherry *et al.*, 2007; Rollinson and Kaye, 2012).

The timing of flowering phenology has important consequences for species interactions and ecosystem functioning. For instance, changes in flowering phenology may not occur in sync with insect phenology (Visser and Both, 2005). This could lead to a lack of food availability for insectivores (Inouye *et al.*, 2000), mismatches between flowering plants and their specific pollinators (Sparks *et al.*, 2005) or a lack of fruit for frugivores and seed dispersers (Rafferty *et al.*, 2015). These potential changes to trophic interactions could upset ecosystem function.

Flowering onset in perennials is triggered mainly by temperature accumulation, though in some species flowering may also be dependent on moisture or photoperiod (Rathcke and Lacey, 1985, and references therein). Given the complexity of phenological cues, understanding how flowering phenology may change due to warmer temperatures combined with altered precipitation is imperative. Furthermore, the unique cues on which flowering plants rely vary by habitat and geographic range (Von Holle, 2010), making studies of different habitats essential to our global understanding of how climate change may impact ecosystems. To our knowledge, flowering phenology in response to warmer temperatures and altered precipitation has not been studied in mesic systems such as temperate or southern boreal forests, which are the focal systems for our study.

Warmer temperatures combined with reduced summer precipitation may change the flowering phenology of herbaceous plants in mesic forests in several ways. While warmer temperatures generally advance flowering (Fitter et al., 1995; Miller-Rushing et al., 2008; Rice et al., 2018), these advances could be negated by a reduction in soil moisture. Soil moisture could be depleted due to reduced precipitation, or by higher evapotranspiration due to higher vapour pressure gradients associated with higher temperatures (Reich et al., 2018). In such situations, herbaceous plants may not have enough resources to produce flowers due to moisture stress. Conversely, if temperature is a stronger driver of flowering phenology than soil moisture (Rathcke and Lacey, 1985; Abu-Asab et al., 2001), then reduced summer precipitation may not have any impact on flowering phenology of herbaceous plants (Cleland et al., 2006; Sherry et al., 2007; Rollinson and Kaye, 2012). Combined with the ability of a perennial plant to store resources, the cue to flower may occur solely based on temperature and not rely on, or become hindered by, lower soil moisture.

Here we tested the response of native herbaceous flowering phenology to ambient and reduced (approx. 27 %) growing season rainfall crossed with three levels of experimental warming in a regenerating southern boreal forest ecosystem. We studied five naturally occurring herbaceous perennial plant species over 3 years; three of the species are early season bloomers and two late season. In addition to the lack of phenology experiments that examine warming combined with reduced rainfall, phenology of herbaceous species in northern forests is also understudied. This study is novel in terms of its contribution to our understanding of how reproductive phenology of the herbaceous plant community will respond to future climate change conditions.

We hypothesized that warming would advance flowering (H1), but enhanced moisture stress from decreased rainfall would delay timing of flowering (H2); moreover, we hypothesized an interaction when both treatments occurred jointly, i.e. that advances in flowering due to warming would be smaller in the lower rainfall treatment (H3). Finally, we expected to see flowering in autumn blooming species most impacted (H4) by both the warming and altered rainfall treatments, given that they had the longest exposure to the (potentially) stressful environment of higher temperatures and limited soil moisture, predicted by H3. Our experiment is a long-term study designed to predict how two important climate variables, warming and altered rainfall, may impact a diverse array of herbaceous perennials.

MATERIALS AND METHODS

Study site and system

Our study took place in the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) project, a long-term, *in situ* experiment in northern Minnesota at the boreal-temperate

forest ecotone (Rich *et al.*, 2015; Reich *et al.*, 2018; Stefanski *et al.*, 2020). First established in 2008, the project has two upland forested sites, in Cloquet (46°40′N, 92°31′W) and Ely (47°56′N, 91°45′W), Minnesota. Cloquet has a mean annual temperature of 4.5 °C and 807 mm of annual precipitation; Ely has a mean annual temperature of 3.0 °C and 722 mm of annual precipitation (Rich *et al.*, 2015).

Each plot has an intact and naturally regenerating community of herbaceous, shrub and fern species growing amongst approx. 120 bare-root tree seedlings planted in the spring of 2012. We studied five species of herbaceous plants from this naturally occurring forest floor community for 3 years, 2012–2014. All the herbaceous species tracked persisted or regenerated from rhizome systems after disturbance from the initial experimental set up. All species occur naturally in the temperate–boreal ecotone of northern Minnesota and throughout much of the USA and Canada.

Heat treatments include unwarmed control plots (ambient) and plots with simultaneous above- (via infrared lamps) and below-ground (via buried resistance cables) warming that, during the years of this study, elevated the temperature to two target levels above ambient, i.e. +1.6 °C and +3.1 °C (Table 1). The heat treatment operates in open-air plots using a feedback control system to maintain the target temperature above ambient throughout the growing season (roughly April to November) of each year of the study; see Rich *et al.* (2015) for more details on the heat treatment manipulation. Each site contains three blocks and, within each block, are two replicates of the three heat treatments. Blocks were established following a clearcut in 2006 (Cloquet) and 2008 (Ely), 4–6 years prior to the beginning of the observations presented herein. In total, we used 36 plots of 7.1 m² divided evenly between the two sites.

The rainfall manipulation treatment, simulating drought, was installed in spring of 2012 in half of the plots within each block. Thus, each block contains one replicate of each warming treatment with and without rainfall manipulation (Stefanski et al., 2020). Rainfall removal starts on 1 June [day of year (DOY) 153] and ends on 30 September (DOY 274) of each year. The rainfall manipulation relies on custom-made 20 m² heavy duty tarps on a furling system to be deployed only during individual rainfall events. Manual rain gauges above the vegetation within the plots and time-domain reflectometry probes approx. 20 cm below the soil surface record rainfall and soil moisture within each plot to estimate efficacy of the rainfall removal. The rainfall manipulation treatment removes approx. 40 % of the total rainfall from June to September and 27 % of the total rainfall from May to September (Table 1). Temperate and boreal forests are likely to be effectively drier in the future than in the past (Seager et al., 2014; Wang et al., 2014), because climate warming will exacerbate water loss by increasing evapotranspiration (Seager et al., 2014; Wang et al., 2014); in addition there will be heightened variability in precipitation (Wang et al., 2014). As a result, the importance of water availability to climate responses may become greater in the future. Our treatments were set up to test whether effects of warming would differ in wetter vs. drier years, or in a future notably drier than historic conditions.

The years 2012–2014 had relatively high summer rainfall. Rainfall from 1 May to 30 September in 2012–2014 in ambient

	2012			2013			2014		
Site	Ambient rainfall (mm)	Rainfall in low rain treatment (mm)	Total rainfall removed	Ambient rainfall (mm)	Rainfall in low rain treatment (mm)	Total rainfall removed	Ambient rainfall (mm)	Rainfall in low rain treatment (mm)	Total rainfall removed
Cloquet	711.7	625.9	12.1 %	482.6	339.34	29.7 %	563.4	372.6	33.9 %
Ely	417.1	343.9	17.5 %	471.9	312.2	33.9 %	463.0	314.5	32.1 %
Site	Ambient	+1.6 °C	+3.1 °C	Ambient	+1.6 °C	+3.1 °C	Ambient	+1.6 °C	+3.1 °C
	warming	warming	warming	warming	warming	warming	warming	warming	warming
Cloquet	15.7 ± 8.3 °C	16.6 ± 7.8 °C	17.9 ± 7.6 °C	15.2 ± 8.5 °C	16.8 ± 8.5 °C	18.6 ± 8.2 °C	14.9 ± 8.1 °C	16.5 ± 8.1 °C	$18.1 \pm 8.1 ^{\circ}C$
Ely	16.6 ± 9.0 °C	17.5 ± 8.0 °C	19.3 ± 8.2 °C	15.8 ± 8.2 °C	17.2 ± 8.2 °C	18.6 ± 8.2 °C	15.4 ± 7.9 °C	17.0 °C ± 7.9	$18.6 ^{\circ}C \pm 8.0$

Warming treatment names are based on average achieved treatment differentials for our study period.

205

and reduced rainfall treatments at Cloquet represent the 87th and 44th percentile highest rainfall when compared with ambient rainfall in Cloquet for those months over a 100 year period (1912-2011); hence the two treatments represent moderately rainy growing seasons and slightly drier than average growing seasons.

Data collection

We recorded weekly flowering phenology observations for three growing seasons in 2012–2014. Observations began after snowmelt and were recorded as DOY records, with DOY 1 correlating with 1 January. Every 7 d, observers visited the research plots to observe the flowering phenology of focal species. Observers recorded the DOY that the first individual of each study species in each plot had an open flower, such that reproductive structures were available for pollination. Data collectors were assigned to particular sites and communicated definitions of phenophases through written protocols and electronic communication for consistency of data collection.

Study species

The five herbaceous perennial plants we studied include data from three individual species and two genera. We followed individuals of naturally established species of herbaceous perennials, namely Fragaria virginiana, Lathyrus venosus, Hieracium aurantiacum, Hieracium pretsense, Solidago altissima, Solidago gigantean and Eurybia macrophylla. Two Solidago species, Solidago altissima L. and Solidago gigantean Aiton, and two Hieracium species, Hieracium aurantiacum L. and Hieracium pretsense Tausch, were present within the experimental plots but were tracked and assessed at the genus level due to inability to identify these species in their immature stages, similar life history traits and phenology between species, and limited replication among treatments.

Under ambient conditions at these sites, F. virginiana generally flowers at the end of May, while *Hieracium* spp. and *L. ven*osus flower in mid-June. Solidago spp. and E. macrophylla generally flower in mid-August and are often called 'autumn flowering'; we will adopt that nomenclature throughout the text.

Statistical analysis

We used mixed-effects analysis of variance (ANOVA) models to assess the response of average DOY of flowering phenology to reduced rainfall combined with warming. First, we used a mixed-effects model to test the individual effects of year, warming treatment, rainfall treatment and species, and their two- and three-way interactions (Table 2). Block nested in site was a random effect. To effectively understand individual species' responses, and to isolate the effects of the rainfall treatment and its interaction with warming, we ran mixedeffects models for each individual species. Model effects included warming treatment, rainfall treatment and their two-way interaction (Table 3). Block nested in site and year were the random effects. We examined the significant results (P < 0.05) of each ANOVA using Tukey's HSD post-hoc tests to evaluate significant differences among treatment variables at $\alpha = 0.05$. Analyses were conducted using JMP 10 statistical software (SAS Institute, Cary, NC, USA).

Finally, we investigated how the rainfall treatment, combined with the warming treatment, altered species' temporal overlap. Specifically, we tested whether the experimental treatments led to convergence or divergence in timing of flowering among paired species. Due to the unbalanced nature of the data (e.g. not all species were found in each plot), we created bootstrapped distributions of the DOY when flowering occurred for each species in the particular treatment combination. As described in Rice et al. (2018), we resampled the data with replacement using 200 iterations, which was the minimum number of iterations needed for the majority of bootstrapped values to fall within the 95 % confidence interval (CI) of the raw mean. Once we had calculated the difference in the DOY when flowering occurred between the two examined species, we created a distribution of species pair differences in each examined treatment combination for all possible species pairs. Next, we conducted two-sample *t*-tests to test for significant differences

TABLE 2. Mixed-effect ANOVA results for flowering response over 3 years for five herbaceous species (n = 312)

$R^2 = 0.94$				
Source of variation	d.f.	d.f. _{Den}	F	Р
Warming	2	233.2	21.07	<0.0001
Year	2	233.2	70.53	<0.0001
Species	4	236.0	641.84	<0.0001
Rainfall	1	233.7	0.17	0.6826
Warming × Year	4	232.6	1.06	0.3755
Warming × Species	8	233.6	2.37	0.0179
Warming × Rainfall	2	234.6	3.65	0.0276
Year × Species	8	232.8	4.56	<0.0001
Year × Rainfall	2	232.4	0.41	0.6666
Species × Rainfall	4	233	3.46	0.0091
Warming × Year × Species	16	232.6	1.01	0.4514
Warming × Year × Rainfall	4	232.6	0.91	0.4614
Warming × Species × Rainfall	8	233.8	3.53	0.0007
Year × Species × Rainfall	8	232.4	8.86	0.5484

Block nested in site is the random effect. Significant results are indicated in bold (P < 0.05).

between the ambient and experimental treatments. Finally, if the absolute difference in DOY between each paired species was significantly larger in the experimental treatment compared with ambient, we deemed this to be divergent phenology. Conversely, smaller absolute differences in the DOY on which flowering occurred in the experimental treatment compared with ambient were deemed convergent. These analyses were conducted using R (R Core Team, 2013) and all figures were made using ggplot2 (Wickham, 2009).

RESULTS

Species differed in flowering phenology, which was strongly influenced by warming. Altered precipitation did not have a strong impact on flowering phenology of the herbaceous plant community (i.e. our study species) as a whole but did elicit strong changes in phenology in particular species. These changes were exacerbated by experimental warming. Overall, phenological responses to rainfall manipulation and experimental warming altered flowering synchrony among species.

Flowering: response of individual species and of the community

The herbaceous plant community showed main effects of warming, year and species, as well as several two- and threeway interactions among year, species, warming and rainfall manipulation (Table 2). Species was by far the strongest fixed effect in the model, followed by year. Warming was a strong driver of change in flowering phenology despite the strong main effect of species and year, supporting H1. For the community, warming advanced flowering by 7.7 ± 1.5 d and 9.2 ± 1.5 d in +1.7 °C and in +3.4 °C warming, respectively. On the other hand, the rainfall treatment did not have a main effect on flowering phenology across species, contrary to H2.

However, there were significant interactions of rainfall with warming and with species, as well as a three-way interaction of species × warming × rainfall (Table 2). In general, flowering phenology in the ambient warming treatment did not differ between the reduced or ambient rainfall conditions. In ambient rainfall conditions, warming advanced flowering linearly, by 5.4 ± 2.0 d and 11.4 ± 2.2 d in +1.6 °C and +3.1 °C, respectively. In reduced rainfall conditions, warming advanced

TABLE 3. Mixed-effect ANOVA results for flowering response over 3 years

Species	\mathbb{R}^2		Sourc	Source of Variation										
		N	Warming			Rainfall				Warming × Rainfall				
			d.f.	d.f. _{den}	F	Р	d.f.	d.f. _{den}	F	Р	d.f.	d.f. _{den}	F	Р
Fragaria virginiana	0.81	78	2	66.5	4.94	0.0100	1	67.2	1.75	0.1907	2	66.8	0.69	0.5042
Lathyrus venosus	0.72	55	2	43.9	1.71	0.1929	1	42.8	0.01	0.9251	2	44.8	0.58	0.5619
Hieracium spp.	0.49	52	2	41.1	6.98	0.0025	1	39.9	0.02	0.8825	2	42.3	0.03	0.9686
Solidago spp.	0.71	37	2	28.4	7.75	0.0021	1	28.7	4.79	0.0368	2	28.4	9.90	0.0005
Eurybia macrophylla	0.46	90	2	77.1	9.23	0.0003	1	76.4	7.84	0.0065	2	76.7	0.98	0.3800

Each row provides results for response of flowering for one individual species. Species are in descending order of timing of flowering in ambient conditions. Five mixed-effects models were run in total. Block nested in site and year are the random effects. Significant results are indicated in bold (P < 0.05).

flowering non-linearly, by 10.0 ± 2.3 d and 6.9 ± 2.3 d in +1.6 °C and +3.1 °C, respectively. The three-way interaction of species × warming × rainfall indicated that *E. macrophylla* and, in particular, *Solidago* spp. generally flowered at the same time in ambient conditions but experienced different magnitude and direction of phenology responses depending on the rainfall and warming combination (Fig. 1).

We examined how warming and rainfall, and their combination, impacted each species individually (Table 3). Of the five studied species, four showed a main effect of warming, two showed a main effect of rainfall manipulation and one showed a warming × rainfall interaction. Warming was the strongest main effect for all species (Table 3), and tended to advance flowering, again consistent with H1.

The two species that responded significantly to the rainfall manipulation differed in direction of response (Fig. 1). On average, *Solidago* spp. generally delayed flowering in the reduced rainfall conditions, by 7.6 ± 3.5 d, supporting H2. Conversely, *E. macrophylla* advanced flowering by 7.0 ± 2.5 d in reduced rainfall compared with ambient.

Solidago spp. was the only species with a significant warming × rainfall interaction (Table 3), exhibiting a pattern that was partially consistent with H3. Under ambient rainfall, this late blooming species advanced flowering by an average of 4.2 ± 4.3 d in +1.6 °C and by 19.5 ± 4.8 d in +3.1 °C warming. In the reduced rainfall treatment combined with +1.6 °C warming, *Solidago* spp. flowering advanced by 17.5 ± 6.5 d. However, when reduced rainfall was combined with +3.1 °C warming, there was no longer any difference in timing between the ambient temperature and +3.1 °C warming with reduced rainfall. The other late blooming species, *E. macrophylla*, had a somewhat different response to warming × rainfall compared with *Solidago* spp. *Eurybia macrophylla* advanced flowering compared with

ambient in both +1.6 °C (by 14.5 \pm 4.4 d) and +3.1 °C (by 16.4 \pm 4.2 d) warming, but this response was not sensitive to rainfall treatment. Overall, the two late bloomers had stronger responses to treatments than the early bloomers, supporting H4.

Changes in species temporal overlap

Compared with ambient conditions, reduced rainfall alone and reduced rainfall with warmer temperatures both consistently altered species temporal overlap (Table 4). Due to reduced rainfall alone (i.e. in ambient temperature conditions), nine out of ten species pairs showed a significant change in synchrony, with four species pairs converging and five pairs diverging (Fig. 2). The pattern of divergence was mainly due to the delayed flowering response of *Solidago* spp. in reduced rainfall (Fig. 2).

In +1.6 °C warming and reduced rainfall, all species pairs showed a significant change in synchrony, with eight pairs converging and two diverging. Most of this response can be explained by the relatively large advance in flowering of *E. macrophylla* and the lack of, or modest, advance in flowering of most other species in +1.6 °C warming and reduced rainfall. In +3.1 °C warming and reduced rainfall, all ten species pairs showed a significant change in synchrony, with half converging and half diverging.

The species pair that showed the largest absolute difference in timing of flowering were the autumn blooming species, *E. macrophylla* and *Solidago* spp. These species primarily diverged in timing of flowering (Fig. 2). The change in synchrony of the spring blooming species was dependent on the species pair, as temporal overlap both converged and diverged in the experimental treatments.





Pairwise species comparison	Difference (d) in ambient temperature and ambient rainfall (± s.e.)	Difference (d) in ambient temperature and reduced rainfall (± s.e.)	Change in synchrony in ambient temperature and reduced rainfall (± s.e.)	Difference (d) in +1.6 °C and reduced rainfall (± s.e.)	Change in synchrony in +1.6 °C and reduced rainfall	Difference (d) in +3.1 °C and reduced rainfall $(\pm$ s.e.)	Change in synchrony in +3.1 °C and reduced rainfall
Eurybia macrophylla– Solidago spp	0.6 ± 1.0	23.8 ± 1.0	Diverge	3.3 ± 1.0	Diverge	31.5 ± 0.8	Diverge
Fragaria virginiana– Lathyrus venosus	18.2 ± 1.3	25.0 ± 1.4	Diverge	24.2 ± 1.4	Diverge	28.4 ± 1.7	Diverge
Hieracium spp.– Lathyrus venosus	14.2 ± 1.2	6.3 ± 1.6	Converge	2.9 ± 1.1	Converge	2.6 ± 1.3	Converge
Fragaria virginiana– Hieracium spp.	31.7 ± 1.0	34.5 ± 2.0	_	25.7 ± 1.1	Converge	28.6 ± 1.3	Converge
Hieracium spp.– Solidago spp.	53.5 ± 1.0	59.2 ± 1.7	Diverge	40.5 ± 0.9	Converge	72.6 ± 0.7	Diverge
Eurybia macrophylla– Hieracium spp.	50.0 ± 0.9	40.1 ± 1.6	Converge	44.9 ± 0.8	Converge	40.8 ± 0.9	Converge
Lathyrus venosus– Solidago spp.	63.2 ± 1.3	70.2 ± 1.0	Diverge	41.9 ± 1.2	Converge	74.0 ± 1.2	Diverge
Eurybia macrophylla– Lathyrus venosus	64.6 ± 1.1	44.6 ± 1.3	Converge	48.3 ± 1.1	Converge	44.8 ± 1.3	Converge
Fragaria virginiana– Solidago spp.	81.9 ± 1.2	93.9 ± 1.2	Diverge	70.0 ± 1.3	Converge	100.3 ± 1.2	Diverge
Eurybia macrophylla– Fragaria virginiana	81.8 ± 1.0	72.6 ± 1.6	Converge	73.4 ± 1.2	Converge	68.7 ± 1.2	Converge

TABLE 4. Pairwise species differences in days on which flowering occurred in treatments

Within each treatment, mean bootstrapped differences ± 1 s.e. (200 iterations) were calculated as the difference in mean DOY on which flowering occurred between the paired species. These differences were compared between ambient and +1.6 °C and between ambient and +3.1 °C warming using *t*-tests. Species are considered convergent (divergent) if the absolute difference between the mean DOY start of senescence which occurred in warming is smaller (larger) than the absolute difference between DOY start of senescence which occurred in ambient. A dash indicates no significant difference in the synchrony between the two species in warming.

DISCUSSION

Flowering phenology responses to warming and rainfall manipulation were species specific in magnitude and direction. These differing responses altered the synchrony between species, leading to both convergence and divergence in timing of flowering between co-occurring species. Such responses to future climate conditions may lead to altered ecological relationships in forest communities.

As hypothesized, warming advanced flowering for four out of five species. On average, flowering advanced by 2.7–4.5 d °C⁻¹. The two species that advanced the most in warming and ambient rainfall conditions, *Solidago* spp. and *E. macrophylla*, flower in the autumn (Farnsworth *et al.*, 1995; Rice *et al.*, 2018). Species-specific responses may be due to individual species following particular cues. For instance, autumn blooming species may be more sensitive to air and soil temperature than spring blooming species, the latter possibly responding more strongly to photoperiod (Bazzaz and Bliss, 1971).

The three spring blooming herbaceous species did not alter flowering phenology in response to altered rainfall. This lack of response is not surprising and may be due to the experimental design of the study system. In this experiment, rainfall removal began approx. 2 weeks before flowering generally occurs in ambient conditions. The timing of this manipulation leaves little time for the plants to respond to the imposed conditions, as flower development has already occurred by 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 corresponding flowering response to be in accordance with future conditions. Additionally, the lack of response to altered rainfall may be related to the mesic habitat in our study. In mesic systems, warming has been shown to be a stronger driver of phenology than precipitation (Khan *et al.*, 2018). However, in moisture-limited systems, such as alpine habitats, soil moisture is a stronger driver of phenology than temperature (Iler *et al.*, 2013).

Autumn flowering species showed divergent responses to rainfall exclusion and to altered rainfall combined with warming. Generally, *E. macrophylla* and *Solidago* spp. flower within days of each other in mid-August. Warmer temperatures with ambient rainfall advanced flowering of both species at a similar rate. However, in +3.1 °C with reduced rainfall, the hottest and driest conditions, *E. macrophylla* advanced flowering by 3 weeks (into the end of July) while *Solidago* spp. delayed flowering by almost 1 week (into the end of August). Thus, in the +3.1 °C rainfall exclusion plots, there was a 1 month



FIG. 2. Average day of year (DOY) on which flowering occurred in manipulated conditions for five herbaceous species. Averages were calculated over 3 years, over two sites and in all plots of the respective treatments. Bars indicate ± 1 s.e.

difference between when *E. macrophylla* flowered compared with *Solidago* spp. For these two species, which generally flower within the same week, the combination of warming plus rainfall reduction led to radically different responses compared with warming alone.

Eurybia macrophylla was unique in its response of advancing flowering in the warmest, driest treatment conditions. This response may be related to this species' ability to cope with hot, dry environments. For instance, it is one of the first species to rebound after a fire (Ahlgren, 1960). 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, dry conditions as a means of phenological escape (Sonesson and Eriksson, 2003). Thus, this species may avoid flowering to conserve water resources until soil moisture is restored, or delay flowering due to an inability to acquire enough soil moisture until later in the season.

One caveat to our results is the unknown impact winter warming may have on these studied plants. Our experimental design implemented continuous above- and below-ground warming for approx. 8 months of the year. While warmer winter temperatures are predicted (Wuebbles and Hayhoe, 2004), warming was not implemented in the winter due to the low levels of biological activity below 0 °C and the potential artefactual effect of warming in the winter, as it would artificially spur snowmelt and potentially change soil structure via freeze-thaw cycles. Despite the advance in flowering we observed, warmer winters may delay flowering of species with vernalization requirements (Cook et al., 2012; Williams et al., 2014). However, Cook et al. (2012) found that far fewer species showed a strong response to winter warming compared with the number of species that responded to spring warming.

Species-specific responses may indicate future changes to ecological relationships. In simulated climate change conditions, paired species showed a mixture of either convergent or divergent timing of first flowering. Changes in temporal overlap may alter community composition (Memmott *et al.*, 2007; Sherry *et al.*, 2007), competition (Elzinga *et al.*, 2007) and, ultimately, trophic interactions (Hegland *et al.*, 2009; Warren *et al.*, 2010; Rafferty *et al.*, 2015). In particular, the striking divergence in flowering of previously synchronous *E. macrophylla* and *Solidago* spp. could reduce pollen availability for autumn pollinators (Moeller, 2004) or allow invasive species to acquire a new temporal niche (Sherry *et al.*, 2007). At the same time, this divergence may also release these species from competition for generalist pollinators (Elzinga *et al.*, 2007).

Changes in first flowering dates of earliest flowering individuals, the focus of our study, may not predict populationlevel responses or how other aspects of flowering phenology may change (CaraDonna *et al.*, 2014), which would truly alter ecological relationships. Further research is needed to understand how other aspects of southern boreal forest flowering phenology may change, such as peak flowering and flowering duration.

Our observations highlight the importance of examining a range of co-occurring species and observing responses in the wide range of climate conditions predicted to change. For instance, we observed different responses depending on the time of year during which the species bloomed. Furthermore, examining the responses of autumn blooming species in warming alone did not fully capture the response of these species. Examining the flowering response in the context of warming combined with altered rainfall provided a fuller picture of responses expected in a changing climate (Hyvönen *et al.*, 2007; Luo *et al.*, 2008). More studies that combine warming with reduced rainfall are needed to enhance our understanding of the phenological shifts which may take place in temperate and boreal forests of North America, pinpointing sensitive species and elucidating potential changes in ecological relationships. Furthermore, studies of ecophysiology coupled with phenology are needed to understand the mechanisms behind such responses to increased warming and altered rainfall.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the hard work and dedication of the many interns and colleagues who made this work possible, especially Kyle Gill and Ruffine Le Villain for their work in the field. Special thanks to Leslie Brandt, Aaron David, the Montgomery lab group and two anonymous reviewers for thoughtful comments and feedback on drafts of the manuscript.

FUNDING

The B4WarmED project was funded by the US Department of Energy (grant no. DE-FG02-07ER64456), and the College of Food, Agricultural and Natural Resources Sciences (CFANS) at the University of Minnesota. R.A.M. and P.B.R. were also supported by Minnesota Agricultural Experiment Station Projects MIN-42-060 and MIN-42-077.

LITERATURE CITED

- Abu-Asab MS, Peterson PM, Shetler SG, Orli SS. 2001. Earlier plant flowering in spring as a response to global warming in the Washington, DC area. *Biodiversity Conservation* **10**: 597–612.
- Ahlgren CE. 1960. Some effects of fire on reproduction and growth of vegetation in northeastern Minnesota. *Ecology* 41: 431–445.
- Bazzaz FA, Bliss LC. 1971. Net primary production of herbs in a central Illinois deciduous forest. *Bulletin of the Torrey Botanical Club* 98: 90–94.
- CaraDonna PJ, Iler AM, Inouye DW. 2014. Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy* of Sciences, USA 11: 4916–4921.
- Cleland EE, Chiariello NR, Loarie SR, Mooney HA, Field CB. 2006. Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences, USA* 103: 13740–13744.
- Cleland EE, Chuine I, Menzel A, Mooney HA, Schwartz MD. 2007. Shifting plant phenology in response to global change. *Trends in Ecology* & *Evolution* 22: 357–365.
- Cook BI, Wolkovich EM, Parmesan C. 2012. Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings* of the National Academy of Sciences, USA 109: 9000–9005.
- Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G. 2007. Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution* 22: 432–439.
- Farnsworth EJ, Nunez-Farfan J, Careaga SA, Bazzaz FA. 1995. Phenology and growth of three temperate forest life forms in response to artificial soil warming. *Journal of Ecology* 83: 967–977.
- Fitter AH, Fitter RSR, Harris ITB, Williamson MH. 1995. Relationships between 1st flowering date and temperature in the flora of a locality in central England. *Functional Ecology* **9**: 55–60.
- Hegland SJ, Nielsen A, Lázaro A, Bjerknes AL, Totland Ø. 2009. How does climate warming affect plant–pollinator interactions? *Ecology Letters* 12: 184–195.

- **Hyvönen R, Agren GI, Linder S,** *et al.* 2007. The likely impact of elevated [CO₂], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist* **173**: 463–480.
- Iler AM, Høye TT, Inouye DW, Schmidt NM. 2013. Nonlinear flowering responses to climate: are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society B: Biological Sciences* 368: 20120489.
- Inouye DW, Barr B, Armitage KB, Inouye BD. 2000. Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences, USA* 97: 1630–1633.
- **IPCC. 2014.** Climate change 2014: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK and New York, USA: Cambridge University Press.
- Karl TR, Trenberth KE. 2003. Modern global climate change. Science 302: 1719–1723.
- Khan MAS, Beierkuhnlein C, Kreyling J. et al. 2018. Phenological sensitivity of early and late flowering species under seasonal warming and altered precipitation in a seminatural temperate grassland ecosystem. *Ecosystems* 21: 1306–1320.
- Luo Y, Gerten D, Le Maire G, et al. 2008. Modeled interactive effects of precipitation, temperature, and CO₂ on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* 14: 1986–1999.
- Memmott J, Craze PG, Waser NM, Price MV. 2007. Global warming and the disruption of plant–pollinator interactions. *Ecology Letters* 10: 710–717.
- Menzel A. 2002. Phenology: its importance to the global change community an editorial comment. *Climatic Change* 54: 379–385.
- Miller-Rushing AJ, Inouye DW, Primack RB. 2008. How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency. *Journal of Ecology* 96: 1289–1296.
- Moeller DA. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85: 3289–3301.
- R Core Team. 2013. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.Rproject.org/.
- Rafferty NE, CaraDonna PJ, Bronstein JL. 2015. Phenological shifts and the fate of mutualisms. *Oikos* 124: 14–21.
- Rathcke B, Lacey EP. 1985. Phenological patterns of terrestrial plants. Annual Review of Ecological Systems 39: 179–219.
- Reich PB, Sendall KM, Stefanski A, Rich RL, Hobbie SE, Montgomery RA. 2018. Effects of climate warming on photosynthesis in boreal tree species depend on soil moisture. *Nature* 562: 263–267.
- Rice KE, Montgomery RA, Stefanski A, Rich RL, Reich PB. 2018. Experimental warming advances phenology of groundlayer plants at the boreal-temperate forest ecotone. *American Journal of Botany* 105: 851–861.
- Rich RL, Stefanski A, Montgomery RA, Hobbie SE, Kimball BA, Reich PB. 2015. Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. *Global Change Biology* 21: 2334–2348.
- Rollinson CR, Kaye MW. 2012. Experimental warming alters spring phenology of certain plant functional groups in an early successional forest community. *Global Change Biology* 18: 1108–1116.
- Seager R, Neelin D, Simpson I, et al. 2014. Dynamical and thermodynamical causes of large-scale changes in the hydrological cycle over North America in response to global warming. Journal of Climate 27: 7921–7948.
- Sherry RA, Zhou X, Gu S, et al. 2007. Divergence of reproductive phenology under climate warming. Proceedings of the National Academy of Sciences, USA 104: 198–202.
- Sonesson J, Eriksson G. 2003. Genetic variation in drought tolerance in *Picea abies* seedlings and its relationship to growth in controlled and field environments. *Scandanavian Journal of Forest Research* 18: 7–18.
- Sparks TH, Roy DB, Dennis RLH. 2005. The influence of temperature on migration of Lepidoptera into Britain. *Global Change Biology* 11: 507–514.
- Stefanski A, Bermudez R, Sendall KM, Montgomery RA, Reich PB. 2020. Surprising lack of sensitivity of biochemical limitation of

photosynthesis of nine tree species to open-air experimental warming and reduced rainfall in a southern boreal forest. *Global Change Biology* **26**: 746–759.

- Visser ME, Both C. 2005. Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences* 272: 2561–2569.
- Von Holle B, Wei Y, Nickerson D. 2010. Climatic variability leads to later seasonal flowering of floridian plants. PLoS One 5: e11500.
- Wang Y, Hogg EH, Price DT, Edwards J, Williamson T. 2014. Past and projected future changes in moisture conditions in the Canadian boreal forest. *The Forestry Chronicle* 90: 678–691.
- Walsh J, Wuebbles D, Hayhoe K, et al. 2014. Our changing climate. In: Melillo JM, Richmond TC, Yohe GW, eds. *Climate change impacts in the*

United States: the third national climate assessment. U.S. Global Change Research Program, 19–67.

- Warren RJ, Bahn V, Bradford MA. 2010. Temperature cues phenological synchrony in ant-mediated seed dispersal. *Global Change Biology* 17: 2444–2454.
- Wickham H. 2009. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag.
- Williams CM, Henry HAL, Sinclair BJ. 2014. Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biological Reviews* 90: 214–235.
- Wuebbles DJ, Hayhoe K. 2004. Climate change projections for the United States Midwest. *Mitigation and Adaptation Strategies for Global Change* 9: 335–363.