PHENOLOGICAL AND DEMOGRAPHIC PLANT RESPONSES TO GLOBAL CHANGE

By

Meredith Ann Zettlemoyer

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Plant Biology—Doctor of Philosophy
Ecology, Evolutionary Biology, and Behavior—Dual Major

2020

ABSTRACT

PHENOLOGICAL AND DEMOGRAPHIC PLANT RESPONSES TO GLOBAL CHANGE

By

Meredith Ann Zettlemoyer

Extinction rates exceed any in recent history. Simultaneously, invasive species are invading new areas and increasing in abundance. The leading proposed drivers of biodiversity loss include habitat destruction, urban and agricultural development, climate change, and biological invasions. Despite evidence that these anthropogenic changes influence both extinction of native species and invasions by non-native species, quantifying the role of different proposed mechanisms of diversity loss remains a challenge, in part due to lack of information on species responses to anthropogenic change, a need to examine species traits associated with both invasiveness and decline, and discrepancies between historical and current extinction patterns. In this dissertation, I use a combination of field experiments, historical datasets, and population modeling to examine how the dominant environmental changes facing natural populations, including habitat loss, climate change, and nutrient deposition, influence local species invasions and extinctions. Each chapter addresses a different potential cause of biodiversity decline (habitat loss, climate change, increasing herbivore densities, and nitrogen deposition) or compares the responses of more versus less successful species (i.e., invasive vs. native or locally extinct vs. extant). This approach will help us understand the species traits and responses to anthropogenic change associated with either invasiveness or extinction. I examined (i) how native and nonnative species differ in their phenological responses to climate warming and (ii) how locally extinct (i.e., species that have disappeared at a small spatial scale) and extant species differ in their species characteristics, their phenological responses to changing temperature and

precipitation regimes over the past century, and their demographic responses to increasing levels of nitrogen and deer herbivory in threatened prairie habitats. I found that while non-native species flower earlier under warming temperatures, native species' flowering time does not respond to warming, potentially putting them at a disadvantage as the climate warms. I also found that locally extinct species, which are often rare, native prairie specialist species, differ from extant species in their phenological responses to climate warming and their demographic responses to nitrogen fertilization. Specifically, locally extinct species did not advance flowering under warmer temperatures to the same extent as extant species, a response consistent with the hypothesis that appropriate phenological responses correlate with species success. Finally, locally extinct species experienced higher mortality and fewer benefits to growth and reproduction under nitrogen addition than extant species, suggesting that increasing nitrogen levels may influence species extinctions in threatened prairie habitat. By providing evidence of differences in phenological and demographic responses to global change between locally extinct and extant species as well as native vs. non-native species, my research allows us to evaluate the mechanisms underlying contemporary biodiversity change.

For my grandmother, Freda Zettlemoyer

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Jen Lau, for her encouragement, enthusiasm, and support throughout my time in graduate school. She always pushed everyone in her lab to become a better scientist, always asking questions and thinking of new ideas. Her endless optimism for science encouraged me to pursue large experiments and big questions. She was extremely supportive and compassionate, and welcomed me into not only her lab but her home. I truly could not have asked for a better advisor.

My committee, Lars Brudvig, Elena Litchman, and Elise Zipkin, provided valuable feedback on my research and were always willing to answer questions about statistics and manuscripts. Discussions with them greatly improved my work and my ability to think critically as a scientist. I would also like to thank Kay Gross and Nick Haddad for acting as my KBS hosts, making sure I had a lab group and resources at KBS after the rest of the Lau lab moved to Indiana University. Finally, the Lau lab has been an incredible group of collaborators, colleagues, and friends. From discussing papers to playing in the Sheagles, the lab's all female Eagles cover band, I could always count on them for support and a laugh. I am especially grateful to Mark Hammond, who, despite telling me my experiments were "ambitious", was patient and realistic, always ready to lend a helpful hand and ear.

Many projects would not have been possible without the help of amazing undergraduate researchers, including Cameron Andrews, Brendan Canavan, Karina Cortijo-Robles, Sarah Johnson, Katy Renaldi, and Nick Srodes. These amazing students worked hard and were always motived despite long hours in the field. They each developed independent research questions and

mentoring each of them through their first research experiences was a highlight of graduate school. Thank you to the many KBS volunteers who helped with field and lab work as well.

Thank you to the Kellogg Biological Station, the Lauff, Porter and Maxey research scholarships, the KBS Long-Term Ecological Research site, the Michigan Botanical Foundation, and the Hanes Foundation for research funding. I also received support from the University Distinguished Fellowship, Plant Sciences Fellowship, AgBio Research Fellowship, College of Natural Sciences Recruiting Fellowship, Dr. Martin Hensley Endowed Fellowship, and the Department of Plant Biology and Ecology, Evolutionary Biology, and Behavior program. I would particularly like to thank Kara Haas and Misty Klotz for their mentorship during my time a Science Education and Outreach Fellow at KBS – this experience made a much better communicator and teacher, and I am extremely grateful for their guidance.

Thank you to Kellogg Biological Station. KBS is a wonderful community, with such a fun, supportive environment. KBS provides so much support with research funds, logistical support, and field sites, but the community was also welcoming, helpful, and close-knit.

Thank you to my parents for your constant love and support. I'm sure twenty-two years of school wasn't entirely expected, but you have always encouraged me to work hard and pursue my goals. Finally, thank you to my partner, Michael Muzyka, for being by my side through all the rejections and successes of graduate school. I cannot image doing this without you, and I cannot wait to continue our adventures.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Species extinction rates are predicted to rise by an order of magnitude over the next few hundred years (Mankga and Yessoufou, 2017). However, there is large variability in predictions of extinction risk. For example, anywhere from 0.17-42.5% of plant species could go extinct within a century due to climate change and land use (Pereira et al., 2010). Yet simultaneously, widespread, tolerant, exotic plants are spreading, becoming dominant, and often replacing those same declining species (McKinney and Lockwood, 1999). We are currently limited in our ability to predict species loss, largely because most studies infer extinction risk from observed species declines in the field rather than from demographic responses to hypothesized drivers of extinction (e.g. habitat loss, global warming, nitrogen deposition, or increased browsing from deer) and because of discrepancies between historical and current extinction patterns (Pereira et al., 2010; Mondanaro et al., 2019). Combining three approaches may improve the accuracy of predicting extinctions, however. First, incorporating historical data from herbaria and field studies documenting species extinctions over the past century could be a valuable resource to help understand contemporary extinction events (Lang et al., 2018). These records provide a recent record of local, species-specific extinction events and likely reflect localized changes to the environment that have recently driven species to local extinction (defined here as extinctions at a small spatial scale; Pimm et al., 2014). As such, local extinctions likely reflect global declines and could therefore help address the current biodiversity crisis (Davies, 2019). Second, comparing more successful vs. declining species (e.g., native vs. nonnative or extirpated vs. extant) could provide clues about the traits and responses of both at-risk and expanding species (van Kleunen and Richardson, 2007). Conservation and invasion biology use similar approaches to look for determinants of rarity and invasiveness, and the traits and responses associated with

potential extinction or invasiveness likely lie on opposing ends of a spectrum. For instance, long-distance dispersal frequently facilitates invasion, but insufficient long-distance dispersal also threatens native species (Trakhtenbrot et al., 2005). Phenological plasticity (i.e., the ability to shift the timing of life-history events such as flowering in response to environmental change) has also been associated with both invasiveness (Wolkovich et al., 2013) and population declines (Willis et al., 2008). Detecting attributes of threatened rare species or locally extinct species could help predict the fate of introduced species and vice versa (van Kleunen and Richardson, 2007). Finally, experimental manipulations and longer-term studies of population dynamics can provide useful tests of hypothesized drivers of invasion and extinction (Collen et al., 2011; Murray et al., 2014). Although most work on species losses is observational, a more mechanistic approach incorporating experiments and demography would highlight the ecological processes underling relationships between species loss and the local environment and improve our ability to produce more predictive models of biodiversity loss (Merow et al., 2014). Despite the potential gains of combining these approaches, few studies have combined historical, demographic, trait, and experimental data to better evaluate the roles of proposed drivers of biodiversity change and more accurately predict the impacts of future environmental changes on species extinctions and invasions.

This dissertation focuses on the use of historical datasets, experimental manipulations, and population modeling to quantitatively examine the effects of various anthropogenic changes on invasive species, native species, and species that have already disappeared. I do this using two approaches:

(1) Examining patterns of regional species and trait diversity loss using historical data, and

(2) Experimentally manipulating proposed drivers of species loss to evaluate potential mechanisms underpinning both invasions and local extinction events.

Each chapter addresses a different potential cause of biodiversity decline or compares the responses of more vs. less successful species (e.g., native vs. nonnative and extirpated vs. extant). This approach will help us understand the species traits and responses to anthropogenic change associated with either invasiveness or extinction. In chapter one, I use historical botanical data from Kalamazoo County, Michigan, to examine whether species characteristics or phylogenetic relatedness explain local species loss at the county level. I found that rare, specialist species occupying threatened prairie habitat were most vulnerable to loss but detected no evidence of a phylogenetic signal to extinction. In chapter two, I address whether phenological responses to warming, which often influences species success under climate change, differ between native vs. nonnative species. I found that invasives tended to be more responsive to warming temperatures than native species, potentially providing them with an advantage under global warming. Native species' phenology did not respond to warming temperatures, suggesting that native species may not respond to climate change as effectively as invasive species and so may be more vulnerable to extinction as global temperatures rise. In chapter three, I use herbarium records from across the Midwestern United States to investigate whether locally extinct vs. extant species once found in Michigan prairies (identified in chapter one) differ in their phenological responses to temperature and precipitation experienced during winter and spring or in their magnitude of flowering time shift over the past century. I found that locally extinct and extant species differ in their phenological responses to warming. Warmer spring temperatures advanced flowering, but locally extinct species advanced flowering less in response to warmer springs than extant species (or even responded with delayed flowering). In contrast,

locally extinct species advanced flowering under warmer winter temperatures to a greater extent than their extant pairs. These results implicate differences in phenological responses as a potential mechanism underlying local extinction events. In chapter four, I experimentally manipulate two common drivers of species losses in prairies, nitrogen fertilization and deer herbivory, in the field to test the effects of nitrogen and herbivory on the population demography of the same confamilial pairs of locally extinct and extant species. I found that nitrogen decreases plant survival, particularly in locally extinct species, and nitrogen increases growth and reproduction for extant species more than locally extinct species. This suggests that nitrogen affects locally extinct and extant species' vital rate responses differently, with nitrogen providing more of a benefit to extant species. However, population modeling revealed that nitrogen results in lower population growth rates across species, suggesting that increasing nitrogen levels influence population declines in native prairie forbs. By providing evidence for the role of environmental changes in recent extinctions and invasions, my research goes beyond observational studies of species loss to quantitatively explore the role of global change in historical, and potentially future, population declines and will inform predictions of extinction risk for threatened species.

LITERATURE CITED

LITERATURE CITED

Collen, B., A Purvis, and G.M. Mace. 2010. When is a species really extinct? Testing extinction inference from a sighting record to inform conservation assessment. *Diversity and Distributions* 16: 755-764.

Davies, T.J. 2019. The macroecology and macroevolution of plant species at risk. *New Phytologist* 222: 708-713.

Lang, P.L.M., F.M. Willems, J.F. Scheepens, H.A. Burbano, and O. Bossdorf. 2018. Using herbaria to study global environmental change. *New Phytologist* 221: 110-122.

Mankga, L.T., and K. Yessoufou. 2017. Factors driving the global decline of cycad diversity. *AoB Plants* 9: 1-10.

McKinney, M.L., and J.L. Lockwood. 1999. Biotic homogenization: a few winners replacing many losers in the next mass extinction. *Trends in Ecology and Evolution* 14: 450-453.

Merow, C., A.M. Latimer, A.M. Wilson, S.M. McMahon, A.G. REbelo, and J.A. Silander Jr. 2014. On using integral projection models to generate demographically driven predictions of species' distributions: development and validation using sparse data. *Ecography* 37: 1167-1183.

Mondanaro, A., M. Di Febbraro, M. Melchionna, F. Carotenuto, S. Castiglione, C. Serio, S. Danisi, et al. 2019. Additive effects of climate change and human hunting explain population decline and extinction in cave bears. *Boreas* 48: 607-615.

Murray, K.S., L.D. Verde Arregoitia, A. Davidson, M. di Marco, and M.M.I. di Fonzo. 2014. Threat to the point: improving the value of comparative extinction risk analysis for conservation action. *Global Change Biology* 20: 483-494.

Pereira, H.M., P.W. Leadley, V. Proença, R. Alkemade, J.P. Scharlemann, J.F. Fernandez-Mangarrés, M.B. Araújo, et al. 2010. Scenarios for global biodiversity in the 21st century. *Science* 330: 1496-1501.

Pimm, S.L., C.N. Jenkin, R. Abell, T.M. Brooks, J.L. Gittleman, L.N. Goppa, P.H. Raven, et al. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344: 987.

Trakhtenbrot, A., R. Nathan, G. Perry, and D.M. Richardson. 2005. The importance of long-distance dispersal in biodiversity conservation. *Diversity and Distributions* 11: 173-181.

van Kleunen, M., and D.M. Richardson. 2007. Invasion biology and conservation biology: time to join forces to explore the link between species traits and extinction risk and

invasiveness. *Progress in Physical Geography* 31: 447-450.

Willis, C.G., R.B. Primack, A.J. Miller-Rushing, and C.C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences* 105: 17029-17033.

Wolkovich, E.M., T.J. Davies, H. Schaffer, E.E. Cleland, B.I. Cook, S.E. Travers, C.G. Willis, and C.C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany* 100: 1407-1421.

CHAPTER ONE:

Species characteristics affect local extinctions[1]

ABSTRACT

Human activities threaten thousands of species with extinction. However, it remains difficult to predict extinction risk for many vulnerable species. Species traits, species characteristics such as rarity or habitat use, and phylogenetic patterns are associated with responses to anthropogenic environmental change and may help predict likelihood of extinction. We use historical botanical data from Kalamazoo County, Michigan, USA, to examine whether species traits (growth form, life history, nitrogen-fixation, photosynthetic pathway), species characteristics (community association, species origin, range edge, habitat specialization, rarity), or phylogenetic relatedness explain local species loss at the county level. Across Kalamazoo County, prairie species, species at the edge of their native range, regionally rare species, and habitat specialists were most likely to become locally extinct. Prairie species experienced the highest local extinction rates of any habitat type, and among prairie species, regionally rare and specialist species were most vulnerable to loss. We found no evidence for a phylogenetic pattern in plant extinctions. Our study illustrates the value of historical datasets for understanding and potentially predicting biodiversity loss. Not surprisingly, rare, specialist species occupying threatened habitats are most at risk of local extinction. As a result, identifying mechanisms to conserve or restore rare or declining species and preventing further habitat destruction may be the most effective strategies for reducing future extinction.

---

[1] Zettlemoyer, M.A., D. D. McKenna, and J.A. Lau. 2019. Species characteristics affect local extinctions. *American Journal of Botany* 106(4), 1-13.

INTRODUCTION

Species extinction rates are predicted to rise by an order of magnitude over the next few hundred years (Mace et al., 2005; Pimm et al., 2014; Mankga and Yessoufou, 2017). However, there is large variability in predictions of extinction risk. For example, anywhere from 0.17-42.5% of plant species could go extinct within a century (Pereira et al., 2010). Two factors could improve our ability to predict extinction risk and which taxa are most vulnerable to extinction: trait-based approaches (Sodhi et al., 2008, Saar et al., 2012; Luiz et al., 2016) and phylogenetic analyses (are some clades more susceptible to extinction than others?) (Fitzpatrick et al., 2008; Davies et al., 2011; Parhar and Mooers, 2011; Yessoufou et al., 2012; Davies and Yessoufou, 2013). Studies of historical and recent data on plant distributions and abundance can test the effectiveness of both traits and phylogeny for predicting extinction (Primack et al., 2004; Fitzpatrick et al., 2008; Willis et al., 2008; Nualart et al. 2017; Lang et al., 2018; Meineke et al. 2018).

Species traits and characteristics have emerged as a valuable framework for predicting responses to global changes (McGill et al., 2006; Lavorel et al., 2007; Mouillet et al., 2013). For example, short plants with large leaf areas are associated with negative responses to climate warming (Venn et al., 2011), and species characteristics that reflect aspects of rarity and habitat affinity influence how species respond to habitat conversion and disturbance (Farnsworth and Ogurcak, 2008; Sodhi et al., 2008; Leão et al. 2014; Palma et al., 2016). Such species traits and characteristics also likely influence extinction risk, as species with biological and ecological characteristics that are ill-suited to survival in altered habitats will likely be at high risk of extinction (Brook et al., 2008; Leão et al. 2014; Palma et al. 2016). In butterflies, for example, species with narrow niche breadth, restricted resource use, poor dispersal ability, and low

reproductive rates are at high risk of extinction (Kotiaho et al., 2005; Öckinger et al., 2010), while in mammals, characteristics such as geographic range and life history strategy have been associated with extinction risk (Davidson et al., 2017). In plants, native species (Suding et al., 2005; Weigmann and Waller, 2006; Rogers et al., 2008), forbs (Leach and Givnish, 1998; Weigmann and Waller, 2006; Smart et al., 2006; Soons and Heil, 2002), perennials (Grashof-Bokam, 1997; Verheyen et al., 2003; Suding et al., 2005), habitat specialists (Rich and Woodruff, 1996; Fischer and Stöcklin, 1997; Preston, 2000; Preston et al., 2002; Kolb and Diekmann, 2004; Davies et al., 2004), and species experiencing high rates of habitat loss (Duncan and Young, 2000; Lienert et al., 2002; Aedo et al., 2015; Auffrett et al., 2018), among others, experience high rates of local extinction. Identifying traits associated with species that have been lost from a given geographic area or habitat may help elucidate the characteristics that help or harm species in the face of global change and may aid in the development of strategies to manage and conserve species with similar characteristics (Cardillo et al., 2006; Farnsworth and Ogurcak, 2008; Razgour et al., 2013; Romeiras et al., 2014; Bai et al., 2018).

Phylogenetic signatures in extinction events can also provide insights into patterns of species loss (Jones et al., 2005; Purvis, 2008). A strong phylogenetic signal implies that certain families are more susceptible to loss than others (Purvis et al., 2000a; Mankga and Yessoufou 2017). Phylogenetic patterns in extinction risk have been detected in birds (Bennett and Owens, 1997; Russell et al., 1998; Purvis et al., 2000b; von Euler, 2001; Fritz and Purvis, 2010), mammals (McKinney, 1997; Harcourt, 1998; Russell et al., 1998; Purvis et al., 2000b; Johnson, 2002), amphibians (Stuart et al., 2004; Bielby et al., 2006), insects (Kotiaho et al., 2005), marine taxa (McKinney, 1997; Roy et al., 2009), and plants (Schwartz and Simberloff, 2001; Sjöström and Gross, 2006; Davies et al., 2011; Yessoufou et al., 2012; Leão et al. 2014). In cases where

there is a phylogenetic signal in extinction risk, species traits likely influence extinction (McKinney, 1997; Fisher and Owens, 2004; Willis et al., 2008; Fritz and Purvis, 2010; Saar et al., 2012, Loza et al., 2017), and phylogeny can help predict extinction risk even when the relevant traits are unknown (Fritz and Purvis, 2010; Davies et al., 2011; Yessoufou et al., 2012). Alternatively, a random phylogenetic pattern of extinction implies that extinction events are not determined by traits conserved among related species. Instead, extinction may be influenced by an unmeasured, non-phylogenetically conserved trait or by an external mechanism that does not strongly select against particular traits, such as habitat loss (Fritz and Purvis, 2010; Daru et al., 2013; Yessoufou and Davies, 2016). Although uncommon (McKinney, 1997; Yessoufou and Davies, 2016), random phylogenetic patterns of extinction have been observed in mammals (Arregoitia et al., 2013) and plants (Fréville et al., 2007; Fitzpatrick et al., 2008; Lapiedra et al., 2015; Yessoufou et al., 2017). However, there are fewer studies of the phylogenetic structure of plant distributions and extinction relative to those of well-studied vertebrates (Mankga and Yessoufou, 2017; Loza et al., 2017).

Incorporating historical data on species losses could be a valuable resource for detecting trait and phylogenetic patterns in recent species extinctions (Primack et al., 2004; Grass et al., 2014; Nualart et al., 2017; Lang et al., 2018; Meineke et al., 2018). Mass species extinctions spanning back hundreds of millions of years have commonly been examined using the paleontological record, in which fossils provide the approximate date of last occurrence before an extinction event (Jablonksi, 1994). However, understanding contemporary extinction events may require examining more contemporary, local species records due to two discrepancies between the paleontological record and more recent extinctions. First, comparing causes of extinction over geological time scales versus more recent time is difficult (Jablonski, 1994;

Barnosky et al., 2011; Pimm et al., 2014; De Vos et al., 2015; Plotnick et al., 2016). For example, in amphibians, habitat-based extinction risk was reversed in contemporary taxa relative to fossil taxa: fossil amphibian taxa declined in stagnant waters while contemporary amphibian taxa declined in flowing waters (Tietje and Rodel, 2017). Second, the paleontological record is often used to examine species extinction on a global scale rather than for studies of local species losses because widely distributed and common species are more likely to appear in the fossil record (Liow et al., 2008; Plotnick et al., 2016) and because the fossil record is often too incomplete to be analyzed at the species level or on local scales (Pereira et al., 2012; Plotnick et al., 2016). Herbarium and museum records provide a more recent record of such local, species-specific extinction events (Lang et al., 2018), span centuries, include a large sample of species (Primack et al., 2004; Meineke et al., 2018), and likely reflect localized changes to the environment that have recently driven species to local extinction (defined here as disappearance at a small spatial scale, such as within a given county) (Pelini et al., 2011; Pimm et al., 2014). As a result, herbarium records have been used to identify characteristics associated with extirpations ranging from local to continental scales (Duncan and Young, 2000, 2011; Bertin, 2002; Blomqvist et al., 2003; DeCandido et al., 2004; Primack et al., 2004; Williams et al., 2005; Smart et al., 2006; Miller-Rushing et al. 2006; Willis et al., 2008, 2010, 2017; Pyke and Ehrlich, 2010; Knapp et al., 2010; Dolan et al., 2011; Gregor et al., 2012; Wolkovich et al., 2013; Grass et al., 2014; Palma et al., 2016; Nualart et al., 2017).

Here, we use historical data from Kalamazoo County, Michigan, USA, to examine patterns of local extinction events. The flora of Kalamazoo County was surveyed extensively from the 1890s-1947 and again from 1994-2003. Over this time period, it has experienced both urbanization and intensified agricultural land use, reflecting similar changes across historically

grassland-dominated sites in central North America. Using these two datasets, which record species presence and absence in the county, we address two questions: (1) Is local extinction influenced by species traits and characteristics? and (2) Is there a phylogenetic pattern to local extinction?

MATERIALS AND METHODS

Study system

Kalamazoo County covers approximately 1492 km^2 in southwestern Michigan (MI), USA. Rivers, streams, and lakes cover about 3.2% of the area. The surrounding land consists of forests, wetlands, prairie remnants, and land developed for urban and agricultural use (McKenna, 2004).

Kalamazoo County boasts a diverse and well-documented flora, with more species reported (1651; McKenna, 2004) than most other county-level floras in North America (Jarnevich et al., 2006). The county was first surveyed from the 1890s to 1940s (Hanes and Hanes, 1947) and was resurveyed in the 1990s (primarily from 1994-2003) (McKenna, 2004). These historical records describe the various community types and presence/absence of native and introduced vascular plants in Kalamazoo County. The 1136 species included in our study were recorded in both the Hanes and Hanes (1947) and McKenna (2004) records.

Historical dataset

During the original surveys, C. and F. Hanes surveyed sites across Kalamazoo County, took detailed field notes and collected plant samples, and eventually compiled their data into a checklist of species in the county's first published flora (hereafter referred to as "original surveys"). In the 1990s (1994-2003), D. McKenna expanded the species checklist by surveying

the same sites across Kalamazoo County, examining more than 5000 herbarium specimens, referencing the Hanes' field notes and vegetation maps completed by the General Land Office in the mid-1800s (Comer et al., 1995, 1997), and communicating with local botanists (McKenna, 2004) (hereafter referred to as "1990s surveys"). We note that different survey methods and sampling intensities across these two periods may affect the data available. Given the extensive sampling across at least a decade during both surveys, we believe that the county-level botanical record is of high quality (Jarnevich et al., 2006; Fréville et al., 2007; Niissalo et al., 2017). In addition, McKenna (2004) found 133 new species, including some native species likely missed during the original surveys.

Using these two records, we designated species as locally extinct (designated as "0") or non-extinct in Kalamazoo County (designated as "1") (McKenna, 2004). Locally extinct species were recorded in the county during the original surveys but were no longer found in the county during the 1990s surveys. For species listed as locally extinct, we cross-referenced with herbarium records to check whether the species had been found in Kalamazoo since the 1990s (http://michiganflora.net/specimen-search.aspx).

Species in Kalamazoo County occur in several unique, discrete plant communities (defined here as in McKenna [2004]: an "assemblage of species in a given habitat type with characteristic growth form, structure, seasonality, dynamics and composition"). Each species included in McKenna (2004) included a notation for the plant community(s) in which the plant was historically reported or found during the 1990s surveys. Specific community types were also more broadly categorized as prairie, wetlands, or forests (categories described in McKenna [2004]; Table S1.1). Kalamazoo County has been exposed to varying degrees of human alteration (post-settlement, i.e., excluding alterations made by Native Americans), such as road

development, urbanization, and intensive agricultural use. When a plant was associated with an anthropogenic feature of the landscape, McKenna (2004) denoted its habitat as “old field”, “roadside”, “railroad right-of -way”, or “garden”; we grouped these species into a man-made habitat category.

We determined the geographic rarity of each species by calculating the proportion of Michigan counties in which a species is found (USDA PLANTS [https://plants.usda.gov/java/]). For scale, Michigan covers 250490 km^2, and most counties in lower Michigan each cover 905—2486 km^2 (www.indexmundi.com). We use this regional rarity metric as a proxy for local rarity because our knowledge of historical population sizes in Kalamazoo County is minimal or lacking for most species, and a standardized scale is needed for comparing between species (Hartley and Kunin, 2003).

We classified each species by a number of categorical characteristics and traits.

1. *Community association:* Defined as the community type (forest, prairie, wetland, or man-made habitat) that a species is most commonly found in, determined from McKenna (2004) (Table S1.1). For species that had gone extinct, community association was based on where it was historically reported (McKenna, 2004). We hypothesize that habitats that have experienced high rates of degradation and loss (e.g., prairies) will experience high rates of species loss (Duncan and Young, 2000; Lienert et al., 2002; Walker and Preston, 2006; Aedo et al., 2015; Auffret et al., 2018).

2. *Species origin:* Classified as native or non-native in Michigan, determined from the USDA PLANTS database. We hypothesize that native species are more likely to be lost than non-native species, as native species are more often lost than non-native species in several other habitat types (Suding et al., 2005; Weigmann and Waller, 2006; Farnsworth and Ogurcak, 2008) and

invasive species may thrive in the face of human disturbance and anthropogenic environmental change (Dukes and Mooney, 1999).

3. *Range edge:* Classified as "edge" or "central" species, determined from the USDA PLANTS database. A species was considered at the edge of its range if Michigan is a state at the border of its native range. We hypothesize that edge species will be more vulnerable to species loss, due to evidence of increased local extinction rates at the edge of species' ranges (Lienert et al., 2002; Doherty et al., 2003; Farnsworth and Ogurcak, 2008; Boakes et al., 2017).

4. *Habitat specialization:* Defined as the number of unique habitat types in which a species was found in Kalamazoo County, as determined by McKenna (2004). This serves as an indicator of whether a species is a specialist that persists in only a few community types, or a generalist that persists in several different community types. We hypothesize that habitat specialists are more likely to succumb to local extinction, as has been reported in other studies of forests and grasslands (Pimm, 1991; Fischer and Stöcklin, 1997; Preston, 2000; Kolb and Diekmann, 2004; Davies et al., 2004; Kotiaho et al., 2005).

5. *Life history:* Classified as annual, annual/biennial, annual/perennial, biennial, or perennial, determined from the USDA PLANTS database. We hypothesize that perennial species will be more susceptible to local extinction, because annuals are often better colonizers of urbanized environments (Palma et al., 2016) and have been shown to persist longer in small patches of habitat (Collins et al., 2009).

6. *Growth form:* Classified as forbs, ferns, vines, woody (trees, shrubs), or graminoid (grasses, sedges, rushes), determined from the USDA PLANTS database. We hypothesize that forb species are more vulnerable to loss, as forbs are lost more often than other growth forms in other

forest and grassland systems (Leach and Givnish, 1996; Smart et al., 2005; Sjöström and Gross, 2006; Weigmann and Waller, 2006).

7. *Nitrogen-fixation:* Classified as a "N-fixer" (a species that can fix nitrogen) or not, determined using state wildflower websites. We hypothesize that N-fixers are more likely to disappear because in grasslands, soil nitrogen levels increase due to deposition and fertilization (Sala et al., 2000) and N-addition experiments commonly reduce the abundance of species with N-fixing symbionts (Leach and Givnish, 1996; Suding et al., 2005).

8. *Photosynthetic pathway ("C3/4"):* Classified as C3 or C4, determined using state wildflower websites. We hypothesize that C3 species are more likely to become locally extinct, as previously found in Minnesota grasslands (Suding et al., 2005).

Data Analysis

We present two sets of data analyses. First, we examine extinction in all of the habitat types across Kalamazoo County ("Kalamazoo County"). Then, because prairies represent the most vulnerable habitat type in the area (Chapman and Brewer, 2008) and experience the highest proportion of extinction events, we present analyses wherein only prairie species extinctions are considered ("Prairie Species").

Kalamazoo County

We tested for correlations between all traits and characteristics using Chi-Square Tests of Independence, which determine whether two categorical variables are correlated. We considered characteristics correlated if $p \leq 0.05$.

We used a generalized linear model with a binomial distribution to examine the effect of each species characteristic on the status (locally extinct/non-extinct) of prairie species in Kalamazoo County. We included status as the response variable, and species origin, range edge,

habitat specialization, life history, growth form, N-fixation, and photosynthetic pathway as categorical predictor variables. Rarity was included as a continuous covariate to control for the likelihood that rare species should be lost more often than common ones. We hypothesized that rare native species, rare habitat specialists, rare N-fixers, and rare species at the edge of their native range might respond differently than rare invasive species, rare generalists, rare non-N-fixers, and rare central species, so interactions between rarity and those characteristics were included.

Because the majority of the traits and characteristics considered were correlated (Table S1.2), we used backwards elimination to simplify the regression. In backwards elimination, the predictor with the highest p-value greater than alpha (α=0.05) is removed. The model is refit, and this procedure repeats until no collinear predictors are included and all p-values are less than α. We provide Akaike Information Criterion values for the models and p-values of removed variables in Table S1.3. The final model included community association, rarity, N-fixation, growth form, range edge, habitat specialization, and the interactions of community association x rarity and N-fixation x rarity as predictor variables. Post-hoc tests were used to evaluate differences between treatment levels when the effect of a species trait or characteristic was significant ($p \leq 0.05$).

We also performed a Multiple Correspondence Analysis (MCA). MCA is an explanatory/descriptive analysis technique that reduces large sets of associated categorical variables into smaller sets of components that summarize the information in the data without any underlying assumptions about the data's distribution (Abdi and Valentin, 2007). This analysis, although it does not allow for use of continuous data, allows us to consider all categorical characteristics rather than removing correlated variables. Results from the two analyses were

similar, but because of the benefits and shortcomings of both of these methods we present the backwards elimination results in the main text and the MCA in Tables S1.4 and S1.5.

Prairie Species

Because prairies represent the most threatened habitat type in Kalamazoo County and because prairie species (species commonly found in, but not necessarily exclusive to, prairie habitats) experience more extinction events than species found in other community types (see Results), we used a generalized linear model with a binomial distribution to examine the effect of each species characteristic on the status (locally extinct/non-extinct) of prairie species in Kalamazoo County. As described above, we included status as the response variable, and species origin, range edge, habitat specialization, life history, growth form, N-fixation, and photosynthetic pathway as categorical predictor variables, rarity as a continuous covariate, and the interactions between rarity x species origin, rarity x habitat specialization, rarity x N-fixation, and rarity x range edge. We again used backwards elimination to simplify the regression and provide Akaike Information Criterion values for the models and p-values of removed variables in Table S1.6. The final model included rarity, habitat specialization, growth form, N-fixation, and the interaction of rarity x N-fixation as predictor variables. Post-hoc tests were used to evaluate differences between treatment levels when the effect of a species trait or characteristic was significant ($p \leq 0.05$).

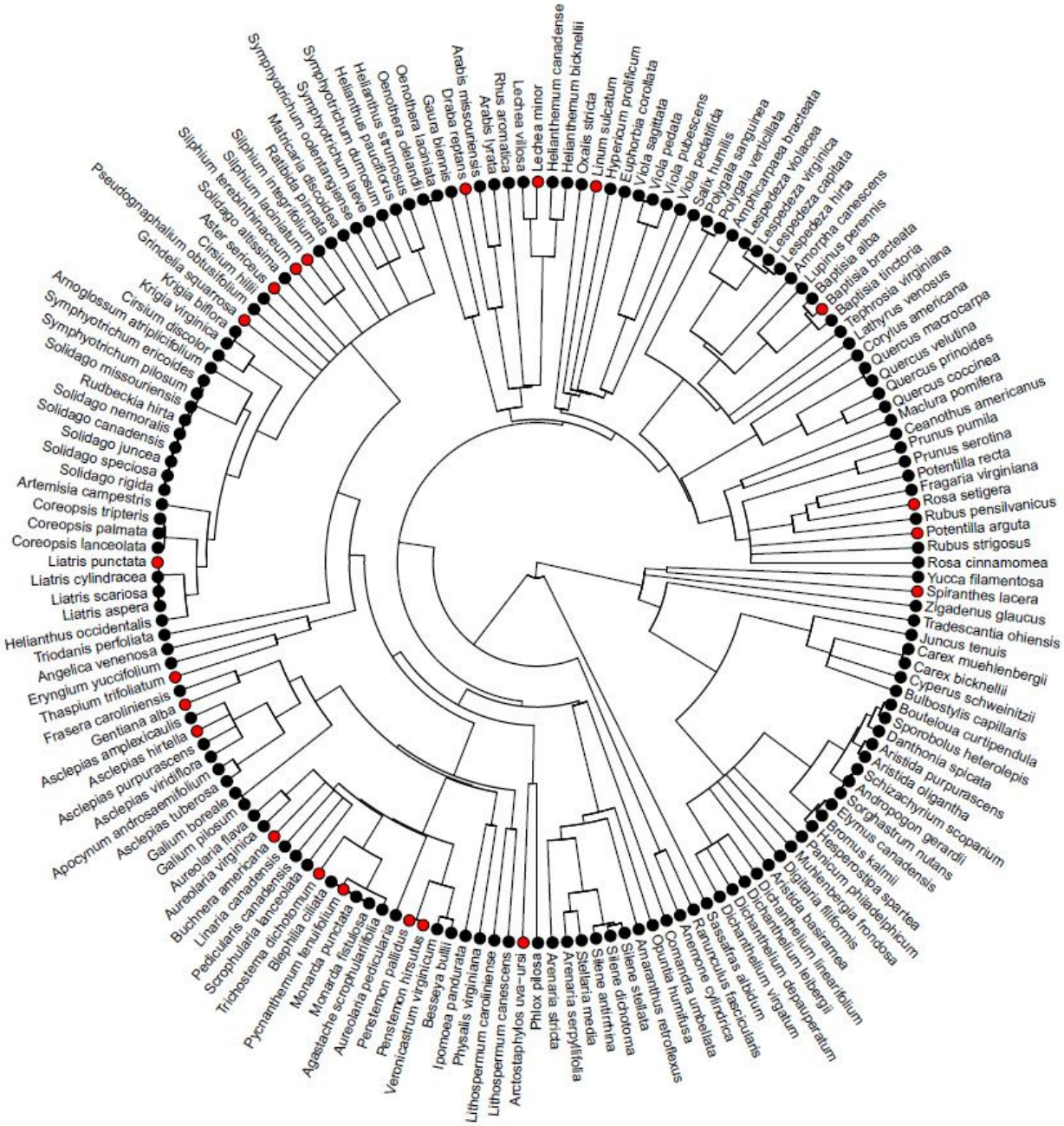
To control for phylogenetic non-independence, we obtained a phylogenetic tree for the prairie species of Kalamazoo County from Phylomatic (phylodiversity.net/phylomatic), using the Zanne et al. (2014) tree (Fig. 1.1; Webb and Donohue, 2005; Webb et al., 2008). We only provide a phylogenetic analysis of prairie species due to incomplete phylogenetic data for species from the other habitat types. We first tested whether each binary species characteristic

was phylogenetically conserved the 'phylo.d' function in the R package 'caper' (v1.0.1; Fritz and Purvis, 2010). 'Phylo.d' calculates the *D* statistic, a test statistic that compares the observed phylogenetic signal in a binary trait with the signal under a continuous Brownian motion model of trait evolution and applies a threshold: if species have a continuous trait value above the threshold, they are assigned a score of 0 and those whose trait value is below the threshold are assigned a score of 1 (Fritz and Purvis 2009). D=1 indicates a random signal while D=0 indicates conservatism. Pr(Brownian) provides the probability that the binary trait state results from a Brownian (non-random) phylogenetic structure. For non-binary traits and characteristics, we tested for phylogenetic conservatusm using the 'phylosignal' function in the package 'picante' in R (version 1.3.0; Kembel et al., 2010), following Fitzpatrick et al. (2008) and Saar et al. (2012). 'Phylosignal' measures Blomberg's K, a test statistic that also compares the observed phylogenetic signal in a trait with the signal under a Brownian motion model of trait evolution (Blomberg et al., 2003). K=0 indicates random or convergent evolution; K=1 indicates trait conservatism; K>1 indicates species being more similar than expected. Groups with a PIC.variance of $p \leq 0.05$ show phylogenetic signal (Blomberg et al., 2003).

To control for phylogenetic correlations, we performed phylogenetic logistic regression (Paradis and Claude, 2002; Ives and Garland, 2010; Daru et al., 2013). We again performed backwards elimination, in a manner similar to Purvis et al. (2000a). The original model again included status as the response variable and rarity, species status, range edge, habitat specialization, life history, growth form, N-fixation, and photosynthetic pathway, as well as interactions between rarity x species origin, rarity x habitat specialization, rarity x N-fixation, and rarity x range edge (the interactions described above) as predictor variables. Our final model

included rarity and habitat specialization as predictor variables (Table S1.6). The model was fit using the 'phyloglm' function in the 'phylolm' package in R (version 2.5; Ho and Ane, 2014).

Figure 1.1. Phylogeny of prairie species in Kalamazoo County, MI. Red circles indicate species that went locally extinct in the county between 1890-1990. Proportion of species extinct within a family are as follows: Asclepiadaceae 1/5, Cistaceae 1/4, Compositae 5/37, Ericaceae 1/1, Fabaceae 1/12, Gentianaceae 1/2, Labiatae 2/6, Linaceae 1/1, Orchidaceae 1/1, Rosaceae 2/9, Schrorphulariaceae 3/11, Umbelliferae 1/3.



RESULTS

Kalamazoo County

43 species (3.79% of the flora) are documented to have disappeared from Kalamazoo County from the early to late twentieth century (McKenna, 2004).

Species characteristics were associated with extinction across the county. Prairie species experience high rates of loss, as do habitat specialists, species at the edge of their native range (Table 1.1; Fig. 1.2a-c), and regionally rare species (Table 1.1; Fig. 1.3a). Forbs and vines tend to experience high rates of loss (Table 1.1; Fig. 1.2d). Prairie species become locally extinct even when relatively common (Table 1.1; Fig. 1.4), and the local extinction of N-fixing species depends on rarity (Table 1.1). For both N-fixers and non-N-fixers, rare species tend to go extinct more often; this is especially true for N-fixers. However, this rarity x N-fixation interaction should be interpreted cautiously because only two N-fixing species went extinct in Kalamazoo County from 1890-2003, and growth form and N-fixation are highly correlated (Table S1.2).

Table 1.1. Results from three separate analyses testing effects of species characteristics and traits on the status (locally extinct/non-extinct) of species in Kalamazoo County, MI. The analyses include: 1) all species (backwards elimination on a generalized linear model (GLM), binomial distribution); 2) prairie species (backwards elimination on a GLM, binomial distribution); 3) prairie species (phylogenetic logistic regression (phyloglm), binomial distribution). ***p≤0.0001; **p≤0.01; *p≤0.05; ·p≤0.1.

Source	df	All species (GLM) χ^2	Prairie species (GLM) χ^2	Prairie species (phyloglm) Z-value
Community association	3	12.20 **		
Rarity	1	11.25 ***	6.04 *	-2.63 **
Habitat specialization	3	25.10 ***	18.87 ***	-3.12 **
N-fixation	1	3.63 ·	2.46	
Growth form	4	14.34 **	13.96 **	
Range edge	1	3.97 *		
Rarity x community association	1	14.41 **		
Rarity x N-fixation	3	5.82 *	4.98 *	

Figure 1.2. Proportion of species (least square means ± SE from the backwards elimination-generalized linear model for Kalamazoo County) that went locally extinct in Kalamazoo County from 1890-1990 by each species characteristic: (a) community association, (b) habitat specialization (number of habitat types occupied), (c) range edge (position of MI relative to the edge of a species' native range), and (d) growth form. Letters represent significant pairwise differences at the α=0.05 level. Values in parentheses represent the number of extinct species over the total number of species in that group.

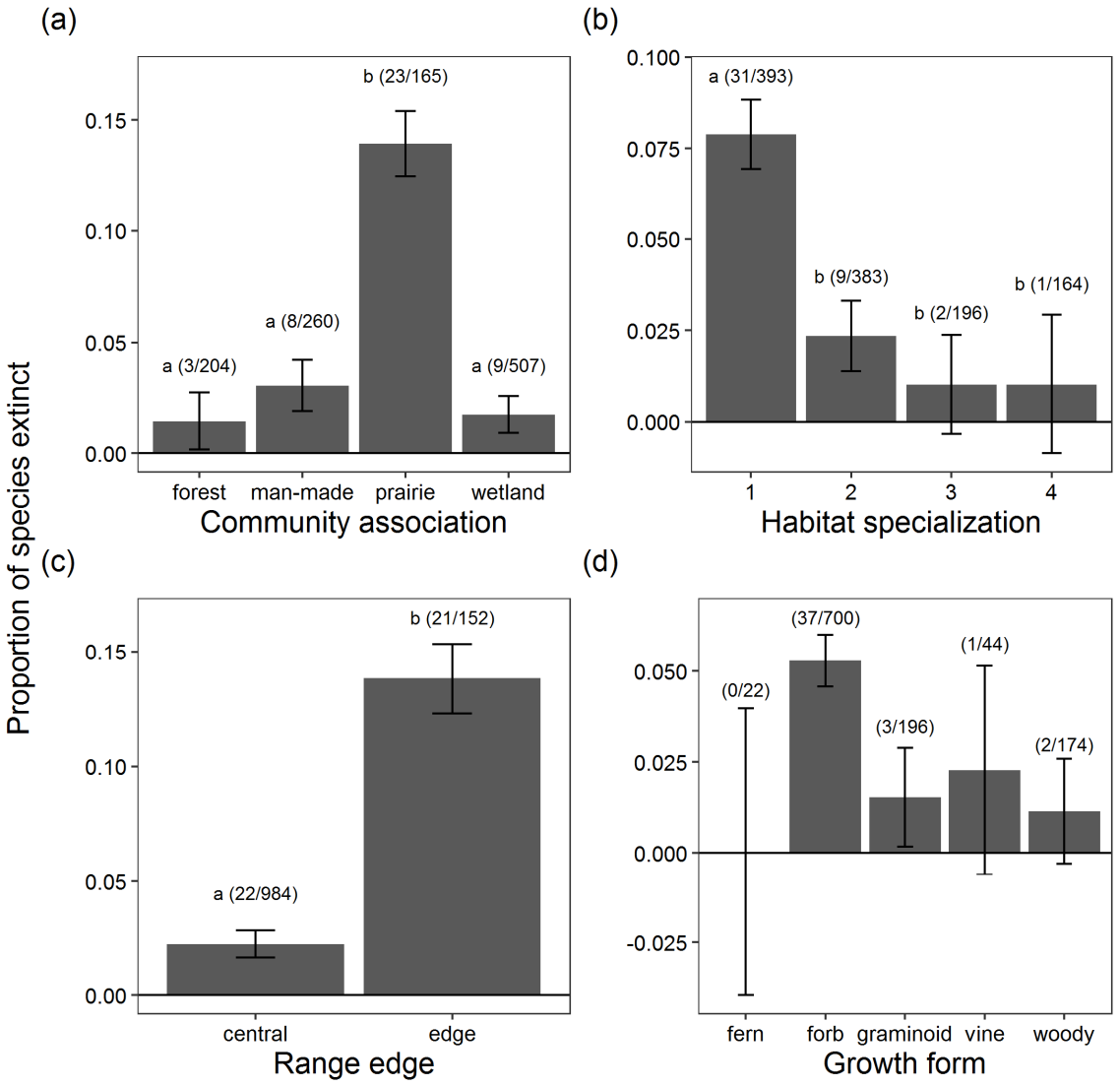


Figure 1.3. Effect of rarity (proportion of MI counties a species is found in) on the status (locally extinct/non-extinct) of (a) all species in Kalamazoo County and (b) prairie species in Kalamazoo County.

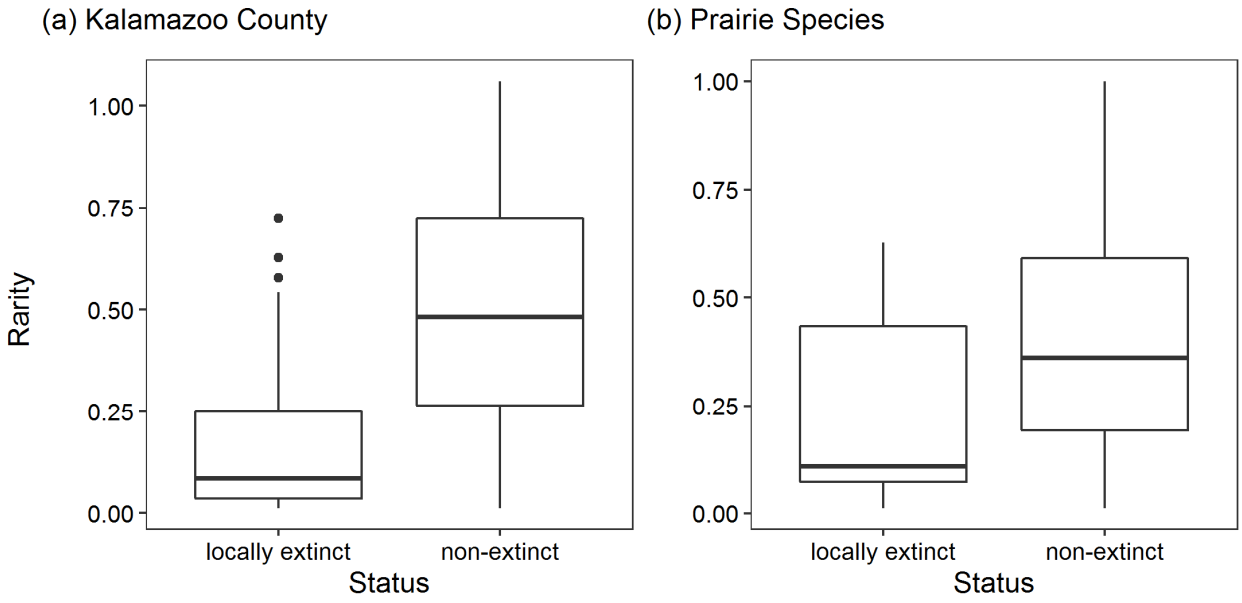
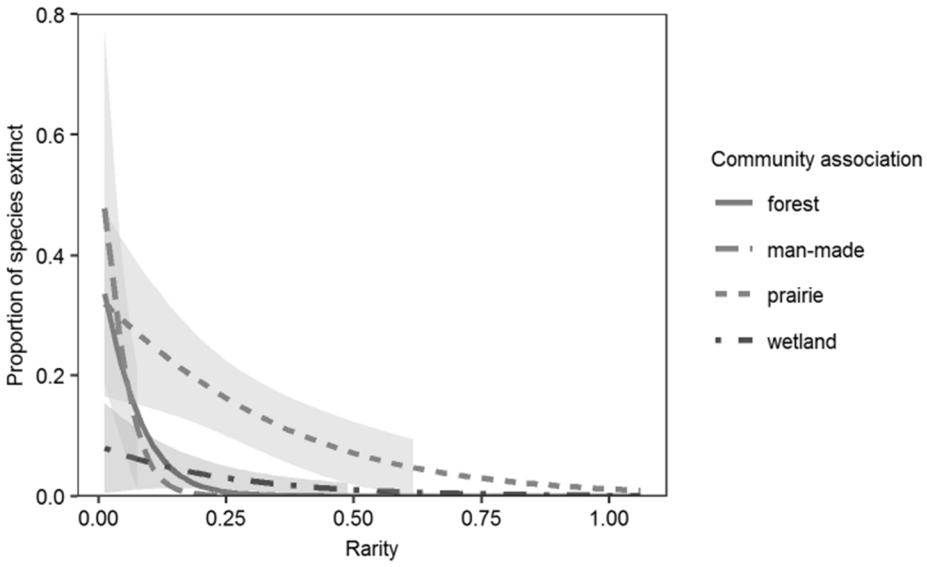


Figure 1.4. Effect of rarity (proportion of MI counties a species is found in) and community association on the proportion of species that went locally extinct in Kalamazoo County from 1890-1990. Shaded areas represent 95% confidence intervals.



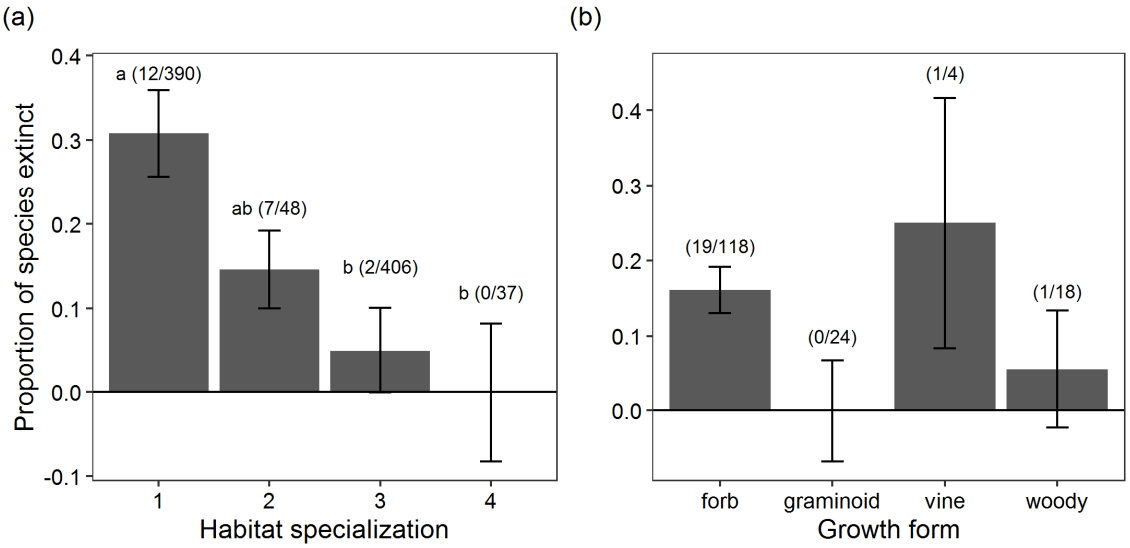
Prairie Species

Prairie species experience more extinction events than species found in forests, wetlands, and man-made community types (Table 1.1; Fig. 1.2a). Of the 164 prairie species found in Kalamazoo County, 23 (14.02%) became locally extinct between the 1890s-1990s. In comparison, 0.03% of species found in man-made habitats, 0.02% of wetland species, and 0.01% of forest species disappeared in the same timeframe (Fig. 1.2a).

Regionally rare species and habitat specialists (species found in 1-2 habitat types) are more likely to become locally extinct than more common and generalist prairie species (Table 1.1; Figs. 1.3b, 1.5a). Growth form also significantly affected extinction, with forbs and vines tending to have higher extinction rates than graminoids and woody species (Table 1.1; Fig. 1.5b). Rare non-nitrogen fixing species are more at risk of extinction (Table 1.1), but N-fixation is again highly correlated with growth form (Table S1.2), so this finding should be interpreted cautiously.

When accounting for phylogeny, only rarity and habitat specialization influence prairie species' extinction. Extinction status is randomly distributed across the phylogeny (status D=0.827; Fig. 1.1; Table S1.7). Life history, growth form, N-fixation, and photosynthetic pathway are phylogenetically conserved, but range edge, native origin, habitat specialization, and rarity are not (Table S1.7).

Figure 1.5. Proportion of prairie species (least square means ± SE from the backwards elimination-generalized linear model for prairie species) that went locally extinct in Kalamazoo County between 1890-1990 by (a) habitat specialization (number of habitat types occupied) and (b) growth form. Letters represent differences at the α=0.05 level. Values in parentheses represent the number of extinct species over the total number of species in that group.



DISCUSSION

Community association, habitat specialization, and regional rarity influence local plant extinctions in Kalamazoo County, Michigan. Across the county, prairie species, forbs and vines, species at the edge of their native range, and rare species experience high rates of loss. Among prairie species, the habitat type experiencing half of the observed extinctions, rare species and habitat specialists become extinct most often when controlling for evolutionary relationships. Despite the fact that most species traits are phylogenetically conserved, we detect no phylogenetic signal in extinction. By using historical botanical records, this work documents regional extinction events and identifies species traits and characteristics associated with extinctions in grassland habitats. Furthermore, it demonstrates how herbaria, which are still underutilized in studies of biodiversity loss and habitat conversion (Meineke et al. 2018), can help identify at-risk species and guide conservation of rare species.

Habitat loss as a driver of extinction

Prairie species were most likely to become extinct between 1890-1990 in Kalamazoo County relative to species from forest, wetland, and man-made habitats. Once among the most abundant plant communities in Kalamazoo County (consisting of more than 149302 acres in the 1820s [McKenna, 2004]), prairie and savanna habitat is now one of the most threatened in southwestern Michigan, as nearly all of Michigan's prairies were destroyed or altered by agriculture or development by 1980 (Chapman, 1984). Habitat succession due to lack of fire has also contributed to loss of prairie habitat (Chapman, 1974). Today, prairie remnants constitute less than 0.1% of Michigan's historical acreage (Chapman and Brewer, 2008), and prairies and savannas are essentially extinct in Kalamazoo County (McKenna, 2004). For reference, terrestrial forest and wetlands covered 154445 and 131600 acres prior to European settlement, respectively, and Kalamazoo has lost approximately 50% of its forests and 35% of its wetlands (www.landscope.org/michigan/overview). Habitat loss and destruction represent the leading cause of biodiversity loss (Vitousek et al., 1997; Wilcove et al., 1998, Mace et al., 2005; Newbold et al., 2012); they contribute to loss of suitable area, fragment the landscape and degrade habitat quality (Fahrig, 1997), all of which may affect species survivorship, establishment, and spread (Pimm, 1991; Tilman et al., 1994; Thompson et al., 1998; Leckie et al., 2000; Baskin and Baskin, 2001; Bellemare et al., 2002; Henle et al., 2004; Honnay et al., 2005; Halley et al., 2016; Nualart et al., 2017; Ceia-Hasse et al., 2018). Therefore, similar to studies wherein species experiencing high rates of habitat loss disappeared from New Zealand (Duncan and Young, 2000), Spain (Aedo et al., 2015), Switzerland (Lienert et al., 2002), England (Walker and Preston, 2006), and European grasslands (Auffret et al., 2018), the loss of 14% of the county's prairie species is likely due to the disproportionate amount of prairie habitat

lost in the 19th -20th centuries. This loss of natural grasslands and their biodiversity reflects the ongoing conversion of historically prairie-dominated landscapes across the Midwestern US, and the effects of past and continuing habitat loss and changes in land use will likely cause further contemporary declines of vulnerable species (Watson et al., 2016; Auffret et al., 2018).

Species characteristics and extinction risk

Habitat specialists were more likely to disappear from Kalamazoo County, consistent with the hypothesis that specialization correlates with extinction risk (Jablonski, 1994; Erwin and Anstey, 1995; McKinney, 1997; Purvis et al., 2000b) and supporting results from previous work on plant extinctions (Rich and Woodruff, 1996; Fisher and Stöcklin, 1997; Preston, 2000; Preston et al., 2002; Kolb and Diekmann, 2004; Davies et al., 2004; Walker and Preston, 2006). The highest predicted extinction rate for generalist species is 7%, while 43% of specialists are predicted to go extinct, as determined from an analysis of extinction risk for endemic plant and vertebrate species based on habitat specificity (Malcolm et al., 2006; Pereira et al., 2010). Species with smaller range sizes have also been shown to be at higher risk, which may be correlated with habitat specificity (Bennett and Owens, 1997; Russell et al., 1998; Purvis et al., 2000a; Lienert et al., 2002; Cardillo, 2003; Fisher and Owens, 2004; Cooper et al., 2008). This decline in habitat specialists could be due to habitat rarity, given that prairie, while once abundant in Kalamazoo County, had a limited range across Michigan (Chapman and Brewer, 2008). Alternatively, specialist declines could be due to habitat loss: as prairie habitat remnants disappear or are altered by agriculture and invasion, prairie specialists that are unable to disperse to and survive in other habitat types slowly disappear (Diamond, 1984; Lawton and May, 1995; Owens and Bennett, 2000; Purvis et al., 2000c; Kotze and O'Hara, 2003; Kotiaho et al., 2005; Auffret et al., 2018).

The random-loss hypothesis predicts that rare species account for most species' declines, partially due to random loss of individuals as density declines (Goldberg and Miller, 1990; Oksanen, 1996; Stevens and Carson, 2002). Although we estimated rarity based on geographic spread rather than population density, as the cited studies do, our results support the idea that rare species are often lost regardless of their characteristics, both at the county level and within prairie species. This is consistent with other studies in New Zealand (Duncan and Young, 2000), rural and semi-urban grasslands in Australia (Williams et al., 2005), Minnesota grasslands (Suding et al., 2005), and the Balearic Islands (Lapiedra et al., 2015). However, some rare species may have been more likely to appear extinct due to observation error.

Finally, species at the edge of their native range may be at higher risk of extinction, as found in Switzerland (Lienert et al., 2002) and New England (Farnsworth and Ogurcak, 2008). This may be because southwestern Michigan is at the edge of a floristic zone: it is both the northeastern-most edge of tallgrass prairie habitat and a climatic transition zone between northern oak-hickory forests and southern hardwood forests (McCann, 1979).

Species traits and extinction risk

Growth form also may influence extinction. Forbs and vines tend towards higher rates of loss than woody or graminoid species for both the county and prairie species. Forbs and low growth forms also are more prone to loss in Wisconsin forests and grasslands, Britain, and Australia (Leach and Givnish, 1996; Blomqvist et al., 2003; Williams et al., 2005; Weigmann and Waller, 2006; Smart et al., 2005; Sjöström and Gross, 2006; Fréville et al., 2007; Saar et al., 2012), and vines had a higher probability of extinction over a 122-year period in New York (Robinson et al., 1994) and in Brazilian rain forests (Leão et al., 2014). Meanwhile, graminoid and woody species tend to persist and/or increase in abundance (Robinson et al., 1994; Turner et

al., 1996; Williams et al., 2005). In our study, the relationship between growth form and extinction disappeared when phylogenetic relationships were controlled for, likely due to the high phylogenetic conservatism of growth form. Although some of the above cited studies considered phylogenetic patterns of extinction, many do not account for phylogenetic conservatism in their analyses, so the tendency for growth form to influence extinction should be investigated further (Leão et al., 2014). We also found that rare non-nitrogen fixing species are at risk, but nitrogen-fixation and growth form are highly correlated traits and this association may reflect loss of forbs.

An avenue for future research in this system is to examine how the species traits associated with local extinctions compare to those of species introduced during the same timeframe. Here we do not examine species introductions due to the potential bias of missing species during the original surveys. However, the 1990s surveys report that more than 400 species are non-native, and 133 species found in the 1990s may represent new invasions as they were not reported in 1947, although they may have been missed during the original survey (McKenna, 2004). Comparing the functional traits of invasive versus extinct species would inform whether invasive species are replacing extinct species or filling a vacant niche in the invaded habitat, as functional diversity is predicted to either remain the same (Tecco et al., 2009) or decline with the extinction of local plant species and addition of invasive species (Carvallo and Castro, 2017).

Phylogenetic patterns of extinction

Phylogenetic patterns did not explain extinction of prairie species in Kalamazoo County. Fréville et al. (2007) and Fitzpatrick et al. (2008) detected no phylogenetic pattern to extinction in 93 species over 60 years and 100 species over 80 years, respectively; we similarly detect no

phylogenetic pattern in 164 prairie species over approximately 100 years. It is possible that our failure to detect a phylogenetic signal in extinction risk resulted from small sample size and short timeframe (most studies detecting phylogenetic signal exceed 500 species). However, extinction may show less phylogenetic signal if species are highly susceptible to a general driver of risk such as habitat loss (Fritz and Purvis, 2010; Daru et al., 2013) rather than to specific anthropogenic changes that might select against particular traits (e.g., nitrogen addition selecting against nitrogen-fixers [Suding et al., 2005]), although phylogenetic signals in extinction risk have been detected when habitat loss is suspected to be a primary driver of extinction in some cases (e.g., Schachat et al., 2016, Mankga and Yessoufou, 2017). Alternatively, the traits important to extinction in this area may not be phylogenetically conserved and may not be measured in this study. We find that several non-conserved traits related to species distribution, including rarity and habitat specialization, predict extinction in our dataset, which may explain the random phylogenetic pattern to extinction in Kalamazoo County.

Conclusions

Our results illustrate how historical collections can be used more extensively to examine patterns of regional and local species losses and to help identify species characteristics and traits associated with susceptibility to loss.

Given the susceptibility of prairie species to extinction and the likely importance of land use change as an extinction driver in this region, restoration may be one mechanism to prevent further extinctions or even to reintroduce many of the extinct taxa in this region. Indeed, locally extinct species have been planted into restored prairies in Kalamazoo County. In a study of 29 prairies across southwest Michigan, eight extinct species were included in restoration seed

mixes, and three of the eight (*Silphium laciniatum, Silphium terebinthinaceum,* and *Echinacea purpurea)* were able to establish in a substantial proportion of sites (7 of 13, 4 of 10, and 22 of 23, respectively) (Grman et al., 2015). It remains to be seen whether the recent increase in prairie restoration will slow or reverse the declines of these taxa.

ACKNOWLEDGEMENTS

The authors thank T. Barkman for advice on Kalamazoo botanical records, M. Weber for advice on phylogenetic analyses, M. Muzyka for help with data visualization, and L. Brudvig, E. Zipkin, E. Litchman, the Lau lab, and three anonymous reviewers for feedback on this manuscript. This is Kellogg Biological Station contribution no. 2123.

APPENDIX

Table S1.1. Plant community types. Plant community types found in Kalamazoo County, MI, following McKenna (2004).

Community type	Habitats categorized into broader community types
Forest	Sugar Maple Forest, Oak Hardwood Forest
Prairie	Prairie, Black Oak Barren, Bur Oak Savanna, White Oak Savanna
Wetland	Submergent/Emergent/Coastal Plain Marsh, Wet Meadow, Bog, Fen, Wet Prairie, Shrub Swamp, Shrub Car, Tamarack/Red Maple/Black Ash/Hardwood Swamp, Floodplain Forest
Man-made	Roads, railroad right-of-ways, old fields, gardens, degraded or urban landscapes

Table S1.2. Chi-Square Tests of Independence. Results of Chi-Square Tests of Independence. Characteristics were considered correlated if $p \leq 0.05$ (bold and indicated by *).

	Species origin	**Life history**	**Growth form**	**Range edge**	**Habitat specialization**	**N-fixation**	**C3/4**	**Rarity**
Kalamazoo County								
Community association	**496.58***	**164.31***	**57.04***	15.35	**141.76***	**20.28***	**32.72***	281.53
Species origin		**122.98***	**12.44***	**12.74***	**41.26***	**4.06***	$1.61e^{-29}$	100.09
Life history			**86.32***	**11.03***	**40.55***	**16.60***	**59.89***	315.50
Growth form				3.98	**29.73***	**59.18***	**229.81***	345.76
Range edge					**28.09***	$9.31e^{-31}$	0.2018	**281.64***
Habitat specialization						2.94	**14.21***	**423.69***
N-fixation							1.972	**112.78***
C3/4								94.50
Prairie Species								
Species origin		6.05	1.36	$3.01e^{-30}$	3.25	$9.23e^{-29}$	0.04	71.79
Life history			7.99	0.82	11.55	1.76	5.63	**333.3***
Growth form				2.65	16.3	**12.03***	**103.11***	174.31
Range edge					5.11	0.33	0.58	73.361
Habitat specialization						3.51	**9.38***	297.54
N-fixation							0.7345	49.14
C3/4								59.88

Table S1.3. Backwards elimination for Kalamazoo dataset. Akaike Information Criterion (AIC) and p-values from each model run during backwards elimination on the generalized linear model (binomial distribution) for the Kalamazoo dataset. The predictor with the highest p-value was sequentially removed until all $p \leq 0.05$, although a main effect was not removed if an interaction term with that variable was still included.

Model	**AIC**	**Variable removed for next model (p-value)**
Model 1. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Rarity:Edge + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix + C3/4	250.67	Rarity:Specialization (0.94)
Model 2. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Rarity:Edge + Specialization + N-fix + Rarity:N-fix + C3/4	250.68	Life history (0.62)
Model 3. Rarity + Origin + Rarity:Origine + Growth form + Edge + Rarity:Edge + Specialization + N-fix + Rarity:N-fix + C3/4	245.32	Rarity:Origin (0.47)
Model 4. Rarity + Origin + Growth form + Edge + Rarity:Edge + Specialization + N-fix + Rarity:N-fix + C3/4	243.84	Origin (0.59)
Model 5. Rarity + Growth form + Edge + Rarity:Edge + Specialization + N-fix + Rarity:N-fix + C3/4	242.13	Rarity:Edge (0.33)
Model 6. Rarity + Growth form + Edge + Specialization + N-fix + Rarity:N-fix + C3/4	241.09	C3/4 (0.23)
Model 7. Rarity + Growth form + Edge + Specialization + N-fix + Rarity:N-fix	240.51	Final model.

Table S1.4. Multiple Correspondence Analysis Dimensions. The 5 dimensions identified by a Multiple Correspondence Analysis (MCA), which reduces large sets of associated categorical variables into smaller sets of components that summarize the information in the data without any underlying assumptions about the data's distribution[2]. Because a MCA cannot include continuous variables, we binned species into three rarity categories: rare (found in 1-25% of counties in MI), occasional (found in 26-75% of MI counties), and common (found in 76-100% of MI counties).Values represent the contribution of each categorical species characteristic to each dimension; higher values represent more importance on that dimension. We also provide the eigenvalue and cumulative proportions of the variance in status (locally extinct/non-extinct) explained by each dimension. The first dimension represents the largest deviation from independence[3], and the number of dimensions used in analysis should represent > 70% of the variance[4].

Species characteristic	**Dimension 1**	**Dimension 2**	**Dimension 3**	**Dimension 4**	**Dimension 5**
Community	0.6723	0.1355	0.1028	0.3185	0.1485
Species origin	0.5595	0.0915	0.0042	0.0109	0.0099
Range edge	0.0082	0.2359	0.2109	0.0676	0.0400
Habitat specialization	0.2266	0.1217	0.1396	0.3385	0.0974
Life history	0.4486	0.1040	0.0575	0.1637	0.1263
Growth form	0.1009	0.3668	0.3038	0.1985	0.5985
N-fixer	0.0061	0.0151	0.2230	0.1338	0.1753
C3/4	0.0937	0.3244	0.1666	0.0418	0.0278
Rarity	0.0669	0.3311	0.2337	0.0497	0.0033
Eigenvalue proportion of variance explained	0.2426	0.1918	0.1602	0.1450	0.1363
Cumulative proportion of variance explained	0.2426	0.4344	0.5946	0.7396	0.8759

[2] Abdi, H., and D. Valentin. 2007. Multiple Correspondence Analysis. *In* N. Salkind [ed.], Encyclopedia of Measurement and Statistics. Sage, Thousand Oaks, CA, USA.

[3] Sourial, N., C. Wolfson, B. Zhu, J. Quail, J. Fletcher, S. Karunananthan, K. Bandeen-Roche, et al. 2010. Correspondence analysis is a useful tool to uncover the relationships among categorical variables. *Journal of Clinical Epidemiology* 63: 638-646.

[4] Higgs, N.T. 1991. Practical and innovative uses of correspondence analysis. *The Statistician* 40: 183-194.

Table S1.5. Multiple Correspondence Analysis Results. Effects of Multiple Correspondence Analysis Dimensions 1-5 (see Table S1.4) on species status (locally extinct/non-extinct) in Kalamazoo County, MI (generalized linear model, binomial distribution). We performed MCA analyses using the 'FactoMineR' and 'ade4' packages in R[5,6,7,8]. ***p≤0.0001.

Source	df	Chi-Square
Dimension 1	1	2.394
Dimension 2	1	12.790 ***
Dimension 3	1	36.515 ***
Dimension 4	1	1.059
Dimension 5	1	11.212 ***

[5] Tenenhaus, M., and F.W. Young. 1985. An analysis and synthesis of multiple correspondence analysis, optimal scaling, dual scaling, homogeneity analysis and other methods for quantifying categorical multivariate data. *Psychometrika* 50: 91-119.
[6] Lebart, L., A. Morineau, and M. Piron. 1995. *Statistique exploratoire multidimensionnelle*. Dunod, Paris.
[7] Le, S., J. Josse, and F. Husson. 2008. FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software* 25: 1-18.
[8] R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Table S1.6. Backwards elimintation for prairie species dataset. Akaike Information Criterion (AIC) and p-values from each model run during backwards elimination on the (A) generalized linear model (binomial distribution) and (B) phylogenetic logistic regression for prairie species. The predictor with the highest p-value was sequentially removed until all $p \leq 0.05$, although a main effect was not removed if an interaction term with that variable was still included. Note that AIC is not used to select models during backwards elimination.

(A) Generalized Linear Model		
Model	**AIC**	**Variable removed for next model (p-value)**
Model 1. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Rarity:Edge + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix + C3/4	100.81	Rarity:Origin (1)
Model 2. Rarity + Origin + Growth form + Life history + Edge + Rarity:Edge + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix + C3/4	98.81	Rarity:Edge (0.8)
Model 3. Rarity + Origin + Growth form + Life history + Edge + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix + C3/4	96.86	Edge (0.66)
Model 4. Rarity + Origin + Growth form + Life history + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix + C3/4	95.04	C3/4 (0.49)
Model 5. Rarity + Origin + Growth form + Life history + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix	93.50	Life history (0.41)
Model 6. Rarity + Origin + Growth form + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix	89.51	Rarity:Specialization (0.35)
Model 7. Rarity + Origin + Growth form + Specialization + N-fix + Rarity:N-fix	88.35	Origin (0.45)
Model 8. Rarity + Growth form + Specialization + N-fix + Rarity:N-fix	86.94	Final model.
(B) Phylogenetic Logistic Regression		
Model	**AIC**	**Variable removed for next model (p-value)**
Model 1. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Rarity:Edge + Specialization + Rarity:Specialization + N-fix + Rarity:N-fix + C3/4	120.80	Rarity:N-fix (0.98)
Model 2. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Rarity:Edge + Specialization + Rarity:Specialization + N-fix + C3/4	116.81	Rarity:Edge (0.97)
Model 3. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Specialization + Rarity:Specialization + N-fix + C3/4	117.28	Rarity:Origin (0.95)
Model 4. Rarity + Origin + Rarity:Origin + Growth form + Life history + Edge + Specialization + Rarity:Specialization + N-fix + C3/4	111.93	Rarity:Specialization (0.85)
Model 5. Rarity + Origin + Growth form + Life history + Edge + Specialization + Rarity:Specialization + N-fix + C3/4	116.92	Life history (0.88)
Model 6. Rarity + Origin + Growth form + Edge + Specialization + Rarity:Specialization + N-fix + C3/4	108.57	N-fixation (0.9)
Model 7. Rarity + Origin + Growth form + Edge + Specialization + Rarity:Specialization + C3/4	104.84	Growth form (0.99)

Table S1.6. (cont'd)

Model 8. Rarity + Origin + Edge + Specialization + Rarity:Specialization + C3/4	100.24	Origin (0.25)
Model 9. Rarity + Edge + Specialization + Rarity:Specialization + C3/4	112.22	Edge (0.26)
Model 10. Rarity + Specialization + Rarity:Specialization + C3/4	100.25	C3/4 (0.13)
Model 11. Rarity + Specialization + Rarity:Specialization	103.34	Final model.

Table S1.7. Phylogenetic signal of species traits and characteristics. Phylogenetic signal of species traits and characteristics. We provide the *D* statistic for binary characteristics. *D*=1 indicates a random signal while *D*=0 indicates conservatism. Pr(Brownian) provides the probability that the binary trait state results from a Brownian (non-random) phylogenetic structure. We provide Blomberg's *K* statistic for non-binary characteristics. *K*=0 indicates a random pattern of evolution, *K*=1 indicates conservatism of traits, and *K*>1 indicates greater similarity than expected. PIC.variance tests for greater phylogenetic signal than expected; *p≤0.05 indicates non-random signal.

Functional group	D statistic	Pr(Brownian)	K statistic	PIC.variance (p-value)
Status	0.827	0.001		
Species origin	0.798	0.097		
Range edge	1.072	0.000		
Nitrogen fixation	-1.330	0.998		
Photosynthetic pathway (C3/4)	-0.781	0.976		
Life history			0.066	0.046*
Growth form			1.108	0.001*
Habitat specialization			0.026	0.286
Rarity			0.029	0.099

LITERATURE CITED

LITERATURE CITED

Abdi, H., and D. Valentin. 2007. Multiple Correspondence Analysis. *In* N. Salkind [ed.], Encyclopedia of Measurement and Statistics. Sage, Thousand Oaks, CA, USA.

Aedo, C., L. Medina, P. Barberá, and M. Fernández-Albert. 2014. Extinctions of vascular plants in Spain. *Nordic Journal of Botany* 33: 83-100.

Arregoitia, L.D.V., S.P. Blomberg, and D.O. Fisher. 2013. Phylogenetic correlates of extinction risk in mammals: species in older lineages are not at greater risk. *Proceedings of the Royal Society B* 280: 20131092.

Auffret, A.G., A. Kimberley, J. Plue, and E. Waldén. 2018. Super-regional land-use change and effects on the grassland specialist flora. *Nature Communications* 9: 3464.

Bai, Y.J., X.P. Wei, and C.Q. Li. 2018. Distributional dynamics of a vulnerable species in response to past and future climate change: a window for conservation prospects. *Peer J* 6: e4287.

Barnosky, A.D., N. Matzke, S. Tomiya, G.O.U. Wogan, B. Swartz, T.B. Quental, C. Marshall, et al. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51-57.

Baskin, C.C., and J.M. Baskin. 2001. *Seeds: ecology, biogeography, and evolution of dormancy and germination.* Academic Press, San Diego, CA, USA.

Bellemare, J., G. Motzkin, and D.R. Foster. 2002. Legacies of the agricultural past on forested present: an assessment of historical land-use effects on rich mesic forests. *Journal of Biogeography* 29: 1401-1420.

Bennett, P.M., and I.P.F. Owens. 1997. Variation in extinction risk among birds: chance or evolutionary predisposition? *Proceedings of the Royal Society B* 264: 401-408.

Bertin, R.I. 2002. Losses of native plant species from Worcester, Massachusetts. *Rhodora* 104: 325-349.

Bielby, J., A.A. Cunningham, and A. Purvis. 2006. Taxonomic selectivity in amphibians: ignorance, geography or biology? *Animal Conservation* 9: 135-143.

Blomberg, S.P., T. Garland, and A.R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717-745.

Blomqvist, M.M., P. Vos, P.G.L. Klinkhamer, and W.J. ter Keurs. 2003. Declining plant species richness of grassland ditch banks—a problem of colonisation or extinction? *Biological Conservation* 109: 391-406.

Boakes, J.E.J.H., N.J.B. Issac, R.A. Fuler, G.M. Mace, and P.J.K. McGowan. 2017. Examining the relationship between local extinction risk and position in range. *Conservation Biology* 32: 229-239.

Brook, B.W., N.S. Sodhi, and C.J.A. Bradshaw. 2008. Synergies among extinction drivers under global change. *Trends in Ecology and Evolution* 23: 453-460.

Cardillo, M. 2003. Biological determinants of extinction risk: why are smaller species less vulnerable? *Animal Conservation* 6: 63-69.

Cardillo, M., G.M. Mace, J.L. Gittleman, and A. Purvis. 2006. Latent extinction risk and the future battlegrounds of mammal conservation. *Proceedings of the National Academy of Sciences* 103: 4157-4161.

Carvallo, G.O., and S.A. Castro. 2017. Invasions but not extinctions change phylogenetic diversity of angiosperm assemblage on southeastern Pacific Oceanic islands. *PLoS ONE* 12: e0182105.

Ceia-Hasse, A., L.M. Navarri, L. Borda-de-Água, and H.M. Pereira. 2018. Population persistence in landscapes fragmented by roads: Disentangling isolation, mortality, and the effect of dispersal. *Ecological Modelling* 375: 45-53.

Chapman, K.A. 1984. An ecological investigation of native grassland in southern Lower Michigan. M.A. Thesis, Western Michigan University, Kalamazoo, MI, USA.

Chapman, K.A., and R. Brewer. 2008. Prairie and savanna in southern lower Michigan: history, classification, ecology. *The Michigan Botanist* 47: 1-48.

Collins, C.D., R.D. Holt, and B.L. Foster. 2009. Patch size effects on plant species decline in an experimentally fragmented landscape. *Ecology* 90: 2577-2588.

Comer, P.J., D.A. Alberts, H.A. Wells, B.I. Hart, J.B. Raub, D.L. Price, D. M. Kashian, et al. 1995. Michigan's Native Landscape. As Interpreted from the General Land Office Surveys 1816-1856. 78 pp. + digital map. Michigan Natural Features Inventory, Lansing, MI, USA.

Comer, P.J., and D.A. Albert. 1997. Cartography: Michael B. Austin. Vegetation of Michigan Circa 1880: An Interpretation of the General Land Office Surveys 1816-1856. 2-Map Set, Scale 1:500000. Michigan Natural Features Inventory, Lansing, MI, USA.

Daru, N.H., K. Yessoufou, L.T. Mankga, and T.J. Davies. 2013. A global trend towards the loss of evolutionary unique species in Mangrove ecosystem. *PLoS ONE* 8: e66686.

Davidson, A.D., K.T. Shoemaker, B. Weinstein, G.C. Costa, T.M. Brooks, G. Ceballos, V.C. Radeloff, et al. 2017. Geography of current and future global mammal extinction risk. *PLoS ONE* 12: e0186934.

Davies, K.F., C.R. Margules, and J.F. Lawrence. 2004. A synergistic effect puts rare, specialized species at greater risk of extinction. *Ecology* 85: 265-271.

Davies T.J., G.F. Smith, D.U. Bellstedt, J.S. Boatwright, B. Bytebier, R.M. Cowling, F. Forest, et al. 2011. Extinction risk and diversification are linked in a plant biodiversity hotspot. *PLoS Biology* 9: e1000620.

Davies, T.J., and K. Yessoufou. 2013. Revisiting the impacts of non-random extinct on the tree-of-life. *Biology Letters* 20130343.

DeCandido, R., A.A. Muir, and M.B. Gargiullo. 2004. A first approximation of the historical and extant vascular flora of New York City: Implications for native plant species conservation. *Journal of the Torrey Botanical Society* 131: 243-251.

De Vos, J.M., L.N. Joppa, J.L. Gittleman, P.R. Stephens, and S.L. Pimm. 2015. Estimating the normal background rate of species extinction. *Conservation Biology* 29: 452-462.

Diamond, J. 1984. "Normal" extinctions of isolated populations. *In* M.H. Nitecki [ed.], Extinctions, pp. 191-246.

Doherty, P.F., T. Boulinier, and J.D. Nichols. 2003. Local extinction and turnover rates at the edge and interior of species' ranges. *Annales Zoologici Fennici* 40: 145-153.

Dukes, J.S., and H.A. Mooney. 1999. Does global change increase the success of biological invaders? *Trends in Ecology and Evolution* 14: 135-139.

Dolan, J.A., C.S. Moreau, and M.L.J. Stiassny. 2017. Digitization of museum collections holds the potential to enhance researcher diversity. *Nature Ecology and Evolution* 1: 1789-1790.

Duncan, R.P., and J.R. Young. 2000. Determinants of plant extinction and rarity 145 years after European settlement of Auckland, New Zealand. *Ecology* 81: 3048-3061.

Duncan, R.P., S.E. Clemants, R.T. Corlett, A.K. Hahs, M.A. McCarthy, M.J. McDonnell, M.W. Schwartz, et al. 2011. Plant traits and extinction in urban areas: a meta-analysis of 11 cities. *Global Ecology and Biogeography* 20: 509-519.

Erwin, D.H., and R.L. Anstey. 1995. Speciation in the fossil record. *In* D.H. Erwin and R.L. Anstey [eds.], New Approaches to Speciation in the Fossil Record, pp. 11-38. Columbia University Press, NY, USA.

Fahrig, L. 1997. Relative effects of habitat loss and fragmentation on population extinction. *The*

Journal of Wildlife Management 61: 603-610.

Farnsworth, E.J., and D.E. Ogurcak. 2008. Functional groups of rare plants differ in levels of imperilment. *American Journal of Botany* 95: 943-953.

Fischer, M., and J. Stöcklin. 1997. Local extinctions of plants in remnants of extensively used calcareous grasslands 1950-1985. *Conservation Biology* 11: 727-737.

Fisher, D.O., and I.P.F. Owens. 2004. The comparative method in conservation biology. *Trends in Ecology and Evolution* 19: 391-398.

Fitzpatrick, M.C., A.D. Gove, N.J. Sanders, and R.R. Dunn. 2008. Climate change, plant migration, and range collapse in a global biodiversity hotspot: the *Banksia* (Proteaceae) of Western Australia. *Global Change Biology* 14: 1-16.

Fréville, H., K. McConway, M. Dodd, and J. Silvertown. 2007. Prediction of extinction in plants: interaction of extrinsic threats and life history traits. *Ecology* 88: 2662-2672.

Fritz, S.A., and A. Purvis. 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology* 24: 1042-1051.

Goldberg, D.E., and T.E. Miller. 1990. Effects of different resource additions of species diversity in an annual plant community. *Ecology* 71: 213-225.

Grashof-Bokdam, C. 2009. Forest species in an agricultural landscape in the Netherlands: effects of habitat fragmentation. *Journal of Vegetation Science* 8: 21-28.

Grass, A., K. Tremetzberger, R. Hössinger, and K. Bernhardt. 2014. Change of species and habitat diversity in the Pannonian region of eastern lower Austria over 170 years: using herbarium records as a witness. *Natural Resources* 5: 583-596.

Gregor, T., D. Bönsel, I. Starke-Ottich, and G. Zizka. 2012. Drivers of floristic change in large cities-A case study of Frankfort/Main (Germany). *Landscape and Urban Planning* 104: 230-237.

Grman, E., T. Bassett, C.R. Zirbel, and L.A. Brudvig. 2015. Dispersal and establishment filters influence the assembly of restored plant communities. *Restoration Ecology* 23: 892-899.

Halley, J.M., N. Monogrousos, A.D. Mazaris, W.D. Newmark, and D. Vokou. 2016. Dynamics of extinction debt across five taxonomic groups. *Nature Communications* 7: 12283.

Hanes, C.R., and F.N. Hanes. 1947. *Flora of Kalamazoo County, Michigan: Vascular Plants.* Anthoensen Press, Portland, ME, USA.

Harcourt, A.H. 1998. Ecological indicators of risk for primates, as judged by species'

susceptibility to logging. *In* T. Caro [ed.], Behavioral Ecology and Conservation Biology, pp. 56-79. Oxford University Press, NY, USA.

Hartley, S., and W.E. Kunin. 2003. Scale dependency of rarity, extinction risk, and conservation priority. *Conservation Biology* 17: 1559-1570.

Henle, K., K.F. Davies, M. Kleyer, C. Margules, and J. Settele. 2004. Predictors of species sensitivity to fragmentation. *Biodiversity and Conservation* 13: 207-251.

Ho, L.S.T., and C. Ane. 2014. A linear-time algorithm for Gaussian and non-Gaussian trait evolution models. *Systematic Biology* 63: 397-408.

Honnay, O., H. Jacquemyn, B. Bossuyt, and M. Hermy. 2005. Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. *New Phytologist* 166: 723-736.

Ives, A.R., and T. Garland. 2010. Phylogenetic logistic regression for binary dependent variables. *Systematic Biology* 59: 9-26.

Jablonski, D. 1994. Extinctions in the fossil record. *Philosophical Transactions of the Royal Society B* 344: 11-16.

Jarnevich, C. S., T.J. Stohlgren, D. Barnett, and J. Kartesz. 2006. Filling in the gaps: modelling native species richness and invasions using spatially incomplete data. *Diversity and Distributions* 12: 511–520.

Johnson, C.N. 2002. Determinants of loss of mammal species during the Late Quaternary 'megafauna' extinctions: life history and ecology, but not body size. *Proceedings of the Royal Society B* 269: 2221-2227.

Jones, K.E., W. Sechrest, and J.L. Gittleman. 2005. Age and area revisited, identifying global patterns and implications for conservation. In: A. Purvis, J.L. Gittleman, T. Brooks [eds.], Phylogeny and Conservation, pp. 141-165. Cambridge University Press, Cambridge, UK.

Kembel, S.W., P.D. Cowan, M.R. Helmus, W.K. Cornell, H. Morlon, D.D. Ackerly, S.P. Blomberg, and C.O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463-1464.

Knapp, S., I. Kuhn, J. Stolle, and S. Klotz. 2010. Changes in the functional composition of a Central European urban flora over three centuries. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 235-244.

Kolb, A., and M. Diekmann. 2004. Effects of environment, habitat configuration and forest continuity on the distribution of forest plant species. *Journal of Vegetation Science* 15: 199-208.

Kotiaho, J.S., V. Kaitala, A. Komonen, and J. Päivinen. 2005 Predicting the risk of extinction from shared ecological characteristics. *Proceedings of the National Academy of Sciences* 102: 1963-1967.

Kotze, D.J., and R.B. O'Hara. 2003. Species decline—but why? Explanations of carabid beetle (Copeoptera, Carabidae) declines in Europe. *Oecologia* 135: 138-148.

Lang, P.L.M., F.M. Willems, J.F. Scheepens, H.A. Burbano, and O. Bossdorf. 2018. Using herbaria to study global environmental change. *New Phytologist* 221: 110-122.

Lapiedra, O., D. Sol, A. Traveset, and M. Vilà. 2015. Random processes and phylogenetic loss caused by plant invasions. *Global Ecology and Biogeography* 24, 774-785.

Lavorel, S., S. McIntyre, J. Landsberg, and T.D.A. Forbes. 2007. Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends in Ecology and Evolution* 12: 474-478.

Lawton, J.H., and R.M. May. 1995. Extinction. *Philosophical Transactions of the Royal Society B* 344: 1-104.

Leach, M.K., and T.J. Givnish. 1996. Ecological determinants of species loss in remnant prairies. *Science* 273: 1555-1558.

Leão, T.C.C., C.R. Fonseca, C.A. Peres, and M. Tabarelli. 2014. Predicting extinction risk of Brazilian Atlantic forest angiosperms. *Conservation Biology* 28: 1349-1359.

Leckie, S., M. Vellend, G. Bell, M.J. Waterway, and M.J. Lechowicz. 2000. The seed bank in an old-growth, temperature deciduous forest. *Canadian Journal of Botany* 78: 181-192.

Lienert, J., M. Fischer, and M. Diemer. 2002. Local extinctions of the wetland specialist *Swertia perennis* L. (Gentianaceae) in Switzerland: a revisitation study based on herbarium records. *Biological Conservation* 103: 65-76.

Liow, L.H., M. Fortelius, E. Bingham, K. Linktulaakso, H. Mannila, L. Flynn, et al. 2003. Higher origination and extinction rates in larger mammals. *Proceedings of the National Academy of Sciences* 105: 6097-6102.

Luiz, O.J., R.M. Woods, E.M.P. Madin, and J.S. Madin. 2016. Predicting IUCN extinction risk categories for the world's data deficient groupers (Teleostei: Epinephelidae). *Conservation Letters* 9: 342-350.

Mace, G.M., H. Masundire, J.E.M. Baillie, T.H. Ricketts, T.M. Brooks, M. Hoffman, S. Stuart, et al. 2005. Biodiversity. *In* Millennium Ecosystem Assessment: Current Status and Trends: Findings of the Conditions and Trends Working Group. Ecosystems and Human Well-Being, pp. 53-98. Island Press, Washington, D.C., USA.

Malcolm, J.R., C. Liu, R.P. Neilson, L. Hansen, and L. Hannah. 2005. Global warming and extinctions of endemic species from biodiversity hotspots. *Conservation Biology* 20: 538-548.

Mankga, L.T., and K. Yessoufou. 2017. Factors driving the global decline of cycad diversity. *AoB Plants* 9: 1-10.

McCann, M.T. 1979. The Plant Tension Zone in Michigan. ScholarWorks at WMU, Western Michigan University, Kalamazoo, MI.

McGill, B.J., B.J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21: 178-185.

McKenna, D.D. 2004. Flora and vegetation of Kalamazoo County, Michigan. *The Michigan Botanist* 43: 137-359.

McKinney, M.L. 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. *Annual Review of Ecology, Evolution, and Systematics* 8: 495-516.

Meineke, E.K., C.C. Davis, and T.J. Davies. 2018. The unrealized potential of herbaria for global change biology. *Ecological Monographs* doi:10.1002/ecm.1307.

Miller-Rushing, A.J., R.B. Primack, D. Primack, and S. Makunda. 2006. Photographs and herbarium specimens as tools to document phenological changes in response to global warming. *American Journal of Botany* 93: 1667-1674.

Mouillot, D., N.A.J. Graham, S. Villéger, N.W.H. Mason, and D.R. Bellwood. 2013. A functional approach reveals community responses to disturbance. *Trends in Ecology and Evolution* 28: 167-177.

Newbold, T., L.N. Hudson, A.P. Arnell, S. Contu, A. Palma, S. Ferrier, et al. 2016. Has land use pushed terrestrial biodiversity beyond the planetary boundary? A global assessment. *Science* 353: 288-291.

Niissalo, M.A., J. Leong-Škorničková, G.S. Khew, and E.L. Webb. 2017. Very small relic populations suggest high extinction debt of gingers in primary forest fragments of a tropical city. *American Journal of Botany* 104 182-189.

Nualart, N., N. Ibáñez, P. Luque, J. Pedrol, L. Vilar, and R. Guàrdia. 2017. Dataset of herbarium specimens of threatened vascular plants in Catalonia. *PhytoKeys* 77: 41-62.

Öckinger, E., S. Schweiger, T.O. Crist, D.M. Debinski, M. Kuusaaari, J.D. Petersen, J. Pöyry, et al. 2010. Life-history traits predict species responses to habitat area and isolation: a cross-continental synthesis. *Ecology Letters* 13: 969-979.

Oksanen, J. 1996. Is the humped relationship between species richness and biomass an artefact

due to plot size? *Journal of Ecology* 84: 293-295.

Owens, P.P.F., and P.M. Bennett. 2000. Ecological basis of extinction in birds: habitat loss versus human persecution and introduced predators. *Proceedings of the National Academy of Sciences* 97: 12144-12148.

Palma, E., J.A. Catford, R.T. Corlett, R.P. Duncan, A.K. Hahs, M.A. McCarthy, M.J. McDonnell, et al. 2016. Functional trait changes in the flora of 11 cities across the globe in response to urbanization. *Ecography* 7: 875-886.

Paradis, E., and J. Claude. 2002. Analysis of comparative data using generalized estimating equations. *Journal of Theoretical Biology* 218: 175-185.

Parhar, R.K., and A.Ø. Mooers. 2011. Phylogenetically clustered extinction risks do not substantially prune the tree of life. *PLoS One* 6: e23528.

Pereira, H.M., P.W. Leadley, V. Proença, R. Alkemade, J.P. Scharlemann, J.F. Fernandez-Mangarrés, M.B. Araújo, et al. 2010. Scenarios for global biodiversity in the 21st century. *Science* 330: 1496-1501.

Pereira, H.M., L.M. Navarro, and I.S. Martins. 2012. Global biodiversity change: the bad, the good, and the unknown. *Annual Review of Environment and Resources* 37: 25-50.

Pimm, S.L. 1991. *The Balance of Nature? Ecological issues in the conservation of species and communities.* University of Chicago Pres, Chicago, IL, USA.

Pimm, S.L., C.N. Jenkin, R. Abell, T.M. Brooks, J.L. Gittleman, L.N. Goppa, P.H. Raven, et al. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344: 987.

Plotnick, R.E., F.A. Smith, and S.K. Lyons. 2016. The fossil record of the sixth extinction. *Ecology Letters* 19: 546-553.

Preston, C.D. 2000. Engulfed by suburbia or destroyed by the plough: the ecology of extinction in Middlesex and Cambridgeshire. *Watsonia* 23: 13-26.

Preston, C.D., M.G. Telfer, D.B. Roy, P.D. Carey, M.O. Hill, W.R. Meek, P. Rothery, et al. 2002. The Changing Distribution of the Flora of the United Kingdom: Technical Report. Centre for Ecology and Hydrology, National Environment Research Council, Huntingdon, Cambridge, UK.

Primack, D., C. Imbres, R.B. Primack, A.J. Miller-Rushing, and P. del Tredici. 2004. Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *American Journal of Botany* 91: 1260-1264.

Purvis, A, P-M. Agapow, J.L. Gittleman, and G.M. Mace. 2000a. Nonrandom extinction risk and

the loss of evolutionary history. *Science* 288: 328-330.

Purvis, A., J.L. Gittleman, G. Cowlishaw, and G.M. Mace. 2000b. Predicting extinction risk in declining species. *Proceedings of the Royal Society B* 26: 1947-1952.

Purvis, A., K.E. Jones, and G.M. Mace. 2000c. Extinction. *BioEssays* 22: 1123-1133.

Purvis, A. 2008. Phylogenetic approaches to the study of extinction. *Annual Review of Ecology, Evolution, and Systematics* 39: 301-319.

Pyke, G.H., and P.R. Ehrlich. 2010. Biological collections and ecological/environmental research: a review, some observations and a look to the future. *Biological Reviews* 85: 247-266.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Razgour, O., J. Juste, C. Ibáñez, A. Kiefer, H. Rebelo, S. Peuchmaille, R. Arlettaz, et al. 2013. The shaping of genetic variation in edge-of-range populations under past and future climate change. *Ecology Letters* 16: 1258-1266.

Rich, T.C.G, and E.R. Woodruff. 1996. Changes in the vascular plant floras of England and Scotland between 1930-1960 and 1987-1988: the BSBI monitoring scheme. *Biological Conservation* 75: 217-229.

Robinson, G.R., M.E. Yurlina, and S.N. Handel. 1994. A century of change in the Staten Island flora: ecological correlates of species losses and invasions. *Bulletin of the Torrey Botanical Club* 121: 119-129.

Rogers, D.A., T.P. Rooney, D. Olson, and D.M. Waller. 2008. Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* 89: 2482-2492.

Romerias, M.M., R. Figueira, M.C. Duarte, P. Beja, and I. Darbyshire. 2014. Documenting biogeographical patterns of African timber species using herbarium records: a conservation perspective based on native trees from Angola. *PLoS One* 9: e103403.

Roy, K., G. Hunt, and D. Jablonski. 2009. Phylogenetic conservatism of extinctions in marine bivalves. *Science* 325: 733-737.

Russell, G.J., T.M. Brooks, M.M. McKinney, and C.G. Anderson. 1998. Extinction selectivity of birds and mammals. *Conservation Biology* 12: 1365-1376.

Saar, L., K. Takkis, M. Pärtel, and A. Helm. 2012. Which plant traits predict species loss in calcareous grasslands with extinction debt? *Diversity and Distributions* 18: 808-817.

Sala, O.E., F.S. Chapin, J.J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, et al. 2000. Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Sachat, S.R., D.G. Mulcahy, and J.R. Mendelson III. 2016. Conservation threats and the phylogenetic utility of IUCN Red List rankings in *Incilius* toads. *Conservation Biology* 30: 72-81.

Schwartz, M.W., and D. Simberloff. 2001. Taxon size predicts rates of rarity in vascular plants. *Ecology Letters* 4: 464-469.

Sjöström, A., and C.L. Gross. 2006. Life-history characters and phylogeny are correlated with extinction risk in the Australian angiosperms. *Journal of Biogeography* 33: 271-290.

Smart, S.M., R.G.H. Bunce, R. Marrs, M. LeDuc, L.G. Firbank, LM.C. Maskell, W.A. Scott, et al. 2005. Large-scale changes in the abundance of common higher plant species across Britain between 1978, 1990 and 1998 as a consequence of human activity: Tests of hypothesized changes in trait representation. *Biological Conservation* 124: 355-371.

Sodhi, N.S., L.P. Koh, K.S.H. Peh, H.T.W. Tan, R.L. Chazdon, R.T. Corlett, T.M. Lee, et al. 2008. Correlates of extinction proneness in tropical angiosperms. *Diversity and Distributions* 14: 1–10.

Soons, M.B., and G.W. Heil. 2002. Reduced colonization capacity in fragmented populations of wind-dispersed grassland forbs. *Journal of Ecology* 90: 1033-1043.

Stevens, M.H.H., and W.P. Carson. 2002. Resource quantity, not resource heterogeneity, maintains plant diversity. *Ecology Letters* 5: 420-426.

Stöcklin, J., and M. Fischer. 1999. Plants with longer-lived seeds have lower local extinction rates in grassland remnants 1950-1985. *Oecologia* 120: 539-543.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and D.M. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 5702: 1783-1786.

Suding, K.N., S.L. Collins, L. Gough, C. Clark, E.E. Cleland, K.L. Gross, D.G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences* 102: 4387-4901.

Tecco, P.A., S. Díaz, M. Cabido, and C. Urcelay. 2009. Functional traits of alien plants across contrasting climatic and land-use regimes: do aliens join the locals or try harder than them? *Journal of Ecology* 98: 17-27.

Thompson, K., J.P. Bakker, R.M. Bekker, and J.G. Hodgson. 1998. Ecological correlates of seed persistence in soil in the north-west European flora. *Journal of Ecology* 86: 163-169.

Tietje, M., and M.O. Rodel. 2017. Contradicting habitat type-extinction risk relationships between living and fossil amphibians. *Royal Society Open Science* 4: 170051.

Tilman, D., R.M. May, C. Lehman, and M.A. Nowak. 1994. Habitat destruction and the extinction debt. *Nature* 371: 65-66.

Turner, I.M., K.S. Chua, J.S.Y. Ong, B.C. Soong, and H.T.W. Tan. 1996. A century of plant species loss from an isolated fragment of lowland tropical rain forest. *Conservation Biology* 10: 1229-1244.

Venn, S.E., K. Green, C.M. Pickering, and J.W. Morgan. 2011. Using plant functional traits to explain community composition across a strong environmental filter in Australian alpine snowpatches. *Plant Ecology* 212: 1491-1499.

Verheyen, K., O. Honnay, G. Motzkin, M. Hermy, and D.R. Foster. 2003. Response of forest plant species to land-use change: a list-history trait-based approach. *Journal of Ecology* 91: 563-577.

Vitousek, P.M., H.A. Mooney, J. Lubchenco, and J.M. Melillo. 1997. Human domination of earth's ecosystems. *Science* 277: 494-499.

von Euler, F. 2001. Selective extinction and rapid loss of evolutionary history in the bird fauna. *Proceedings of the Royal Society B* 268: 127-130.

Walker, K.J., and C.D. Preston. 2006. Ecological predictors of extinction risk in the flora of lowland England, UK. *Biodiversity and Conservation* 15: 1913-1942.

Webb, C.O., and M.J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* 5: 181-183.

Webb, C.O., D.D. Ackerly, and S.W. Kembel. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24: 2098-2100.

Weigmann, S.M., and D.M. Waller. 2006. Fifty years of change in northern upland forest understories: identity and traits of "winner" and "loser" plant species. *Biological Conservation* 129: 109-123.

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. *BioScience* 48: 607-615.

Williams, N.S.G, J.W. Morgan, M.J. McDonnel, M.C. McCarthy. 2005. Plant traits and local extinctions in natural grasslands along an urban-rural gradient. *Journal of Ecology* 93: 1203-1213.

Williams, N.S.G, A.K. Hahs, and P.A. Vesk. 2015. Urbanisation, plant traits and the composition of urban floras. *Perspectives in Plant Ecology, Evolution, and Systematics* 17: 78-86.

Willis, C.G, R. Primack, A.J. Miller-Rushing, and C.C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences* 105: 17029-17033.

Willis, C.G., E.R. Ellwood, R.B. Primack, C.C. Davis, K.D. Pearson, A.S. Gallinat, J.M. Yost, et al. 2017. Old plants, new tricks: phenological research using herbarium specimens. *Trends in Ecology and Evolution* 32: 531-546.

Wolkovich, E.M., T.J. Davies, H. Schaffer, E.E. Cleland, B.I. Cook, S.E. Travers, C.G. Willis, and C.C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany* 100: 1407-1421.

Yessoufou, K., B.H. Daru, and T.J. Davies. 2012. Phylogenetic patterns of extinction risk in the eastern arc ecosystem an African biodiversity hotspot. *PLoS ONE* 7: e47082.

Yessoufou, K., and T.J. Davies. 2016. Re-considering the loss of evolutionary history: how does non-random extinction prune the tree-of-life? In: R. Pellens and P. Grandcolas [eds.], *Biodiversity Conservation and Phylogenetic Systematics*, pp. 57-80. Springer Nature, USA.

Yessoufou, K., B.H. Daru, R. Tafirei, H.O. Elansary, and I. Rampedi. 2017. Integrating biogeography, threat and evolutionary data to explore extinction crisis in the taxonomic group of cycads. *Ecology and Evolution* 7: 2735-2746.

Zanne, A.E., D.C. Tank, W.K. Cornwell, J.M. Eastman, S.A. Smith, R.G. FitzJohn, D.J., et al. 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* 506: 89-92.

CHAPTER TWO:

Phenology in a warming world: differences between native and non-native plant species[9]

ABSTRACT

Phenology is a harbinger of climate change, with many species advancing flowering in response to rising temperatures. However, there is tremendous variation among species in phenological response to warming, and any phenological differences between native and non-native species may influence invasion outcomes under global warming. We simulated global warming in the field and found that non-native species flowered earlier and were more phenologically plastic to temperature than natives, which did not accelerate flowering in response to warming. Non-native species' flowering also became more synchronous with other community members under warming. Earlier flowering was associated with greater geographic spread of non-native species, implicating phenology as a potential trait associated with the successful establishment of non-native species across large geographic regions. Such phenological differences in both timing and plasticity between native and non-natives are hypothesized to promote invasion success and population persistence, potentially benefiting non-native over native species under climate change.

INTRODUCTION

Phenology, or the timing of life-history events, both responds to and serves as a major indicator of climate change (Peñuelas and Filella, 2001; Fitter and Fitter, 2002; Menzel, 2002; Cleland et al., 2007; Parmesan, 2007; Ovaskainen et al., 2013; CaraDonna et al., 2014;

[9] Zettlemoyer, M.A., E.H. Schultheis, and J.A. Lau. 2019. Phenology in a warming world: differences between native and non-native plant species. *Ecology Letters* 22: 1253-1263.

Thackeray et al., 2016). For plants, the timing of germination, leaf-out (or green-up), flowering, and fruiting, are frequently determined at least in part by environmental conditions likely to be affected by climate change (Bradshaw, 1965; Sparks et al., 2000; Parmesan and Yohe, 2003; Badeck et al., 2004; Visser, 2008; Forrest and Miller-Rushing, 2010; Wolkovich et al., 2013). Because phenology influences interspecific competition, resource access, vulnerability to herbivores, mating success, and ultimately, population and community dynamics (Rathcke and Lacey, 1985; Visser and Both, 2005; Parmesan, 2007; Forrest and Miller-Rushing, 2010; Wolkovich and Cleland, 2011; Cleland et al., 2012; Thackeray et al., 2016), it is also likely to influence population persistence in the face of future climate change (Møller et al., 2008; Willis et al., 2008, 2010; Donnelly et al., 2011; Cleland et al., 2012; Wolkovich et al., 2013; Thackeray et al., 2016).

Both observational and experimental studies document shifts in phenology in response to global warming, with many species advancing leaf-out, flowering, or both (Arft et al., 1999; Bradley et al., 1999; Fitter and Fitter, 2002; Dunne et al., 2003; Parmesan and Yohe, 2003; Menzel et al., 2006; Cleland et al., 2007; Jarrad et al., 2008; Amano et al., 2010; Hoffman et al., 2010; Fridley, 2012; Ovaskainen et al., 2013; Whittington et al., 2015; Thackeray et al., 2016; König et al., 2017; Zohner and Renner, 2017). However, the direction and magnitude of these shifts differ, and some species exhibit delayed phenological responses to warming (Peñuelas et al., 2002; Sherry et al., 2007; Dunnell and Travers, 2011; Cook et al., 2012; Liancourt et al., 2012) or no response to warming (Bradley et al., 1999; Peñuelas et al., 2002; Liancourt et al., 2012; CaraDonna et al., 2014). Variable responses to warming may result from differential effects of climate change on early- versus late-season flowering species (Sherry et al., 2007; Park et al., 2018) or variation among species in the degree to which phenology is regulated by

photoperiod vs. temperature (Chuine et al., 2010). Furthermore, because species respond differently to climate change, global warming also may alter phenological synchrony, or the degree of overlap in the flowering times of interacting species (Harrington et al., 1999; Stenseth and Mysterud, 2002; Visser et al., 2004; CaraDonna et al., 2014; Kharouba et al., 2018; Zohner et al., 2018).

Interestingly, some evidence suggests that native and non-native species may differ in both phenology and phenological responses to warming in ways that could influence biological invasions and favor non-native species in warmer environments (Willis et al., 2010; Wolkovich et al., 2013). Here, we experimentally simulate global warming to test four non-mutually exclusive hypotheses on the role of phenology in non-native species' success developed by Wolkovich and Cleland (2011), all of which may be influenced by global warming: vacant niche, priority effects, niche breadth, and plasticity. (1) The *vacant niche hypothesis* extends Elton's (1958) theory to predict that non-native plants invade when there is a temporally empty niche to exploit. In this scenario, non-native species leaf, flower, and/or fruit earlier or later than native species, allowing them to better utilize temporally available resources. As a result, if global warming increases phenological differences between non-native and native species because they differ in either the magnitude or direction of response, then global warming may increase the availability of vacant niches. A pattern of more asynchronous flowering for non-native species with other community members (i.e., filling more temporally available niches) would further support this hypothesis. (2) *Priority effects* predict that non-native species establish earlier in the season than native species, sequester resources first, and thus may be more competitive (Sale, 1977). Consistent with this hypothesis, multiple studies find that non-native species leaf and flower earlier than native species (Crawley et al., 1996; Seabloom et al., 2003; DeFalco et al.,

2007; Resasco et al., 2007; Xu et al., 2007; Pyšek and Richardson, 2007; Godoy et al., 2009; Pearson et al., 2012; Wolkovich et al., 2013). Priority effects for non-native species may become more prevalent if non-natives exhibit stronger phenological advances in response to warming than natives. (3) The *niche breadth hypothesis* suggests that non-native species occupy a broader niche space, or have longer phenological phases (e.g., leaf or flower for longer periods) than native species and thus gain extended access to nutrients, light, and pollinators. Consistent with this hypothesis, in some systems non-natives flower longer than native species and extend their growing seasons later into the year (Gerlach and Rice, 2003; Lake and Leishman, 2004; Cadotte et al., 2006). If global warming causes non-native species to extend their growing season or flowering period more than natives, then global warming may increase non-native niche breadth to a greater extent than native species. Finally, (4) the *plasticity hypothesis* proposes that phenological plasticity may provide invaders an advantage in the warmer and increasingly variable climates predicted in the future (Nicotra et al., 2010). In two studies using observational long-term records, non-native species exhibit more plastic flowering times in response to temperature compared to native species (Willis et al., 2008, 2010; Wolkovich et al., 2013).

We experimentally simulated global warming in the field to test the effects of warming (+3°C) on flowering phenology of 42 native and non-native species that are common in western Michigan grasslands and old fields. We also compiled data from the literature and local botanical records to determine time since introduction to North America, current extent (geographic distribution), and reconstructions of species' phylogenetic relationships. Our approach complements prior studies using long-term observations to compare phenological responses of native vs. non-native taxa by allowing us to differentiate phenological responses to warming from other variables that have also changed over the past century. In addition to considering

differences between native and non-native species' phenology, we consider differences in the responses of non-invasive exotic and invasive (here defined as widespread and damaging) species, which may help address the question of why only some non-native species become invasive and identify traits associated with increased invasiveness and spatial spread (Pyšek and Richardson, 2007; Gallagher et al., 2015; Divíšek et al., 2018). We address the following specific questions: (1) Does the phenology of native and non-native species differ, as predicted by the vacant niche, priority effects, and niche breadth hypotheses, and does warming influence these differences? (2) In accordance with the plasticity hypothesis, do native and non-native species differ in their phenological responses to warming? (3) Do native and non-native species differ in phenological synchrony at the community level as predicted by the vacant niche hypothesis, and how does warming influence phenological synchrony? Finally, because phenology may influence non-native species success and because the ecological and evolutionary processes that influence invasion can change over space and time (Dietz and Edwards, 2006; Schultheis et al., 2015), we ask (4) Are flowering time and phenological plasticity correlated with spread (geographic distribution in the introduced range) of non-native species, and is there evidence that non-native species have evolved increased phenological plasticity to temperature since their introduction?

MATERIALS AND METHODS

Field warming experiment

We established this experiment within the warming array at the Kellogg Biological Station (KBS), which has run constantly over the growing season (April-October) since its establishment in 2008. The warming array uses infrared heaters to elevate temperatures 3°C

above ambient temperatures, matching regional predictions for climate warming in this area by the end of the 21st century (0.3°C-4.8°C) (Stocker et al., 2013). The array consists of four 3m-diameter plots, each surrounded by six infrared ceramic heaters (Model FTE-1000, Kalglo, Inc.) that evenly raise temperature across similar heating arrays (Kimball et al., 2008). Dummy heaters are suspended above four additional control plots to control for shading effects. Heaters are regulated by a proportional-integrative-derivative (PID) control system, which allows for a consistently elevated temperature relative to focal control (no heater) plots {see Kimball et al. (2008) for a full description of the heating apparatus}. Such heating designs have been shown to be effective at maintaining temperatures within 0.5°C of the target level 75% of the time (Kimball et al., 2008; Fig. S2.1).

In spring 2012, we planted 52 species (25 native, 12 exotic, 15 invasive) into the background early successional community in each plot (n=3 replicates/species/plot). Of these, 42 species (20 natives, 22 non-natives {7 exotic, 15 invasive}) survived to flower in 2013 and were included in this study. Study species were all forb and grass species found in old field or grassland habitats and, when possible, were selected congener or confamilial triplets of native, exotic, and invasive species representing a broad range of phylogenetic diversity (Schultheis et al., 2015). To avoid unintentional introduction of new invasive species to the area, we only included species reported in Kalamazoo County (McKenna, 2004). When possible, we chose species that had local seed available, either through our own collections or commercial seed sources (Table S2.1). Variation among seed sources did not influence results as analyses that excluded seeds sourced from outside the Midwest or that controlled for seed source by including a factor for seed source both yielded qualitatively similar results to those presented below (data not shown). Species were considered native if they were present in Michigan prior to European

settlement (McKenna, 2004). The non-native species are all from outside the United States, based on herbarium or historical records (Michigan Flora [http://michiganflora.net], Consortium of Midwest Herbaria [http://midwestherbaria.org/portal/]). We further categorized non-native species as invasive or non-invasive exotic because differentiating between these two types of non-native species can yield important information on the drivers of invasiveness (Agrawal et al., 2005; Stricker and Stiling, 2014; Schultheis et al., 2015). Species were characterized as invasive (here defined as widespread and damaging non-native species) if they were listed on one or more of the following as of June 2014: (1) Michigan Natural Features Inventory (Borland 2009), (2) Czarapata (2005) list of "major invader[s] of natural areas" not needing disturbance to establish, (3) Wild Type Plants (http://www.wildtypeplants.com), and (4) the Michigan Seed Law (Act 329 of 1965) (http://www.michigan.gov/). Inclusion on these lists means a species has been categorized as invasive in the midwestern United States based on reports from land managers, inclusion on government invasive species lists, or published documentation of their impacts on native plant and animal communities (Schultheis et al., 2015). We note that there can be substantial disagreement about an "invasive" classification and that invasive status often depends on local biotic and abiotic factors. Because of these concerns, we present results for the native vs. non-native comparison in the main text and results for native, exotic, and invasive comparisons in the Appendices.

We germinated seeds of all species in low-nutrient potting media in the greenhouse and then transplanted seedlings into randomly selected locations within each field plot. Seedlings were planted 20cm apart and watered as needed to facilitate establishment. During the 2013 growing season, we recorded the flowering stage of each plant (bud, flower, or fruit) at weekly intervals (starting 21 May 2013). From this data we determined four phenological variables

relevant to the hypotheses proposed by Wolkovich and Cleland (2011): (1) days to first flower, (2) days to last flower, (3) duration of flowering period, and (4) days to first fruit.

Data analysis

Because of the nested structure of our experimental design and potential phylogenetic non-independence of our study species, we analyzed our data in two ways. First, we determined the effects of warming and status (native or non-native) on phenology using a linear mixed model (SAS Institute, 2011; PROC MIXED). We included days to first flower, days to last flower, flowering period length, or days to first fruit as four separate response variables. We included warming (ambient or elevated), status (native or non-native) and the warming by status interaction as predictor variables in each model. Plot (nested within warming treatment) and species (nested within status) were included as random factors. Post-hoc contrasts were used to evaluate differences between statuses and warming treatments when the warming by status interaction was significant ($p \leq 0.05$). We used similar models to test the effects of warming, species, and the warming by species interaction to examine variation among species independent of status, with plot within warming treatment included as a random effect.

To control for phylogenetic non-independence between species in our study, we conducted additional analyses that accounted for phylogenetic relatedness. First, we retrieved nucleotide sequences for *ITS, matK*, and *rbcL* from NCBI Genbank for each species (accessed November 2016) (Table S2.1). Using the MUSCLE algorithm in Geneious v6.1.8 (Kearse et al., 2012) we aligned gene sequences. We trimmed the ends of each sequence and concatenated the three genes using the R function phyutility (Smith and Dunn, 2008). We determined the optimal model of molecular evolution for the alignment using the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Performance Based Selection (DT) using ModelTest2

v2.1.7 (Darriba et al., 2012). All three methods selected the General Time Reversible model, with rate heterogeneity including invariable sites and the rate of evolution at other sites as a gamma distribution (GTR + I + Γ), as the optimal model. Maximum likelihood (ML) analysis with 100 bootstrap replicates was implemented with the high-performance computing version of RAxML v8.1.17 (Stamatakis, 2014). We included a partition file for ML analysis to account for gene regions in the concatenated alignment.

We then performed phylogenetic generalized least squares (PGLS) analyses with Brownian motion models of trait evolution (Garland et al., 1993, Martins and Hansen, 1997). PGLS was implemented by incorporating the constructed phylogeny (Fig. S2.2) into the covariance structure using the R package ape (v3.1-4; Paradis, 2012), after which the linear models were fit using the gls function in the R package nlme (v3.1-119; Pinheiro et al., 2015). Each of the four phenological measurements were included as separate response variables and warming, status, and the warming by status interaction were included as fixed predictor variables.

Results from the two analyses were similar, so for clarity we present mixed model results in the main text because they use the appropriate nested field replication and report PGLS results in Supporting Information (Table S2.3).

Phenological synchrony

We examined the effects of warming and status on phenological synchrony between individuals at the community level using Augspurger's (1983) method, which measures synchrony (X) as the amount of overlap between an individual's flowering days with those of all other individuals within some defined population or community. A score of X=1 indicates complete synchrony; a score of X=0 indicates complete asynchrony. We calculated phenological

synchrony at the community level as the amount of overlap of a given individual's flowering days with all hetero- and conspecific individuals within the same warming treatment (X). We used a linear mixed model to examine the effects of status, warming, and their interaction on X and included species (nested within status) and plot (nested within treatment) as random factors. We performed all synchrony analyses in R (R Core Team, 2015; v3.3.2).

Phenological plasticity, invasion spread, and invasion time

We examined whether phenological plasticity in flowering time is correlated with species' geographic spread. We calculated the phenological plasticity of each species as the difference in mean days to first flower between elevated and ambient temperatures. Geographic spread was determined by counting all United States counties in which a species is found and indicated as "introduced" in the USDA PLANTS database (https://plants.usda.gov). We determined the effects of phenological plasticity and status on geographic spread using a linear model with geographic spread (number of US counties) as the response variable and status (native or non-native), phenological plasticity, and the status by plasticity interaction as predictor variables. Flowering time bears on the role of priority effects in invasion, so we also examined whether flowering time is correlated with geographic spread. We used a linear model to examine the effects of days to first flower, status, and the status by days to first flower interaction on geographic spread (number of US counties).

We then examined whether time since introduction is correlated with phenological plasticity in non-native species. We calculated time since introduction as the number of years a species has been found in Michigan, based on the date of first collection recorded in the Michigan Flora database (http://michiganflora.net). We determined the effects of time since introduction and status (exotic or invasive) on phenological plasticity using a linear model,

including phenological plasticity as the response variable and status, time since introduction, and the status by time interaction as predictor variables.

To account for shared ancestry, we performed PGLS with Brownian models of trait evolution using the same linear models for geographic spread and time since introduction described above. We performed all geographic spread and time analyses in R (R Core Team, 2015; v3.3.2).

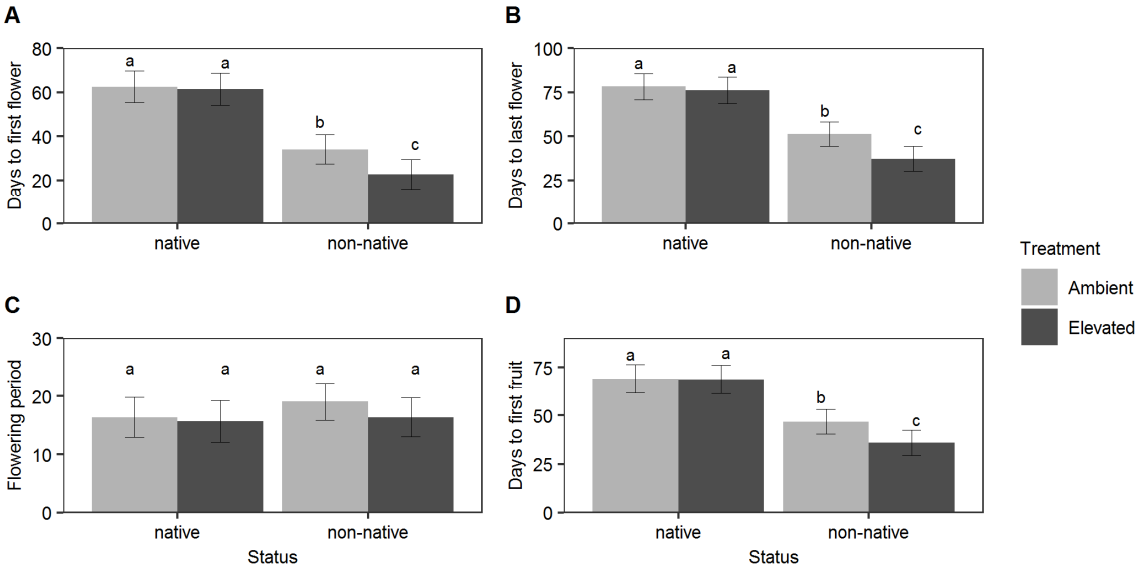
RESULTS

Effects of warming on native and non-native species' phenology

Non-native species exhibited advanced phenologies compared to native species (days to first flower, days to last flower, and days to first fruit (all $p \leq 0.05$; Fig. 2.1; Table S2.2) and accelerated their phenology in response to warming more than native species (status × warming: flowering $F_{1,283}=4.73$, $p=0.03$; days to last flower $F_{1,283}=5.70$, $p=0.02$; days to first fruit $F_{1,281}=6.03$, $p=0.02$; Fig. 2.1; Table S2.2). Similar results were observed even after accounting for phylogeny (Table S2.3). For non-native species, warming significantly accelerated flowering by 11.42 ± 6.79 days ($F_{1,283}=12.42$, $p=0.0005$), days to last flower by 14.12 ± 6.95 days ($F_{1,283}=16.65$, $p \leq 0.0001$), and days to first fruit by 10.91 ± 6.47 days ($F_{1,281}=14.83$, $p=0.0001$). Native species did not respond phenologically to warming (all $p \geq 0.6$; Fig. 2.1) and thus flowered 38.76 ± 7.12 days later and fruited 32.95 ± 6.97 days later than non-native species under warming (compared to 28.45 ± 7.00 and 22.38 ± 6.91 days later than non-natives under ambient temperatures for flowering and fruiting respectively). Finally, because species shifted days to first and last flower similarly, no effects on flowering period were observed (Table S2.2). However, when phylogenetic relationships are accounted for, native, and non-native species

differed in how flowering period responded to warming (Table S2.3). Nonnative species shortened their flowering periods by 2.74 ± 3.26 days while native species tended to maintain the same flowering periods regardless of temperature.

Figure 2.1. Effect of warming on (A) days to first flower, (B) days to last flower, (C) flowering period duration (days), and (D) days to first fruit for native and non-native species (least square means ± SE; N = 20 native and 22 non-native species). Letters represent significant differences between groups (adjusted for multiple comparisons with a Tukey test, $p \leq 0.05$).



These differences between non-native and native species were likely driven by the strong phenological responses of invasive relative to exotic species (Fig. S2.3, Table S2.4-S2.5). Of the 8 species that significantly accelerated flowering in response to warming, 5 were invasive, 0 were exotic, and 3 were native (Fig. 2.2, Table S2.6).

It is possible that these patterns were driven by the Poaceae because in this family all of the non-native species included in our study happen to be C_3 grasses while most included natives are C_4 grasses (with the exception of C_3 native *Bromus kalmii*); C_3 species may advance flowering in response to warming more so than C_4 species, as shown in C_3 *Chenopodium album* relative to C_4 *Setaria viridis* (Lee, 2011). However, results were qualitatively similar when C_3 Poaceae species were excluded from analyses (data not shown). It is also possible that native

origin of the non-native species influenced phenology; however, most species included in our study originated from Europe or Eurasia, and flowering dates did not differ between species from these regions ($F_{1,18}$=0.93, p=0.35).

Effects of warming and status on phenological synchrony

Warming increased the phenological synchrony of non-native, but not native, species with other community members (warming × status $\chi^2_{1,311}$=17.61, p≤0.0001; Fig. 2.3). As a result, non-native species flowered more synchronously with other community members than native species did in the elevated temperature treatment but not in the ambient temperature treatment. This pattern was likely driven by the increased synchrony of exotic species under elevated temperatures (Fig. S2.4).

Figure 2.2. The effect of warming on flowering phenology of invasive, exotic, and native species. Each line represents the period between the Julian calendar date of first flower (DFF, left point) and the date of last flower (DLF, right point) (LSmeans ± SE). Gray and black bars represent ambient and elevated temperatures, respectively. Only species with data available for both DFF and DLF are included. * indicates a significant advance and ǂ represents a significant delay in DFF ($p \leq 0.05$).

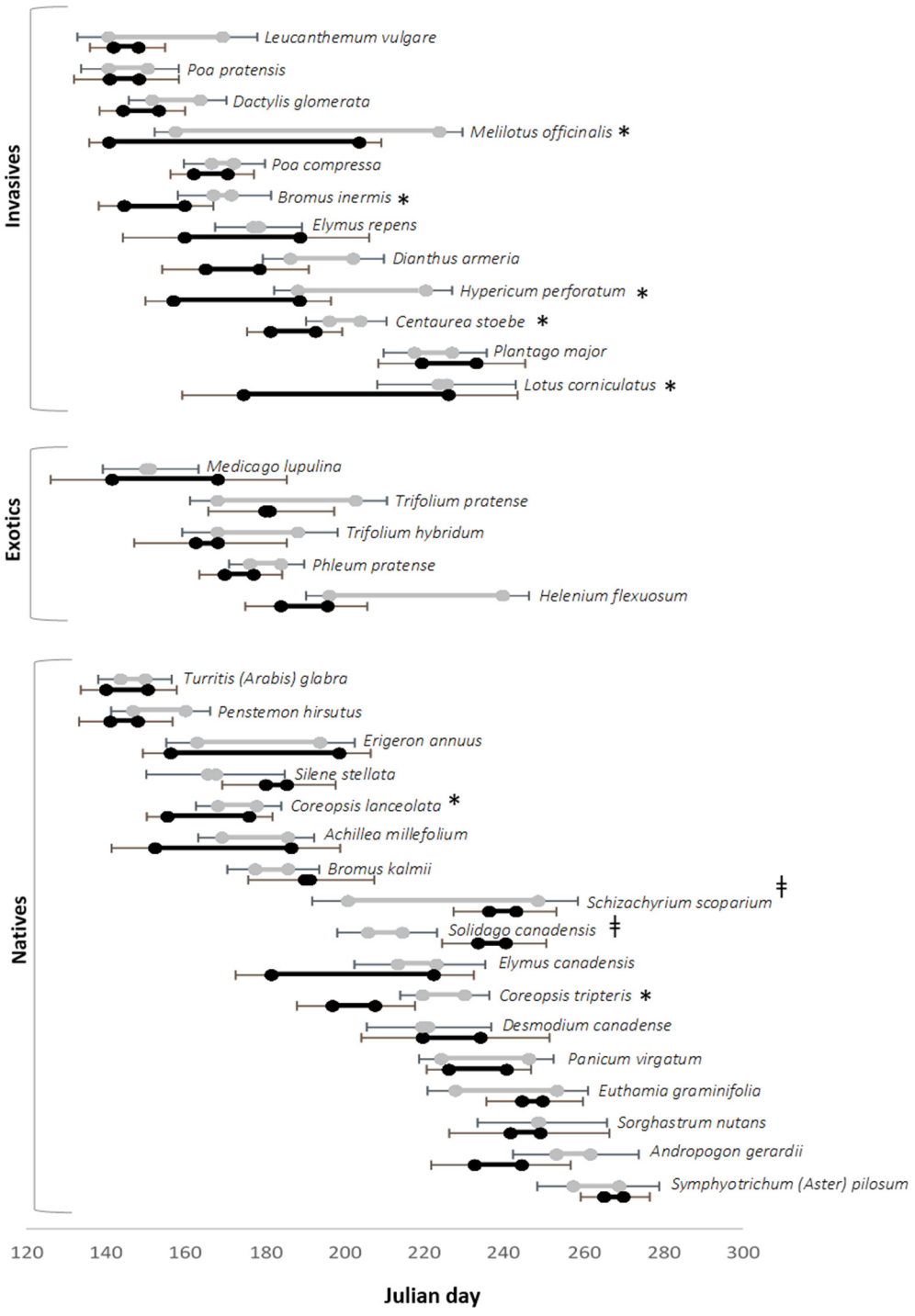
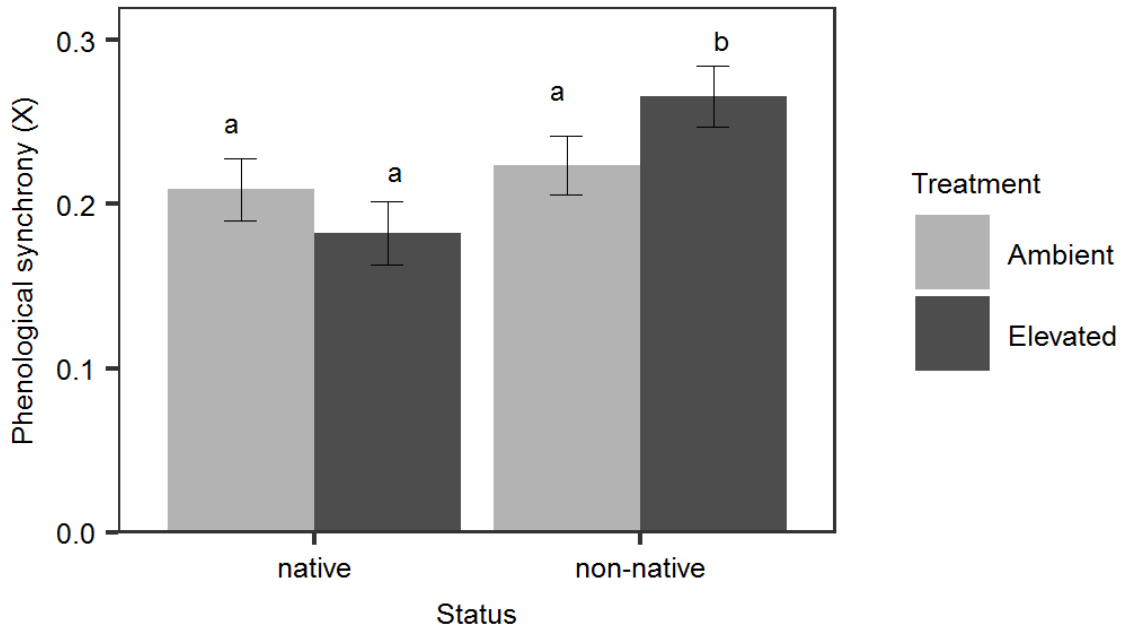


Figure 2.3. Phenological synchrony (X) (least square means ± SE) of native and non-native species under ambient and elevated (+3°C) temperatures. A phenological synchrony score of X=1 indicates complete synchrony among all individuals experiencing the same warming treatment, where all species start flowering at the same time and for the same length of time. A score of X=0 indicates complete asynchrony, or no overlap in flowering. Letters represent significant differences between groups (adjusted for multiple comparisons with a Tukey test, $p \leq 0.05$).



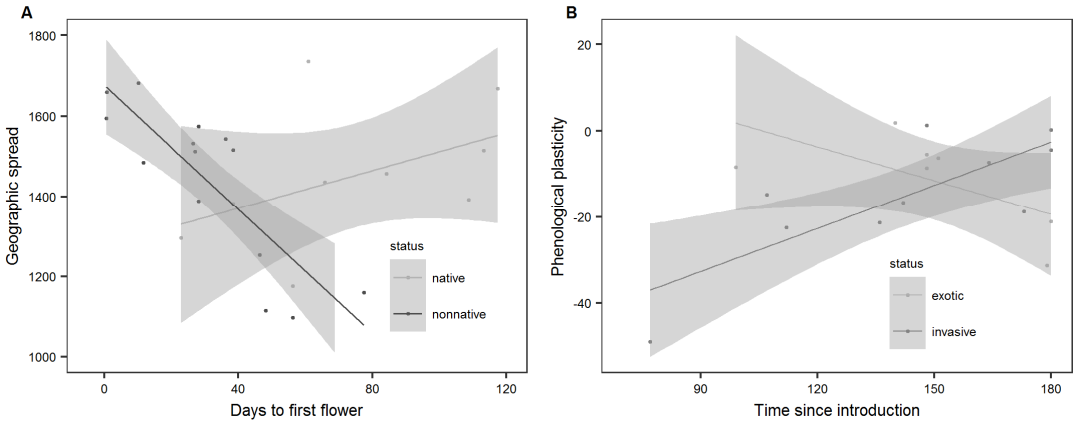
Phenological plasticity, invasion spread, and invasion time

In non-native species, earlier flowering was significantly associated with wider geographic spread, whereas native species' flowering time was not correlated with their geographic distributions (status × days to first flower $F_{3,33}=9.66$, $p=0.004$; non-native $R^2=0.37$, $p=0.004$; native $R^2=0.13$, $p=0.16$; Fig. 2.4A; Table S2.7A). Phenological plasticity was not associated with geographic spread ($F_{3,30}=0.19$, $p=0.66$; $R^2=0.23$; Table S2.7B). Results for both phenological plasticity and flowering time were similar when controlling for phylogeny (Table S2.8A-B) and when excluding C_3 grasses (days to first flower [DFF]: status × DFF $F_{1,25}=7.64$, $p=0.01$; plasticity: status $F_{1,22}=6.80$, $p=0.02$). Our choice of scale may influence these patterns (e.g., northern ranges are truncated by not including Canada). Results are non-significant when

we used number of Michigan counties as a local measure of geographic spread (Table S2.9), likely because many native species occupy more Michigan counties than non-native species do. Exotic and invasive species exhibited similar relationships between earlier flowering and spread (Table S2.10A).

We detected some evidence that longer time since introduction was associated with increased phenological plasticity for invasive species but not for exotic species (status × time $F_{1,14}$=4.04, p=0.06; Fig. 4B; invasive R^2=0.62, p=0.007; exotic R^2=0.02, p=0.7). This pattern remains significant after controlling for phylogeny (Table S2.8) and is not driven by invasive C_3 grasses (when excluded, patterns were similar but non-significant, likely because of the reduced power resulting from the exclusion of 13 species {Fig. S2.5}). While removing the highly plastic and early-invading outlier, *Lotus corniculatus,* eliminated the significant status × time interaction in the mixed model, suggesting that the pattern was heavily influenced by this outlier, the status × time interaction in the phylogenetically-controlled analysis remained significant even when this outlier was removed ($t_{1,12}$=5.87, p=0.03).

Figure 2.4. Phenological plasticity, geographic spread, and time since invasion. (A) Effect of flowering time (days to first flower under ambient conditions) on the geographic spread of native and non-native species (non-native R^2=0.38, p=0.004; native R^2=0.13, p=0.16). (B) Effect of time since introduction to Michigan (MI) (years) on phenological plasticity for invasive and exotic species (invasive R^2=0.62, p=0.007; exotic R^2=0.02, p=0.7). Gray areas represent 95% confidence intervals.



DISCUSSION

In the 42 species studied here, non-native species flower and fruit earlier than native species, and warming increases these differences. Warming significantly accelerated both flowering and fruiting and increased phenological synchrony of non-native species. In contrast, warming did not alter native species' phenology. Earlier flowering, but not phenological plasticity, was associated with the geographic spread of non-native species, potentially suggesting that early phenologies may help promote successful establishment across large geographic ranges. Together these findings suggest potentially important differences in native and non-native species' phenologies and phenological responses to climate change, which may have implications for the future success of native vs. non-native species in a warming world.

Vacant niche/priority effects hypothesis

Non-native species flower and fruit earlier than native species, particularly under warming, consistent with the priority effects hypothesis proposed by Wolkovich and Cleland (2011). Earlier flowering may allow earlier access to pollinators and resources (Sale, 1977; Wolkovich and Cleland, 2011), help introduced species avoid warmer temperatures and limited precipitation later in the season (DeFalco et al., 2007; Sherry et al., 2007; Craine et al., 2012), and allow non-native, particularly widespread invasive, species to become more competitive within the invaded community. Early phenologies have been observed in several of the most problematic invasive species, including *Lonicera maackii* (Resasco et al., 2007; Xu et al., 2007), *Centaurea solstitialis* (Gerlach and Rice, 2003), *Bromus tectorum* (DeFalco et al., 2007), California annual grasses (Seabloom et al., 2003), and exotic species dominating US grasslands (Wilsey et al., 2018). Other work suggests that non-native species benefit from priority effects by beginning growth earlier in the season than natives (Dickson et al., 2012; Fridley, 2012, Wilsey

et al., 2015). Supporting these studies, we find that non-native species with earlier flowering times have wider geographic distributions, suggesting that priority effects may play a role in invasion success. Global warming may increase the strength of priority effects favoring non-native species as non-natives shifted flowering earlier in response to rising temperatures while native species did not respond to warming, increasing the magnitude of difference in flowering time between native and non-native species.

While advanced flowering of non-native species may also be consistent with the vacant niche hypothesis, native and non-native species did not exhibit different patterns of phenological synchrony under ambient temperatures, perhaps suggesting that non-natives are not occupying vacant phenological niches for much of their flowering periods even though their phenologies are shifted substantially earlier than native species. Non-native species' (particularly exotics') flowering became even more synchronous under elevated temperatures. Synchronous flowering with other community members can increase pollinator visitation, thereby increasing reproduction and seed set (Bawa, 1977; Augspurger, 1981; Ollerton and Lack, 1992, 1998; Brown and Mitchell, 2001; Donnelly et al., 2011; Burkle et al., 2013), but also may increase competition for pollinators (Memmot et al., 2007; Cleland et al., 2012; Burkle et al., 2013). In contrast to our finding, other studies have detected decreased synchrony under warming in grassland plant species, European herbaceous and woody species, and bird populations (Sherry et al., 2007; Reed et al., 2013; Wang et al., 2016; Zohner et al., 2018). Further work is needed to understand how phenological synchrony will shift with climate change (Kharouba et al., 2018) and how synchrony changes will influence community composition and the success of individual populations under global warming.

Niche breadth hypothesis

Though a few species shifted the length of their flowering periods with warming, we find no evidence generally supporting the niche breadth hypothesis. Native and non-native species' flowering periods did not differ, and because species shifted days to first and last flower similarly under warmed and ambient treatments, warming minimally affected flowering duration (non-natives did significantly increase flowering period under warming when controlling for evolutionary history).

Plasticity hypothesis

Non-native (and especially invasive) species accelerated flowering in response to warming more than native species, supporting Wolkovich and Cleland (2011)'s plasticity hypothesis, a potentially worrisome result given previous observational work demonstrating that phenological plasticity was associated with increased abundance and/or performance over the past decades of warming temperatures (Willis et al., 2008, 2010; Cleland et al., 2012; Wolkovich et al., 2013; Lamarque et al., 2015). For example, Willis et al. (2010) found that non-native, but especially invasive, species shift flowering time more than native species in response to interannual variation in temperature and that this plasticity correlated with increases in abundance over a 100-year time-span, characterized by a 2.4°C temperature increase (Willis et al., 2008). Similarly, in cross-continental comparisons, *Acer negundo* populations from the invasive range demonstrate greater phenological sensitivity to temperature and increased growth than native range populations (Lamarque et al., 2015). Enhanced phenological plasticity in non-native and particularly widespread invasive species may be part of a broader pattern of increased phenotypic plasticity in a variety of traits that may enhance invasion success (Davidson et al., 2011), but studies linking phenological plasticity to fitness and population growth are needed.

Interestingly, early colonizing non-native species exhibited greater phenological plasticity than more recent colonizers, possibly as a result of post-introduction evolution as populations are selected to shift phenological cues to those that are more relevant to the novel invaded environment. However, this pattern was influenced by *Lotus corniculatus,* an exceptionally plastic invasive species that established early, and there are several additional viable hypotheses for this pattern. First, species that rely more on temperature than photoperiod as a flowering cue may be more successful at matching their phenology to novel conditions and may have established more quickly and earlier than other invaders. Second, phenological plasticity or early flowering may not be the target of selection; instead phenological traits may be correlated with another trait under strong selection post-invasion (e.g., height or specific leaf area) (Anderson and Gezon, 2014; Cooper, 2018). Third, early-flowering species have been shown to shift flowering earlier under warming temperatures relative to late-flowering species (Sherry et al., 2007). Because invaders flower earlier than natives, this general pattern could also explain the difference in plasticity between invaders and natives: however, early- and late-flowering species do not differ in their warming responses in our study (i.e., days to first flower was not correlated with phenological plasticity, R^2=-0.03 p=0.99).

In our study, we did not detect any effect of warming on the reproductive phenology of native species. Similar to the decline of bird species' whose spring migration does not track climate change (Møller et al., 2008), inability to track climate and adjust flowering time has been shown to be associated with declines in native plant species' abundance (Stenseth and Mysterud, 2002; Willis et al., 2008) and biodiversity (Wolf et al., 2017). This may be due to challenges associated with maintaining mutualistic interactions with pollinators or dispersers that are also responding to climate change (Memmot et al., 2007; Cleland et al., 2012; Burkle et al., 2013) or

avoiding negative interactions with predators and competitors, including invasive species (Tikkanen and Julkunen-Tiitto, 2003; Willis et al., 2008). If species with weak phenological responses are more prone to population declines (Willis et al., 2008), then native species may be at higher risk of extinction as the climate warms.

Conclusion

Our results show that non-native species flower and fruit earlier than native species and that non-native, but especially invasive, species accelerate phenology under warming temperatures, providing support for the priority effects and plasticity hypotheses (Wolkovich and Cleland, 2011) and suggesting that warming may promote invasion success. As a group, native species in our study did not significantly advance flowering under simulated warming. This may affect seed set and fitness if a failure to accelerate flowering disrupts interactions with pollinators or causes other mismatches between ideal abiotic conditions for flowering and flowering time (e.g., temperature stress can inhibit pollen viability; Brown and Mitchell, 2001). Further experimental work is needed to determine whether phenological plasticity is associated with plant fitness and demographic effects of climate change in long-lived species and to investigate the relative importance of plasticity and adaptation in phenological responses. However, this study of 42 species suggests that native and non-native taxa differ in key phenological traits and that global warming magnifies these phenological differences. Our findings illustrate the potential importance of phenology to invasion success and also prompt concerns that these phenological differences could be a mechanism by which global warming will advantage non-native species and disadvantage natives.

ACKNOWLEDGEMENTS

The authors thank Mark Hammond, Zoe Getman-Pickering, and Jeremy Jubenville for their help in the field. Jason Winters provided assistance with phenological synchrony analyses, and Andrea Berardi helped with phylogenetic analyses. L. Brudvig, J. Conner, K. Gross, the Lau, Conner, and Fitzpatrick labs, and three anonymous reviewers provided valuable feedback on this manuscript. Support for this work was provided by the W.K. Kellogg Biological Station and the NSF Long-Term Ecological Research Program (DEB 1637653) at the Kellogg Biological Station and by Michigan State University AgBioResearch. This is Kellogg Biological Station contribution no. 2124.

APPENDIX

Figure S2.1. Air temperature in the warming array. Air temperature data (°C) in ambient and elevated plots in the warming array over the 2013 growing season (June-August). Heaters are set to raise temperatures by approximately 3°C. Sensors are hung above the center of the plot and measure daily mean temperatures.

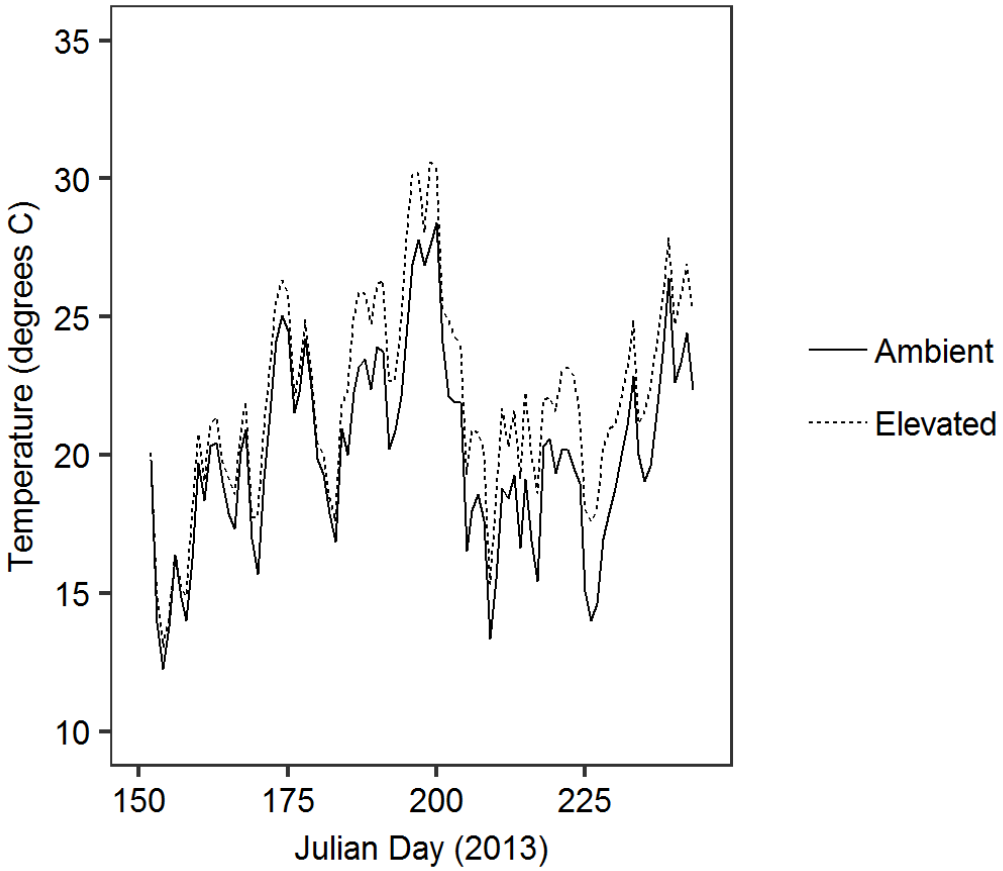


Figure S2.2. Phylogenetic relationships of native, exotic, and invasive species. The best-scoring ML tree from a rapid bootstrap analysis in RAxML from the analysis of concatenated sequences of *ITS, maK, rbcL.* ML bootstrap frequencies are the numbers associated with nodes, and branch lengths are proportional to the number of nucleotide changes.

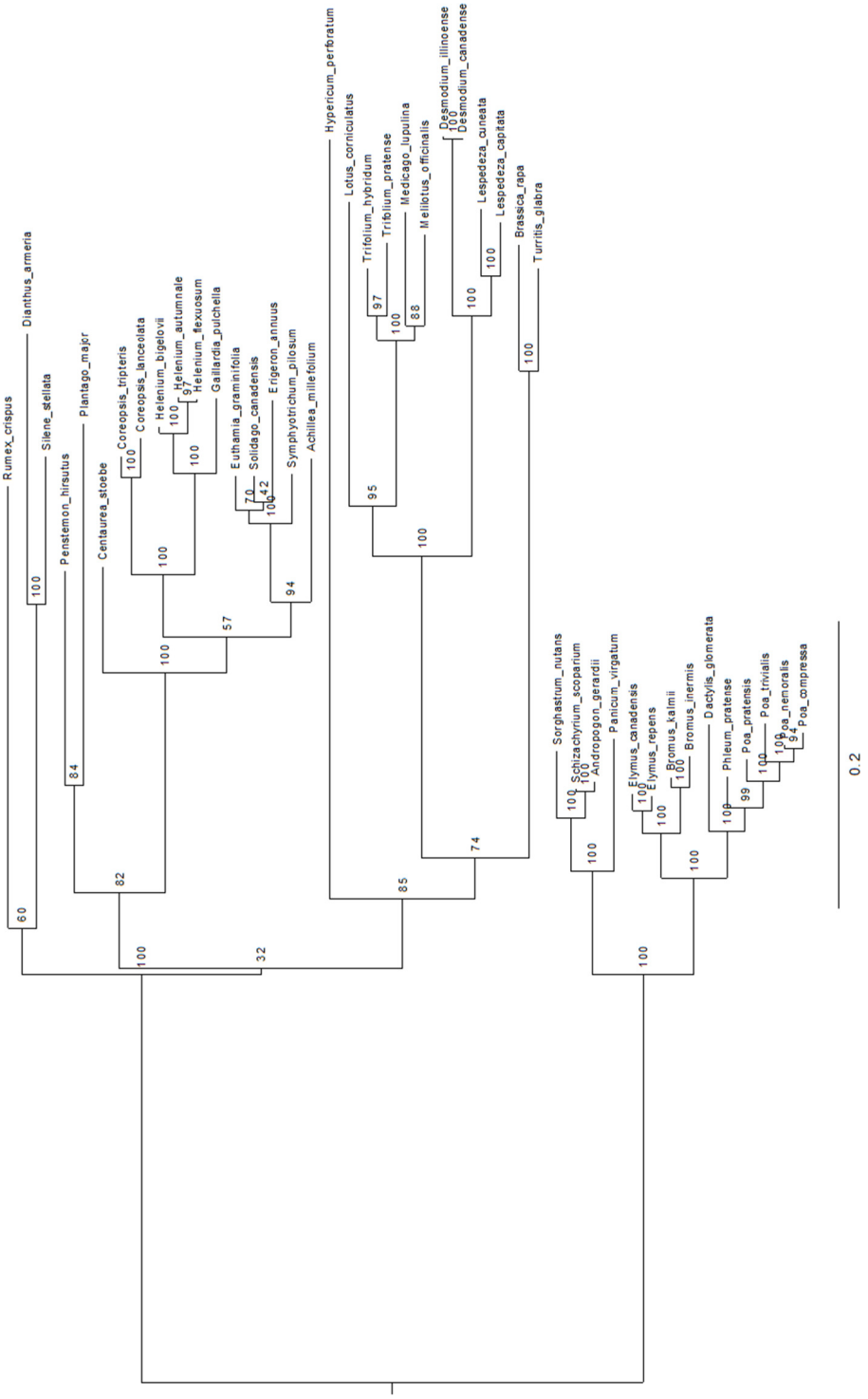


Figure S2.3. Effect of warming on the phenology of native, exotic, and invasive species. Effect of warming on (A) days to first flower, (B) days to last flower, (C) flowering period duration (days), and (D) days to first fruit for native, exotic, and invasive species (least square means ± SE; N = 20 native, 7 exotic, and 15 invasive species). Letters represent significant differences between groups (adjusted for multiple comparisons with a Tukey test, $p \leq 0.05$).

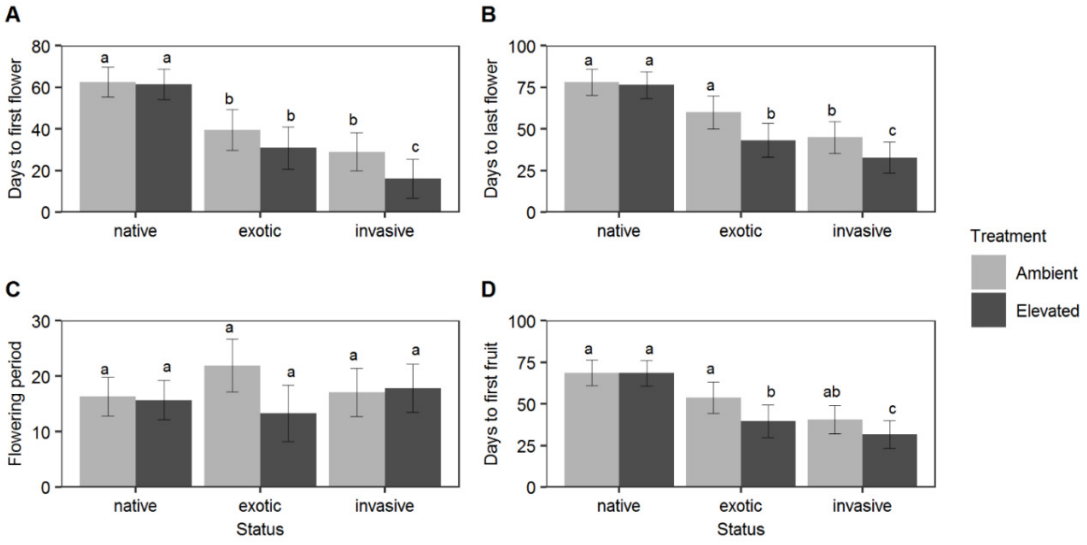


Figure S2.4. Phenological synchrony of native, exotic, and invasive species. Phenological synchrony (X) (least square means ± SE) of native, exotic, and invasive species under ambient and elevated (+3°C) temperatures. A phenological synchrony score of X=1 indicates complete synchrony among all individuals experiencing the same warming treatment, where all species start flowering at the same time and for the same length of time. A score of X=0 indicates complete asynchrony, or no overlap in flowering. Letters represent significant differences between groups (adjusted for multiple comparisons with a Tukey test, $p \leq 0.05$).

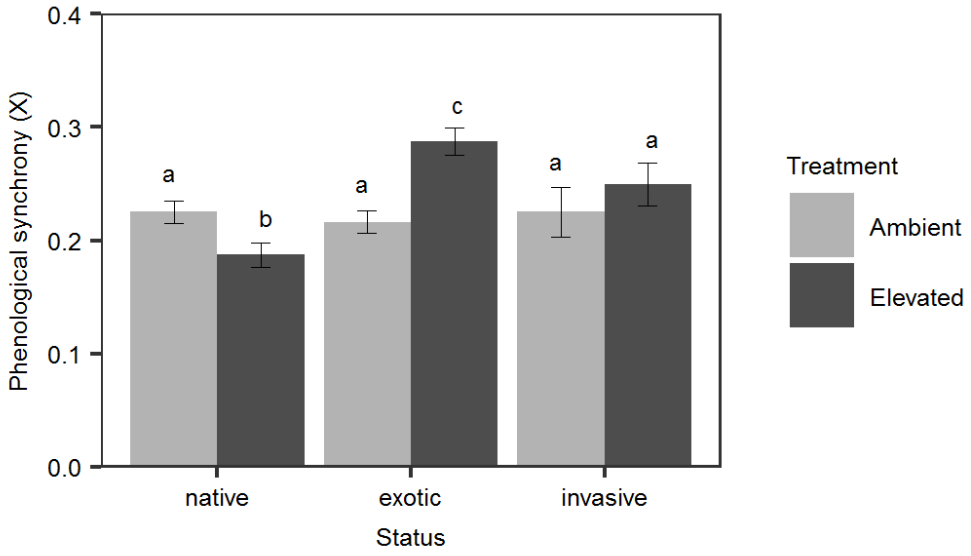


Figure S2.5. Effect of time since introduction on phenological plasticity, (no C_3 grasses). Effect of time since introduction to Michigan (MI) (years) on phenological plasticity for invasive and exotic species, excluding C_3 grasses (invasive R^2=0.62, p=0.07; exotic R^2=-0.16, p=0.6). Gray areas represent 95% confidence intervals.

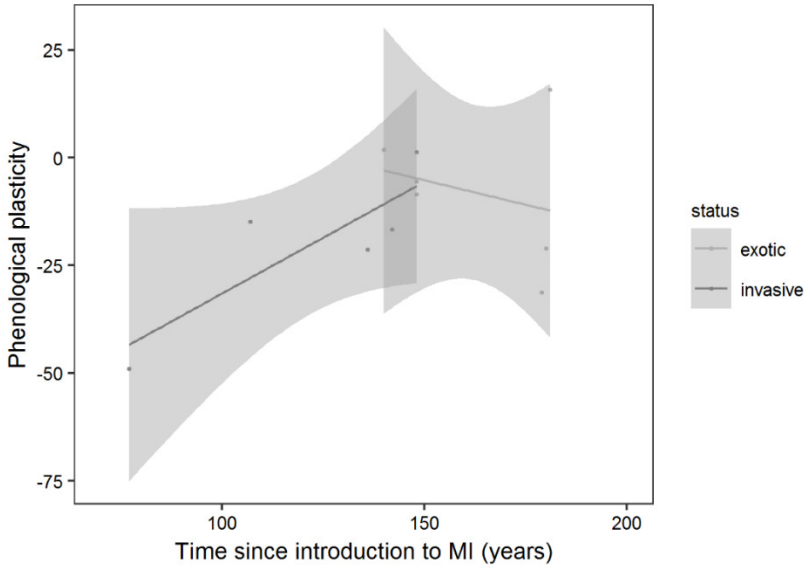


Table S2.1. Seed and phylogenetic information. Characteristics of the 42 species planted into the heating ring experimental plots in April 2012, including family, status (native, exotic, or invasive), and seed source. Field-collected seeds were from plants growing at the WK Kellogg Long-Term Ecological Research site. Purchased seed was from sources originally collected from MI (Michigan Wildflower Farm); OH, MN (Prairie Moon); PA, TX, CN, OR, WA (Ernst Seeds); NE (GRIN). GenBank accession numbers of genes (ITS, makK, rbcL) used for phylogenetic reconstruction are also provided.

Species name	Family	Status	Source	ITS	makK	rbcL
Achillea millefolium	Asteraceae	native	Field-collected	AY603185.1	EU385315.1	JX848399.1
Symphyotrichum (Aster) pilosum	Asteraceae	native	Field-collected	JQ360419.1	EU749444.1	EU677053.1
Centaurea stoebe	Asteraceae	invasive	Field-collected	JF914072.1	KC969492.1	KJ746252.1
Coreopsis lanceolata	Asteraceae	native	Michigan Wildflower Farm	KM347947.1	AY551495.1	HM849915.1
Coreopsis tripteris	Asteraceae	native	Michigan Wildflower Farm	KM347917.1	AY551499.1	
Erigeron annuus	Asteraceae	native	Field-collected	GU724302.1	HM989796.1	KJ841309.1
Euthamia graminifolia	Asteraceae	native	Field-collected	HQ142624.1	KJ592944.1	HQ590098.1
Gaillardia pulchella	Asteraceae	exotic	Ernst Seeds	KF607074.1	HM989787.1	HQ590105.1
Helenium autumnale	Asteraceae	native	Michigan Wildflower Farm	GU818553.1	GU817467.1, KJ772823.1	KJ773547.1
Helenium flexuosum	Asteraceae	exotic	Prairie Moon Nursery	KF607070.1	AY215804.1	AY215123.1
Leucanthemum vulgare	Asteraceae	invasive	Ernst Seeds	EF091600.1	HQ593344.1	KJ841377.1
Solidago canadensis	Asteraceae	native	Field-collected	HQ142591.1	EU749415.1	EU677023.1
Brassica rapa	Brassicaceae	invasive	Ernst Seeds	KF704394.1	AY541619.1	GQ184370.1
Turritis (Arabis) glabra	Brassicaceae	native	Prairie Moon Nursery	DQ310526.1	KP210444.1	HQ589958.1
Dianthus armeria	Caryophyllaceae	invasive	Field-collected	KX167086.1	KP210382.1	KT695582.1
Silene stellata	Caryophyllaceae	native	Prairie Moon Nursery	HQ334912.1	FJ589561.1	KP643867.1
Hypericum perforatum	Clusiaceae	invasive	Field-collected	JN811136.1	AB698447.1	HQ590139.1
Desmodium canadense	Fabaceae	native	Michigan Wildflower Farm	KM098891.1	HQ593266.1	KJ841264.1
Desmodium illinoense	Fabaceae	native	Ernst Seeds	KT459271.1	KT456906.1	KT458042.1
Lespedeza capitata	Fabaceae	native	Michigan Wildflower Farm	GU572172.1	KJ772888.1	KT695592.1
Lespedeza cuneata	Fabaceae	invasive	Ernst Seeds	GU572172.1	EU717416.1	EU717275.1
Lotus corniculatus	Fabaceae	invasive	Ernst Seeds	JN861076.1	HM049505.1	KJ841388.1

Table S2.1. (cont'd)

Medicago lupulina	Fabaceae	exotic	GRIN	JQ858257.1	HE966952.1	KJ841412.1
Melilotus officinalis	Fabaceae	invasive	Ernst Seeds	KJ999362.1	HE970723.1	KJ841414.1
Trifolium hybridum	Fabaceae	exotic	Ernst Seeds	AF053159.1	AF522125.1	KJ841632.1
Trifolium pratense	Fabaceae	exotic	Ernst Seeds	AF053171.1	EU749448.1	KJ841633.1
Plantago major	Plantaginaceae	invasive	Field-collected	AY101861.1	EU749328.1	EU676935.1
Andropogon gerardii	Poaceae	native	Michigan Wildflower Farm	DQ005015.1	AF144577.1	AJ784818.1
Bromus inermis	Poaceae	invasive	Field-collected	KF713194.1	AF164398.1	KJ841141.1
Bromus kalmii	Poaceae	native	Prairie Moon Nursery	AY367916.1		KT695565.1
Dactylis glomerata	Poaceae	invasive	Ernst Seeds	KJ598940.1	KF713137.1	HQ590058.1
Elymus canadensis	Poaceae	native	Michigan Wildflower Farm	KJ526335.1	HM770807.1	KC237138.1
Elymus repens	Poaceae	invasive	Field-collected	GQ365145.1	KF713125.1	HQ590076.1
Panicum virgatum	Poaceae	native	Michigan Wildflower Farm	DQ005062.1	EU434294.1	EF125135.1
Phleum pratense	Poaceae	exotic	Field-collected	HQ600524.1	HQ593382.1	KJ841460.1
Poa compressa	Poaceae	invasive	Ernst Seeds	KJ598896.1	KJ599232.1	KJ599121.1
Poa pratensis	Poaceae	invasive	Ernst Seeds	KJ598925.1	KJ599261.1	KJ599150.1
Poa trivialis	Poaceae	exotic	Ernst Seeds	GQ342555.1	FJ395369.1	JN893080.1
Schizachyrium scoparium	Poaceae	native	Michigan Wildflower Farm	DQ005072.1	FR832830.1	HE577863.1
Sorghastrum nutans	Poaceae	native	Michigan Wildflower Farm	DQ005080.1	EF137473.1	EF125121.1
Rumex crispus	Polygonaceae	invasive	Field-collected	KR537778.1	HQ593423.1	HQ590251.1
Penstemon hirsutus	Schrophulariaceae	native	Michigan Wildflower Farm	DQ531111.1		

Table S2.2. **Effect of warming and status on phenology of native vs. non-native species.** F-statistics and associated p-values for the effects of warming (ambient or elevated) and status (native or non-native) on reproductive phenology (days to first flower, days to last flower, flowering period, and days to first fruit) (linear mixed models, Gaussian distributions). Plot (nested in warming treatment) and species (nested in status) were included as random effects (estimates given as χ^2-values). Denominator degrees of freedom ranged from 6.91-283 for warming, from 32.4-40.6 for status, and from 281-294 for the interaction, depending on response variable. ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1.

Source	**df**	**Days to first flower F**	**Days to last flower F**	**Flowering period F**	**Days to first fruit F**
Warming	1	6.97**	10.09**	0.72	6.86**
Status	1	11.99**	11.12**	0.15	8.30**
Warming x Status	1	4.73*	5.70*	0.28	6.03*
Plot(treatment) (χ^2)		0.00	0.00	0.04	0.00
Species(status) (χ^2)		832.47	858.45	133.37	728.20
Residual		391.51	447.35	278.32	315.97

Table S2.3. **Phylogenetic analyses of the effect of warming and status on phenology of native vs. non-native species.** Results from phylogenetic generalized least squares (PGLS) testing the effects of status (native or non-native) and warming (ambient or elevated) on days to first flower, days to last flower, flowering period, and days to first fruit, while controlling for variance due to shared ancestry. ***p<0.0001, **p<0.01, *p≤0.05, ·p≤0.1.

Source **Brownian Motion**	**df**	**Days to first flower** **t**	**Days to last flower** **t**	**Flowering period** **t**	**Days to first fruit** **t**
Warming	1	-2.25*	-9.12***	-5.53***	-3.26**
Status	1	-0.03	-0.20	-0.14	0.00
Warming x Status	1	2.25*	11.52***	7.55***	2.34*
Residual	187	1135.65	1013.52	1207.57	17.06

Table S2.4. **Effect of warming and status on phenology of native, exotic, and invasive species.** F-statistics and associated p-values for the effects of warming (ambient or elevated) and status (native, exotic, or invasive) on reproductive phenology (days to first flower, days to last flower, flowering period, and days to first fruit) (linear mixed models, Gaussian distributions). Plot (nested in warming treatment) and species (nested in status) were included as random effects (estimates given as χ^2-values). Denominator degrees of freedom for warming ranged from 7.09-284 for warming, from 30.1-40.4 for status, and from 81.7-287 for the interaction, depending on response variable. ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1.

Source	**df**	**Days to first flower F**	**Days to last flower F**	**Flowering period F**	**Days to first fruit F**
Warming	1	8.85**	9.33*	1.88	10.02**
Status	2	6.41**	5.67**	0.06	4.55*
Warming x Status	2	2.55·	3.06*	1.54	2.66·
Plot(treatment) (χ^2)		0.00	10.68	0.00	2.32
Species(status) (χ^2)		833.46	874.85	134.54	716.85
Residual		392.46	296.2	258.9	129.1

Table S2.5. Phylogenetic analyses of the effect of warming and status on phenology of native, exotic, and invasive species. Results from phylogenetic generalized least squares (PGLS) testing the effects of status (native, exotic, or invasive) and warming (ambient or elevated) on days to first flower, days to last flower, flowering period, and days to first fruit, while controlling for variance due to shared ancestry. ***p<0.0001, **p<0.01, *p≤0.05, ·p≤0.1.

Source Brownian Motion	df	Days to first flower t	Days to last flower t	Flowering period t	Days to first fruit t
Warming	1	-0.05	-0.06	-0.16	-0.08
Status	2	-1.81·	-3.13**	-4.54***	-4.56***
Warming x Status	2	1.81·	3.84***	8.53***	3.25**
Residual	153	1134.42	1053.92	1237.70	17.07

Table S2.6. Species-specific phenological responses to temperature. Species-specific phenological responses to temperature (linear mixed model, Gaussian distribution; plot nested in status included as a random factor). N is the number of individuals for each species that flowered. Values (least square means ± SE) represent the difference in each phenological variable (days to first flower DFF, days to last flower DLF, flowering period FP, and days to first fruit DFFr) between elevated and ambient temperatures. Negative values indicate that phenology was accelerated under elevated temperatures and positive values indicate that phenology was delayed under elevated temperatures. Significant values are in bold; ·p<0.1, *p<0.05, **p<0.01, ***p<0.0001 (Tukey's tests for warming x species).

Species name	N	Difference in DFF	Difference in DLF	Difference in FP	Difference in DFFr
Invasive species					
Bromus inermis	9	**-22.43 ± 7.67***	-11.69 ± 8.63	+10.82 ± 7.97	-10.58 ± 4.71
Centaurea stoebe	14	**-14.80 ± 5.91·**	-11.20 ± 6.63	+3.22 ± 6.09	**-10.67 ± 4.45·**
Dactylis glomerata	14	-7.29 ± 5.91	-10.32 ± 6.63	-3.00 ± 6.09	**-23.40 ± 4.10*****
Dianthus armeria	7	-21.30 ± 9.01	-23.49 ± 10.14	-2.86 ± 9.37	+5.90 ± 5.40
Elymus repens	3	-18.67 ± 13.27	+11.82 ± 14.94	+29.20 ± 13.87	+21.50 ± 9.65
Hypericum perforatum	12	**-31.25 ± 6.49****	**-31.69 ± 7.30****	-0.58 ± 6.70	**-39.23 ± 4.53*****
Leucanthemum vulgare	11	+1.25 ± 6.90	**-21.12 ± 7.75 ·**	**-21.85 ± 7.14 ***	-12.90 ± 5.40
Lotus corniculatus	2	**-48.98 ± 15.46***	+0.45 ± 17.42	+50.73 ± 16.20	
Melilotus officinalis	19	**-16.67 ± 6.12***	**-20.13 ± 5.74***	-3.33 ± 5.25	-7.60 ± 3.67
Plantago major	6	+1.83 ± 9.36	+6.09 ± 10.53	+4.06 ± 9.76	+9.60 ± 8.18
Poa compressa	12	-4.43 ± 6.47	-1.56 ± 7.27	+3.07 ± 6.69	-4.25 ± 3.88
Poa pratensis	8	+0.23 ± 7.94	-2.21 ± 8.93	-2.14 ± 8.26	+1.86 ± 4.96
Rumex crispus	6	+15.84 ± 11.24	-5.76 ± 12.65	-19.80 ± 11.74	+1.75 ± 8.48
Exotic species					
Helenium flexuosum	10	-12.20 ± 7.46	**-44.01 ± 8.38****	-32.06 ± 7.73	**-28.33 ± 5.40****
Medicago lupulina	3	-8.57 ± 13.20	+17.10 ± 14.87	+25.96 ± 13.81	
Phleum pratense	15	-6.32 ± 5.80	-6.81 ± 6.51	-0.59 ± 5.98	-5.54 ± 4.02
Trifolium hybridum	4	-5.49 ± 12.20	-20.12 ± 13.73	-14.94 ± 12.75	+1.33 ± 8.92
Trifolium pratense	6	+13.09 ± 11.25	-22.72 ± 12.66	**-34.12 ± 11.72·**	**+23.50 ± 8.48·**
Native species					
Achillea millefolium	9	-16.86 ± 8.44	+0.87 ± 9.50	+18.02 ± 8.77	+1.00 ± 8.92
Andropogon gerardii	4	-20.54 ± 10.94	-17.17 ± 12.31	+3.87 ± 11.43	-13.00 ± 9.65
Bromus kalmii	6	+13.69 ± 11.23	4.28 ± 12.64	-7.89 ± 11.71	-0.50 ± 6.31
Coreopsis lanceolata	17	**-12.62 ± 5.39·**	-1.84 ± 6.04	+10.70 ± 5.53	-5.97 ± 3.42
Coreopsis tripteris	11	**-22.58 ± 7.24***	**-22.43 ± 8.14·**	-0.43 ± 7.51	+2.83 ± 7.96
Desmodium canadense	2	-1.35 ± 15.47	14.65 ± 17.42	_14.33 ± 16.02	
Elymus canadensis	5	**-31.77 ± 9.97***	-0.68 ± 11.22	**+30.03 ± 10.40***	+3.25 ± 6.82
Erigeron annuus	9	-6.62 ± 7.42	4.88 ± 8.34	+12.18 ± 7.69	-0.45 ± 5.36
Euthamia graminifolia	8	+16.77 ± 7.98	-3.55 ± 8.97	**-20.82 ± 8.29·**	**+19.00 ± 6.53***
Helenium autumnale	5				-15.00 ± 8.92
Panicum virgatum	16	+1.89 ± 5.54	-5.62 ± 6.22	-7.50 ± 5.70	-1.66 ± 4.67
Penstemon hirsutus	12	-5.77 ± 6.66	-11.98 ± 7.48	-6.56 ± 6.89	**-24.22 ± 4.41****
Schizachyrium scoparium	6	**+35.46 ± 9.01****	-5.47 ± 10.13	**-40.98 ± 9.37****	+1.50 ± 7.26
Silene stellata	3	+14.61 ± 13.20	+17.79 ± 14.86	+4.67 ± 13.81	-5.00 ± 8.00
Solidago canadensis	7	**+27.64 ± 8.47***	**+25.98 ± 9.53·**	-2.89 ± 8.78	**+37.50 ± 9.65****
Sorghastrum nutans	2	-7.16 ± 15.46	+0.55 ± 17.41	+7.12 ± 16.21	
Symphyotrichum (Aster) pilosum	10	+7.78 ± 7.46	+1.03 ± 8.39	-6.11 ± 7.75	
Turritus (Arabis) glabra	14	-3.52 ± 5.98	+0.69 ± 6.92	+4.52 ± 6.36	+1.11 ± 3.49

Table S2.7. Geographic spread, phenological plasticity, and time since introduction. Effect of status (native or non-native) and (A) days to first flower (DFF) and (B) phenological plasticity (difference in DFF between elevated and ambient temperatures) on the geographic spread (number of US counties) of native and non-native species (linear models, Gaussian distributions). (C) Effect of status (exotic or invasive) and time since introduction to Michigan (years) on phenological plasticity for non-native species (linear model, Gaussian distribution). **p≤0.01, *p≤0.05, ·p≤0.1.

A) Source	**df**	**F**
Status	1	11.57**
DFF	1	3.01·
Status x DFF	1	9.66**
Residual	33	415.2
B) Source	**df**	**F**
Status	1	8.19**
Plasticity	1	0.19
Status x Plasticity	1	1.41
Residual	30	432.6
C) Source	**df**	**F**
Status	1	4.57*
Time since introduction	1	0.21
Status x Time	1	4.04·
Residual	14	12.2

Table S2.8. Phylogenetic analyses of geographic spread, phenological plasticity, and time since introduction. Results from phylogenetic generalized least squares (PGLS) testing the effects of status (native or non-native) and (A) days to first flower (DFF) and (B) phenological plasticity (difference in DFF between elevated and ambient temperatures) on the geographic spread (number of US counties) of native and non-native species. (C) Effect of status (exotic or invasive) and time since introduction to Michigan (years) on phenological plasticity for non-native species, while controlling for variance due to shared ancestry. **p≤0.01, *p≤0.05.

A) Source	**df**	**t**
Status	1	10.74**
DFF	1	1.75
Status x DFF	1	4.59*
Residual	28	625.55
B) Source	**df**	**t**
Status	1	10.74**
Plasticity	1	1.75
Status x Plasticity	1	4.59*
Residual	28	672.50
C) Source	**df**	**t**
Status	1	1.27
Time since introduction	1	5.68*
Status x Time	1	5.92*
Residual	12	16.22

Table S2.9. Effect of flowering time and phenological plasticity on geographic spread in Michigan. Effect of status (native or non-native) and (A) days to first flower (DFF) and (B) phenological plasticity (difference in days to first flower between elevated and ambient temperatures) on the geographic spread (number of MI counties) of native and non-native species (linear models, Gaussian distributions).

A) Source	**df**	**F**
Status	2	0.01
Phenological plasticity	1	0.16
Status x Plasticity	2	0.08
Residual	33	21.6
B) Source	**df**	**F**
Status	2	0.00
DFF	1	0.09
Status x DFF	2	0.14
Residual	30	19.7

Table S2.10. Effects of flowering time and phenological plasticity on the geographic spread of native, exotic, and invasive species. Effect of status (native, exotic, or invasive) and (A) days to first flower (DFF) and (B) phenological plasticity (difference in DFF between elevated and ambient temperatures) on the geographic spread (number of US counties) of native, exotic, and invasive species (linear models, Gaussian distributions). Exotic and invasive species demonstrate similar patterns for both plasticity and DFF (DFF: Tukey test for status × DFF p=0.45; plasticity: Tukey test for status p=0.97). **p≤0.01, *p≤0.05, ·p≤0.1.

A) Source	**df**	**F**
Status	2	2.46
DFF	1	5.62**
Status x DFF	2	4.58*
Residual	31	425.50
B) Source	**df**	**F**
Status	2	3.84*
Plasticity	1	2.76·
Status x Plasticity	2	0.86
Residual	28	444.50

LITERATURE CITED

LITERATURE CITED

Agrawal, A.A., P.M. Kotanen, C.E. Mitchell, A.G. Power, W. Godsoe, and J. Klironomos. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology* 86: 2979-2989.

Amano, T., R.J. Smithers, T.H. Sparks, and W.J. Sutherland. 2010. A 250-year index of first flowering dates and its response to temperature changes. *Proceedings of the Royal Society B.* 266: 2451-2457.

Anderson, J.T., and Z.J. Gezon. 2014. Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Global Change Biology* 21: 1689-1703.

Arft, A.M., M.D. Walker, J. Gurevitch, J.M. Alatalo, M.S. Bret-Harte, M. Dale, M. Diemer, et al. 1999. Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecological Monographs* 69: 4991-5110.

Augspurger, C.K. 1981. Reproductive synchrony of a tropical shrub: experimental studies of effects of pollinators and seed predators on *Hybanthus prunifolius* (Violaceae). *Ecology* 62: 775-788.

Augspurger, C.K. 1983. Phenology, flowering synchrony, and fruit set of six neotropical shrubs. *Biotropica* 15: 257-267.

Badeck, F.W., A. Bondeau, K. Bottcher, D. Doktor, W. Lucht, J. Schaber, et al. 2004. Responses of spring phenology to climate change. *New Phytologist* 162: 295-309.

Bawa, K.S. 1977. The reproductive biology of *Cupania guatemalensis* Radlk. (Sapindaceae). *Evolution* 31: 52-63.

Borland K., S. Campbell, R. Schillo, and P. Higman. 2009. *A field identification guide to invasive plants in Michigan's natural communities*. Michigan Natural Features Inventory, Lansing, Michigan, USA.

Bradley, N.L., A.C. Leopold, J. Ross, and W. Huffaker. 1999. Phenological changes reflect climate change in Wisconsin. *Proceedings of the National Academy of Sciences* 96: 9701-9704.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics* 13: 115-155.

Brown, B.J., and R.J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia* 129: 34-49.

Burkle, L.A., J.C. Marlin, and T.M. Knight. 2013. Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science* 339: 1611-1615.

Cadotte, M.W., B.R. Murray, and J. Lovett-Doust. 2006. Ecological patterns and biological invasions: using regional species inventories in macroecology. *Biological Invasions* 8: 809-821.

CaraDonna, P.J., A.M. Iler, and D. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences* 111: 4916-4921.

Chuine, I., X. Morain, and H. Bugmann, H. 2010. Warming, photoperiods, and tree phenology. *Science* 329: 277-278.

Cleland, E.E., I. Chuine, A. Menzel, H.A. Mooney, and M.D. Schwartz. 2007. Shifting plant phenology in response to global change. *Trends in Ecology and Evolution* 22: 357-365.

Cleland, E.E., S.E. Travers, E.S. Zavalta, T.M. Crimmins, J.A. Dunne, S. Pau, et al. 2012. Phenological tracking enables positive species responses to climate change. *Ecology* 93: 1765-1771.

Cook, B.I., E.M. Wolkovich, and C. Parmesan. 2012. Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings of the National Academy of Sciences* 109: 9000-9005.

Cooper, H.F. 2018. Integrating plant functional traits, genetics, phenotypic plasticity, and community structure to assess the impact of climate change on native plants in the Southwestern US. Order No. 10817213 Northern Arizona University. Ann Arbor: *ProQuest.* Web. 15 Jan. 2019.

Craine, J.M., E.M. Wolkovich, E.G. Towne, and S.W. Kembel. 2012. Flowering phenology as a functional trait in a tallgrass prairie. *New Phytologist* 193: 673-682.

Crawley, M.J., P.H. Harvey, and A. Purvis. 1996. Comparative ecology of the native and alien floras of the British Isles. *Philosophical Transactions of the Royal Society B* 351: 1251-1259.

Czarapata, E.J. 2005. *Invasive plants of the Upper Midwest*. University of Wisconsin Press, Madison, Wisconsin, USA.

Darriba, D., G.L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

DeFalco, L.A., G.C.J. Fernandez, and R.S. Novak. 2007. Variation in the establishment of a non-

native annual grass influences competitive interactions with Mojave Desert perennials. *Biological Invasions* 9: 293-307.

Dickson, T.L., J.L. Hopwood, and B. Wilsey. 2012. Do priority effects benefit invasive plants more than native plants? An experiment with six grassland species. *Biological Invasions* 14: 2617-2624.

Dietz, H., and P.J. Edwards. 2006. Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* 87: 1359-1367.

Divíšek, J., M. Chytrý, B. Beckage, N.J. Gotelli, Z. Lososová, P. Pyšek, et al. 2018. Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. *Nature Communications* 9: 1-10.

Donnelly, A., A. Caffarra, and B.F. O'Neill. 2011. A review of climate-driven mismatches between interdependent phenophases in terrestrial and aquatic ecosystems. *International Journal of Biometeorology* 55: 805-817.

Dunne, J., J. Harte, and K. Taylor. 2003. Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs* 73: 69-86.

Dunnell, K.L., and S.E. Travers. 2011. Shifts in the flowering phenology of the northern Great Plains: Patterns over 100 years. *American Journal of Botany* 98: 935-945.

Elton, C.S. 1958. *The ecology of invasions by animals and plants*. Ed. 2000. University of Chicago Press, Chicago, IL, USA.

Fitter, A.H., and R.S.R. Fitter. 2002. Rapid changes in flowering time in British plants. *Science* 296: 1689-1691.

Forrest, J., and A.J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society London B* 365: 3101-3112.

Fridley, J.D. 2012. Extended leaf phenology and the autumn niche in deciduous forest invasions. *Nature* 485: 359-362.

Gallagher, R.V., R.P. Randall, and M.R. Leishman. 2015. Trait differences between naturalized and invasive plant species independent of residence time and phylogeny. *Conservation Biology* 29: 360-390.

Garland, T., A.W. Dickerman, C.M. Janis, and J.A. Jones. (1993). Phylogenetic analysis of covariance by computer simulation. *Systematic Biology* 42: 265-292.

Gerlach, J.D., and K.J. Rice. 2003. Testing life history correlates of invasiveness using congeneric plant species. *Ecological Applications* 13: 167-179.

Godoy, O., D.M. Richardson, F. Valladares, and P. Castro-Diez. 2009. Flowering phenology of invasive alien plant species compared with native species in three Mediterranean-type ecosystems. *Annals of Botany* 103: 485-494.

Harrington, R., I. Woiwod, and T. Sparks. 1999. Climate change and trophic interactions. *Trends in Ecology and Evolution* 14: 146-150.

Hoffman, A., J. Camac, R.J. Williams, W. Papst, F.C. Jarrad, and C.H. Wahren. 2010. Phenological changes in six Australian subalpine plants in response to experimental warming and year-to-year variation. *Journal of Ecology* 98: 927-937.

Jarrad, F., C.H. Wahren, E.J. Williams, and M.A. Burgman. 2008. Impacts of experimental warming and fire on phenology of subalpine open-heath species. *Australian Journal of Botany* 56: 617-629.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Surrock, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.

Kharouba, H.M., J. Ehrlén, A. Gelman, K. Bolmgren, J.M. Allen, S.E. Travers, et al. 2018. Global shifts in the phenological synchrony of species interactions over recent decades. *Proceedings of the National Academy of Sciences* 115: 5211-5216.

Kimball, B.A., M.M. Conley, S. Wang, X. Lin, C. Luo, I. Morgan, and D. Smith. 2008. Infrared heater arrays for warming ecosystem field plots. *Global Change Biology* 14: 309-320.

König, P., S. Tautenhahn, H.C. Cornelissen, J. Kattge, G. Bönisch, and C. Römermann. 2017. Advances in flowering phenology across the Northern Hemisphere are explained by functional traits. *Global Ecology and Biogeography* 27: 310-321.

Lake, J.C., and M.R. Leishman. 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation* 117: 215-226.

Lamarque, L.J., C.J. Lortie, A.J. Porté, and S. Delzon. 2015. Genetic differentiation and phenotypic plasticity in life-history traits between native and invasive populations of invasive maple trees. *Biological Invasions* 17: 1109-1122.

Lee, J-S. 2011. Combined effect of elevated CO_2 and temperature on the growth and phenology of two annual C_3 and C_4 weedy species. *Agriculture, Ecosystems & Environment* 140: 484-491.

Liancourt, P., L. Spence, B. Boldgiv, A. lkhagva, B.R. Helliker, B.B. Casper, et al. 2012. Vulnerability of the northern Mongolian steppe to climate change: insights from flower production and phenology. *Ecology* 93: 815-824.

Martins, E.P., and T.F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* 98: 778-789.

McKenna, D.D. 2004. Flora and Vegetation of Kalamazoo County, Michigan. *The Michigan Botanist* 43: 137-359.

Memmot, J., P.G. Craze, N.M. Waser, and M.V. Price. 2007. Global warming and the disruption of plant-pollinator interactions. *Ecology Letters* 10: 710-717.

Menzel, A., T. Sparks, N. Estrella, E. Koch, A. Aasa, R. Ahas, et al. 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology* 12: 1969-1976.

Menzel, A. 2002. Phenology: its importance to the global change community. *Climatic Change* 54: 379-385.

Møller, A.P., D. Rubolini, and E. Lehikoinen, E. 2008. Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proceedings of the National Academy of Sciences* 105: 16195-16200.

Nicotra, A.B., O.K. Atkin, S.P. Bonser, A.M. Davidson, E.J. Finnegan, U. Mathesius, et al. 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* 15: 684-692.

Ollerton, J., and A.J. Lack. 1992. Flowering phenology: an example of relaxation of natural selection? *Trends in Ecology and Evolution* 7: 274-276.

Ollerton, J., and A.J. Lack. 1998. Relationship between flowering phenology, plant size and reproductive success in shape *Lotus corniculatus* (Fabaceae). *Plant Ecology* 139: 35-47.

Ovaskainen, O., S. Skorokhadova, M. Yakovleva, A. Sukhov, A. Kutenkov, N.Kutenkova, et al. 2013. Community-level phenological response to climate change. *Proceedings of the National Academy of Sciences* 110: 13434-13439.

Paradis, E. 2012. Analysis of phylogenetics and evolution with R. Ed. 2. Springer, New York, New York, USA.

Park, D.S., I. Breckheimer, A.C. Williams, E. Law, A.M. Ellison, and C.C. Davis. 2018. Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. *Philosophical Transactions of the Royal Society B.* 374: 20170394.

Parmesan, C. 2007. Influences of species, latitudes, and methodologies on estimates of phenological response to global warming. *Global Change Biology* 13: 1860-1872.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.

Pearson, D.R., Y.K. Ortega, and S.J. Sears. 2012. Darwin's naturalization hypothesis up-close: Intermountain grassland invaders differ morphologically and phenologically from native community dominants. *Biological Invasions* 14: 901-913.

Peñuelas, J., and I. Filella. 2001. Responses to a warming world. *Science* 294: 793-795.

Peñuelas, J., I. Filella, and P. Comas. 2002. Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biology* 8: 531-544.

Pinheiro, J., D. Bates, S. DebRoy, D. Sakar, and R Core Team. 2015. nlme: linear and nonlinear mixed effects models. R package version 3.1-119.

Pyšek, P., and D.M. Richardson. 2007. Traits associated with invasiveness in alien plants: where do we stand? *Ecological Studies* 193: 97-125.

Rathcke, B., and L.P. Lacey. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology, Evolution, and Systematics* 16: 179-214.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Reed, T.E., S. Jenouvrier, and M.E. Visser, M.E. 2013. Phenological mismatch strongly affects individual fitness but not population demography in a woodland passerine. *Journal of Animal Ecology* 82: 131-144.

Resasco, J., A.N. Hale, M.C. Henry, and D. Gorchov. 2007. Detecting an invasive shrub in a deciduous forest understory using late-fall Landsat sensor imagery. *International Journal of Remote Sensing* 29: 3739-3745.

Sale, P.F. 1977. Maintenance of high diversity in coral reef fish communities. *American Naturalist* 111: 337-359.

SAS Institute. 2011. SAS 9.3 for Windows. SAS Institute, Cary, North Carolina, USA.

Schultheis, E.H., A.E. Berardi, and J.A. Lau. 2015. No release for the wicked: enemy release is dynamics and no associated with invasiveness. *Ecology* 96: 2446-2457.

Seabloom, E., W. Harpole, O. Reichman, and D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences* 104: 13384-13389.

Sherry, R.A., X. Zhou, S. Gu, J.A. Arnone, D.S. Schimel, P.S. Verburg, L.L. Wallace, and Y. Luo. 2007. Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences* 104: 198-202.

Smith, S.A., and C.W. Dunn. 2008. Phyutility: a phyloinformatics tool for trees, alignments, and molecular data. *Bioinformatics* 24: 715-716.

Sparks, T.H., E.P. Jeffree, and C.E. Jeffree, C.E. 2000. An examination of the relationship between flowering times and temperature at the national scale using long-term phenological records from the UK. *International Journal of Biometeorology* 44: 82-87.

Stamatakis, A. 2014. RaxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313.

Stenseth, N.C., and A. Mysterud. 2002. Climate, changing phenology, and other life history and traits: nonlinearity and mismatch to the environment. *Proceedings of the National Academy of Sciences* 99: 13379-13381.

Stocker, T.F., D. Qin, G.K. Plattner, M. Tignor, S.K. Allen, J. Boschung, et al. 2013. Technical Summary. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, UK and New York, NY, USA.

Stricker, K.B., and P. Stiling. 2014. Release from herbivory does not confer invasion success for *Eugenia unifora* in Florida. *Oecologia* 174: 817-826.

Thackeray, S.J., P.A. Henrys, D. Hemming, J.R. Bell, M.S. Botham, S. Burthe, P. Helauoet, et al. 2016. Phenological sensitivity to climate across taxa and trophic levels. *Nature* 535: 241-245.

Tikkanen, O-P., and R. Julkunen-Tiitto. 2003. Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operpphtera brumata. Oecologia* 136: 3187-3199.

Visser, M.E., C. Both, and M.M. Lambrechts. 2004. Global climate change leads to mismatched avian reproduction. *Advances in Ecological Research* 25: 89-110.

Visser, M., and C. Both. 2005. Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society London B* 272: 2561-2569.

Visser, M. E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society London B* 275: 649-659.

Wang, C., Y. Tang, and J. Chen. 2015. Plant phenological synchrony increases under rapid within-spring warming. *Scientific Reports* 6: 25460.

Whittington, H.R., D. Tilman, P.D. Wragg, and J.S. Powers. 2015. Phenological responses of prairie plants vary among species and year in a three-year experimental warming study. *Ecosphere* 6: 1-15.

Willis, C.G., R.B. Primack, A.J. Miller-Rushing, and C.C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences* 105: 17029-17033.

Willis, C.G., B. Ruhfel, R.B. Primack, A.J. Miller-Rushing, J.B. Losos, and C.C. Davis. 2010. Favorable climate change response explains non-native species' success in Thoreau's woods. *PLoS One* 5(1): e8878.

Wilsey, B.J., K. Barber, and L. Martin, L. 2015. Exotic grassland species have stronger priority effects than natives regardless of whether they are cultivated or wild genotypes. *New Phytologist* 205: 928-937.

Wilsey, B.J., L.M. Martin, and A.D. Kaul. 2018. Phenology differences between native and novel exotic-dominated grasslands rival the effects of climate change. *Journal of Applied Ecology* 55: 863-873.

Wolf, A.A., E.S. Zavaleta, and P.C. Selmants. 2017. Flowering phenology shifts in response to biodiversity loss. *Proceedings of the National Academy of Sciences* 114: 3463-3468.

Wolkovich, E.M., and E.E. Cleland. 2011. The phenology of plant invasions: a community ecology perspective. *Frontiers in Ecology and Evolution* 9: 287-294.

Wolkovich, E.M., T.J. Davies, H. Schaffer, E.E. Cleland, B.I. Cook, S.E. Travers, C.G. Willis, and C.C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany* 100: 1407-1421.

Xu, C.Y., K.L. Griffin, and W.S.F. Schuster. 2007. Leaf phenology and seasonal variation of photosynthesis of invasive *Berberis thunbergii* (Japanese barberry) and two co-occurring native understory shrubs in a northeastern United States deciduous forest. *Oecologia* 154: 11-21.

Zohner, C.M., and S.S. Renner. 2017. Innately shorter vegetation periods in North American species explain native-non-native phenological asymmetries. *Nature Ecology and Evolution* 1: 1655-1660.

Zohner, C.M., L. Mo, and S.S. Renner. 2018. Global warming reduces leaf-out and flowering synchrony among individuals. *eLife* 7: e40214.

CHAPTER THREE:

Extinct and extant species differ in their phenological responses to warming[10]

ABSTRACT

Shifting phenology in response to climate and other factors is one mechanism that can promote population persistence and geographic spread; therefore, species with limited ability to phenologically track changing environmental conditions may be more susceptible to population declines. Alternatively, apparently nonresponding species may demonstrate divergent responses to multiple environmental conditions experienced across seasons. Capitalizing on herbarium records from across the Midwestern United States and detailed botanical surveys documenting local extinctions over the past century, we investigate whether extinct and extant taxa differ in their phenological responses to temperature and precipitation experienced during winter and spring (during flowering and the growing season prior to flowering) or in their magnitude of flowering time shift over the past century. Although warmer temperatures across seasons advanced flowering, locally extinct and extant species differed in the magnitude of their phenological responses to winter and spring warming. Locally extinct species advanced flowering in response warmer spring temperatures to a lesser extent than extant species. In contrast, locally extinct species advanced flowering more than extant species in response to warmer winter temperatures. Greater spring precipitation tended to delay flowering for both extinct and extant taxa. Finally, both extinct and extant taxa delayed flowering over time. This study highlights the importance of understanding phenological responses to seasonal warming and indicates that locally extinct species differ from extant species in their phenological

[10] Co-authors: K. Renaldi, M.D. Muzyka, and J.A. Lau

responses to temperature, a finding consistent with the hypothesis that appropriate phenological responses may reduce species' likelihood of extinction.

INTRODUCTION

Phenology, or the timing of life history events, is critical to fitness and population persistence (Parmesan and Yohe, 2003; Cleland et al., 2007). While some species and populations exhibit little phenological plasticity (i.e., shift their phenology little in response to environmental variation), other species and populations respond strongly to both temperature and precipitation (Visser and Both, 2005; Miller-Rushing and Primack, 2008; Matthews and Mazer, 2016; Thackeray et al., 2016; Cremonense et al., 2017), as well as other environmental variables like nutrient availability or competition (Smith et al., 2012; Xia and Wan, 2013; Du et al., 2019; Wang and Tang, 2019). Phenological plasticity may promote population growth (or limit population declines) in the face of climate change and has been associated with invasiveness and range size (Crawley et al., 1996; DeFalco et al., 2007; Willis et al., 2008; 2010; Cleland et al., 2012; Pearson et al., 2012; Wolkovich et al., 2013; Zettlemoyer et al., 2019b), suggesting that species that are less phenologically plastic may be more at risk of population declines and eventual extinction (Willis et al., 2008; Møller et al., 2008; Forrest and Miller-Rushing, 2010; Miller-Rushing et al., 2010).

If failure to adjust phenology is in fact correlated with population decline and extinction (or if phenological plasticity correlates with species success), phenological traits could be key predictors of extinction risk, particularly under future climates. However, whether a failure to shift phenology is linked to local extinction events remains uncertain. Traits of locally extinct species may correspond with historical responses of those species to environmental change,

thereby informing predictions of extinction risk for these species across their ranges as well as for similar threatened species (Purvis et al., 2000; Collen et al., 2010). Herbarium specimens and other historical datasets, like repeated botanical surveys, provide valuable records of local extinction events (Lang et al., 2018; Meineke et al., 2018; Zettlemoyer et al., 2019a). They span decades, include dozens of species replicates, and often contain species that have recently declined due to contemporary changes such as rising temperatures (Primack et al., 2004; Meineke et al., 2018). Individual plants contained in herbaria also provide a valuable record of how phenology shifts over time in response to climate (Willis et al., 2017; Ellwood et al., 2019). The time span and geographic area encompassed by herbarium specimens represent greater climatic variation than traditional observational or manipulative studies, thus providing a more complete picture of phenological shifts (Davis et al., 2015).

The bulk of studies investigating phenological plasticity have focused on phenological responses to temperature in the context of warming. However, a surprising number of species do not appear to advance flowering in response to warming (Rafferty and Ives, 2011; Cook et al., 2012; Parmesan and Hanley, 2015). Rather than being insensitive to rising temperature, species may respond to climate changes beyond spring warming, like changing precipitation patterns or winter warming, and temperate species may demonstrate contrasting responses to environmental conditions in different seasons (Cook et al., 2012). Warmer winters and earlier snowfall generally accelerate phenology (Arft et al., 1999; Bjorkman et al., 2016). Drought can accelerate (Cremonense et al., 2017) or delay flowering (Cui et al., 2017), while heavy rainfall can extend the growing season later into the year (Schuster et al., 2017). Finally, species may delay flowering in response to warming during some seasons while advancing flowering in response to warming in other seasons (Parmesan and Hanley, 2015). For example, 81 out of 490 studied

species from the US and UK delayed flowering due to winter warming and advanced flowering due to spring warming, resulting in no observable overall phenological response to temperature (Cook et al., 2012). Phenological shifts in response to winter warming or shifts in precipitation also could prove maladaptive if it makes plants more susceptible to late frost events or other harsh environmental conditions (Elzinga et al., 2007). Failing to consider climatic conditions in seasons other than spring may result in underestimating the proportion of species able to shift their phenology in response to climate change.

We used herbarium specimens from across the Midwestern United States to examine the flowering phenologies of 8 confamilial (often congeneric) pairs of locally extinct (defined here as species that have disappeared from a particular county) (Zettlemoyer et al., 2019a) and extant prairie species. We investigated how extinct vs. extant species' phenologies have shifted over the last 155 years (ca. 1860-2015) and in response to temperature and precipitation experienced during both spring (during flowering and the growing season prior to flowering) and winter. By considering the responses of both locally extinct and extant species, we investigate whether the inability to shift phenology in response to temperature or precipitation or over time is associated with local extinction. By considering responses to both spring and winter climatic variables, we address the importance of seasonal variation on phenology. We ask the following questions: (1) How does the phenology of locally extinct and extant species respond to temperature and precipitation experienced during the month of flowering, during the growing season prior to flowering, and during the winter prior to flowering? (2) Does the phenology of locally extinct and extant species differ, and do those differences influence phenological responses to climate? (3) Has the phenology of locally extinct and extant species shifted over time? If the hypothesis

that failure to adjust phenology contributes to population decline holds, we expect that locally extinct species will be less sensitive to temperature and precipitation than extant species.

MATERIALS AND METHODS

Study System

Kalamazoo County, covering 1492 km^2 in southwestern Michigan, boasts a diverse and well-documented flora that was surveyed from ca. 1890-1940 (McKenna, 2004) and was resurveyed from ca. 1994-2003 (Hanes and Hanes, 1947). These historical records describe the presence/absence of native and introduced vascular plants in Kalamazoo County (note that no historical abundance data are available). Rare, prairie specialist species are at high risk of extinction in the county (Zettlemoyer et al., 2019a). From these species we selected 17 native prairie species in which one species within a family is locally extinct ("locally extinct") while the other persists ("extant") (7 pairs and 1 triplet; Table 3.1). The species selected represent all available pairs of native, perennial, prairie specialist forbs. Although the locally extinct species are not extinct across their entire range, they are rare species likely susceptible to population declines elsewhere (Daru et al., 2018). We limit our study taxa to prairie specialist species because they are at higher risk of loss than species that can persist in other habitat types (Kuussaari et al., 2009; Zettlemoyer et al. 2019a) and so that differences in habitat use (e.g., the ability to use other grossly different habitat types) would not be confounded with extinction. However, we acknowledge that many other differences between taxa may be associated with extinction (e.g., abundance or niche breadth differences beyond broad habitat type preferences). Our approach is akin to comparisons of native vs. non-native species in that it identifies traits and responses associated with shifts in abundance or range size, in this case rarity and eventual

extinction (Murray, 2002; van Kleunen and Richardson, 2007). This combination of historical datasets documenting local extinctions and herbarium records for assessing phenology provides us with the capacity to test the hypothesis that phenology influences not only population declines but local extinctions.

Table 3.1. Species, plant family, mean flowering date, range of years represented by herbarium samples, and sample size for the 7 confamilial pairs and 1 triplet (*Penstemon*) included in this study. * indicates locally extinct species.

Species	Family	Mean flowering date (Julian day)	Year range	Sample size
Aster ericoides	Asteraceae	Sept 21 (264.23)	1888-2006	100
*Aster sericeus**	Asteraceae	Sept 16 (259.84)	1860-2008	99
Baptisia tinctoria	Fabaceae	July 17 (198.20)	1870-2015	50
*Baptisia bracteata**	Fabaceae	May 27 (147.10)	1981-2010	100
Eryngium yuccifolium	Apiaceae	July 27 (208.86)	1880-2010	50
*Thaspium trifoliatum**	Apiaceae	June 12 (163.40)	1876-1998	50
Liatris aspera	Asteraceae	Sept 3 (246.45)	1902-2011	49
*Liatris punctata**	Asteraceae	Aug 26 (238.74)	1890-2014	39
Monarda fistulosa	Lamiaceae	July 22 (203.97)	1889-2015	108
*Pycnanthemum tenuifolium**	Lamiaceae	Aug 4 (216.63)	1896-2012	50
Penstemon digitalis	Scrophulariaceae	June 25 (176.03)	1892-2011	48
*Penstemon hirsutus**	Scrophulariaceae	June 10 (161.69)	1890-2008	99
*Penstemon pallidus**	Scrophulariaceae	June 3 (154.69)	1895-2005	50
Ratibida pinnata	Asteraceae	July 27 (208.76)	1887-2011	50
*Ratibida columnifera**	Asteraceae	July 14 (195.11)	1896-2011	50
Silphium perfoliatum	Asteraceae	Aug 8 (220.09)	1882-2013	50
*Silphium terebinthinaceum**	Asteraceae	Aug 16 (228.76)	1891-2011	48

Phenological Data

We examined 1,090 herbarium specimens from locations spanning the Midwestern USA. Although this phenological scale (the Midwest) differs from the scale of extinction (Kalamazoo County), we included samples from Michigan, Ohio, Indiana, Illinois, and Wisconsin to increase sample size (Table S3.1). Due to the difference in scale, we performed all analyses on the full dataset and on specimens collected in Michigan. Because the results of both the full and the spatially restricted model are qualitatively similar, we present the results from the broader Midwestern dataset in the main text and report Michigan-only results in Table S3.2.

We visited the Michigan State University and University of Michigan herbaria to examine specimens in person and refine protocols; we found all other specimens online in the Consortium of Midwest Herbaria (http://midwestherbaria.org/portal/). We excluded specimens that had no reproductive structures present. From each specimen, we recorded the number of buds, flowers, senescing flowers, and fruits. For species with flowering heads, we visually estimated the proportion of each head in bloom, multiplied that by the average number of flowers and buds per head, and calculated approximate numbers of flowers and buds. For each specimen, we also noted date and year collected (ranging from 1860-2015), and location (latitude and longitude).

Herbarium specimens, while useful in phenological studies, pose challenges due to biases (Daru et al., 2018). Specimens may have been more intensively collected in different years; however, we detect little evidence that any bias in collection efforts across time differed for extinct and extant species (status x decade $F_{1,28}$=0.01, p=0.91). Since error in phenology estimated from a specimen can be high (Schmidt-Lebuhn et al., 2013), we examined at least 50 specimens per species. To make the dataset more robust than records from a single herbarium, we incorporated records from 27 herbaria across the Midwest.

We conducted all analyses on two response variables currently debated as the most appropriate phenological metrics from herbarium data: discrete phenology vs. a continuous estimate (Pearson, 2019). First, we used collection day of year as a proxy for flowering day of year ("day of year" or "DOY"), following Park et al. (2018). However, herbarium specimens represent a discrete life stage and are often biased towards mature flowers (Schmidt-Lebuhn et al., 2013), resulting in later first-flowering estimates than detected in the field (Davis et al., 2015). To compare this discrete flowering date to a phenological estimate spanning budding to

fruiting specimens, we also quantified phenology along a continuum (Moussus et al., 2010; Panchen and Gorelick, 2017). We calculated a Developmental Index (DI) for each specimen based on number of different reproductive structures as:

$$Developmental\ Index\ =\ (\frac{number\ of\ reproductive\ structures}{1(buds)+2(flowers)+3(senescing\ flowers)+4(fruits)})*\ln\ (DOY)$$

DI accounts for variation in phenology by incorporating a continuum of phenological phases from budding to fruiting across specimens collected on varying dates. The coefficients preceding each floral stage (buds, flowers, senescing flowers, and fruits) make it so that species farther along in their phenology (i.e., a greater proportion of fruits than buds; flowered earlier) have a lower DI. For example, a specimen with 10 buds collected on Julian day 200 has a lower DI (5.3) than a specimen with 10 buds collected on Julian day 300 (5.7) and a higher DI than a specimen collected on Julian day 200 but with 10 fruits instead of buds (DI=1.32). DI and DOY estimates were correlated (Pearson's r=0.11, p=0.0003; correlations ranged from r=-0.03 to r=0.42 across taxa).

Temperature data

We used the CLIMOD database (http://climod2.nrcc.cornell.edu/) and the Applied Climate Information System (rcc-acis.org) to collect all temperature and precipitation data. Weather data was queried from the ACIS using the tidyr, httr, sqldf, jsonlite, and lubridate packages in R v.3.0.2 (Grolemund and Wickham, 2011; Ooms, 2014; R Core Team, 2015; Grothendieck, 2017; Wickham, 2019; Wickham and Henry, 2019). We used each specimen's date, year, and county of collection to search all available records from contemporaneously operating weather stations and calculated the mean of each climatic variable across weather stations.

Given that temperature commonly influences flowering time (Fitter and Fitter, 2002) and has been associated with phenological shifts in a wide range of both plant and animal taxa (Thackeray et al., 2016) and that precipitation can also influence flowering phenology (Schuster and Dukes, 2017), we collected temperature and precipitation data for three seasonal time periods: during flowering ($T_{flowering}$ or $P_{flowering}$), during the growing season ($T_{growing}$ or $P_{growing}$), and during the winter prior to flowering (T_{winter} or P_{winter}). Each species was assigned a set range of dates for each seasonal metric based on its mean flowering date (date (e.g., *Monarda fistulosa*'s mean flowering date across the entire dataset was July 22 so all *Monarda fistulosa* specimens had a flowering season of 30 days prior to July 22, a growing season of April 1 – July 22, and a winter season of November – March). Specifically, $T_{flowering}$ and $P_{flowering}$ were calculated as the means of daily temperatures (°C) or precipitation (mm) experienced at the specimen's location during the year in which a specimen was collected 30 days prior to the species' mean flowering date. $T_{growing}$ and $P_{growing}$ were calculated as the mean of daily temperatures (°C) or precipitation (mm) experienced between April 1 (which we denote as approximately the beginning of the Midwest growing season based on average last frost dates [MSU CANR]) and the species' mean flowering date. T_{winter} and P_{winter} were calculated as the mean of daily temperatures (°C) or precipitation (mm) experienced during the winter season prior to flowering (November – March, based on when snowfall occurs across the region represented in this study [Fig. S3.1]).

Data analysis

We first tested for relationships between year, geography, and each individual climatic variable using linear models. We included $T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, and P_{winter} as

separate response variables and year, latitude, and longitude as predictor variables (interactions were never significant ($p>0.1$) and removed from analyses) (Tables S3.3-S3.4).

To test for shifts in phenology due to variation in temperature or precipitation and whether extant and extinct species differ in phenological responses to climate, we used random slopes linear mixed models to determine the effects of $T_{flowering}$ $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}, status (locally extinct vs. extant), and interactions of status with each climatic variable (e.g., status x $T_{flowering}$, status x $T_{growing}$, etc.) on flowering phenology (response variables: DOY or DI). Models were fit using the lme4 package in R (Bates et al., 2015; R Core Team, 2015; v3.3.2). We included latitude and longitude as covariates to control for spatial variation in phenological responses from cooler, high latitude populations to warmer, low latitude populations and from wetter eastern populations to drier western populations, respectively. Because of the high number of potential model terms, we did not include all interactions and instead only included interactions between latitude, longitude, climatic variables, and status when there was a biologically reasonable hypothesis for the interaction based on prior studies (Table S3.5). We fit random slopes for each species' response to each climatic variable (i.e., each species varied in its slope to reflect species-specific phenological responses to climate) (Bliese and Ployhart, 2002). We then used backwards elimination to simplify the two models (one for DOY and another for DI), sequentially removing predictors with the highest p-value greater than alpha ($\alpha=0.1$) until no collinear predictors were included and all p-values were greater than α. We provide Akaike Information Criterion values for sequential models for DOY produced via backwards elimination in Table S3.6 (procedures for DI models were similar). The final model for DOY included status, latitude, longitude, $T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing,}$ status x $T_{flowering}$, status x $T_{winter,}$ latitude x $T_{growing}$, latitude x T_{winter}, latitude x $P_{growing}$, and longitude x

T_{winter} as predictor variables. The final model for DI included status, latitude, longitude, $T_{flowering}$, $T_{growing}$, T_{winter}, $P_{growing,}$ status x longitude, status x $T_{flowering}$, status x $T_{growing}$, latitude x $T_{growing}$, longitude x $T_{flowering}$, and longitude x T_{winter} as predictor variables.

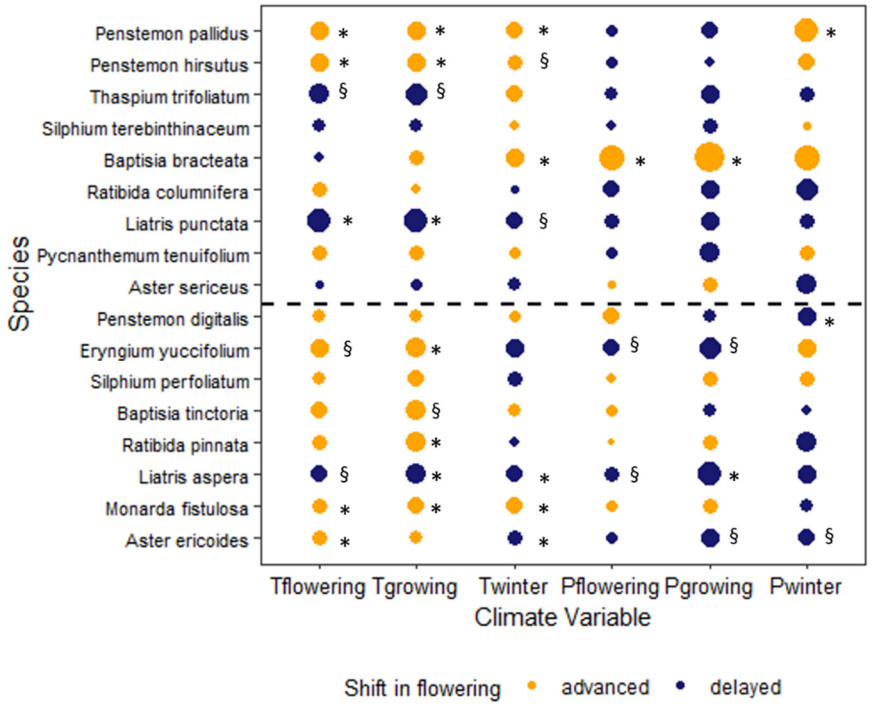
Earlier-flowering species often respond more strongly to climate change (Park et al., 2018). To determine whether any phenological differences between extinct and extant taxa could be attributed to relative flowering time, we re-conducted the analyses described above including each species' mean flowering date (calculated as mean DOY) as a covariate. We again included hypothesized interactions between Mean Flowering Date ("MFD"), latitude, longitude, status, and climatic variables (Table S3.5). The final model for DOY included status, latitude, longitude, $T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing,}$ MFD, status x $T_{flowering}$, latitude x MFD, longitude x MFD, $T_{flowering}$ x MFD, T_{winter} x MFD, $P_{flowering}$ x MFD, latitude x $P_{flowering}$, longitude x $T_{growing}$, and longitude x $P_{flowering}$ as predictor variables.

Since these are complex models with many interactions, we also examined DOY and DI as a function of latitude and longitude then conducted downstream analyses on the residuals, thereby removing variation associated with geography. Using this method, individuals with a negative residual value flower earlier than expected after controlling for geography and vice versa. Models included the six climatic variables, status, MFD, and their interactions as predictor variables and the random slopes described above. Results are quantitatively similar, so we present residual models in Table S3.7. We chose to present DOY and DI models in the main text because they explicitly test for differences in phenological responses across space, which we expect based on spatial variation in responses to climate change (Bradshaw and Holzapfel, 2006).

To test for phenological changes over time independently from responses to climate (Panchen and Gorelick, 2017), we used general linear models with DOY or DI included as separate response variables, and year, status, and their interactions as predictor variables and latitude and longitude as covariates. As above, we fit random slopes for each species' phenological response over time.

To further investigate how extinct vs. extant taxa differ in phenological responses to climate, we examined differences in phenological responses to year and climatic variables within each confamiliar pair of locally extinct and extant species by fitting separate models for each species pair. We included DOY and DI as two separate response variables, and status, $T_{flowering}$ $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}, latitude, longitude, the interactions of status with each climatic variable, status x latitude, and status x longitude as predictor variables. MFD was not included as a covariate due to its collinearity with status. To investigate how extinct vs. extant taxa differ in phenological responses over time, we again fit separate models for each species pair; we included DOY and DI as separate response variables, status, year, and the interaction of status x year as predictor variables, and latitude and longitude as covariates. We applied a Bonferroni correction for multiple comparisons.

Figure 3.1. Locally extinct and extant species vary in the direction and magnitude of their phenological responses to climate. Effect of climatic variables ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter},) on flowering phenology (day of year) of locally extinct and extant species. Dot size represents the number of days shifted per 1°C or per 1 mm precipitation. Blue circles indicate delayed flowering; orange circles indicate advanced flowering. Species above the black dashed line are locally extinct; species below the line are extant. *p<0.05, §p<0.1.



RESULTS

Extinct vs. extant species' responses to temperature and precipitation

Locally extinct and extant species differed in their phenological responses to warming (Fig. 3.1). Under warmer temperatures during month of flowering ($T_{flowering}$), both locally extinct and extant species accelerated flowering. However, on average locally extinct species accelerated phenologies in response to $T_{flowering}$ less than extant species (DOY: status x $T_{flowering}$ $\chi^2_{1,973}$=29.85, p<0.0001; extant response = –4.98 ± 0.51 days/°C; extinct response = -1.41 ± 0.62 days/°C; Table S3.8; Fig. 3.2A-B). This difference in the mean responses of extinct and extant taxa was largely due to more consistent advances in flowering time for extant taxa. Seven of

eight extant species advanced flowering under warmer temperatures experienced during the month of flowering (two significantly advanced; $p<0.05$), while for locally extinct species, only four of nine advanced flowering (two significantly) and another five tended to delay flowering under warmer temperatures (Fig. 3.1, 3.3A). Under warmer growing season temperatures, both locally extinct and extant species advanced flowering by an average of 3.14 ± 0.99 days/°C (DOY: $T_{growing}$ $\chi^2_{1,973}=21.65$, $p<0.0001$; Table S3.8; Fig. 3.2C-D). Twelve out of seventeen species tended to advance flowering under warmer growing season temperatures (five significantly advanced) (Table S3.9; Fig. 3.1; Fig. 3.3B).). Under warmer winter temperatures, locally extinct species generally advanced flowering while, on average, extant species did not respond to warmer winter temperatures (DOY: status x T_{winter} $\chi^2_{1,973}=13.38$, $p=0.0005$; extant response = 0.20 ± 0.68 days/°C; extinct response = −1.86 ± 0.69 days/°C; Table S3.8; Fig. 3.2E-F). Six of nine locally extinct species tended to advance flowering under warmer winter temperatures (two significantly advanced), while for extant species, two species significantly delayed and another three tended to delay flowering under warmer winter temperatures (Table S3.9; Fig. 3.1; Fig. 3.3C).

Although locally extinct species flowered earlier than extant species (DOY: status $\chi^2_{1,973}=39.55$, $p<0.0001$) and early-flowering species tended to advance flowering more than late-flowering species under warmer temperatures during flowering and winter (DOY: MFD x $T_{flowering}$ $\chi^2_{1,973}=2.84$, $p=0.09$; MFD x T_{winter} $\chi^2_{1,958}=2.60$, $p=0.1$; Table S3.8; Fig. 3.2B), including MFD as a covariate did not qualitatively change results. This suggests that differences in mean flowering time between locally extinct and extant species do not entirely explain differences between extinct and extant taxa in phenological responses.

Greater precipitation during flowering and the growing season delayed flowering by 0.16 ± 0.70 and 1.44 ± 1.17 days/mm, respectively (DOY: $P_{flowering}$ $\chi^2_{1,973}$=3.69, p=0.05; $P_{growing}$ $\chi^2_{1,973}$=3.63, p=0.08; Table S3.8, Figs. S3.4-S3.5; Fig. 3.4). We also detected a pattern for early-flowering species to advance flowering under increased precipitation during flowering (DOY: MFD x $P_{flowering}$ $\chi^2_{1,1088}$=8.51, p=0.003), but this effect was driven by one early-flowering species, *Baptisia bracteata.*

Results for differences in responses to temperature and precipitation between locally extinct and extant species are quantitatively similar using DI as an indicator of phenology (Table S3.8; Figs. S3.2-S3.3), with two exceptions. First, extant species advanced flowering while locally extinct species did not shift flowering under warmer growing season temperatures (DI: status x $T_{growing}$ $\chi^2_{1,973}$=5.43, p=0.02; extant response = -0.05 ± 0.01 days/°C; extinct response = −0.005 ± 0.007 days/°C; Fig. S3.2). Second, both locally extinct and extant species accelerated flowering under warmer winter temperatures by 0.34 ± 0.15 days/°C (DI: T_{winter} $\chi^2_{1,973}$=4.42, p=0.03; Fig. S3.2).

Phenological shifts over time and variation across space

Locally extinct and extant species both delayed flowering over time (year: DOY $\chi^2_{1,1016}$=10.32, p=0.001; Table S3.8; Fig. 3.2G-H). Over space, specimens from southern latitudes delayed flowering under warmer growing season temperatures more so than those from northern latitudes (DOY: latitude x $T_{growing}$ $\chi^2_{1,973}$=13.17, p=0.0003). This was true for six of the seventeen species studied (Fig. S3.6). Specimens from eastern populations advanced flowering under warmer winter temperatures while more western populations did not respond to variation in winter temperature (DOY: latitude x T_{winter} $\chi^2_{1,973}$=26.33, p<0.0001; longitude x T_{winter} $\chi^2_{1,958}$=6.22, p=0.01); this pattern held in in seven species (Fig. S3.7). Finally, early-flowering

species flowered later while late-flowering species flowered earlier in more northern and western populations (DOY: MFD x latitude $\chi^2_{1,973}$=7.31, p=0.007; MFD x longitude $\chi^2_{1,973}$=7.95, p=0.005; Table S3.8). Other interactions with geography were model-specific (Table S3.8).

Figure 3.2. Locally extinct and extant species differ in the magnitude of their phenological responses to temperature and over time. Left: Phenological sensitivity to (A) $T_{flowering}$, (C) $T_{growing}$, and (E) T_{winter} (°C), and (G) year in locally extinct (red) vs. extant (grey) species. Sensitivity is defined here as the slope (days/°C or year) (± standard error) of extinct vs. extant species' overall phenological response to temperature and over time (Park et al., 2018). Positive values indicate delayed flowering; negative values indicate advanced flowering. Asterisks over a bar indicate a significant response to temperature; asterisks over a bracket indicate a significant difference between locally extinct and extant species; "n.s." indicates a non-significant response. Right: Effect of (B) $T_{flowering}$, (D) $T_{growing}$, (F) T_{winter}, and (H) year on flowering phenology (day of year) of all species included in this study. Red and grey lines show locally extinct and extant species, respectively. We fit random slopes for each species' response to $T_{flowering}$, $T_{growing}$, and T_{winter}.

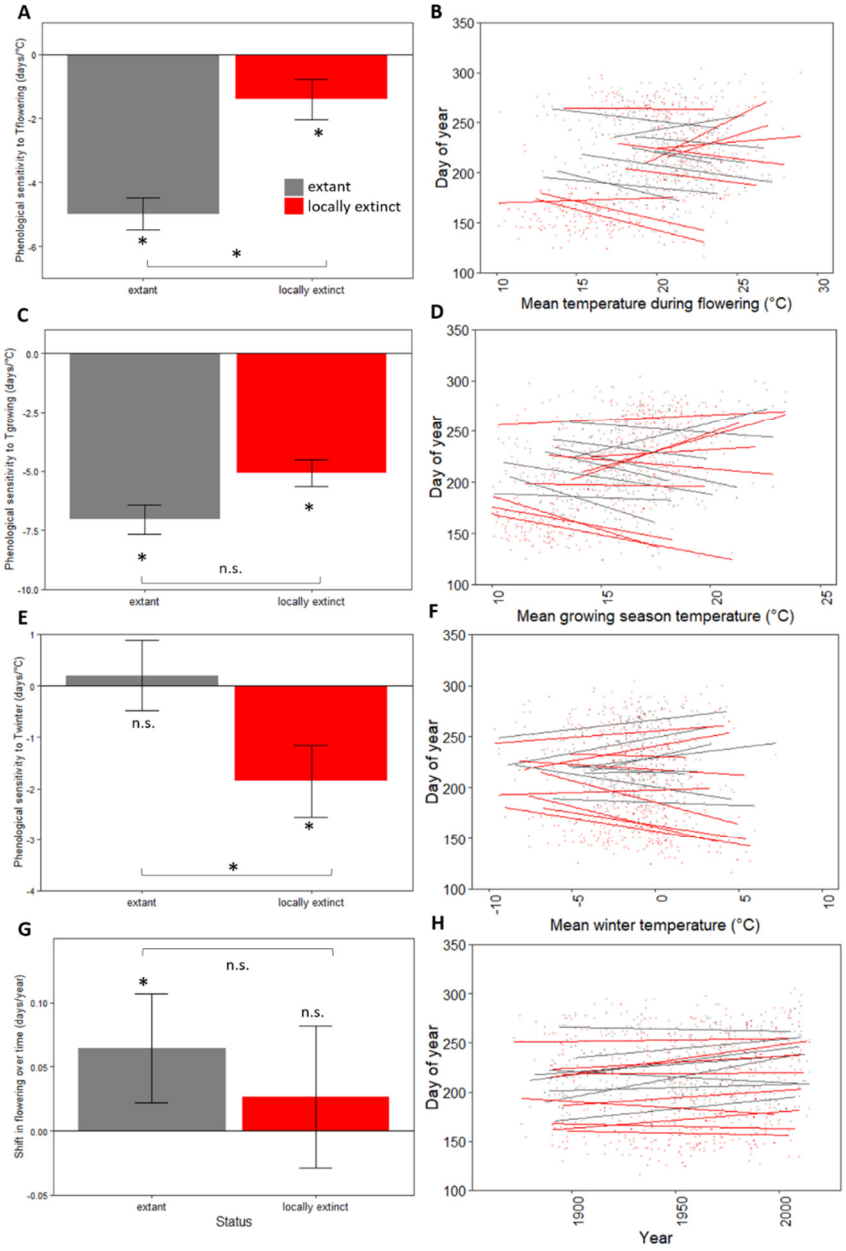


Figure 3.3. Effect of (A) $T_{flowering}$, (B) $T_{growing}$, and (C) T_{winter} (all °C) on flowering phenology (day of year) of all species pairs (and 1 triplet; *Penstemon*) included in this study. Each panel represents one congeneric (or confamilial) pair. Red and grey lines show locally extinct and extant species within a pair, respectively. Gray areas represent 95% confidence intervals.

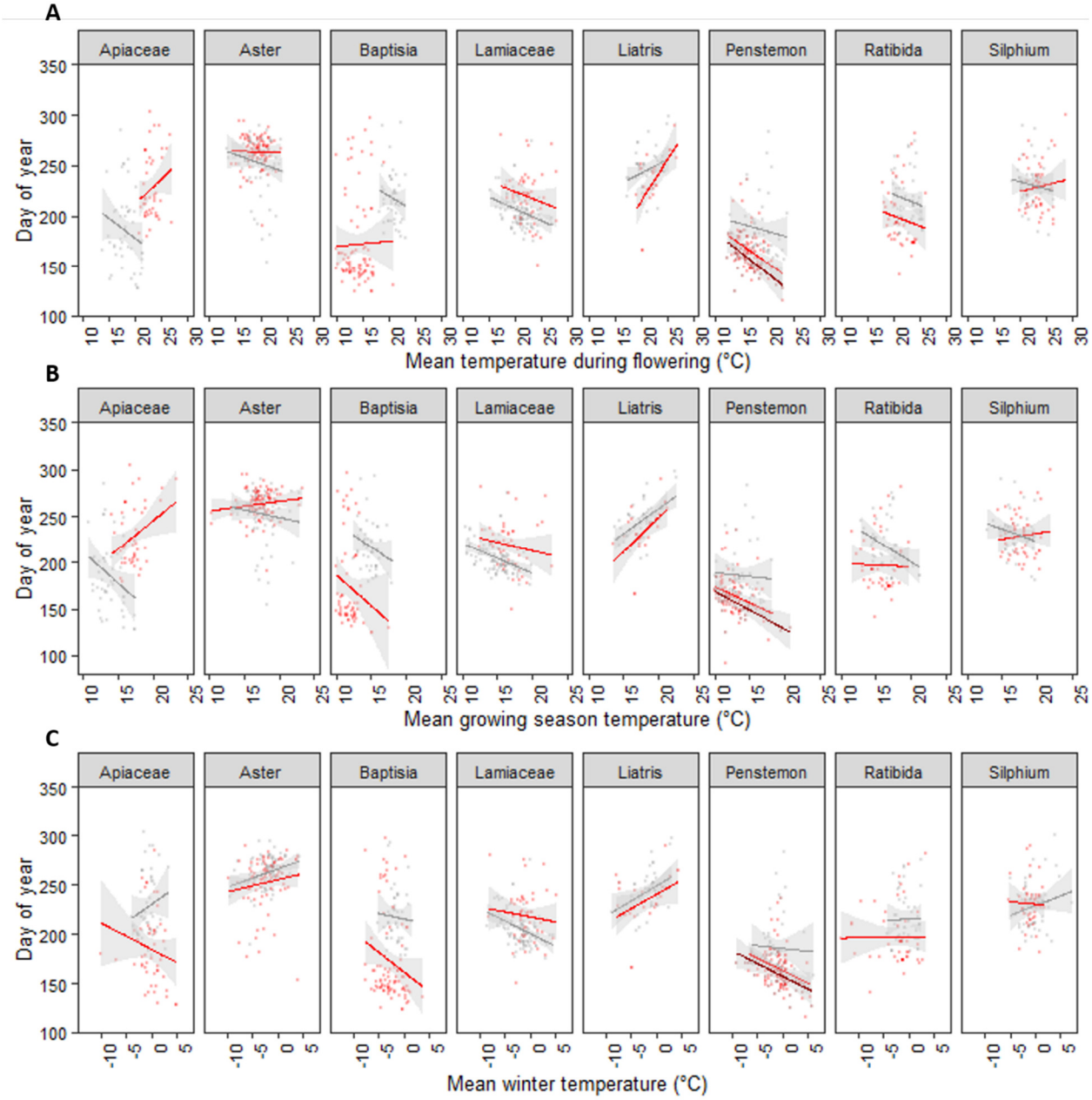
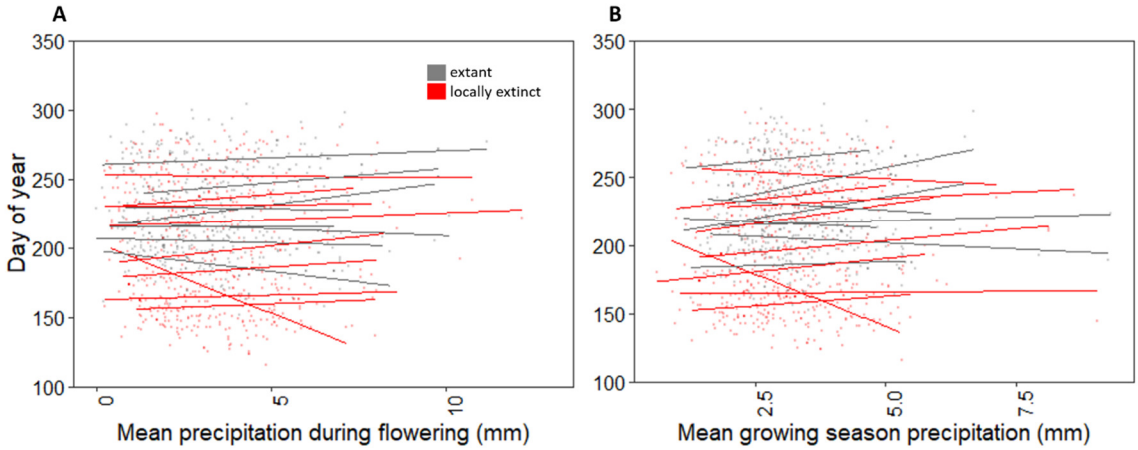


Figure 3.4. Effect of (A) $P_{flowering}$ and (B) $P_{growing}$ (mm) on flowering phenology (day of year) of all species included in this study. Red and grey lines show locally extinct and extant species, respectively. We fit random slopes for each species' response to $P_{flowering}$ and $P_{growing}$. The species advancing flowering in both panels is locally extinct *Baptisia bracteata.*



DISCUSSION

Consistent with previous findings of advancing phenology under climate warming, the 17 native prairie species studied here advanced flowering under warmer spring and winter temperatures. However, locally extinct species advanced flowering less in response to warmer spring temperatures (during flowering and during the growing season) than extant species. These results support previous work positing that species that do not respond to rising spring temperatures experience population declines (Willis et al., 2008; 2010; Miller-Rushing et al., 2010; Thackeray et al., 2016). In contrast, locally extinct species advanced flowering more than extant species as winter temperatures warmed. This result illustrates the increasing need to examine warming across seasons when examining phenological shifts. This study, by highlighting differences in phenological responses between locally extinct and extant taxa, implicates phenology as a potential response to global change underlying local extinction events.

In our study, species advance flowering under spring temperatures (during flowering and the growing season), but locally extinct species on average advanced flowering less than extant

species. Two things could explain the reduced average response of locally extinct species to spring warming. First, locally extinct species' responses might be more idiosyncratic. While extant species demonstrated consistent responses to spring warming, locally extinct species were just as likely to delay as to advance flowering in response to spring warming. Alternatively, locally extinct species might be less phenologically plastic to spring temperature. Lack of phenological responses have been detected in species ranging from North American grasslands and mountains to the United Kingdom to the Mongolian steppe (Bradley et al., 1999; Dunnell and Travers, 2011; Cook et al., 2012, Liancourt et al., 2012), and there are several hypotheses for why temperate species might not shift their phenology under rising temperatures. First, other abiotic factors such as moisture may regulate flowering more than temperature (Körner and Basler, 2010; Caffarra et al., 2011; Crimmins et al., 2011; Chuine et al., 2012). In our study, increasing amounts of spring precipitation generally delayed flowering, similar to phenological patterns detected in other forbs such as *Trillium obvatum* (Matthews and Mazer, 2006). Second, warming might affect early- and late-flowering species differently (Sherry et al., 2007; Cornelius et al., 2013; CaraDonna et al., 2015; Park et al., 2015) and extinct species typically flowered earlier than extant species in our dataset. In our case, we find that earlier-flowering species advanced flowering under warmer temperatures during flowering more than later-flowering species so this is unlikely to explain observed the reduced phenological responses of extinct taxa. Third, species may delay flowering if they do not experience sufficient winter chilling requirements (vernalization): if winter warming delays vernalization, species may flower later in the spring (Schwartz and Hanes, 2010; Yu et al., 2010; Cook et al., 2012; Hart et al., 2014). Finally, variable temperatures, altered snowmelt timing, and frost events may select for delayed phenology if accelerated flowering leads to increased risk of reproductive consequences under

novel environmental conditions (Elzinga et al., 2007; Cooper et al., 2011; Rafferty et al., 2013; Iler et al., 2019).

While locally extinct species shifted flowering less consistently in response to spring temperatures than their extant congeners, they advanced flowering in response to winter warming more than extant species. As described above, flowering earlier under warmer winter temperatures can expose plants to disproportionately harsh abiotic conditions from earlier snowmelt as plants are exposed to cold air and soil temperatures, resulting in negative consequences for growth, survival, and reproduction (Rosa et al., 2015; CaraDonna and Bain, 2016). Since the early-flowering, locally extinct species studied here accelerated flowering in response to warmer winters, they may have experienced such losses and subsequent population declines. This finding illustrates the need to examine species' responses to seasonal temperatures, as failures to identify phenological responses across seasons may incorrectly identify some species as insensitive to climate. For example, Cook et al. (2012) reanalyzed species responses to spring vs. winter warming in species previously found to exhibit non-responding phenology in the UK and US. They found that 17% of species advanced flowering under spring warming and delayed flowering under winter warming, but these patterns were obscured by previous use of a single environmental variable. Similarly, winter warming decreased the effects of spring warming on phenological advancement in the Alps (Vitasse et al., 2018) and Switzerland (Güsewell et al., 2017). However, none of these studies addressed whether species' responses to warm spring vs. winter temperatures had consequences for population persistence. Here, locally extinct species are more consistent in their responses to winter rather than spring warming. This suggests that rare and or locally extinct species' phenology might respond to different seasonal temperatures than more common species. We

might have misidentified one set of taxa as unresponsive to climate had we examined either winter or spring temperature independently. Ultimately, variable responses to different environmental conditions could lead to an equivocal conclusion that species do not respond to warming temperatures when in fact they respond to diverse temperatures cues across seasons.

Earlier flowering is often associated with species success (Willis et al., 2010; Cleland et al., 2012), particularly in invasive species (Willis et al., 2010; Wolkovich et al., 2013; Zettlemoyer et al., 2019b). In contrast, our study reveals that locally extinct species flower earlier than extant species. Early-flowering species advanced flowering under warmer spring temperatures more so than later-flowering species, similar to other studies in the Great Plains (Sherry et al., 2007) and the Rocky Mountains (CaraDonna et al., 2014) (although other studies find that early-flowering species delay flowering relative to late-flowering species [Cornelius et al., 2013; Park et al., 2015, 2018]). This suggests that changes in phenology (and subsequent effects on population dynamics) might be affected by historical flowering times. Our dataset is skewed towards the Asteraceae, which generally flower later in the season. We reran analyses including only one pair of Asteraceae at a time: in two of four models, the interaction of status x T_{winter} became non-significant and in all four models, effects of monthly and growing season precipitation became nonsignificant (data not shown). It is possible that these late-flowering species respond more strongly to precipitation experienced across growing season, although this could also be due to low power.

Conclusions

Here, on average native prairie species advanced their flowering phenologies in response to both spring and winter warming, a combination of environmental cues that is rarely examined together (Cook et al., 2012). However, locally extinct species accelerated phenology less

consistently than extant species in response to warmer springs but advanced flowering more than extant species in response to warmer winters. This result highlights a need to examine phenological responses to multiple environmental cues to accurately predict phenological shifts under climate change.

Locally extinct species flowered earlier than extant species, suggesting that historical flowering time might contribute to subsequent population declines. By examining historical responses to changing climates in recently extinct species, this study not only supports the hypothesis that ineffective phenological responses correlate with population declines but suggests that phenology plays a role in contemporary extinction events. Our use of locally extinct vs. extant species provides a novel framework for examining mechanisms that might influence species declines and extirpations across a species' range. However, further work is needed to determine whether delayed or nonresponsive phenology is associated with lower fitness or population growth rates (Miller-Rushing et al., 2010; Iler et al., 2019). We note that many other traits likely influence population declines and extinction and that climate change is only one possible cause of extinction. This is highlighted by the fact many extinct species responded similarly to extant taxa, advancing flowering greatly under spring warming and minimally responding or even delaying flowering in response to winter warming; ongoing experimental work is investigating the role of climate warming, nitrogen enrichment, and herbivory on population demography in reintroduced populations of these same locally extinct vs. extant prairie species. As these species are rare prairie specialists, likely at-risk throughout their range, understanding their phenological trends where they will persist may prove a useful tool in their conservation.

ACKNOWLEDGEMENTS

The authors thank S.E. Johnson for help collecting temperature data and A. Fryday and R. Rabeler for collections access. L. Brudvig, E. Zipkin, and the Lau and Haddad labs provided valuable feedback on this manuscript. Support for this work was provided by the National Science Foundation Research Experience for Undergraduates program (K.R.) (Award #1757530).

APPENDIX

Table S3.1. Herbaria information. Name and location (state) of the 27 herbaria included in this study.

Herbaria	Location
Academy of Natural Sciences of Philadelphia	Pennsylvania
Albion College	Michigan
Alma College	Michigan
Austin Peay State University Herbarium	Tennessee
Butler University	Indiana
Calvin College	Michigan
Central Michigan University	Michigan
Chicago Botanic Garden	Illinois
Eastern Michigan University	Michigan
Field Museum of Natural History	Illinois
Grand Valley State University	Michigan
Hope College	Michigan
Huntington University	Indiana
Indiana University	Indiana
Kent Scientific Museum	Ohio
Marygrove College	Michigan
Missouri Botanical Garden	Missouri
Morton Arboretum	Illinois
Michigan State University	Michigan
Oberlin College	Ohio
Ohio Flora	Ohio
Ohio State University	Ohio
Plants of Indiana	Indiana
Stover-Ebinger Herbarium (Eastern Illinois University)	Michigan
University of Illinois	Illinois
University of Michigan	Michigan
University of Wisconsin-Madison (WI State Herbarium)	Wisconsin

Table S3.2. Effects of climate and year on phenology (Michigan specimens). For Michigan specimens only: (A) Results from random slope linear mixed models (Gaussian distributions) testing the effects of mean temperature during flowering (30 days prior to species mean flowering date; $T_{flowering}$, °C), mean growing season temperature (April – species mean flowering date; $T_{growing}$, °C), mean winter temperature (November – March prior to flowering; T_{winter}, °C), mean precipitation during flowering ($P_{flowering}$, cm), mean growing season precipitation ($P_{growing}$, cm), mean winter precipitation (P_{winter}, cm), status (locally extinct vs. extant), and interactions between status and each climatic variable ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}) on each flowering specimen's day of year (DOY) and Developmental Index (DI). Latitude and longitude were included as covariates to control for spatial variation in phenology. We provide the results of models with and without Mean Flowering Date (MFD) included as a covariate, which controls for differences between early- vs. late-flowering species. We included hypothesized interactions between covariates and climatic variables (Table S3.5). We fit random slopes for individual species' responses to each climatic variable. We then sequentially removed predictors via backwards elimination (Table S3.6), and here analyze those models using only specimens from Michigan. (B) Results from linear mixed models (Gaussian distributions) testing the effects of year, status, and year x status on flowering phenology, with latitude and longitude included as covariates and random slopes for individual species' responses to year. ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1.

Source	df	DOY χ^2	DOY (no MFD) χ^2	DI χ^2	DI (no MFD) χ^2
(A) Climate model					
Status	1	0.01	46.16 ***	8.90 **	1.20
Latitude	1	0.91	0.89	2.93 ·	0.01
Longitude	1	0.14	4.60 *	0.17	0.03
$T_{flowering}$	1	0.52	46.07 ***	1.02	0.67
$T_{growing}$	1	5.47 ***	1.22	2.03 ·	0.56
T_{winter}	1	0.00	1.27	0.03	0.00
$P_{flowering}$	1	1.12	4.56 *	-	-
$P_{growing}$	1	0.76	0.93	19.40 ***	17.49 ***
MFD	1	0.98	-	0.86	-
Status x $T_{flowering}$	1	0.00	38.64 ***	15.01 ***	24.00 ***
Status x $T_{growing}$	1	-	-	2.59 ·	18.53 ***
Status x T_{winter}	1	-	4.12 *	-	-
Status x latitude	1	-	-	15.78 ***	-
Status x longitude	1	-	-	1.04	1.78
Status x MFD	1	-	-	1.06	-
MFD x latitude	1	0.34	-	0.98	-
MFD x longitude	1	4.66 *	-	-	-
MFD x $T_{flowering}$	1	0.49	-	-	-
MFD x T_{winter}	1	0.00	-	-	-
MFD x $P_{flowering}$	1	0.07	-	-	-
Latitude x $T_{growing}$	1	-	1.90	-	0.26
Latitude x T_{winter}	1	-	3.64 ·	-	-
Latitude x $P_{flowering}$	1	1.09	-	-	-
Latitude x $P_{growing}$	1	-	0.87	-	-
Longitude x $T_{flowering}$	1	-	-	0.38	0.16
Longitude x $T_{growing}$	1	4.85 *	-	-	-
Longitude x T_{winter}	1	-	0.52	0.08	0.01

Table S3.2. (cont'd)

Longitude x $P_{flowering}$	1	0.15	-	-	-
Residual	423	$4.26e^{+02}$	$4.34e^{+02}$	$2.40e^{-01}$	$2.46e^{-09}$
Species ($T_{flowering}$)		$5.07e^{-02}$	$1.65e^{+00}$	$5.62e^{-05}$	$2.64e^{-09}$
Species ($T_{growing)}$		$0.00e^{+00}$	$1.92e^{+00}$	$1.04e^{-08}$	$1.25e^{-04}$
Species (T_{winter})		$2.01e^{-07}$	$5.19e^{-01}$	$3.02e^{-09}$	$1.26e^{-07}$
Species ($P_{flowering}$)		$4.99e^{+02}$	$8.46e^{+02}$	$1.26e^{+00}$	$7.46e^{-01}$
Species ($P_{growing}$)		$1.68e^{+03}$	$0.00e^{+00}$	$1.95e^{-02}$	$6.14e^{-01}$
Species (P_{winter})		$2.54e^{+02}$	$2.60e^{-04}$	$3.32e^{+00}$	$1.19e^{-01}$
(B) Year model					
Year	1	0.34		0.65	
Status	1	0.72		0.09	
Year x status	1	0.97		0.06	
Latitude	1	6.84 **		2.97 ·	
Longitude	1	0.47		0.00	
Residual	437	$2.35e^{-04}$		$2.33e^{-08}$	
Species (year)		$4.40e^{+02}$		$2.59e^{-01}$	

Table S3.3. Correlations between climate, year, and geography. Pearson's correlations between year, latitude, longitude, and all climatic variables ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}). ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1.

	$T_{flowering}$	$T_{growing}$	T_{winter}	$P_{flowering}$	$P_{growing}$	P_{winter}	year	latitude	longitude
$T_{flowering}$	1	0.79***	0.37***	-0.02	0.09**	0.11**	0.08*	-0.39***	-0.01
$T_{growing}$	0.79***	1	0.36***	0.02	0.10**	0.05	0.06*	-0.43***	0.08*
T_{winter}	0.37***	0.36 **	1	0.02	0.13***	0.42***	0.00	-0.78***	-0.15***
$P_{flowering}$	-0.02	0.02	0.02	1	0.64***	0.1	0.09**	-0.08*	0.01
$P_{growing}$	0.09 *	0.10**	0.13***	0.64***	1	0.18***	-0.06 ·	-0.19***	-0.01
P_{winter}	0.11**	0.05	0.42***	0.01	0.18***	1	-0.01**	-0.36***	-0.21***
year	0.08*	0.06*	0.00	0.09**	-0.06 ·	-0.01**	1	0.04	0.03
latitude	-0.39***	-0.43***	-0.78***	-0.08*	-0.19***	-0.36***	0.04	1	0.10**
longitude	-0.01	0.08 *	-0.15 ***	0.01	-0.01	-0.21***	0.03	0.10**	1

Table S3.4. Effects of time and geography on climate. Effects of year, latitude, and longitude on all climatic variables: (A) $T_{flowering}$, (B) $T_{growing}$, (C) T_{winter}, (D) $P_{flowering}$, (C) $P_{growing}$, (F) P_{winter}. Interactions were not significant ($p>0.05$) and removed from analyses. ***$p\leq0.0001$, **$p\leq0.01$, *$p\leq0.05$.

Source	df	Coefficient	t
(A) $T_{flowering}$			
Year	1	0.009 ± 0.003	3.28 **
Latitude	1	-0.832 ± 0.062	-13.44 ***
Longitude	1	0.021 ± 0.026	0.83
Residual	974		3.06
(B) $T_{growing}$			
Year	1	0.007 ± 0.002	2.87 **
Latitude	1	-0.813 ± 0.052	-15.76 ***
Longitude	1	0.096 ± 0.021	4.42 ***
Residual	976		2.56
© T_{winter}			
Year	1	0.003 ± 0.002	2.06 *
Latitude	1	-1.267 ± 0.032	-39.72 ***
Longitude	1	-0.046 ± 0.013	-3.42 ***
Residual	974		1.58
(D) $P_{flowering}$			
Year	1	0.005 ± 0.002	2.85 **
Latitude	1	-0.092 ± 0.036	-2.55 *
Longitude	1	0.006 ± 0.015	0.40
Residual	1012		1.86
© $P_{growing}$			
Year	1	0.002 ± 0.001	2.123 *
Latitude	1	-0.134 ± 0.021	-6.29 ***
Longitude	1	0.001 ± 0.009	0.09
Residual	1012		1.10
(F) P_{winter}			
Year	1	-0.002 ± 0.001	-2.83 **
Latitude	1	-0.173 ± 0.015	-11.84 ***
Longitude	1	-0.037 ± 0.006	-6.07 ***
Residual	1010		0.75

Table S3.5. Model hypotheses. Hypothesized interactions between mean flowering date (MFD), latitude, longitude, status, and climatic variables ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}).

Interaction	Biological hypothesis
Latitude or longitude x status	Extinct vs. extant species differ in the magnitude and/or direction of their latitudinal or longitudinal cline in flowering time
Latitude or longitude x $T_{flowering}$, $T_{growing}$, or T_{winter}	Phenological responses to temperature during flowering, during the growing season, or during the winter prior to flowering vary in their magnitude and/or direction across a latitudinal or longitudinal cline[1-4]
Latitude or longitude x $P_{flowering}$, $P_{growing}$, or P_{winter}	Phenological responses to precipitation during flowering, during the growth season, or during the winter prior to flowering vary in their magnitude and/or direction across their latitudinal or longitudinal cline[5]
MFD x status	Phenological responses of early vs. late flowering species depend on whether the species is extinct vs. extant[3-4,6]
MFD x latitude or longitude	Early vs. late flowering species differ in the magnitude and/or direction of their latitudinal or longitudinal cline in flowering time[4]
MFD x $T_{flowering}$, $T_{growing}$, or T_{winter}	Early vs. late flowering species differ in the magnitude and/or direction of their phenological responses to temperature during flowering, during the growing season, or during the winter prior to flowering[4,7-9]
MFD x $P_{flowering}$, $P_{growing}$, or P_{winter}	Early vs. late flowering species differ in the magnitude and/or direction of their phenological responses to precipitation during flowering, during the growing season, or during the winter prior to flowering[4-5,7]
MFD x year	Early vs. late flowering species differ in the magnitude of their phenological shifts over time[4]

[1] Debieu, M., C. Tang, B. Stitch, et al. 2013. Co-variation between seed dormancy, growth rate, and flowering time changes with latitude in *Arabidopsis thaliana. PLoS One* 8(5): e6175.

[2] Prendeville, H.R., K. Barnard-Kubow, C. Dai, et al. 2013. Clinal variation for only some phenological traits across a species range. *Oecologia* 173: 421-430.

[3] Park, I.W., and M.D. Schwartz. 2015. Long-term herbarium records reveal temperature-dependent changes in flowering phenology in the southeastern USA. *International Journal of Biometeorology.* 59: 347-355.

[4] Park, D.S., I. Breckheimer, A.C. Williams, et al. 2018. Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. *Philosophical Transactions of the Royal Society B.* 374: 20170394.

[5] Matthews, E., and S. Mazer. 2016. Historical changes in flowering phenology are governed by temperature x precipitation interactions in a widespread perennial herb in North America. *New Phytologist* 210: 157-167.

[6] Willis, C.G., B. Ruhfel, R.B. Primack, et al. 2010. Favorable climate change response explains non-native species' success in Thoreau's woods. *PloS One* 5(1): e8878.

[7] Sherry, R.., X. Zhou, S. Gu, et al. 2007. Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences* 104: 198-202.

[8] Cornelius, C., A. Leingärtner, B. Hoiss, et al. 2013. Phenological response of grassland species to manipulative snowmelt and drought along an altitudinal gradient. *Journal of. Experimental Botany.* 64: 241-251.

[9] CaraDonna, P.J., A.M. Iler, and D. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences* 11: 4916-4921.

Table S3.6. Backwards elimination. Akaike Information Criterion (AIC) values for each model run during backwards elimination. The predictor with the highest p-value was sequentially removed until all $p \leq 0.1$ or until removing terms did not improve model fit. A main effect was not removed if an interaction term with that variable was still included. I provide backwards elimination procedures for the two main models including DOY as a response variable; procedures to remove terms were identical for DI.

Model: DOY (without Mean Flowering Date)	**Removed term (p-value)**	**AIC**
Initial: Status + latitude + longitude + $T_{flowering}$ + $T_{growing}$ + T_{winter} + $P_{flowering}$ + $P_{growing}$ + P_{winter} + status:latitude + status:longitude + status:$T_{flowering}$ + status:$T_{growing}$ + status:T_{winter} + status:$P_{flowering}$ + status:$P_{growing}$ + status:P_{winter} + latitude:$T_{flowering}$ + latitude:$T_{growing}$ + latitude:T_{winter} + latitude:$P_{flowering}$ + latitude:$P_{growing}$ + latitude:P_{winter} + longitude:$T_{flowering}$ + longitude:$T_{growing}$ + longitude:T_{winter} + longitude:$P_{flowering}$ + longitude:$P_{growing}$ + longitude:P_{winter}		6785.78
	Latitude:$T_{flowering}$ (0.71)	6783.91
	Longitude:$T_{flowering}$ (0.75)	6782.02
	Status:longitude (0.67)	6780.20
	Longitude:$P_{flowering}$ (0.63)	6778.43
	Longitude:$P_{growing}$ (0.59)	6776.53
	Latitude:$P_{flowering}$ (0.58)	6774.84
	Longitude:P_{winter} (0.57)	6773.17
	Status:$T_{growing}$ (0.51)	6771.60
	Longitude:$T_{growing}$ (0.65)	6770.27
	Latitude:P_{winter} (0.36)	6769.12
	Status:P_{winter} (0.37)	6767.91
	P_{winter} (0.58)	6766.22
	Status:$P_{flowering}$ (0.35)	6765.12
	Status:latitude (0.23)	6764.57
Final: Status + latitude + longitude + $T_{flowering}$ + $T_{growing}$ + T_{winter} + $P_{flowering}$ + $P_{growing}$ + status:$T_{flowering}$ + status:T_{winter} + latitude:$T_{growing}$ + latitude:T_{winter} + latitude:$P_{growing}$ + longitude:T_{winter}	Status:$P_{growing}$ (0.23)	6764.03
Model: DOY with Mean Flowering Date (MFD)	**Removed term (p-value)**	**AIC**
Status + latitude + longitude + $T_{flowering}$ + $T_{growing}$ + T_{winter} + $P_{flowering}$ + $P_{growing}$ + P_{winter} + MFD + status:latitude + status:longitude + status:$T_{flowering}$ + status:$T_{growing}$ + status:T_{winter} + status:$P_{flowering}$ + status:$P_{growing}$ + status:P_{winter} + MFD:status + MFD:latitude + MFD:longitude + MFD:$T_{flowering}$ + MFD:$T_{growing}$ + MFD:T_{winter} + MFD:$P_{flowering}$ + MFD:$P_{growing}$ + MFD:P_{winter} + latitude:$T_{flowering}$ + latitude:$T_{growing}$ + latitude:T_{winter} + latitude:$P_{flowering}$ + latitude:$P_{growing}$ + latitude:P_{winter} + longitude:$T_{flowering}$ + longitude:$T_{growing}$ + longitude:T_{winter} + longitude:$P_{flowering}$ + longitude:$P_{growing}$ + longitude:P_{winter}		6443.14
	Status:$P_{growing}$ (0.99)	6441.14
	Status:P_{winter} (0.98)	6439.14
	Longitude:$T_{flowering}$ (0.94)	6437.15
	MFD:$T_{growing}$ (0.93)	6435.16
	MFD:P_{winter} (0.87)	6433.18
	MFD:$P_{growing}$ (0.80)	6431.21
	Status:latitude (0.87)	6429.28
	Latitude:$P_{growing}$ (0.78)	6427.36
	Latitude:$T_{growing}$ (0.80)	6425.43
	Longitude:P_{winter} (0.76)	6421.66
	Longitude:$P_{growing}$ (0.71)	6420.00
	Status:longitude (0.68)	6419.84
	Status:$P_{flowering}$ (0.64)	6418.06
	Latitude:T_{winter} (0.56)	6416.40
	Latitude:P_{winter} (0.63)	6414.64

Table S3.6. (cont'd)

	Status:T_{winter} (0.49)	6413.12
	Longitude:T_{winter} (0.53)	6411.47
	Status:$T_{growing}$ (0.29)	6409.88
	Latitude:$T_{flowering}$ (0.18)	6408.62
	P_{winter} (0.46)	6408.01
Final: status + latitude + longitude + $T_{flowering}$ + $T_{growing}$ + T_{winter} + $P_{flowering}$ + $P_{growing}$ + MFD + status:$T_{flowering}$ + MFD:latitude + MFD:longitude + MFD:$T_{flowering}$ + MFD:T_{winter} + MFD:$P_{flowering}$ + latitude:$P_{flowering}$ + longitude:$T_{growing}$ + longitude:$P_{flowering}$	MFD:status (0.85)	6407.48

Table S3.7. Effects of climate and year on phenology (residuals). (A) Results from random slope linear mixed models (Gaussian distributions) testing the effects of mean temperature during flowering ($T_{flowering}$, °C), mean growing season temperature (April – flowering; $T_{growing}$, °C), mean winter temperature (November – March prior to flowering; T_{winter}, °C), mean precipitation during flowering ($P_{flowering}$, cm), mean growing season precipitation ($P_{growing}$, cm), mean winter precipitation (P_{winter}, cm), status (locally extinct vs. extant), and interactions between status and each climatic variable ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}) on the residuals of each flowering specimen's day of year (DOY) and Developmental Index (DI). Residuals were derived from two models testing the effects of geography (latitude and longitude) on (1) DOY and (2) DI, thereby removing variability associated with geography. We provide the results of models with and without Mean Flowering Date (MFD) included as a covariate, which controls for differences between early- vs. late-flowering species. We included hypothesized interactions between covariates and climatic variables (Table S3.5). We fit random slopes for individual species' responses to each climatic variable. (B) Results from linear mixed models (Gaussian distributions) testing the effects of year, status, and year x status on flowering phenology (residuals), with random slopes for individual species' responses to year. ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1.

Source	df	DOY Residuals χ^2	DOY Residuals (no MFD) χ^2	DI Residuals χ^2	DI Residuals (no MFD) χ^2
Status	1	0.67	28.47 ***	0.35	2.31 ·
$T_{flowering}$	1	1.70 ·	53.37 ***	2.05 ·	64.90 ***
$T_{growing}$	1	0.42	127.17 ***	0.75	56.25 ***
T_{winter}	1	12.45 ***	1.25	4.00 *	2.04 ·
$P_{flowering}$	1	2.72 ·	0.05	0.25	0.10
$P_{growing}$	1	0.01	0.02	0.72	0.42
P_{winter}	1	0.01	1.56	0.71	0.51
Mean flowering date (MFD)	1	8.70 **	-	0.92	-
Status x $T_{flowering}$	1	0.37	23.79 ***	14.70 ***	35.26 ***
Status x $T_{growing}$	1	1.43	1.07	7.18 **	28.32 ***
Status x T_{winter}	1	1.94 ·	4.83 *	2.09 ·	0.60
Status x $P_{flowering}$	1	0.00	1.43	0.02	0.01
Status x $P_{growing}$	1	0.00	1.52	0.59	0.15
Status x P_{winter}	1	0.02	1.46	0.42	1.63
Status x MFD	1	1.21	-	3.69 ·	-
MFD x $T_{flowering}$	1	2.06 ·	-	0.16	-
MFD x $T_{growing}$	1	0.66	-	0.07	-
MFD x T_{winter}	1	14.70 ***	-	2.22 ·	-
MFD x $P_{flowering}$	1	3.00 ·	-	0.37	-
MFD x $P_{growing}$	1	0.02	-	0.93	-
MFD x P_{winter}	1	0.11	-	0.99	-
Species ($T_{flowering}$)		$7.88e^{-02}$	0.38	0.00	$9.60e^{-05}$
Species ($T_{growing}$)		$0.00e^{+00}$	2.78	0.00	$1.81e^{-09}$
Species (T_{winter})		$4.92e^{-01}$	1.21	0.00	$8.53e^{-05}$
Species ($P_{flowering}$)		$8.83e^{+02}$	$1.60\ e^{+02}$	0.02	$1.39e^{-02}$
Species ($P_{growing}$)		$1.63e^{+03}$	$8.04e^{+02}$	0.49	$5.57e^{-01}$
Species (P_{winter})		$2.88e^{+02}$	$5.48e^{+02}$	2.12	$1.91e^{+00}$
Residual	973	$7.04e^{+02}$	$6.90e^{+02}$	0.26	$2.62e^{-01}$
(2) Year model					
Year	1	8.88 **		0.08	
Status	1	0.88		0.05	
Status x year	1	1.35		0.08	

Table S3.7. (cont'd)

Species (year)		$4.31e^{-04}$	$1.38e^{-08}$
Residual	1016	$7.04e^{+02}$	$2.58e^{-01}$

Table S3.8. Effects of climate and year on phenology (DOY and DI). Following backwards elimination, (A) Results from random slope linear mixed models (Gaussian distributions) testing the effects of mean temperature during flowering (30 days prior to species mean flowering date; $T_{flowering}$, °C), mean growing season temperature (April – species mean flowering date; $T_{growing}$, °C), mean winter temperature (November – March prior to flowering; T_{winter}, °C), mean precipitation during flowering ($P_{flowering}$, cm), mean growing season precipitation ($P_{growing}$, cm), mean winter precipitation (P_{winter}, cm), status (locally extinct vs. extant), and interactions between status and each climatic variable ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}) on each flowering specimen's day of year (DOY) and Developmental Index (DI). Latitude and longitude were included as covariates to control for spatial variation in phenology. We provide the results of models with and without Mean Flowering Date (MFD) included as a covariate, which controls for differences between early- vs. late-flowering species. We included hypothesized interactions between covariates and climatic variables (Appendix S6). We fit random slopes for individual species' responses to each climatic variable. We then sequentially removed predictors via backwards elimination (see Appendix S7). (B) Results from linear mixed models (Gaussian distributions) testing the effects of year, status, and year x status on flowering phenology, with latitude and longitude included as covariates and random slopes for individual species' responses to year. ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1.

Source	df	DOY χ^2	DOY (no MFD) χ^2	DI χ^2	DI (no MFD) χ^2
(A) Climate model					
Status	1	3.19·	39.55 ***	0.31	4.84 *
Latitude	1	6.42 *	29.88 ***	11.09 ***	6.49 *
Longitude	1	0.91	9.80 **	12.67 ***	12.67 ***
$T_{flowering}$	1	3.01 ·	61.92 ***	19.66 ***	21.78 ***
$T_{growing}$	1	9.97 ***	21.65 ***	5.58 *	3.19 ·
T_{winter}	1	2.54 ·	43.46 ***	4.42 *	6.46 *
$P_{flowering}$	1	8.91 **	3.69 *	-	-
$P_{growing}$	1	2.80 ·	3.63 ·	2.05	2.55 ·
MFD	1	22.01 ***	-	7.25 **	-
Status x $T_{flowering}$	1	4.27 *	29.85 ***	15.89 ***	32.03 ***
Status x $T_{growing}$	1	-	-	5.43 *	32.57 ***
Status x T_{winter}	1	-	13.38 ***	-	-
Status x latitude	1	-	-	4.68 *	-
Status x longitude	1	-	-	4.55 *	5.99 *
Status x MFD	1	-	-	3.25 ·	-
MFD x latitude	1	5.19 *	-	8.92 **	-
MFD x longitude	1	11.85 ***	-	-	-
MFD x $T_{flowering}$	1	2.84 ·	-	-	-
MFD x T_{winter}	1	2.60 ·	-	-	-
MFD x $P_{flowering}$	1	8.51 **	-	-	-
Latitude x $T_{growing}$	1	-	13.15 ***	-	6.88 **
Latitude x T_{winter}	1	-	27.28 ***	-	-
Latitude x $P_{flowering}$	1	7.46 **	-	-	-
Latitude x $P_{growing}$	1	-	3.31 ·	-	-
Longitude x $T_{flowering}$	1	-	-	15.06 ***	15.61 ***
Longitude x $T_{growing}$	1	9.38 **	-	-	-
Longitude x T_{winter}	1	-	6.12 *	3.86 *	5.98 *
Longitude x $P_{flowering}$	1	3.24 ·	-	-	-

Table S3.8. (cont'd)

Residual	973	$6.72e^{+02}$	$6.77e^{+02}$	$2.57e^{-01}$	$2.03e^{-05}$
Species ($T_{flowering}$)		$0.00e^{+00}$	$9.17e^{-01}$	$1.06e^{-04}$	$0.00e^{+00}$
Species ($T_{growing}$)		$0.00e^{+00}$	$2.23e^{+00}$	$0.00e^{+00}$	$8.52e^{-10}$
Species (T_{winter})		$5.65e^{-05}$	$1.07e^{-06}$	$0.00e^{+00}$	$4.80e^{-05}$
Species ($P_{flowering}$)		$0.00e^{+00}$	$3.17e^{+00}$	$2.09e^{-09}$	$7.23e^{-01}$
Species ($P_{growing}$)		$1.28e^{+03}$	$1.37e^{+04}$	$8.46e^{-01}$	$9.59e^{-01}$
Species (P_{winter})		$3.19e^{-02}$	$6.12e^{+02}$	$6.41e^{-01}$	$2.58e^{-01}$
(B) Year model					
Year	1	10.32 **		0.08	
Status	1	1.14		0.05	
Year x status	1	2.22		1.01	
Latitude	1	0.14		0.03	
Longitude	1	1.64		0.09	
Residual	1016	$6.96e^{+02}$		$2.54e^{-01}$	
Species (year)		$2.37e^{-04}$		$5.90e^{-08}$	

Table S3.9. Species-specific phenological responses to climate and over time. Results of general linear mixed models (Gaussian distributions) for each taxonomic group (each lettered table represents a separate confamilial or congeneric pair, with plant family provided in parentheses), testing: (1) Effects of mean temperature during flowering (30 days prior to species mean flowering date; $T_{flowering}$, °C), mean growing season temperature (April – species mean flowering date; $T_{growing}$, °C), mean winter temperature (November – March prior to flowering; T_{winter}, °C), mean precipitation during flowering ($P_{flowering}$, cm), mean growing season precipitation ($P_{growing}$, cm), mean winter precipitation (P_{winter}, cm), status (locally extinct vs. extant), and interactions between status and each climatic variable ($T_{flowering}$, $T_{growing}$, T_{winter}, $P_{flowering}$, $P_{growing}$, P_{winter}) on each specimen's day of year (DOY) or Developmental Index (DI). (2) Effects of year, status, and year x status on flowering phenology. Latitude and longitude were included as covariates to control for spatial variation in phenology. ***p≤0.0001, **p≤0.01, *p≤0.05, ·p≤0.1, after applying a Bonferroni correction for multiple comparisons.

Source	df	DOY F	DI F
(A) Apiaceae (Apiaceae)			
(1) Climate model			
Status	1	3.80 *	0.21
$T_{flowering}$	1	0.03	0.00
$T_{growing}$	1	0.26	0.00
T_{winter}	1	0.00	0.10
$P_{flowering}$	1	0.24	0.02
$P_{growing}$	1	0.09	0.15
P_{winter}	1	1.05	0.03
Latitude	1	1.12	0.03
Longitude	1	0.00	0.92
Status x $T_{flowering}$	1	0.08	0.06
Status x $T_{growing}$	1	0.46	0.01
Status x T_{winter}	1	0.11	0.05
Status x $P_{flowering}$	1	0.03	0.91
Status x $P_{growing}$	1	0.07	0.03
Status x P_{winter}	1	2.10	2.14
Status x latitude	1	3.59 ·	0.78
Status x longitude	1	0.82	0.18
Residual	76	35.14	0.39
(2) Year model			
Year	1	2.98 *	1.89
Status	1	3.48	1.88
Latitude	1	0.76	3.85 *
Longitude	1	0.40	0.43
Status x year	1	3.88 *	1.90
Residual	89	35.09	0.39
(B) Asters (Asteraceae)			
(1) Climate model			
Status	1	4.31 * 0.04	0.14
$T_{flowering}$	1	2.02	0.11
$T_{growing}$	1	0.02	0.05
T_{winter}	1	0.78	0.23
$P_{flowering}$	1	0.38	0.02
$P_{growing}$	1	0.10	0.03
P_{winter}	1	0.08	0.00
Latitude	1	0.86	0.01

Table S3.9. (cont'd)

Longitude	1	3.52 ·	0.01
Status x $T_{flowering}$	1	0.03	0.41
Status x $T_{growing}$	1	4.01 *	1.62
Status x T_{winter}	1	0.02	2.68
Status x $P_{flowering}$	1	2.28	0.94
Status x $P_{growing}$	1	1.05	0.59
Status x P_{winter}	1	0.86	1.20
Status x latitude	1	0.86	2.73
Status x longitude	1	2.22	2.25
Residual	153	19.23	0.51
(2) Year model			
Year	1	0.00	0.13
Status	1	0.20	0.58
Latitude	1	5.84 *	2.08
Longitude	1	7.73 **	0.84
Status x year	1	0.16	0.70
Residual	193	19.73	0.50
(C) Baptisias (Fabaceae)			
(1) Climate model			
Status	1	0.57	0.23
$T_{flowering}$	1	0.01	0.63
$T_{growing}$	1	0.66	0.11
T_{winter}	1	0.02	1.04
$P_{flowering}$	1	0.88	0.07
$P_{growing}$	1	0.76	0.78
P_{winter}	1	0.00	0.75
Latitude	1	0.02	0.68
Longitude	1	0.07	4.62 *
Status x $T_{flowering}$	1	0.12	0.04
Status x $T_{growing}$	1	0.18	0.14
Status x T_{winter}	1	0.09	0.39
Status x $P_{flowering}$	1	0.02	0.03
Status x $P_{growing}$	1	1.14	0.20
Status x P_{winter}	1	0.01	2.88
Status x latitude	1	0.36	0.93
Status x longitude	1	0.08	0.55
Residual	115	38.51	0.56
(2) Year model			
Year	1	0.50	0.75
Status	1	1.11	0.01
Latitude	1	7.06 **	1.04
Longitude	1	0.05	6.42 *
Status x year	1	0.85	0.00
Residual	130	38.64	0.56
(D) Lamiaceae (Lamiaceae)			
(1) Climate model			
Status	1	0.15	0.00
$T_{flowering}$	1	1.11	0.77
$T_{growing}$	1	4.12 *	0.05
T_{winter}	1	1.35	7.00 **
$P_{flowering}$	1	0.24	1.16
$P_{growing}$	1	0.06	2.52
P_{winter}	1	2.56 ·	0.69

Table S3.9. (cont'd)

Latitude	1	0.14	0.24
Longitude	1	0.26	0.26
Status x $T_{flowering}$	1	1.87	0.30
Status x $T_{growing}$	1	1.08	0.02
Status x T_{winter}	1	1.15	1.91
Status x $P_{flowering}$	1	0.23	0.39
Status x $P_{growing}$	1	3.02 ·	0.96
Status x P_{winter}	1	2.61 ·	0.30
Status x latitude	1	0.01	0.02
Status x longitude	1	1.73	0.04
Residual	128	19.4	0.67
(2) Year model			
Year	1	1.37	3.37 ·
Status	1	0.54	1.24
Latitude	1	8.04 **	0.00
Longitude	1	0.06	0.20
Status x year	1	0.45	1.56
Residual	147	20.07	0.67
(E) Liatris (Asteraceae)			
(1) Climate model			
Status	1	8.01 **	1.97
$T_{flowering}$	1	1.19	0.21
$T_{growing}$	1	6.83 *	0.34
T_{winter}	1	0.64	3.42 ·
$P_{flowering}$	1	0.18	0.44
$P_{growing}$	1	2.23	0.03
P_{winter}	1	2.66 ·	0.73
Latitude	1	0.32	1.91
Longitude	1	0.62	0.04
Status x $T_{flowering}$	1	7.39 **	3.85 *
Status x $T_{growing}$	1	0.55	0.27
Status x T_{winter}	1	0.25	1.93
Status x $P_{flowering}$	1	0.74	1.37
Status x $P_{growing}$	1	0.02	0.66
Status x P_{winter}	1	2.54	2.12
Status x latitude	1	2.51	0.02
Status x longitude	1	1.83	0.78
Residual	50	17.78	0.22
(2) Year model			
Year	1	7.99 **	0.03
Status	1	0.14	0.10
Latitude	1	15.94 ***	0.08
Longitude	1	0.12	3.24 ·
Status x year	1	0.12	0.13
Residual	74	17.98	0.22
(F) Penstemons (Scrophulariaceae)			
(1) Climate model			
Status	1	0.00	0.00
$T_{flowering}$	1	0.13	0.51
$T_{growing}$	1	0.14	2.4 ·
T_{winter}	1	0.05	0.07
$P_{flowering}$	1	7.59 **	0.30
$P_{growing}$	1	5.25 *	1.26

Table S3.9. (cont'd)

P_{winter}	1	7.39 **	0.05
Latitude	1	0.35	0.00
Longitude	1	4.15 *	0.21
Status x $T_{flowering}$	1	0.05	0.23
Status x $T_{growing}$	1	0.52	0.67
Status x T_{winter}	1	0.01	0.00
Status x $P_{flowering}$	1	7.75 **	0.02
Status x $P_{growing}$	1	4.14 *	1.06
Status x P_{winter}	1	6.38 *	0.01
Status x latitude	1	0.26	0.09
Status x longitude	1	2.38	0.01
Residual	153	20.56	0.31
(2) Year model			
Year	1	4.34 *	2.73 ·
Status	1	4.86 *	3.04 ·
Latitude	1	14.16 ***	1.86
Longitude	1	1.11	0.42
Status x year	1	5.31 *	2.86 ·
Residual	172	20.99	0.31
(G) Ratibidas (Asteraceae)			
(1) Climate model			
Status	1	0.09	0.02
$T_{flowering}$	1	1.34	0.53
$T_{growing}$	1	3.28 ·	0.51
T_{winter}	1	0.84	0.76
$P_{flowering}$	1	1.16	0.84
$P_{growing}$	1	0.03	0.01
P_{winter}	1	0.44	0.00
Latitude	1	0.18	0.03
Longitude	1	1.66	0.87
Status x $T_{flowering}$	1	2.71 ·	1.52
Status x $T_{growing}$	1	3.74 *	1.15
Status x T_{winter}	1	0.03	0.75
Status x $P_{flowering}$	1	0.03	1.42
Status x $P_{growing}$	1	0.04	0.38
Status x P_{winter}	1	0.47	0.05
Status x latitude	1	0.01	0.00
Status x longitude	1	1.16	0.01
Residual	77	27.14	0.62
(2) Year model			
Year	1	10.78 **	2.15 ·
Status	1	0.89	0.16
Latitude	1	2.17	0.16
Longitude	1	0.24	2.59
Status x year	1	1.05	0.13
Residual	92	25.99	0.58
(H) Silphiums (Asteraceae)			
(1) Climate model			
Status	1	0.34	0.20
$T_{flowering}$	1	0.01	0.01
$T_{growing}$	1	0.14	1.01
T_{winter}	1	2.55 ·	0.09
$P_{flowering}$	1	1.29	1.47

Table S3.9. (cont'd)

$P_{growing}$	1	1.98	2.38
P_{winter}	1	2.38	0.18
Latitude	1	0.01	0.02
Longitude	1	2.74	1.05
Status x $T_{flowering}$	1	0.01	0.55
Status x $T_{growing}$	1	0.17	3.06 ·
Status x T_{winter}	1	0.42	0.02
Status x $P_{flowering}$	1	0.37	2.68
Status x $P_{growing}$	1	2.31	5.16 *
Status x P_{winter}	1	0.27	0.04
Status x latitude	1	0.13	0.00
Status x longitude	1	0.37	0.09
Residual	77	23.33	0.55
(2) Year model			
Year	1	3.88 *	0.74
Status	1	0.13	0.85
Latitude	1	0.36	1.15
Longitude	1	2.72	1.18
Status x year	1	0.12	0.89
Residual	90	22.37	0.56

Figure S3.1. Snowfall. Total number of snowfall events per month across all years and counties represented in this study.

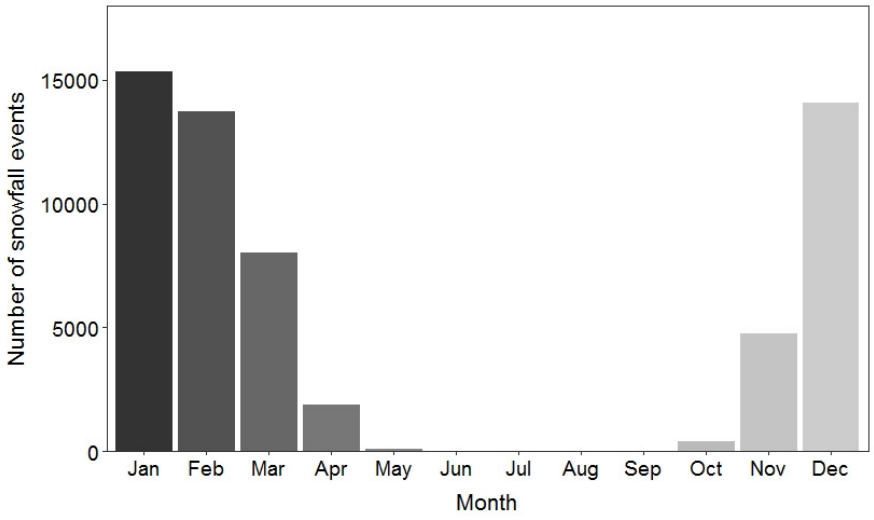


Figure S3.2. Effects of climate and year on Developmental Index. Locally extinct and extant species differ in the magnitude of their phenological responses to temperature and over time. Left: Phenological sensitivity to (a) $T_{flowering}$, (c) $T_{growing}$, and (e) T_{winter} (°C), and (g) year in locally extinct (red) vs. extant (grey) species. Sensitivity is defined as the slope (days/°C or year) (± standard error) of locally extinct vs. extant species' overall phenological response to temperature and over time. Positive values indicate delayed flowering; negative values indicate advanced flowering. Asterisks over a bar indicate a significant response to temperature; asterisks over a bracket indicate a significant difference between locally extinct and extant species; "n.s." indicates a non-significant response. Right: Effect of (b) $T_{flowering}$, (d) $T_{growing}$, (f) T_{winter}, and (h) year on flowering phenology (Developmental Index) of all species included in this study. Red and grey lines show locally extinct and extant species, respectively. We fit random slopes for each species' response to $T_{flowering}$, $T_{growing}$, and T_{winter}.

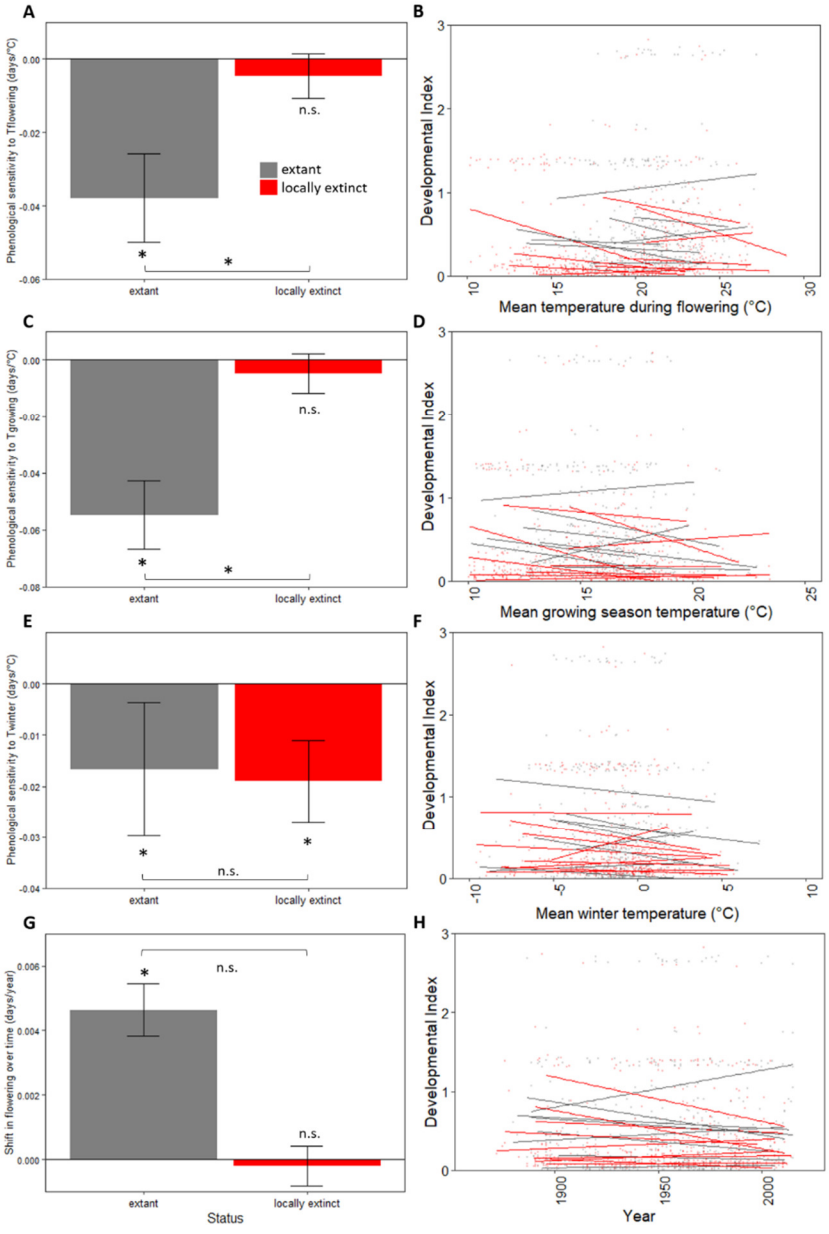


Figure S3.3. Species-specific effects of temperature on Developmental Index. Effect of (a) $T_{flowering}$, (b) $T_{growing}$, and (c) T_{winter} (°C) on flowering phenology (Developmental Index; DI) of all species pairs included in this study. A higher DI indicates later flowering. Each panel represents one congeneric (or confamilial) pair. Red and grey lines show locally extinct and extant species within a pair, respectively. Gray areas represent 95% confidence intervals.

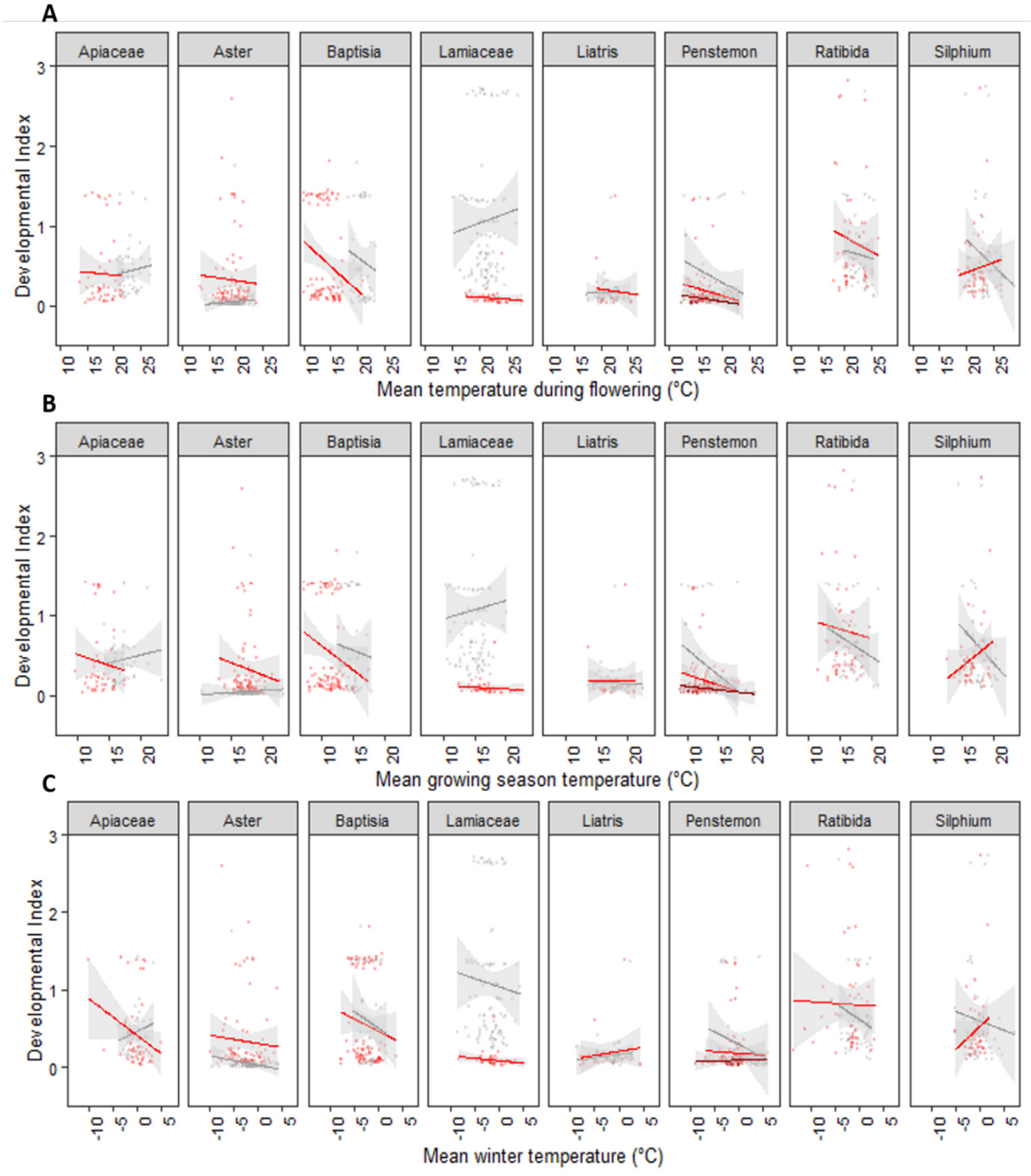


Figure S3.4. Species-specific effects of precipitation on phenology (DOY). Effect of (a) $P_{flowering}$, (b) $P_{growing}$, and (c) P_{winter} (cm) on flowering phenology (day of year) of all species pairs included in this study. Each panel represents one congeneric (or confamilial) pair. Red and grey lines show locally extinct and extant species within a pair, respectively. Gray areas represent 95% confidence intervals.

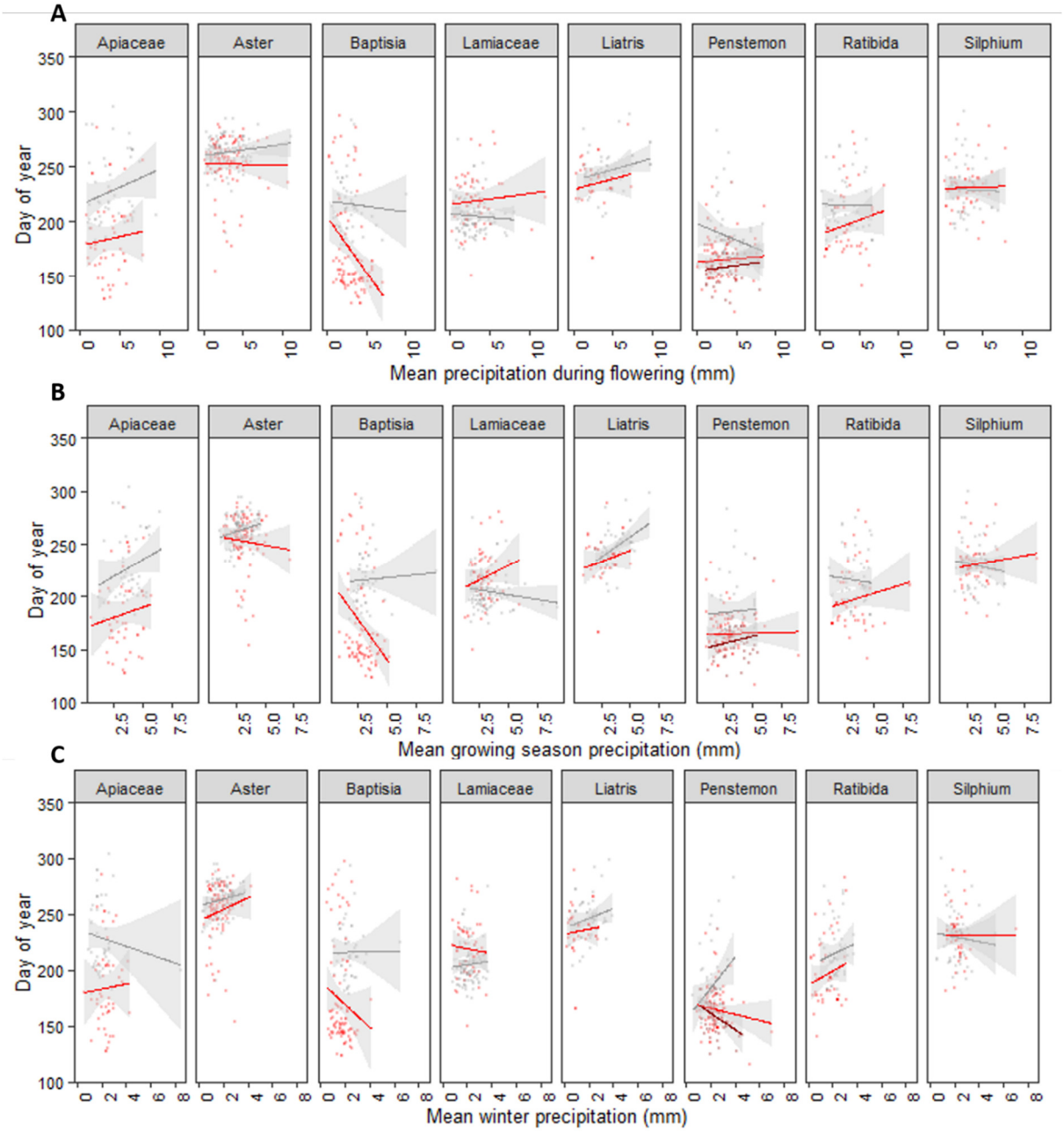


Figure S3.5 Species-specific effects of precipitation on phenology (DI). Effect of (a) $P_{flowering}$, (b) $P_{growing}$, and (c) P_{winter} (cm) on flowering phenology (Developmental Index; DI) of all species pairs included in this study. A higher DI indicates later flowering. Each panel represents one congeneric (or confamilial) pair. Red and grey lines show locally extinct and extant species within a pair, respectively. Gray areas represent 95% confidence intervals.

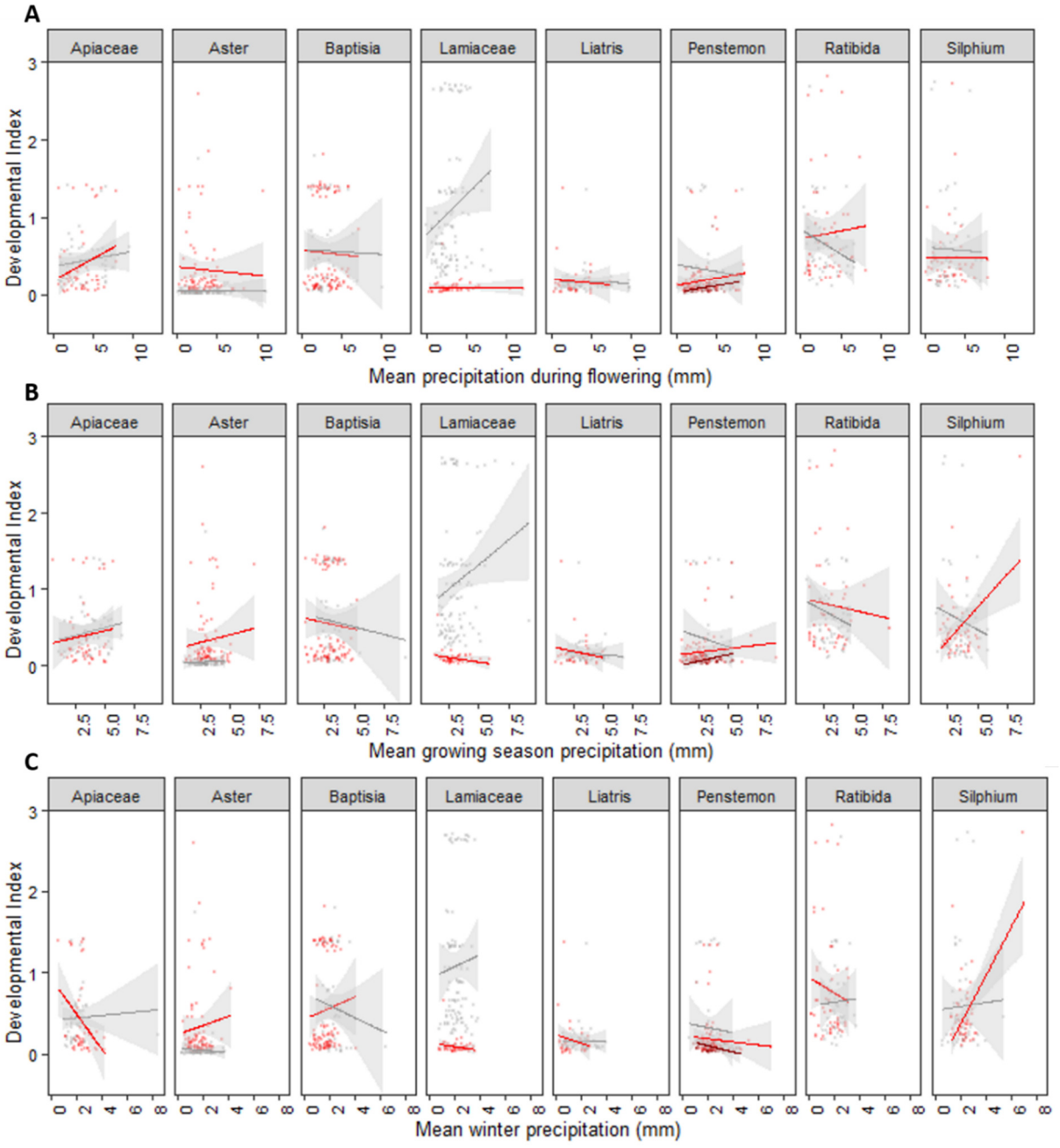


Figure S3.6. Effect of latitude and growing season temperature on phenology. Effect of latitude and growing season temperature ($T_{growing}$; April – mean flowering date; °C) on flowering phenology (day of year). For visualization, latitude is binned into 36-39° ("south", light blue), 40-44° ("mid", medium blue), and 45-48° ("north", dark blue). * indicates a locally extinct species.

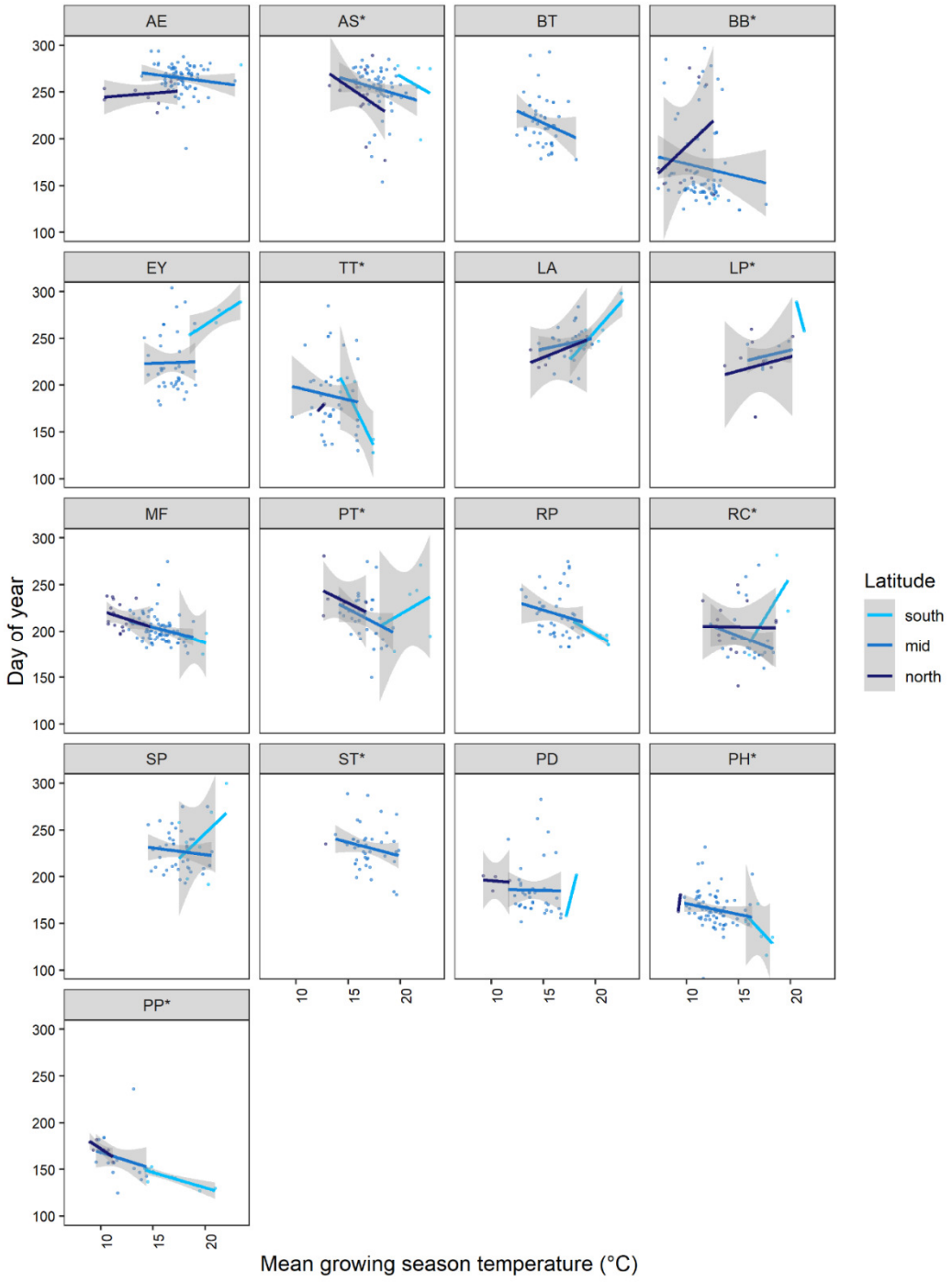
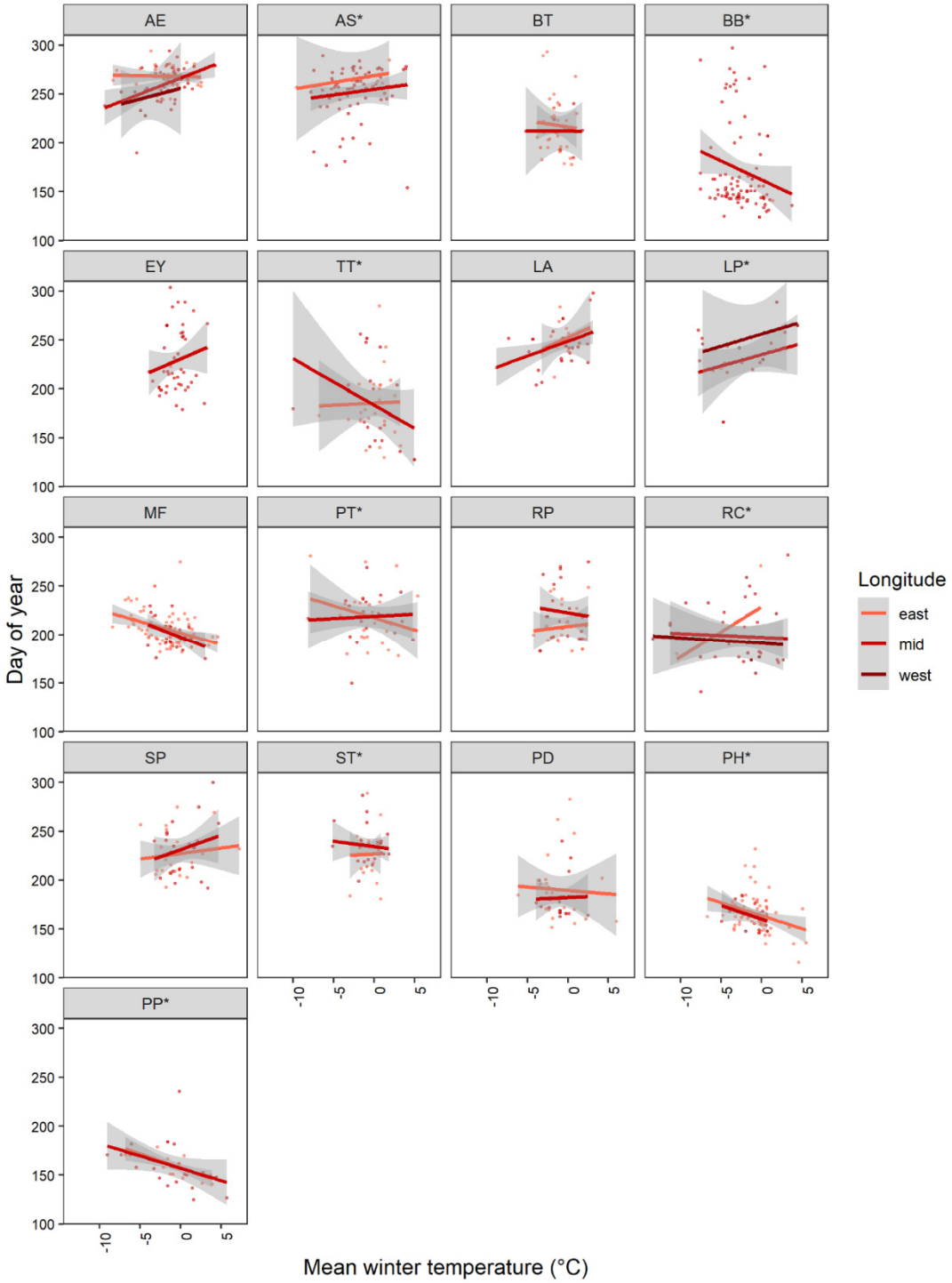


Figure S3.7. Effect of longitude and winter temperature on phenology. Effect of longitude and winter temperature (T_{winter}; November – March prior to flowering; °C) on flowering phenology (day of year). For visualization, longitude is binned into 75-86° ("east", light red), 87-98° ("mid", medium red), and 99-108° ("west", dark red). * indicates a locally extinct species.



LITERATURE CITED

LITERATURE CITED

Arft, A.M., M.D. Walker, J. Gurevitch, J.M. Alatalo, M.S. Bret-Harte, M. Dale, M. Diemer, et al. 1999. Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecological Monographs* 69: 4991-5110.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: https://doi.org/10.18637/jss.v067.i01

Bjorkman, A.D., S.C. Elmendorf, A.L. Beamish, M. Vellend, M., and G.H.R. Henry. 2016. Contrasting effects of warming and increased snowfall on Arctic tundra plant phenology over the past two decades. *Global Change Biology* 21: 4651-4661.

Bliese, P.D., and R.E. Ployhart. 2002. Growing modeling using random coefficient models: model building, testing, and illustrations. *Organizational Research Methods* 5: 362-387.

Bradley, N.L., A.C. Leopold, J. Ross, and W. Huffaker. 1999. Phenological changes reflect climate change in Wisconsin. *Proceedings of the National Academy of Sciences* 96: 9701-9704.

Bradshaw, W.E., and C. Holzapfel. 2006. Climate change. Evolutionary response to rapid climate change. *Science* 9: 1477-1478.

Caffarra, A., A. Donnelly, I. Chuine, I., and M.B. Jones. 2011. Modelling the timing of *Betula pubescens* budburst. I. Temperature and photoperiod: a conceptual model. *Climate Research* 46: 147-157.

CaraDonna, P.J., A.M. Iler, A.M., and D. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences* 111: 4916-4921.

CaraDonna, P.J., and J.A. Bain. 2015. Frost sensitivity of leaves and flowers of subalpine plants is related to tissue type and phenology. *Journal of Ecology* 104, 55-64.

Cleland, E.E., I. Chuine, A. Menzel, H.A. Mooney, and M.D. Schwartz. 2007. Shifting plant phenology in response to global change. *Trends in Ecology and Evolution* 22: 357-365.

Cleland, E.E., J.M. Allen, T.M. Crimmins, J.A. Dunne, S. Pau, S.E. Travers, E.S. Zavaleta, and E.M. Wolkovich. 2012. Phenological tracking enables positive species responses to climate change. *Ecology* 93: 1765-1771.

Collen, B., A. Purvis, A., and G.M. Mace. 2010. When is a species really extinct? Testing extinction inference from a sighting record to inform conservation assessment. *Diversity and Distributions* 16: 755-764.

Cook, B.I., E.M. Wolkovich, and C. Parmesan. 2012. Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings of the National Academy of Sciences* 109: 9000-9005.

Cooper, E.J., S. Dullinger, and P. Semenchuk. 2011. Late snowmelt delays plant development and results in lower reproductive success in the High Arctic. *Plant Science* 180: 157-167.

Cornelius, C., A. Leingärtner, B. Hoiss, J. Krauss, I. Steffan-Dewenter, and A. Menzel. 2013. Phenological response of grassland species to manipulative snowmelt and drought along an altitudinal gradient. *Journal of Experimental Botany* 64: 241-251.

Crawley, M.J., P.H. Harvey, and A. Purvis. 1996. Comparative ecology of the native and alien floras of the British Isles. *Philosophical Transactions of the Royal Society B* 351: 1251-1259.

Cremonese, E., G. Filippa, M. Galvagno, C. Sinisacalco, L. Oddi, U.M. di Cella, and M. Migliavacca. 2017. Heat wave hinders green wave: The impact of climate extreme on the phenology of a mountain grassland. *Agricultural and Forest Meteorology* 247: 320-330.

Crimmins, T.M, M.A. Crimmins, and C.D. Bertelsen. 2011. Onset of summer flowering in a 'Sky Island' is driven by monsoon moisture. *New Phytologist* 191: 468-479.

Cui, T., L. Martz, and X. Guo. 2017. Grassland phenology response to drought in the Canadian prairies. *Remote Sensing* 9: 1258-1279.

Daru, B.H., D.W. Park, R.B. Primack, C.G. Willis, D.S. Barrington, T.J.S. Whitfield, T.J. Seidler, et al. 2018. Widespread sampling biases in herbaria revealed from large-scale digitalization. *New Phytologist* 217: 939-955.

Davis, C.C., C.G., Willis, B. Connolly, C. Kelly, and A.M. Ellison. 2015. Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms. *American Journal of Botany* 102: 1599-1609.

DeFalco, L.A., G.C.J. Fernandez, and R.S. Novak. 2007. Variation in the establishment of a non-native annual grass influences competitive interactions with Mojave Desert perennials. *Biological Invasions* 9: 293-307.

Du, Y., B. Yang, S.C. Chen, and K. Ma. 2019. Diverging shifts in spring phenology in

response to biodiversity loss in a subtropical forest. *Journal of Vegetation Science* 30: 1175-1183.

Dunnell, K.L., and S.E. Travers. 2011. Shifts in the flowering phenology of the northern Great Plains: Patterns over 100 years. *American Journal of Botany* 98: 935-945.

Ellwood, E.R., K.D. Pearson, and G. Nelson. 2019. Emerging frontiers in phenological research. *Applications in Plant Science* 7(3): c1234.

Elzinga, J.A., A. Atlan, A. Biere, L. Gigord, A.E. Weis, and G. Bernasconi. 2007. Time after time: Flowering phenology and biotic interactions. *Trends in Ecology and Evolution* 22: 432-439.

Fitter, A.H., and R.S.R. Fitter. 2002. Rapid changes in flowering time in British plants. *Science* 296: 1689-1691.

Forrest, J., and A.J. Miller-Rushing. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B.* 365: 3101-3112.

Grolemund, G., and H. Wickham. 2011. Dates and times made easy with lubridate. *Journal of Statistical Software* 40: 1-25.

Grothendieck, G. 2017. sqldf: manipulate R data frames using SQL. R package version 0.4-11.

Güsewell, S., R. Furrer, R. Gehrig, and B. Pietragalla. 2017. Changes in temperature sensitivity of spring phenology with recent climate warming in Switzerland are related to shifts of the preseason. *Global Change Biology* 23: 5189-5202.

Hanes, C.R., and F.N. Hanes. 1947. *Flora of Kalamazoo County, Michigan: Vascular Plants.* Anthoensen Press, Portland, ME, USA.

Hart, R., J. Salick, S. Ranjitkar, and J. Xu. 2014. Herbarium specimens show contrasting phenological responses to Himalayan climate. *Proceedings of the National Academy of Sciences* 111: 10615-10619.

Iler, A.M., A. Compagnoni, D.W. Inouye, J.L. Williams, P.J. CaraDonna, A. Anderson, and T.E.X. Miller. 2019. Reproductive losses due to climate change-induced earlier flowering are not the primary threat to plant population viability in a perennial herb. *Journal of Ecology* 107: 1931-1943.

Körner, C., and D. Basler. 2010. Phenology under global warming. *Science* 327: 1461-1462.

Kuussaari, M., R. Bommarco, R.K. Heikkinen, A. Helm, J. Krauss, R. Lindborg, E. Öckinger, et al. 2009. Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology and Evolution* 24: 564-571.

Lang, P.L.M., F.M. Willems, J.F. Scheepens, H.A. Burbano, and O. Bossdorf. 2018. Using herbaria to study global environmental change. *New Phytologist* 221: 110-122.

Liancourt, P., L. Spence, B. Boldgiv, A. lkhagva, B.R. Helliker, B.B. Casper, and P.S. Petraitis. 2012. Vulnerability of the northern Mongolian steppe to climate change: insights from flower production and phenology. *Ecology* 93: 815-824.

Matthews, E.R., and S.J. Mazer. 2016. Historical changes in flowering phenology are governed by temperature x precipitation interactions in a widespread herb in western North America. *New Phytologist* 210: 157-167.

McKenna, D.D. 2004. Flora and vegetation of Kalamazoo County, Michigan. *Michigan Botanist* 43: 137-359.

Meineke, E.K., C.C. Davis, and T.J. Davies. 2018. The unrealized potential of herbaria for global change biology. *Ecological Monographs* 88: 505-525.

Miller-Rushing, A.J., T.T. Høye, D.W. Inouye, and E. Post. 2010. The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B.* 365: 3177-3186.

Miller-Rushing, A.J., and R.B. Primack. 2008. Global warming and flowering times in Thoreau's Concord: a community perspective. *Ecology* 89: 332-341.

Møller, A.P., D. Rubolini, and E. Lehikoinen. 2008. Populations of migratory bird species that did not show a phenological response to climate change are declining. *Proceedings of the National Academy of Sciences* 105: 16195-16200.

Murray, B.R., P.H. Thrall, A.M. Gill, and A.B. Nicotra. 2002. How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology* 27: 291-310.

Moussus, J., R. Julliard, and F. Jiguet. 2010. Featuring 10 phenological estimators using simulated data. *Methods in Ecology and Evolution* 1: 140-150.

MSU CANR. Frost free date table. https://tinyurl.com/yy9efkn3. Web. 2019.

Ooms, J. 2014. The jsonlite package: a practical a consistent mapping between JSON data and R objects. arXiv:1403.2805.

Panchen, Z.A., and R. Gorelick. 2017. Prediction of Arctic plant phenological sensitivity to climate change from historical records. *Ecology and Evolution* 7: 1325-1338.

Park, D.S., I. Breckheimer, A.C. Williams, E. Law, A.M. Ellison, and C.C. Davis. 2018. Herbarium specimens reveal substantial and unexpected variation in phenological

sensitivity across the eastern United States. *Philosophical Transactions of the Royal Society B.* 374: 20170394.

Park, I.W., and M.D. Schwartz. 2015. Long-term herbarium records reveal temperature-dependent changes in flowering phenology in the southeastern USA. *International Journal of Biometeorology* 59: 347-355.

Parmesan, C., and M.E. Hanley. 2015. Plants and climate change: complexities and surprises. *Annals of Botany* 116: 849-864.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.

Pearson, D.R., Y.K. Ortega, and S.J. Sears. 2012. Darwin's naturalization hypothesis up-close: Intermountain grassland invaders differ morphologically and phenologically from native community dominants. *Biological Invasions* 14: 901-913.

Pearson, K.D. 2019. Spring- and fall-flowering species show diverging phenological responses to climate in the Southeast USA. *International Journal of Biometeorology* 63: 481-492.

Primack, D., C. Imbres, R.B. Primack, A.J. Miller-Rushing, and P. del Tredici. 2004. Herbarium specimens demonstrate earlier flowering times in response to warming in Boston. *American Journal of Botany* 91: 1260-1264.

Purvis, A., J.L. Gittleman, G. Cowlishaw, and G.M. Mace. 2000. Predicting extinction risk in declining species. *Proceedings of the Royal Society B.* 26: 1947–1952.

Rafferty, N.E., P.J. CaraDonna, L.A. Burkle, A.M. Iler, and J.L. Bronstein. 2013. Phenological overlap of interacting species in a changing climate: an assessment of available approaches. *Ecology and Evolution* 3: 3183-3193.

Rafferty, N.E., and A.R. Ives. 2011. Effects of experimental shifts in flowering phenology on plant-pollinator interactions. *Ecology Letters* 14: 69-74.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rosa, R.K., S.F. Oberbauer, G. Starr, I.P. La Puma, E. Pop, L. Ahlquist, and T. Baldwin. 2015. Plant phenological responses to a long-term experimental extension of growing season and soil warming in the tussock tundra of Alaska. *Global Change Biology* 21: 4520-4532.

Schmidt-Lebuhn, A.N., N.J. Knerr, and M. Kessler. 2013. Non-geographic collecting biases in herbarium specimens of Australian daisies (Asteraceae). *Biodiversity and Conservation* 22: 905-919.

Schuster, M.J., and J.S. Dukes. 2017. Rainfall variability counteracts N addition by promoting invasive *Lonicera maackii* and extending phenology in prairie. *Ecological Applications* 27: 1555-1563.

Schwartz, M.D., and J.M. Hanes. 2010. Continental-scale phenology: warming and chilling. *International Journal of Climatology* 30: 1595-1598.

Sherry, R.A., X. Zhou, S. Gu, J.A. Arnone, D.S. Schimel, P.S. Verburg, L.L. Wallace, and Y. Luo. 2007. Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences* 104: 198-202.

Smith, J.G., W. Sconiers, M.J. Spasojevic, I.W. Ashton, and K.N. Suding. 2012. Phenological changes in alpine plants in response to increased snowpack, temperature, and nitrogen. *Arctic, Antarctic, and Alpine Research* 44: 135-142.

Thackeray, S.J., P.A. Henrys, D. Hemming, J.R. Bell, M.S. Botham, S. Burthe, P. Helauoet, et al. 2016. Phenological sensitivity to climate across taxa and trophic levels. *Nature* 535: 241-245.

van Kleunen, M., and D.M. Richardson. 2007. Invasion biology and conservation biology: time to join forces to explore the link between species traits and extinction risk and invasiveness. *Progress in Physical Geography* 31: 447-450.

Visser, M., and C. Both. 2005. Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B.* 272: 2561-2569.

Vitasse, Y., C. Signarbieux, and Y.H. Fu. 2018. Global warming leads to more uniform spring phenology across elevations. *Proceedings of the National Academy of Sciences* 115: 1004-1008.

Wang, C., and Y. Tang. 2019. Responses of plant phenology to nitrogen addition: a meta-analysis. *Oikos* 128: 1243-1253.

Wickham, H. 2019. httr: tools for working with IRLs and HTTP. R package version 1.4.1.

Wickham, H., and L. Henry. 2019. tidyr: easily tidy data with ‘spread()’ and ‘gather()’ functions. R package version 0.8.3.

Willis, C.G., R.B. Primack, A.J. Miller-Rushing, and C.C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau’s woods are driven by climate change. *Proceedings of the National Academy of Sciences* 105: 17029-17033.

Willis, C.G., B. Ruhfel, R.B. Primack, A.J. Miller-Rushing, J.B. Losos, and C.C. Davis. 2010. Favorable climate change response explains non-native species’ success in Thoreau’s woods. *PLoS One* 5(1): e8878.

Willis, C.G., E.R. Ellwood, R.B. Primack, C.C. Davis, J.D. Pearson, A.S. Gallinat, J.M. Yost, et al. 2017. Old plants, new tricks: phenological research using herbarium specimens. *Trends in Ecology and Evolution* 32: 531-546.

Wolkovich, E.M., T.J. Davies, H. Schaffer, E.E. Cleland, B.I. Cook, S.E. Travers, C.G. Willis, and C.C. Davis. 2013. Temperature-dependent shifts in phenology contribute to the success of exotic species with climate change. *American Journal of Botany* 100: 1407-1421.

Xia, J., and S. Wan. 2013. Independent effects of warming and nitrogen addition on plant phenology in the Inner Mongolian steppe. *Annals of Botany* 111: 1207-1217.

Zettlemoyer, M.A., D.D. McKenna, and J.A. Lau. 2019a. Species characteristics affect local extinctions. *American Journal of Botany* 106: 1-13.

Zettlemoyer, M.A., E.H. Schultheis, and J.A. Lau. 2019b. Phenology in a warming world: differences between native and non-native plant species. *Ecology Letters* 22: 1253-1263.

CHAPTER FOUR:

Nitrogen reduces population growth rates by decreasing survival in native prairie forbs

ABSTRACT

Species extinctions are predicted to rise by an order of magnitude over the next few centuries. Although contemporary documented extinctions are relatively rare, local population declines and extinction events likely provide hints about global extinction risks. Comparing traits and responses to global change of locally extinct species to still extant species could highlight why certain species are more vulnerable to extinction than others. However, we are still limited in our ability to predict species loss, largely because most studies infer extinction risk from observed species declines in the field rather than from demographic responses to anthropogenic change. Abundances in the field derive from occurrence data, while demographic responses describe specific ecological processes. More direct information on species' responses to changes in their environment would improve our ability to understand and predict population declines in response to anthropogenic change. Moreover, anthropogenic changes likely interact to affect population declines, but multifactorial demographic studies are rare. Here, I use Integral Projection Models and Life Table Response Experiments to examine demographic responses to nitrogen addition and deer herbivory, two major drivers of species losses in native grasslands, in fourteen locally extinct and extant native plant species found in Michigan prairies. Nitrogen consistently reduces survival, especially in locally extinct species, and locally extinct species' growth and reproduction benefit less from nitrogen addition than extant species. Integral Projection Models demonstrate that nitrogen reduces population growth rates across these native species, largely via reductions in survival. Deer herbivory also tended to reduce survival and population growth rates across species. This study highlights the need to study responses to

anthropogenic change across life stages and links community-level of patterns of species loss under nitrogen addition to the population-level processes underlying those losses.

INTRODUCTION

Contemporary rates of extinction are threefold higher than extinctions recorded in the fossil record (Barnosky et al., 2011; Cronk, 2016). Accurately predicting local species declines and ultimately extinction will depend on understanding demographic responses to anthropogenic change. However, relatively few studies examine how anthropogenic factors impact the demography of plant populations across their life cycle, even though demographic responses determine whether a population will persist under anthropogenic change (Campbell, 2019). Instead, most work on anthropogenic stressors has focused on how targeted vital rates (e.g., germination, survival, growth, or reproduction) respond to change, but vital rates trade-off and can respond differently to environmental conditions (Stearns 1989; Sheth and Angert, 2018), making analyses of the net fitness effects of anthropogenic change on threatened species critical. This is particularly important for long-lived native plants whose cumulative demographic responses to changing conditions may lead to long periods of population decline and eventual extinction (Kuussaari et al., 2009; Bialic-Murphy et al., 2019).

We currently have limited insight into the processes of local extinction, which is ultimately the result of a series of reductions in vital rates leading to reduced population growth rates (Doak and Morris, 1999; Collen et al., 2010). Here, I suggest that comparing the responses of locally extinct versus still-extant congeneric species could help reveal whether locally extinct species have generally lower vital rates or respond differently to anthropogenic change than their more successful counterparts (Mack, 1996; MacDowell, 2002; van Kleunen and Richardson,

2007). This approach has four key benefits. First, local population declines likely reflect global extinction risks, making population decline and local extinction events indicators of at-risk species (Menges, 2000; Ceballos and Ehrlich, 2002; Maschinski et al., 2005; Collen et al., 2010; Dirnböck et al., 2011; Davies, 2019). Second, locally extinct species represent realized, local-scale extinctions following contemporary rates of habitat loss and environmental change (Hanski and Ovaskainen, 2002). Therefore, they potentially provide a more relevant picture of recent extinctions than the fossil record (Zettlemoyer et al., 2019a). Finally, examining differences between already extinct vs. extant species could provide novel tests of the traits and ecological processes underlying recent extinctions. Most studies infer extinction risk from observed declines in natural populations (Chazal and Rounsevell, 2009; Mondanaro et al., 2019). Such observational studies, while good indicators of species loss, often focus on general patterns of vulnerability to extrinsic threats (e.g., habitat loss or nutrient deposition) (Fritz et al., 2009). Comparisons of locally extinct vs. still-extant species can focus on intrinsic trait differences between taxa (e.g., vulnerability to extinction) in addition to differences in their responses to global change (Murray et al., 2014). This approach is similar comparisons of native vs. non-native species in that it identifies traits and responses associated with shifts in abundance, in this case decline and eventual extinction (Murray, 2002; van Kleunen and Richardson, 2007).Yet no studies to our knowledge reintroduce locally extinct plants and monitor their responses to anthropogenic change, although this method might explicitly link population declines to extinction events.

As humans continue to alter environments, anthropogenic changes might interact to influence demography. Taking a multifactorial approach to studying population declines has several benefits, but multifactorial demographic studies are rare (Bernardo et al., 2019; Bialic-

Murphy et al., 2019). First, we can capture the effects of multiple global changes on vital rates across the life cycle and on overall population growth. Including multiple interacting stressors like these in population models allows for more accurate estimations of population viability (Tye et al., 2016; Bernando et al., 2019; Morris et al., 2020). For example, in one of the few demographic studies examining multiple drivers of population decline, climate warming threatens *Eurybia furcata,* but only when woody encroachment and deer herbivory are high (Bernardo et al., 2018). Second, examining population-level responses to anthropogenic changes can link species and community responses to finer-scale demographic processes, promoting a more mechanistic examination of biodiversity loss (Gotelli and Ellison, 2002; Simkin et al., 2016). In contrast to observation studies of species loss, mechanistic models incorporating experimental and demographic data can integrate intrinsic differences between taxa and test the roles of multiple, interacting anthropogenic changes in species declines (Urban et al., 2016). Such mechanistic models often outperform correlative approaches when projecting responses to global change (Pagel and Schurr, 2012) because they indicate processes hidden by associations between species loss and the local environment (Buckley et al., 2010; Urban et al., 2016). For example, population growth rates determine how population abundances change through time and in response to environmental change, while population abundance describes an emergent property of those changes (Urban et al., 2016). This lack of direct information about ecological processes limits our ability to build mechanistic models that would help us better understand and predict population responses to global change (Merow et al., 2014).

Here I focus on nitrogen addition and deer herbivory, two anthropogenic factors likely to influence prairie plant population growth rates in temperate North America. Nitrogen is a leading driver of biodiversity loss across the globe (Pennings et al., 2005; Suding et al., 2005, Clark et

al., 2007, Clark and Tilman, 2008; Borer et al., 2014; Hodapp et al., 2018), and high nitrogen levels can depress population growth rates (e.g., *sphagnum* spp. [Press et al., 1986], *Calluna* spp. [Heil and Diemont, 1983], and *Sarrecenia purpurea* [Gotelli and Ellison, 2002] [note that these are unique taxa that are susceptible to nitrogen]). Conversion of land for agriculture, extirpation of large predators, and hunting regulations also result in increased populations of white-tailed deer (*Odocoileus virginianus*) populations; the subsequent herbivory can cause population declines (Rooney and Waller, 2003; Knight et al., 2009; Kalisz et al., 2014; Bialic-Murphy et al., 2019), especially for rare and threatened species (Phillips and Maun, 1996). Moreover, these two drivers likely interact, as herbivores may prevent competitive exclusion by dominant species in high nitrogen systems by increasing ground-level light availability (Hautier et al., 2009; Borer et al., 2014). However, despite many studies examining biodiversity loss in response to nitrogen and herbivory, we have a limited understanding of demographic responses to both nitrogen and herbivory across a plant's entire life cycle.

Differences in demographic responses across taxa are likely influenced by plant functional traits, which are assumed to affect vital rates and fitness (Violle et al., 2007) as well as responses to environmental conditions (McGill et al., 2006; Mouillot et al., 2013). Although many studies correlate functional traits with individual vital rates, the role of traits across plant demography is less understood (Visser et al., 2016). In particular, traits might vary in their effects across a plant's life cycle. For instance, in tropical trees, seed mass is correlated with higher seedling establishment but lower survival (Visser et al., 2016). Additionally, locally extinct and extant species may differ in traits associated with their vital rates or their responses to anthropogenic change. For example, extinct species in Australia have thick, tough leaves relative to extant species (Kyle and Leishman, 2009), and extinct prairie forbs differ from extant

congeners/confamiliars in their phenological responses to temperature (Chapter 3). Ultimately, understanding trait-demography relationships across different vital rates may help improve the potential for plant traits to inform models of biodiversity loss.

I experimentally manipulated nitrogen and deer presence in the field to test how two major global changes affect the population demography of nine confamilial (often congeneric) pairs of still-extant versus locally extinct (defined here as species that have disappeared from a particular county; Pimm et al., 2014) prairie species once found in Michigan prairies and savannas. I ask the following questions: (1) How do vital rates (survival, growth, reproduction, and recruitment) respond to nitrogen addition and deer herbivory, and do these responses differ between locally extinct and extant species? The nitrogen x herbivory experiment includes high (agronomic) levels of fertilization, but the environmental and demographic effects of lower rates of nitrogen addition that characterize most habitats are less well-studied (Clark and Tilman, 2008). To test how even low rates of nitrogen deposition affect demography, I conducted a second nitrogen gradient experiment to tease apart locally extinct vs. extant prairie species' vital rate responses to increasing levels of nitrogen deposition, asking (2) How do vital rates respond to a gradient of nitrogen addition? I then use Integral Projection Models (IPMs) and Life Table Response Experiment (LTRE) analyses to ask (3) how nitrogen addition and deer herbivory affect population growth rates (λ) for a subset of species. The IPMs allow me to estimate how each vital rate contributes to the effects of nitrogen and herbivory on λ. Finally, I examine trait-demography relationships by testing whether four plant traits (specific leaf area, flowering phenology, leaf nitrogen content, and leaf carbon to nitrogen ratio) (4) differ between locally extinct vs. extant species and (5) whether those traits correlate with vital rates.

MATERIALS AND METHODS

Study System

Kalamazoo County, covering 1492 km^2 in southwestern Michigan (MI), USA, has lost 99% of its native prairie habitat from 1800-2004, with only 130 of its original 21500 acres remaining (Chapman and Brewer, 2008; Zettlemoyer and Srodes, 2019). The county has lost 14.01% of its native prairie species during a similar timeframe (1890-2004), mostly rare perennial prairie specialist forbs (Hanes and Hanes, 1947; McKenna, 2004; Zettlemoyer et al., 2019a). For details on historical extinction events in Kalamazoo County, see {Zettlemoyer et al. 2019a}. Although habitat loss is certainly a significant driver of species loss in the area, more species have been lost than predicted (based on an analysis of the species-area relationship, 164 prairie species should remain, but only 141 remain [Zettlemoyer and Srodes, 2019]). This suggests that other anthropogenic factors, such as nitrogen addition and deer herbivory, may play a role in plant extinctions in this area. Soil nitrogen levels in Michigan are high due to nitrogen deposition and agricultural fertilization. Nitrogen deposition in Michigan is higher than the United States average (MI: 5-7 kg N ha^{-1} yr^{-1} [Pardo et al., 2010]; US: 1-4 kg N ha^{-1} yr^{-1} [Fenn et al., 2003]). Nitrogen fertilization recommendations range from 60-180 lbs N $acre^{-1}$ (0.67 – 20 g N m^{-2}) (Warncke et al., 2009). Grassland species are more susceptible to nitrogen-induced declines and disappear at lower levels of nitrogen deposition relative to species from other habitat types (Simkin et al. 2016). Simultaneously, white-tailed deer populations in Michigan began increasing in the 1890s following hunting regulations, rising to 1.5M statewide in the late 1940s and peaking at 2.2M in 1995 (MI DNR), matching the period of species decline examined here (1890-2004).

Studies were conducted in restored prairies at the Boudeman Conservation Farm (Richland, MI) (hereafter "BCF") and the Kalamazoo Nature Center's Kal-Haven prairie (Kalamazoo, MI) (hereafter "KHP"). Both prairies were burned for management in fall 2016. BCF is ca. 20 years old, dominated by *Andropogon gerardii*; KHP is ca. 10 years old, dominated by *Sorghastrum nutans.*

Nitrogen addition x deer herbivory experiment

In 2017, I set up a 2x2 split-plot experiment manipulating deer herbivory and nitrogen addition. To exclude deer, I constructed 10ft-high deer fencing around the perimeter of five 9m^2 whole plots at each site. Five additional 9m2 whole plots per site served as herbivore present controls (n = 10 whole plots/site {5 fenced and 5 unfenced} x 2 sites = 20 whole plots). I applied nitrogen (44% time-release urea) at 10 g m^{-2} yr^{-1} (elemental mass: 22.73 g m^{-2} yr^{-1}) to 4.5m^2 subplots (n = 40 subplots) (Nutrient Network). Nitrogen addition significantly increased productivity (total aboveground biomass) and decreased light availability (Fig. S4.1).

To test how locally extinct vs. extant species' population dynamics respond to nitrogen and deer herbivory, I selected 8 confamiliar pairs and 1 triplet of native, perennial, prairie specialist forbs in which one species is locally extinct ("locally extinct") while the other persists ("extant") (Table 4.1). The species selected are pairs of native, perennial, prairie specialist forbs. I selected prairie specialists because they are at higher risk of loss than species that can persist in other habitat types (Zettlemoyer et al., 2019a) and so that differences in habitat affinity (i.e., the ability to persist in different habitat types) are not confounded with extinction. I selected perennial species because they are more likely to demonstrate delayed extinctions following environmental change (Vellend et al., 2006) and because annuals are extremely rare in older prairies. Seeds were sourced as locally to Michigan as possible and always from a Midwestern

seed source (nurseries in order of selection: Michigan Wildflower Farm [Portland, Michigan], Naturally Native Nursey [South Bend, Indiana], Agrecol [Edgerton, Wisconsin], Prairie Moon [Winona, Minnesota]). In spring 2017, I sowed all 17 species in low-nutrient potting media (Sunshine Mix LP5) in the greenhouse. Fourteen species successfully germinated. I transplanted 6-week-old seedlings of those species into randomly selected field locations (planted 40cm apart) within each subplot (n = 10 seedlings/species/subplot x 40 subplots x 14 species = 5600 seedlings). I included a 40cm buffer area from the fences to control for edge and shading effects.

I monitored vital rates of all individuals from spring 2017- fall 2019. Vital rates included survival, plant size, reproductive status, reproductive effort, reproductive output, and recruitment. Seedlings were considered dead if they were not found in two subsequent surveys. Plant size was measured as height (cm) to the highest photosynthetic leaf. A plant was considered reproductive if it produced any flowers during a given year. Reproductive effort was measured as the total number of flowers produced by an individual over a single growing season. Reproductive output was measured two ways: (i) number of seeds produced per fruit (to use in population models), estimated by sampling one fruit from each reproductive plant, and (ii) total number of seeds produced per plant, estimated by multiplying seeds/fruit x number of flowers produced.

To determine recruitment (germination), I established a seed addition experiment in October 2019 at BCF. I sowed 100 seeds/species into a circular ring (0.5m diameter x 8cm deep) surrounded with aluminum flashing into each subplot (n = 100 seeds/species/ring x 1 ring/subplot x 20 subplots = 2000 seeds/species x 17 species = 34000 seeds). I left 4cm above the soil to prevent seeds from moving. I marked germinated seedlings weekly in May 2020 (n=4 surveys) to estimate germination (“recruits” = number of seeds germinated/100 seeds sown) and

germinant mortality (number of dead recruits/total recruits over the month) under each nitrogen x herbivory treatment combination.

Table 4.1. Species and plant family for the seven confamilial pairs and one triplet (*Penstemon*) included in this study. * indicates locally extinct species. Species above the line were included in both the nitrogen x herbivory and nitrogen gradient experiments.

Species	Family
Baptisia tinctoria	Fabaceae
*Baptisia bracteata**	Fabaceae
Monarda fistulosa	Lamiaceae
*Pycnanthemum tenuifolium**	Lamiaceae
Penstemon digitalis	Scrophulariaceae
*Penstemon hirsutus**	Scrophulariaceae
*Penstemon pallidus**	Scrophulariaceae
Ratibida pinnata	Asteraceae
*Ratibida columnifera**	Asteraceae
Aster ericoides	Asteraceae
Eryngium yuccifolium	Apiaceae
Liatris aspera	Asteraceae
Silphium perfoliatum	Asteraceae
*Silphium terebinthinaceum**	Asteraceae

Nitrogen gradient experiment

To examine whether nitrogen levels (from natural levels [0 g N added] to 12 g N m^{-2} yr^{-1}, agricultural fertilization) influence population demography, I set up a nitrogen gradient experiment at BCF. Nitrogen was applied to $3m^2$ plots at six levels: 0, 1, 2, 4, 8, and 12 g m^{-2} yr^{-1}. Nitrogen decreased light availability (Fig. S4.2). A subset of species, the three confamiliar pairs and one triplet (n = 9 species) with high survival in the nitrogen x herbivory experiment, were included in this experiment (Table 4.1). Seedlings were germinated in the greenhouse (see above) and transplanted into random locations within each field plot (n = 10 seedlings/species/plot x 6 N levels x 3 plots/N level x 9 species = 1620 seedlings). Vital rates were determined as described previously from 2018-2019 (2020 vital rate data are forthcoming).

Trait-demography relationships

To examine if functional traits (a) differ between locally extinct and extant species and (b) correlate with vital rates, I measured four traits: (1) mean specific leaf area (SLA), (2) mean flowering time, (3) leaf nitrogen content, and (4) leaf carbon to nitrogen (C:N) ratio on all species. SLA was estimated by hole-punching one leaf collected ca. 1cm from the top of all surviving plants in 2018, drying leaf material at 70°C for 48h, weighing leaf material, and calculating leaf area/mass (mm/mg) (n=1913 samples). Mean flowering time (Julian day) was estimated from herbarium records (Chapter 3). Leaf nitrogen and carbon (% content by mass) were determined using C:N combustion analysis (University of Wyoming Stable Isotope Facility). C and N content were estimated for each species in each treatment combination by pooling leaf samples from each species at the subplot level (n = 14 species x 20 subplots = 280 samples).

Data Analysis

Vital rates of locally extinct vs. extant species

I used vital rate data to fit statistical models for six demographic processes that together influence population dynamics: (1) survival (1=alive, 0=dead), (2) growth (height in year $t+1$ – height in year t), (3) probability of flowering (1=yes, 0=no), (4) reproductive effort (number of flowers/plant), (5) reproductive output (see below for details), and (6) recruitment (proportion of seeds germinated). I fit all generalized linear mixed models (GLMMs) using the lme4 package in R v.3.3.1 (Bates et al., 2015; R Core Team, 2016). Due to low survival at the KHP site (subsequently limiting sample size and affecting growth and reproduction estimates), I present vital rate models using only data from BCF in the main text; models including KHP are included in Appendix Table S4.1. Results were quantitatively similar, but locally extinct species were less likely to survive or flower at KHP (site x status: survival $\chi^2_{1,8717}=14.62$, $p<0.0001$; probability of

flowering $\chi^2_{1,4367}$=2.80, p=0.09). Nitrogen also tended to benefit growth and seed production less at KHP than at BCF (N x site: growth $\chi^2_{1,4387}$=3.41, p=0.06; seeds $\chi^2_{1,115}$=3.02, p=0.06). Before running models on individual vital rates, which are likely correlated, I first ran a MANOVA including all vital rates (except recruitment, which was estimated in a separate experiment) as a multivariate response variable, with nitrogen (control vs. nitrogen), herbivory (deer present vs. absent), status (locally extinct vs. extant), and their interactions as predictor variables. Following significant effects of nitrogen and status (both p<0.01; Table S4.2), I proceeded to analyze individual vital rates.

To test treatment effects on survival, I included survival as a binomial response variable and nitrogen, herbivory, status, and their interactions as predictor variables. I included species (nested within status), subplot (nested within plot), and the interaction of height x species as random effects. To test treatment effects on growth, I used a linear mixed model with the same predictors and random effects described. I tested treatment effects on reproduction using four metrics: (1) probability of flowering (binomial distribution), (2) reproductive effort (number of flowers produced; Poisson distribution), (3) reproductive output (Poisson distribution), and (4) recruitment. For 1-3, I only analyzed species that flowered (*Monarda fistulosa, Pycnanthemum tenuifolium, Penstemon digitalis, P. hirsutus, P. pallidus*). I examined reproductive output as (i) number of seeds produced per fruit (estimate used in Integral Projection Models) and (ii) total seed production per plant. I used the same fixed and random effects described above in all reproductive models.

In the nitrogen gradient experiment, I again analyzed the five vital rates (Pr[survival], growth, Pr[flowering], flower production, and seed production [per fruit and per plant]), first using MANOVA (Table S4.3) then separate GLMMs. I included nitrogen (continuous, 0-12 g N

m^{-2} yr^{-1}), status, and their interactions as predictor variables and species (nested in status), plot, and the interaction of height x species as random factors in all models. I hypothesized that vital rates would demonstrate a threshold response to nitrogen addition and so included a quadratic term (nitrogen^2) in all models.

To examine species-specific differences in vital rates, I examined the same six vital rates described above for each species separately. For the nitrogen x herbivory experiment, I included height, nitrogen, herbivory, and all their interactions as predictor variables, site as an independent fixed effect, and subplot (nested in plot and site), and plot (nested in site) as a random effect in all models. I included height as a covariate in species-specific models because Integral Projection Models (see "Population Modeling") require size-dependent vital rates; I present models without height as a covariate in Table S4.4 for comparison with traditional vital rate models (results are quantitatively similar). The species-specific models include data from both BCF and KHP to increase sample size (needed for the IPMs below), so I included site as a factor to control for differences between sites. For the nitrogen gradient experiment, I included height, nitrogen, nitrogen^2, and height x nitrogen, and height x nitrogen^2 as predictor variables and plot as a random effect in all models. Again, reproductive data was only analyzed for species that flowered.

Population modeling

I used the size-dependent species-specific vital rate models (Table S4.5) to parameterize Integral Projection Models (IPMs) for the five species that had sufficient survival, growth, and reproductive data (Lamiaceae and *Penstemon*). IPMs integrate contributions from vital rates across a continuous range of plant sizes (here, height) to predict population growth in discrete time steps (year t to year $t+1$) (Easterling et al., 2000; Ellner and Rees, 2006). IPMs produce a

projection kernel that describes all possible combinations of size-dependent demographic parameters. IPMs were modeled as follows:

$$n(y, t+1) = \int [p(x,y) + f(x,y)]\, n(x,t)dx$$

p(x,y) represents the survival component, which can be broken down such that

$$p(x,y) = s(x)g(x,y)$$

where *s(x)* is the probability that an *x*-sized individual survives from year *t* to *t+1* and *g(x.y)* is the growth of the plant from size *x* to size *y* over that time period. *f(x,y)* represents the reproduction component, or the production of *y*-sized individuals from *x*-sized individuals. *f(x,y)* can be described as

$$f(x,y) = P_f(x)f(x)P_g f_d(y)$$

where $P_f(x)$ is the probability of an *x*-sized individual producing flowers, P_g is the probability of recruitment, and $f_d(y)$ is the size distribution of new seedlings. I was not able to measure individual seedlings from the seed addition experiment, so I estimated $f_d(y)$ as the mean and standard deviation of seedling size in 2017, their first year (I note that 2017's seedlings were sown in a greenhouse, so this estimate is likely higher than the size of seedlings germinated in the field). *f(x)* represents the fertility of an *x*-sized individual, and can be decomposed into

$$f(x) = f_w(x)f_z(x)$$

where $f_w(x)$ is the flower production (reproductive effort) of an *x*-sized plant and $f_z(x)$ is the seed production per fruit (reproductive output) of an *x*-sized plant. For species without sufficient data to run a vital rate model for reproductive effort or output (*P. hirsutus* and *P. digitalis*), I used mean species estimates for $f_w(x)$ and $f_z(x)$ (Table S4.5). Lastly, I calculated P_g as $P_e(1-P_m)P_s$, where P_e is recruits (number of seeds germinated/100 seeds sown), P_m is the probability of germinant mortality (dead recruits/total recruits; $1-P_m$ represents the probability of a germinant

surviving), and P_s is an estimate of seed predation (i.e., proportion seeds removed by small mammals and arthropods) from a separate study (S.E. Johnson & M.A. Zettlemoyer, unpublished data). I included P_s because plots were fenced but not trenched, allowing small mammals and arthropods to consume seeds. Although P_g includes any seed predation from October – May, P_s was included to estimate summer seed predation (June – July). Because germination did not differ across experimental treatments, I used species means for P_g in IPMs. Together, *p(x,y)* and *f(x,y)* represent the IPM kernel, which describes all demographic possibilities.

To test for consistent effects of nitrogen and herbivory on deterministic population growth rates (λ) and whether λ differs between locally extinct vs. extant species, I used a linear mixed model with λ as the response variable, status, nitrogen, herbivory, and their interactions as predictor variables, and species as a random effect. For the nitrogen gradient experiment, I conducted similar IPMs and examined λ as a function of status, nitrogen, nitrogen^2, status x nitrogen, and status x nitrogen^2, with species included as a random effect. These models have low power, so I also discuss qualitative differences in λ. Because these were small founder populations planted into only two sites and were not at a stable age distribution, I discuss differences between λ, not absolute values of λ, in Results.

Finally, I used a Life Table Response Experiment (LTRE) to quantify the contributions of each vital rate to observed differences in lambda (Horvitz et al., 1996; Caswell, 2001). The difference between λ between the control and a treatment, Δλ, is calculated as

$$\Delta\lambda = \lambda^{t} - \lambda^{c} \approx \sum_{ij} \alpha_{ij}^{t} - \alpha_{ij}^{c} * \frac{\delta\lambda}{\delta\alpha_{ij}}$$

where (α_{ij}^{t} - α_{ij}^{c}) is the difference in a vital rate, α_{ij}, between the treatment and control matrices, and $\delta\lambda/\delta\alpha_{ij}$ is the sensitivity of λ to changes in α_{ij} (here, a perturbation of 0.01). A negative LTRE contribution indicates that the value of that vital rate under that experimental treatment is lower

than the control; i.e., a negative contribution of nitrogen to survival means that the probability of survival from year *t* to *t+1* is lower under nitrogen addition.

Trait-demography relationships

To examine whether locally extinct and extant species differ in their mean trait values, I first used MANOVA with four traits (SLA, flowering time, leaf N content, and C:N) as response variables and status as a predictor variable. Following a significant effect of status (see Results), I examined each trait separately using linear mixed models. Each trait had a different model due to differences in data collection. For SLA, which was collected on every surviving plant in the nitrogen x herbivory experiment, I included SLA as a response variable, status, nitrogen, herbivory, and their interactions as predictor variables, and species (nested in status) and subplot (nested in plot and site) as random factors. For flowering time, which was a species mean, I included flowering date as the response variable and status as the predictor variable. For leaf N content and C:N, which were estimated at the species level within each subplot, I used two linear models with either leaf N content or C:N as the two responses variables, status, nitrogen, herbivory, and their interactions as predictor variables, and subplot (nested in plot) as a random effect. To examine whether these four traits correlate with vital rates, I tested for Pearson's correlation between species mean trait values and each vital rate under each treatment combination (e.g., survival under control, nitrogen, deer absence, and nitrogen x deer absence) (i.e., 4 treatment combinations x 4 traits x 5 vital rates for all 13 species).

RESULTS

Effects of nitrogen and deer herbivory on extinct vs. extant species' vital rates

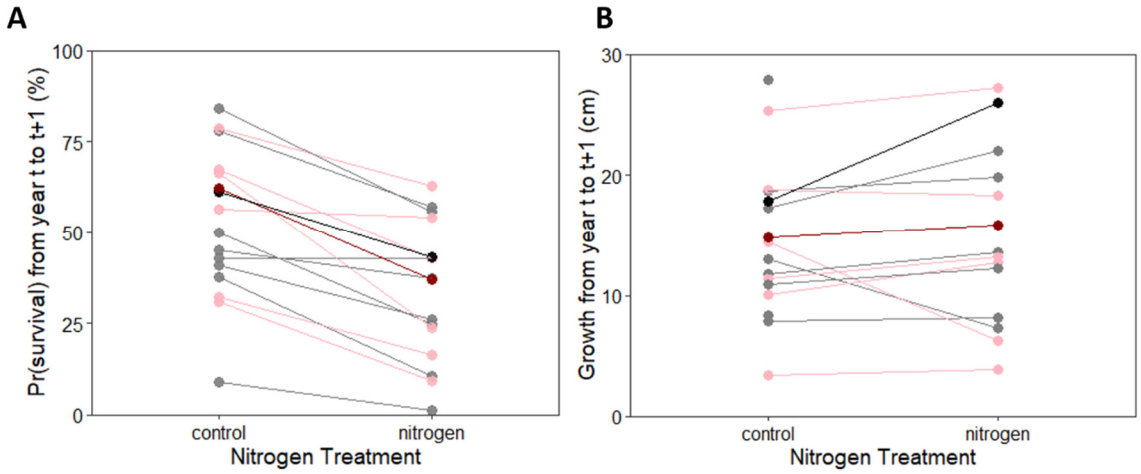
When presenting results, I describe results from the nitrogen x herbivory experiment then compare them to results from the nitrogen gradient experiment.

Nitrogen decreased survival, especially for locally extinct plants (N x status $\chi^2_{1,4919}$=3.69, p=0.03; Table S4.2; Fig. 4.1A). While extinct and extant species both had approximately 60% survival under the control treatment, locally extinct species only had 37.16 ± 8.85% survival compared to 43.05 ± 7.87% survival of extant species under nitrogen addition. Nitrogen significantly decreased survival in five of six locally extinct species and in four of eight extant species (Table S4.5), and the remaining species exhibited non-significant trends towards lower survival under nitrogen addition (Fig. 4.2A). Survival patterns were qualitatively similar between the nitrogen gradient and the nitrogen x herbivory experiments, with nitrogen tending to increase survival until about 6g N m^{-2}, after which point survival declined (although the quadratic term was not significant; N^2 $\chi^2_{1,2877}$=2.28, p=0.13; Table S4.3; Fig. 4.3A). Although the quadratic term was only statistically significant in two species (locally extinct *Baptisia bracteata* and extant *Penstemon digitalis),* this pattern held in all nine species (Table S4.5; Fig. S4.3A).

Extant species' growth benefited more from nitrogen than locally extinct species' growth (N x status $\chi^2_{1,2830}$=5.33, p=0.02; Table S4.2; Fig. 4.1B). Extant species grew 59.64 ± 3.95% larger under nitrogen addition compared to control plots, while locally extinct species only grew 18.04 ± 4.09% larger under nitrogen addition. Although nitrogen generally increased growth (except for the *Baptisia),* four out of eight extant species and only one extinct species' growth significantly benefited from nitrogen (*Penstemon pallidus;* Fig. 4.2B; Table S4.5). Similarly, in the nitrogen gradient, extant species' growth benefited more from nitrogen than extinct species' growth (status x N $\chi^2_{1,2236}$=5.69, p=0.02; Table S4.3; Fig. 4.3B). Nitrogen increased growth of locally extinct *Pycnanthemum tenuifolium*, *Baptisia bracteata,* and *Penstemon pallidus* as well

as extant *Penstemon digitalis* until about 6g N m^{-2}, after which growth benefits tapered off (N^2: PT $\chi^2_{1,330}$=15.56, p<0.0001; BB $\chi^2_{1,43}$=7.18, p=0.007; PD $\chi^2_{1,336}$=2.96, p=0.08; PP $\chi^2_{1,265}$=4.24, p=0.03; N all p<0.05; Table S4.5; Fig. S4.3B).

Figure 4.1. Locally extinct and extant species differ in their survival and growth responses to nitrogen. (A) Probability of survival (Pr[survival], %) and (B) growth (cm) from year *t* to *t+1* for locally extinct (pink) and extant (grey) species in control vs. nitrogen-treated plots. Each connected line represents a confamilial pair. Red and black dots represent overall means for locally extinct and extant species, respectively. The two species that respond negatively to nitrogen in (B) are the two *Baptisia* (Fabaceae).



Herbivory (deer presence) only affected survival and growth in a few species. Herbivory decreased survival of extant *Eryngium yuccifolium, Silphium perfoliatum*, and *Ratibida pinnata* (herbivory: EY $\chi^2_{1,705}$=7.25, p=0.007; SP $\chi^2_{1,693}$=5.23, p=0.02; RP $\chi^2_{1,654}$=2.80, p=0.06; Table S4.5; Fig. 4.2A). Herbivory decreased growth of extant *P. digitalis* and locally extinct *P. pallidus* and *B. bracteata* when N was added (without N, no differences were observed) (nitrogen x herbivory: PD $\chi^2_{1,661}$=10.30, p=0.001; PP $\chi^2_{1,354}$=8.10, p=0.004; BB $\chi^2_{1,214}$=4.69, p=0.03; Table S4.5; Fig. 4.2B).

Nitrogen increased flower production by 66.67 ± 5.08% (N: $\chi^2_{1,114}$ =4.74, p=0.03; Table S4.2; Fig. 4.4A). Tall *Pycnanthemum tenuifolium* individuals produced more flowers under

nitrogen addition (height x N: $\chi^2_{1,57}$=4.86, p=0.03; Table S4.5; Fig. S4.4B). In the nitrogen gradient, *P. tenuifolium, P. digitalis*, and *P. pallidus* exhibited non-linear responses to nitrogen in terms of reproductive effort, such that flower production increased with increasing N until ca. 8 g N m^{-2} (N^2: PT $\chi^2_{1,51}$=3.75, p=0.05; PD $\chi^2_{1,44}$=15.40, p<0.0001; PP $\chi^2_{1,24}$=13.54, p<0.0001; N all p<0.08; Table S4.5; Fig. S4.3D).

Nitrogen increased total seed production (seeds per plant) (N $\chi^2_{1,85}$=2.17, p=0.1; Table S4.2; Fig. 4.4B). Extant species produced 4.32 times more seeds per fruit than locally extinct species (status $\chi^2_{1,82}$=15.98, p<0.0001) (extant = 54.4 ± 15.15 vs. locally extinct = 12.6 ± 4.05 seeds). However, because locally extinct species tended to produce approximately twice as many flowers as extant species, extant species tended to produce only 2.32 times more seeds per plant than locally extinct species (total seed production: status $\chi^2_{1,85}$=2.13, p=0.1) (extant = 789 ± 440 vs. locally extinct = 332 ± 213 seeds). Individually, only *Monarda fistulosa* seed production per fruit responded to nitrogen and herbivory: When deer were present and nitrogen was added, seed production increased with height but stayed consistent regardless of height under control conditions. When deer were absent, seed production was again consistent across plant height under control conditions, but nitrogen addition caused seed production to decrease in taller individuals (height x N x herbivory $\chi^2_{1,43}$=5.44, p=0.02; Fig. S4.5). Total seed production did not differ across treatments, with one exception. Tall *P. tenuifolium* produced more seeds per plant under nitrogen addition, likely due to its increased flower production (height x N $\chi^2_{1,42}$=4.91, p=0.03). In the nitrogen gradient, extant species produced 2.52 times more seeds per fruit than their locally extinct congeners (status $\chi^2_{1,131}$=5.68, p=0.02; Fig. 4.3E). Total seed production increased with increasing N before decreasing at higher levels of nitrogen; this pattern was more pronounced in locally extinct species (N^2 x status $\chi^2_{1,131}$=70.8, p<0.0001; Table S4.3; Fig. 4.3F).

Figure 4.2. Nitrogen reduces survival and increases growth, but locally extinct and extant species vary in the direction and magnitude of their responses to deer herbivory. Effect of nitrogen, deer presence, and their combination on (A) survival and (B) growth of locally extinct and extant species. Dot size represents the effect size of each treatment. Blue and orange circles indicate positive and negative responses, respectively. Species above the black dashed line are locally extinct; species below the line are extant.

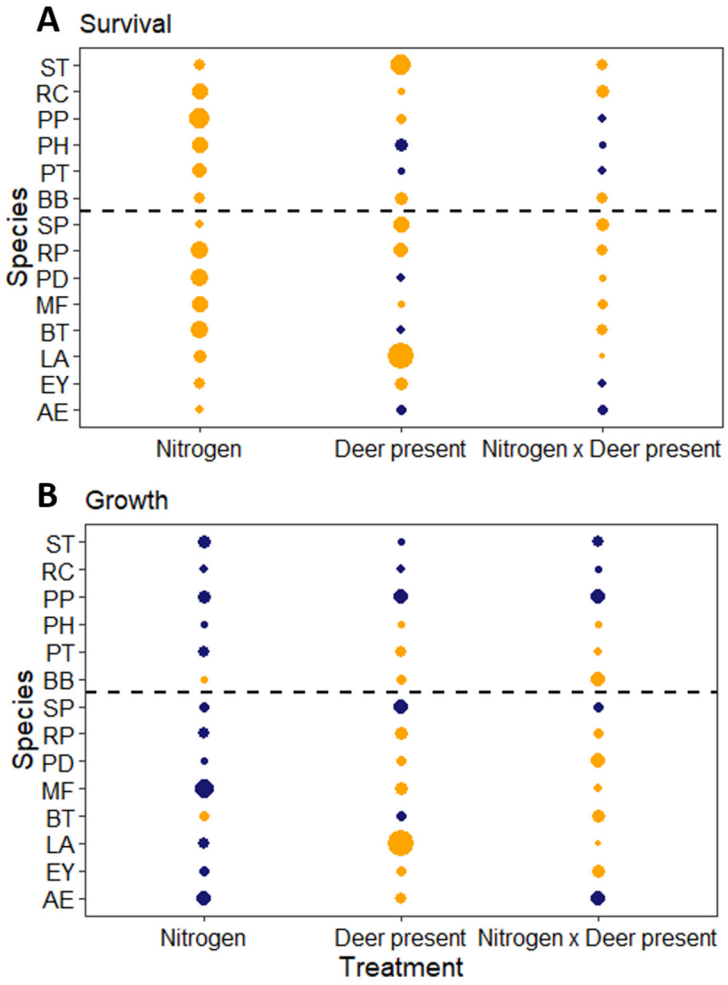


Figure 4.3. Nitrogen decreases survival as well as flower and seed production. Effect of a gradient of nitrogen addition (0-12 g N m^{-2} yr^{-1}) on (A) probability of survival (Pr[survival], %) from year *t* to *t+1*, (B) growth from year *t* to *t+1* (cm), (C) probability of flowering (Pr[flowering], %) in year *t+1*, (D) reproductive effort in year *t+1* (number of flowers produced), (E) reproductive output in year *t+1* (number of seeds per fruit), and (F) reproductive output in year *t+1* (total number of seeds produced per plant) in extant (left) vs. locally extinct (right) species. Dots are jittered values. Lines show linear (B,C,E,F) or quadratic fit (A,D) (Table S4.3). Shaded areas represent 95% confidence intervals.

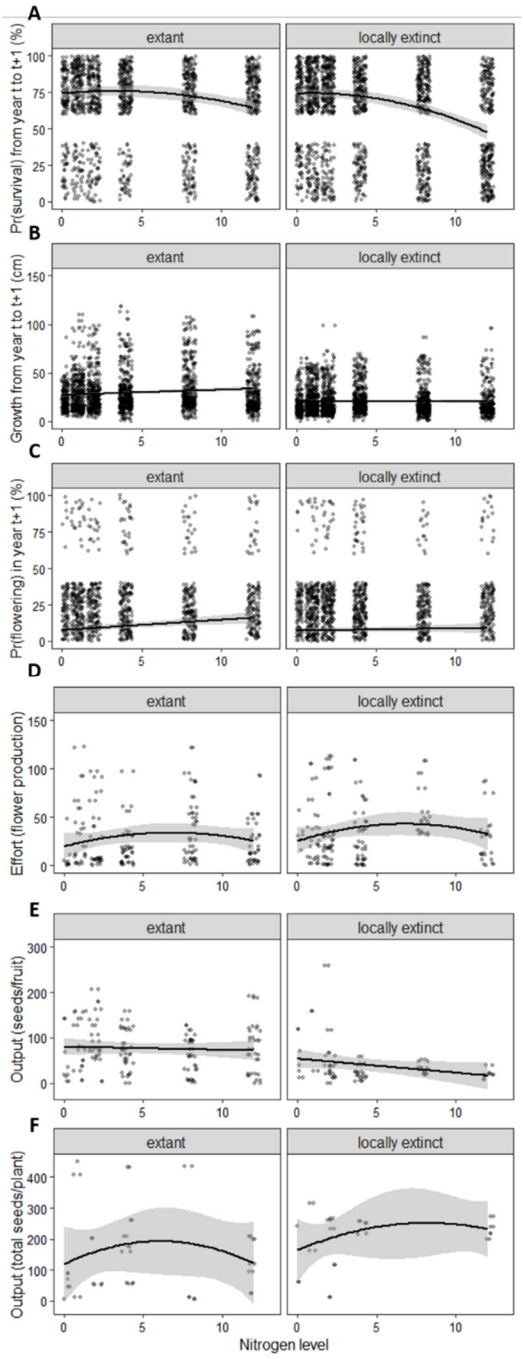
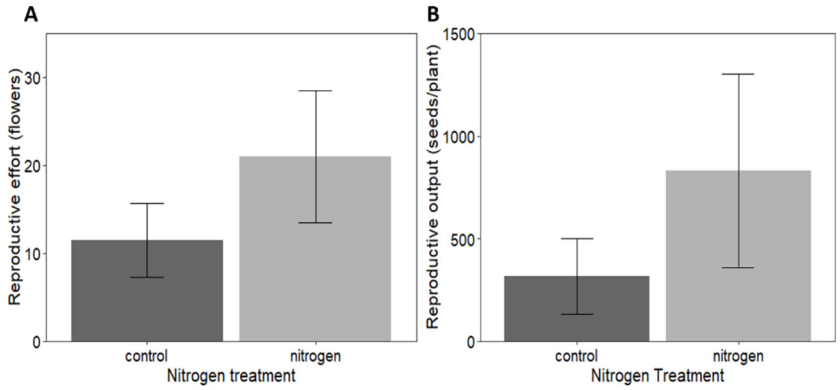


Figure 4.4. Nitrogen addition increases (A) reproductive effort (number of flowers produced) and (B) output (total seed production; estimated as seeds/fruit x number of flowers) (least square means ± standard error).



Population modeling and Life Table Response Experiment

Averaged across all species, nitrogen addition reduced population growth rates by 65% (N $\chi^2_{1,20}$=8.21, p=0.004; $\lambda_{control}$=0.40 ± 0.06 vs. $\lambda_{nitrogen}$=0.26 ± 0.06; Table S4.6; Fig. 4.5A). This effect was independent of status (p>0.05). Reduced survival under nitrogen addition contributed to differences in λ (Δλ) between nitrogen ("N" in superscripts) and control ("C"). In the *Penstemon,* reduced survival under nitrogen addition accounted for more than half of $\Delta\lambda^{N-C}$ (i.e., differences in λ between nitrogen and control conditions) (LTRE; Table S4.7; Fig. 4.6A). Plant growth from year *t* to *t+1* was much lower under nitrogen addition in three out of five species (i.e., growth contributed negatively to $\Delta\lambda^{N-C}$ and had a large contribution to lower λ under nitrogen addition in those species) (Fig. 4.6B). Overall, reproductive vital rates contributed relatively little to $\Delta\lambda^{N-C}$, with one exception. Nitrogen addition benefited flower production in *P. tenuifolium*, resulting in a benefit to λ (although this small benefit did not outweigh the negative contributions of growth and survival) (Fig. 4.6C-E). In the nitrogen gradient, results were qualitatively similar such that population growth rates decreased with increasing nitrogen levels (but this trend was not significant) (Fig. 4.5B; Table S4.6).

Qualitatively, herbivory (deer presence) decreased λ in three of the five species studied (Table S4.7). In the Lamiaceae, lower λ with deer present was largely driven by lower growth from year *t* to *t+1*, followed by reduced flowering and fruit production (output) under herbivory (Fig. 4.6). In the *Penstemon,* different vital rates contributed most to lower λ with deer present. Herbivory decreased λ in *P. hirsutus* largely by decreasing survival. In *P. digitalis,* reduced probability of flowering contributed most to decreases in λ when deer were present. In contrast, herbivory had positive effects on survival, growth, and probability of flowering in *P. pallidus* (Fig. 4.6C).

λ was lower in all species when nitrogen was added and deer were absent ("ND"; Table S4.7). Survival and growth negatively contributed to $\Delta\lambda^{ND-C}$ in all species (Fig. 4.6A-B). Contributions of reproductive vital rates to $\Delta\lambda^{ND-C}$ varied across species (Table S4.7; Fig. 4.6C-E).

Figure 4.5. Nitrogen addition reduces and deer absence increases population growth rates. Population growth rates (λ) for 1 pair (Lamiaceae) and 1 triplet (*Penstemon)* of extant vs. locally extinct (indicated by *) species under (A) a nitrogen x herbivory experiment and (B) a nitrogen gradient experiment. In (B) λ is calculated across a nitrogen gradient (0-12 g N m^{-2} yr-1). Note that λ estimates for (B) are from only two years of demographic data, so results should be interpreted with caution. Grey areas represent 95% confidence intervals. Because these were small experimental populations, I discuss differences in λ between treatments, not absolute values of λ.

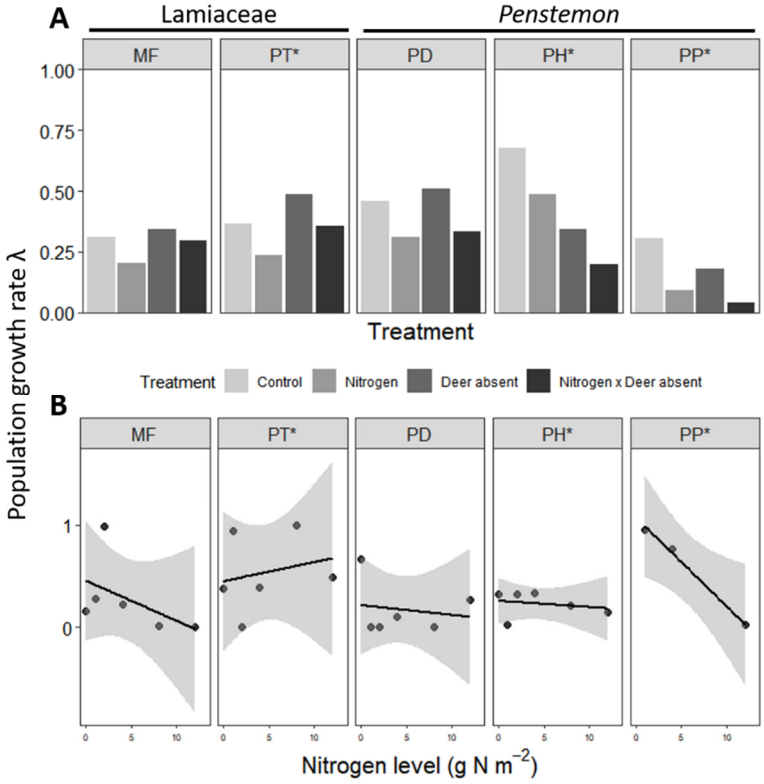
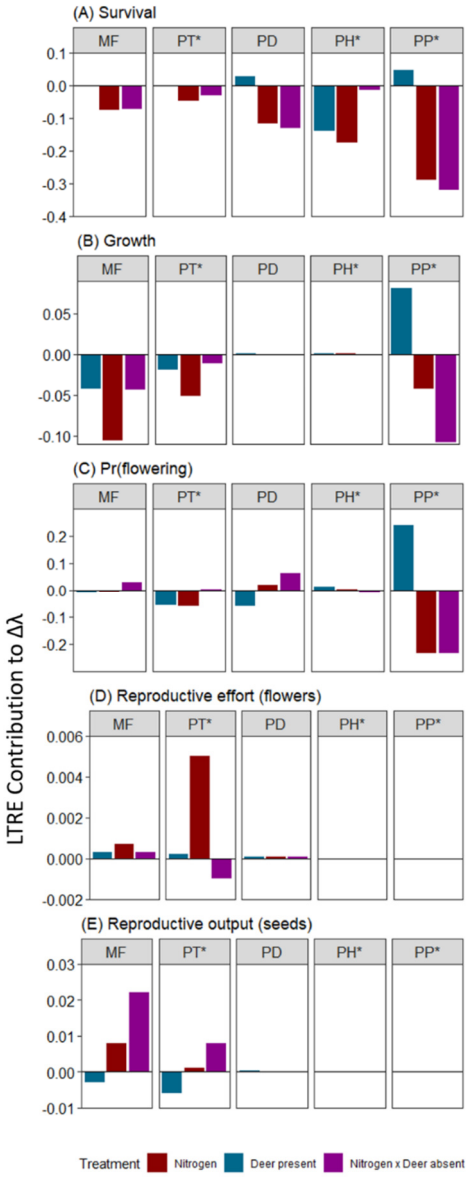


Figure 4.6. Life Table Response Experiment (LTRE) contributions of each vital rate (A: survival; B: growth; C: probability of flowering; D: reproductive effort [flower production]; E: reproductive output [seed production]) to differences in population growth rates λ (Δλ) between control conditions (no nitrogen, deer present) and treatments: (1) deer present (blue), (2) nitrogen addition (red), and (3) nitrogen addition x deer absent (purple). The LTRE contribution of each vital rate α_{ij} to Δλ is estimated as the difference in α_{ij} between the treatment and control multiplied by the sensitivity of λ to changes in α_{ij} (here, a perturbation of 0.01). A negative LTRE contribution indicates that the value of those matrix elements is lower in the treatment than the control. Note that this figure presents deer presence instead of absence (i.e., the inverse of the LTRE contribution for deer absence). C-E do not include species for which I used a mean estimate for vital rates (Table S4.5). Table S4.7 provides values of all LTRE contributions. Asterisks indicate locally extinct species.



Trait-demography relationships

Locally extinct and extant species differed in their mean trait values (MANOVA: status $F_{1,12}$=4.49, p=0.02; Table S4.8). Extinct species had lower specific leaf area (thicker leaves), flowered earlier, and had lower leaf nitrogen content than their extant congeners (status: SLA $\chi^2_{1,1913}$=7.26, p=0.007; flowering time $\chi^2_{1,12}$=3.63, p=0.08; leaf N $\chi^2_{1,230}$=2.09, p=0.1).

Species mean traits were generally correlated with vital rates (although most relationships were non-significant, likely due to the limited power of this dataset to examine relationships between species mean traits and vital rates) (Fig. S4.6). Species with thinner leaves (higher SLA) had significantly higher growth compared to those with thicker leaves, especially in treatments where deer were absent (deer absent: r=0.56; deer absent x nitrogen: r=0.57; both p<0.05; Fig. S4.6). When nitrogen was added, species with later flowering dates and higher leaf nitrogen content (i.e., extant species), tended to demonstrate increased growth relative to early-flowering species and those with lower nitrogen content (flowering time: r=0.48, p=0.08; leaf N: r=0.45, p=0.1). Although non-significant, higher SLA, earlier flowering, and higher leaf nitrogen content tended to correlate with higher probabilities of survival (SLA: r=0.25, p=0.38; flowering date: r=-0.17, p=0.56; leaf N: r=-0.21, p=0.48; Fig. S4.6).

DISCUSSION

I assessed the role of two common hypothesized drivers of biodiversity loss, nitrogen addition and deer herbivory, on the population demography of locally extinct and extant native species found in Michigan prairies. Nitrogen significantly decreased survival, particularly in locally extinct species. Extant species' growth also benefited more from nitrogen addition than locally extinct species. These results indicate that locally extinct and extant taxa differ in their

vital rate responses to nitrogen addition, with nitrogen providing less detriment (or more of a benefit in terms of growth) to extant species. However, Integral Projection Models revealed lower population growth rates under nitrogen addition, mostly driven by reductions in survival, across species regardless of status. This suggests that increasing nitrogen levels influence population declines in native prairie forb species. Herbivory decreased population growth rates, although herbivory effects varied across species. Finally, locally extinct species produced fewer seeds on average than extant species, suggesting a general demographic difference between those species at risk of extirpation and those able to persist. Below, I discuss (i) how this study provides a novel link between species loss under nitrogen addition in grasslands and the population-level processes that cause those losses and (ii) how these results highlight the increasingly recognized need to examine cumulative vital rates across a plant's life cycle to understand processes of population decline in response to anthropogenic change. Lastly, I outline the trait-demography relationships observed here, which point to the potential for plant traits to inform models of biodiversity loss.

Linking community-level species losses to population processes

This study links the commonly observed pattern of species loss under nitrogen addition to the demographic responses causing those declines, connecting community responses to finer-scale demographic processes. Although nitrogen increased plant growth, as commonly found in other studies (Seastedt et al., 1991; Collins et al., 1998), benefits to growth were not enough to overcome nitrogen's negative effects on survival. In the nitrogen x herbivory experiment, all fourteen species show reduced survivorship under nitrogen addition. In the nitrogen gradient experiment, survival increased until ca. 6 g N m^{-2} before survivorship declined (although non-significantly) at the highest levels of nitrogen addition. Furthermore, LTRE analyses indicate that

reduced survival and growth contributed to significantly lower population growth rates under nitrogen addition relative to control plots. This result reflects patterns of species losses in a nitrogen gradient spanning North America (Clark et al., 2007). In that experiment, species losses (mostly of short, locally rare species) followed a nitrogen-induced increase in total community productivity (Aerts and Chapin 2000; Craine et al., 2002; Suding et al., 2005; Clark et al. 2007, 2013; Simkin et al., 2016). In our experiment, background vegetation biomass increased (mostly *Andropogon gerardii, Sorghastrum nutans,* and *Solidago canadensis*) and light availability decreased with N addition (Fig. A1). We therefore hypothesize that competition with surrounding vegetation likely indirectly influences survival rates (Bobbink et al., 2010; Bobbink and Hicks, 2014; Borer et al., 2014). Altogether, this study highlights the population-level processes that could result in biodiversity decline at the ecosystem level (Gotelli and Ellison, 2002; Merow et al., 2014).

Locally extinct and extant species differed in several demographic responses to anthropogenic change. They also demonstrated differences in overall vital rates consistent with differences in fitness between more rare or threatened species vs. more common ones (van Kleunen and Richardson, 2007). First, nitrogen decreased survival more and benefited growth less in locally extinct species than in extant species, potentially implicating nitrogen addition in the local extinction of these prairie forbs. Second, locally extinct species produced fewer seeds, on average, than their closely-related congeners. Similarly, two rare species of mariposa lilies (*Calochortus obispoentis* and *C. tiburonensis*) had lower seed production than a common congener, *C. albus* (Fiedler, 1987). Such a difference in reproductive output could provide an indicator of at-risk species to target via management, although more information on how reproduction contributes to differences in lambda is needed. As the locally extinct species

studied here are likely rare or declining elsewhere in their range, these differences in vital rates and demographic responses to anthropogenic factors might aid in conservation and management to help mitigate their loss elsewhere.

Differing vital rates responses across life stages

The prairie species studied here all demonstrated contrasting responses to nitrogen and herbivory across their life cycles. For instance, vital rate models showed that nitrogen increased plant growth. However, nitrogen decreased λ overall by reducing survival across all species, an effect we would not have detected by measuring biomass or seed production alone (although these two metrics are commonly used as estimates of plant fitness and responses to nitrogen). This result highlights the need to examine differing responses to anthropogenic change across a plant's entire life cycle, as analyses of targeted vital rates might obscure important responses to that change that ultimately influence population decline.

Also demonstrating contrasting effects across the life cycle, herbivory reduced survival in nine of the fourteen prairie species studied here but had variable effects on growth and reproduction. In LTRE analyses, herbivory reduced λ in the Lamiaceae by decreasing growth, probability of flowering, and fruit production. This may be because deer consume flowering stems more often than non-flowering ones, and consumption of flowering stems can have a more negative effect on population growth than foliage consumption (Garcia and Ehlrén, 2002; Flaherty et al., 2017). In contrast, herbivory benefitted growth and survival in several *Penstemon.* Anecdotally, populations of dominant *Solidago canadensis* (Canada goldenrod) and *Cirsium arvense* (Canada thistle) were less dense in plots where deer were able to browse, so deer browsing may increase the amount of light reaching shorter seedlings. Altogether, herbivory qualitatively decreased population growth rates in three of the five species examined here. Deer

herbivory similarly decreases population growth rates in forbs such as *Trillium grandifolium, Polemonium vanbruntaie,* and *Eurybia furcata* (Knight et al., 2009; Bermingham, 2010; Bernardo et al., 2018). We also note that plots were fenced but not trenched, allowing small mammals to enter and browse, so herbivory from other species observed in these prairies (e.g., field mice, voles) was ubiquitous across the experiment. We included an estimate of granivory from a seed predation experiment in our IPMs to account for some damage from small mammals and arthropods, and ongoing work is investigating whether patterns of seed predation and herbivory differ between locally extinct vs. extant species.

Trait-demography relationships

The locally extinct and extant species studied here differed in their traits: extinct species had thicker leaves (lower SLA), flowered earlier, and had lower leaf nitrogen content than extant species. Similarly, locally extinct species in Australia had thick leaves relative to extant species (Kyle and Leishman, 2009). This suggests that differences in plants traits might influence population declines. In terms of trait-demography relationships, SLA correlated with increased plant growth, as plants with thinner leaves (here, extant species) had higher growth than those with thick leaves. This pattern corresponds with previous work on leaf traits demonstrating that species with thin leaves (high SLA) often have higher growth rates (Wright and Westoby, 2000; Falster et al., 2018), although this relationship can be weak (Paine et al., 2015). Additionally, later flowering and higher leaf nitrogen correlated with increased growth under nitrogen addition, consistent with previous studies finding that nitrogen addition selects for delayed phenology across species (Wang and Tang, 2019) and higher nitrogen content (Quétier et al., 2007). Finally, thinner leaves and earlier flowering tended to correlate with higher survival; these traits tend to be more common in widespread invasive species than natives (Grotkopp and

Rejmánek, 2007; Willis et al., 2010; Zettlemoyer et al., 2019b). Here, I used species mean trait values; future work should investigate whether intraspecific trait variation influences demographic rates or, ultimately, lambda.

Conclusions

Using IPMs to compare vital and population growth rates of closely related locally extinct vs. extant species under anthropogenic change provides a framework for two useful comparisons. First, we can examine drivers of contemporary, local extinction events. Modeling the demographic processes that led to decline, particularly in response to multiple and interacting anthropogenic changes, permits more mechanistic explanations of species losses (Merow et al., 2014) and will inform predictions of extinction risk for today's threatened species. Second, by monitoring the population demography of reintroduced, recently extinct species, we can assess demographic differences between more "successful" (i.e., still extant) and extinct species. Together, such models can improve our ability to project population risk of these species in other locations and of other species experiencing similar environmental conditions.

ACKNOWLEDGEMENTS

I thank C. Andrews, B. Canavan, K. Cortijo-Robles, S. Johnson, K. Renaldi, and N. Srodes for help with field work, M. Hammond, T. Cook, R. Logan, S. Magnoli, S. Boshnoyak, R. Ranjan, and R. Robertson for help setting up fencing, and numerous volunteers for help with lab work. The Kalamazoo Nature Center and Boudeman Conservation Farm (Woody Boudeman and Kevin Louden) provided field sites. J.A. Lau and the Lau lab provided valuable feedback on this manuscript. Support for this work was provided by the W.K. Kellogg Biological Station, the Hanes Foundation, and the Michigan Botanical Foundation.

APPENDIX

Table S4.1. Vital rates across sites. Results of generalized linear mixed models for (A) survival (binomial distribution), (B) growth (Gaussian distribution), (C) probability of flowering (Pr[flower]; binomial distribution), (D) reproductive effort (number of flowers produced; Poisson distribution), and (E) reproductive output (Poisson distribution) from the nitrogen x herbivory experiment, including both sites. Reproductive output is estimated as seeds/fruit (E.1) and seeds/plant (seeds/fruit x effort) (E.2). Status (locally extinct vs. extant), herbivory (deer present vs. absent), nitrogen (control vs. addition [10 g N m^{-2} yr^{-1}]), site (BCF vs. KHP), and all their interactions were included as predictor variables. Species (nested in status), subplot (nested in plot and site), and the interaction of height x species were included as random factors. Residual degrees of freedom (df) are provided in parentheses. ***p<0.0001, **p<0.01, *p<0.5, ·p<0.1.

Source	**df**	**(A) Survival χ^2**	**(B) Growth χ^2**	**(C) Pr(flower) χ^2**	**(D) Effort χ^2**	**(E.1) Output (per fruit) χ^2**	**(E.1) Output (per plant) χ^2**
Nitrogen	1	36.79 ***	9.63 **	0.05	2.14 ·	6.17 *	0.01
Herbivory	1	0.02	1.35	0.42	0.10	1.05	1.62
Status	1	0.00	0.55	0.11	0.90	0.03	0.27
Site	1	32.86 ***	0.89	0.17	0.21	0.11	3.95 *
Nitrogen x herbivory	1	0.40	0.22	0.40	1.51	2.61	0.09
Nitrogen x status	1	0.05	1.19	0.16	0.56	0.26	7.40 **
Herbivory x status	1	0.02	0.08	0.21	1.66	0.18	0.28
Nitrogen x site	1	1.43	3.41 ·	0.55	0.05	6.41 *	3.02 ·
Herbivory x site	1	0.02	1.96	0.98	0.02	0.01	0.46
Status x site	1	14.62 ***	1.99	2.80 ·	1.70	2.15	0.38
Nitrogen x herbivory x status	1	1.71	0.00	0.58	0.00	1.10	0.20
Nitrogen x herbivory x site	1	1.89	0.91	3.08 ·	0.17	0.57	0.15
Nitrogen x status x site	1	0.37	1.31	1.56	0.14	4.27 *	4.85 *
Herbivory x status x site	1	0.01	0.06	1.77	1.64	0.29	0.00
Nitrogen x herbivory x status x site	1	0.46	0.57	0.62	0.04	0.48	0.38
Residual		0.19	$1.05e^{+02}$	$8.67e^{-03}$	0.00	0.00	0.00
(df)		(8717)	(4387)	(4367)	(151)	(115)	(115)
Height x species		$7.33e^{-02}$	$1.51e^{+02}$	$4.95e^{-02}$	$1.26e^{+00}$	$4.11e^{+00}$	$4.13e^{+00}$
Species		$8.48e^{-01}$	$2.32e^{+01}$	$4.85e^{-03}$	$2.82e^{-01}$	$1.10e^{+00}$	$1.05e^{+00}$
(status)		($4.45e^{-10}$)	($2.45e^{-05}$)	($1.52e^{-06}$)	($2.64e^{-06}$)	($2.30e^{-05}$)	($4.31e^{-06}$)
Subplot		$1.22e^{-02}$	$5.62e^{-06}$	$6.91e^{-12}$	$7.49e^{-02}$	$3.15e^{-05}$	$7.95e^{-07}$
(plot:site)		($4.31e^{-02}$)	($1.20e^{+00}$)	($6.75e^{-05}$)	($2.34e^{-01}$)	($1.33e^{+00}$)	($1.27e^{+00}$)
(site)		(0.00)	($4.57e^{-08}$)	($2.78e^{-04}$)	($4.55e^{-06}$)	($3.02e^{-05}$)	($9.90e^{-10}$)

Table S4.2. Effects of nitrogen x herbivory on vital rates. Results of generalized linear mixed models for (A) survival (binomial distribution), (B) growth (Gaussian distribution), (C) probability of flowering (Pr[flower]; binomial distribution), (D) reproductive effort (number of flowers produced; Poisson distribution), (E) reproductive output (Poisson distribution), and (F) recruitment (proportion of seeds germinated; Gaussian distribution) from the nitrogen x herbivory experiment (Boudeman Conservation Farm only). Reproductive output is estimated as seeds/fruit (E.1) and seeds/plant (seeds/fruit x effort) (E.2). Status (locally extinct vs. extant), herbivory (deer present vs. absent), nitrogen (control vs. addition [10 g N m^{-2} yr^{-1}]), and their interactions were included as predictor variables. Species (nested in status), subplot (nested in plot), and the interaction of height x species were included as random factors. Residual degrees of freedom (df) are provided in parentheses. The lower table provides output from the initial MANOVA, which examined all vital rates (as a single multivariate response variable) as a function of nitrogen, herbivory, status, and their interactions. ***p<0.0001, **p<0.01, *p<0.5, ·p<0.1.

Source	**df**	**(A) Survival χ^2**	**(B) Growth χ^2**	**(C) Pr(flower) χ^2**	**(D) Effort χ^2**	**(E.1) Output (per fruit) χ^2**	**(E.2) Output (per plant) χ^2**	**(F) Recruit-ment χ^2**
Nitrogen	1	103.00 ***	15.13 ***	2.35	4.74 *	0.01	4.01 *	0.08
Herbivory	1	0.02	0.84	0.15	0.73	0.16	0.09	0.08
Status	1	0.09	0.99	0.15	1.48	15.98 ***	2.13 ·	0.12
Nitrogen x herbivory	1	0.20	0.05	0.75	2.82	0.54	0.69	1.97
Nitrogen x status	1	3.69 *	5.33 *	0.02	0.46	0.10	1.66	0.60
Herbivory x status	1	2.32	0.02	0.15	0.97	0.48	1.67	1.01
Nitrogen x herbivory x status	1	1.64	0.01	0.30	0.40	0.15	0.23	0.21
Residual (df)		0.00 (4919)	$2.32e^{+02}$ (2830)	0.02 (1553)	0.00 (158)	0.00 (82)	0.00 (82)	$1.73e^{-03}$ (339)
Species (status)		$7.58e^{-01}$ ($2.33e^{-10}$)	$1.97e^{+01}$ ($1.60e^{-05}$)	0.005 (0.03)	$3.59e^{-01}$ ($3.61e^{-09}$)	$7.58e^{-07}$ ($1.06e^{-06}$)	$1.02e^{-01}$ ($4.68e^{-08}$)	$2.63e^{-03}$ ($2.04e^{-05}$)
Subplot (plot)		$2.04e^{-02}$ ($6.80e^{-02}$)	$1.40e^{-06}$ ($1.60e^{-05}$)	0.00 ($3.73e^{-04}$)	$1.12e^{-01}$ ($3.94e^{-01}$)	$1.18e^{-04}$ ($5.01e^{-01}$)	$4.42e^{-07}$ ($1.45e^{+00}$)	$5.74e^{-05}$ (0.00)
Height x species		$1.75e^{+02}$	0.03	0.08	$1.26e^{+00}$	$1.10e^{+00}$	$3.31e^{+00}$	-

MANOVA	**df**	**F**
Nitrogen	1	3.72 **
Herbivory		0.74
Status	1	13.70 ***
Nitrogen x herbivory	1	1.37
Nitrogen x status	1	1.48
Herbivory x status	1	0.16
Nitrogen x herbivory x status	1	0.62
Residuals	149	

Table S4.3. Effects of a nitrogen gradient on vital rates. Results of generalized linear mixed models for (A) survival (binomial distribution), (B) growth (Gaussian distribution), (C) probability of flowering (Pr[flower]; binomial distribution), (D) reproductive effort (number of flowers produced; Poisson distribution), and (E) reproductive output (Poisson distribution) from the nitrogen gradient experiment. Reproductive output is estimated as seeds/fruit (E.1) and seeds/plant (seeds/fruit x effort) (E.2). Nitrogen (continuous: 0-12 g N m^{-2} yr^{-1}), status (locally extinct vs. extant), nitrogen^2, and their two-way interactions were included as predictor variables. Species (nested in status), plot, and the interaction of height x species were included as random factors. Residual degrees of freedom (df) are provided in parentheses. The lower table provides output from the initial MANOVA, which examined all the vital rates (as a single multivariate response variable) as a function of nitrogen, nitrogen^2, status, and their interactions. ***p<0.0001, **p<0.01, *p<0.5, ·p<0.1.

Source	**df**	**(A) Survival χ^2**	**(B) Growth χ^2**	**(C) Pr(flower) χ^2**	**(D) Effort χ^2**	**(E.1) Output (per fruit) χ^2**	**(E.2) Output (per plant) χ^2**
Nitrogen	1	0.35	6.95 **	2.45	2.06	4.85 *	0.23
Status	1	0.18	0.29	0.32	0.11	5.68 *	13.96 ***
Nitrogen^2	1	2.28	5.82 *	1.64	0.83	2.54	0.05
Nitrogen x status	1	0.59	5.69 *	0.92	1.19	2.37	209.30 ***
Nitrogen^2 x status	1	0.26	2.58	0.34	0.74	1.26	80.80 ***
Residual (df)		0.00 (2877)	183.33 (2236)	0.00 (2230)	0.00 (201)	0.00 (131)	0.00 (131)
Species (status)		$3.04e^{+00}$ ($1.99e^{-08}$)	93.68 (0.00)	$2.35e^{+00}$ ($1.32e^{-05}$)	$1.48e^{+00}$ ($2.66e^{-07}$)	$2.36e^{-01}$ ($7.13e^{-10}$)	$1.25e^{+00}$ ($7.36e^{-07}$)
Plot		$2.56e^{-01}$	6.82	$3.68e^{-01}$	$2.11e^{-01}$	$3.60e^{-01}$	$3.42e^{+00}$
Height x species		$2.94e^{-02}$	70.13	$1.06e^{+00}$	$4.89e^{-01}$	$1.31e^{+00}$	$5.46e^{+00}$

MANOVA	**df**	**F**
Nitrogen	1	2.12 ·
Status	1	12.67 ***
Nitrogen^2	1	0.05
Nitrogen x status	1	0.95
Nitrogen^2 x status	1	1.14
Residuals	119	

Table S4.4. Species-specific vital rates in the nitrogen x herbivory and nitrogen gradient experiments (without height as a covariate). Results of generalized linear mixed models for (A) survival (binomial distribution), (B) growth (Gaussian distribution), (C) probability of flowering (Pr[flower]; binomial distribution), (D) reproductive effort (number of flowers produced; Poisson distribution), and (E) reproductive output (Poisson distribution) for each species (lettered tables, with plant family provided in parentheses; asterisks indicates a locally extinct species). Reproductive output is estimated as seeds/fruit (E.1) and seeds/plant (seeds/fruit x number of flowers) (E.2). (F) Recruitment (proportion of seeds germinated) did not vary across treatments, status, or species, so I provide mean germination for each species. (1) Results from the nitrogen x herbivory experiment. Nitrogen (control vs. addition [10 g N m^{-2} yr^{-1}]), herbivory (deer present vs. absent), and their interaction were included as predictor variables. Site (BCP vs. KHP) was included as a fixed effect, and subplot (nested in plot and site) was included as a random factor. (2) Results from the nitrogen gradient experiment. Nitrogen (continuous: 0-12 g N m^{-2} yr^{-1}) and nitrogen^2 were included as predictor variables, and plot was included as a random factor. Species that were not included in the nitrogen gradient experiment are marked as "N/A". "Non-est" indicates that there was not sufficient data to fit a model. When possible, I provide the mean (μ) value used in Integral Projection Models (if no mean is provided, no plants flowered). ***p<0.0001, **p<0.01, *p<0.5, ·p<0.1.

Source	df	(A) Survival χ^2	(B) Growth χ^2	(C) Pr(flower) χ^2	(D) Effort χ^2	(E.1) Output (per fruit) χ^2	(E.2) Output (per plant) χ^2	(F) Recruitment χ^2
***(A) Aster ericoides* (Asteraceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	14.31 ***	3.87 *	non-est	non-est	non-est	non-est	μ = 0.00%
Herbivory	1	0.65	0.80					
Site	1	23.60 ***	10.18 **					
Nitrogen x herbivory	1	0.37	3.33 ·					
Residual		0.00	$5.56e^{+01}$					
(df)		(1186)	(217)					
Subplot (plot, site)		$2.22e^{-01}$	3.24					
Plot (site)		$1.84e^{-01}$	0.00					
Site		$1.42e^{-09}$	0.00					
(2) Nitrogen gradient								
N/A								
***(B) Baptisia bracteata** (Fabaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	10.24 **	5.52 *	non-est	non-est	non-est	non-est	μ = 0.47%
Herbivory	1	0.24	2.18					
Site	1	8.27 **	0.01					
Nitrogen x herbivory	1	0.61	0.27					
Residual		0.00	$9.98e^{+01}$					
(df)		(1183)	(248)					
Subplot (plot, site)		0.11	$9.13e^{-06}$					
Plot (site)		0.00	$1.31e^{+00}$					
Site		0.00	$7.67e^{-07}$					
(2) Nitrogen gradient								
Nitrogen	1	0.86	6.50 *	non-est	non-est	non-est	non-est	
Nitrogen^2	1	1.39	4.67 *					
Residual (df)		0.00 (360)	25.31 (68)					
Plot		1.62	0.56					
***(C) Baptisia tinctoria* (Fabaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	28.37 ***	21.53 ***	non-est	non-est	non-est	non-est	μ = 0.33%
Herbivory	1	0.89	2.38					
Site	1	23.64 ***	0.17					
Nitrogen x herbivory	1	0.12	3.07					

Table S4.4. (cont'd)

Residual		0.00	89.51					
(df)		(1192)	(179)					
Subplot (plot, site)		0.00	0.00					
Plot (site)		$1.68e^{-01}$	0.00					
Site		$1.53e^{-10}$	0.00					
(2) Nitrogen gradient								
Nitrogen	1	0.04	1.51	non-est	non-est	non-est	non-est	
$Nitrogen^2$	1	0.14	0.46					
Residual (df)		0.00 (360)	7.26 (47)					
Plot		1.66	0.00					
***(D) Eryngium yuccifolium* (Apiaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	2.77 ·	1.35	non-est	non-est	non-est	non-est	μ = 0.05%
Herbivory	1	1.67	0.01					
Site	1	46.73 ***	36.92 ***					
Nitrogen x herbivory	1	0.87	0.26					
Residual		0.00	$2.70e^{+02}$					
(df)		(1181)	(340)					
Subplot (plot, site)		0.00	$2.16e^{+00}$					
Plot (site)		0.14	$2.05e^{+00}$					
Site		0.00	$3.56e^{-07}$					
(2) Nitrogen gradient								
N/A								
***(E) Liatris aspera* (Asteraceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	1.63	3.99 *	non-est	non-est	non-est	non-est	μ = 0.24%
Herbivory	1	2.22	1.35					
Nitrogen x herbivory	1	0.12	2.86					
Residual (df)		0.00 (474)	16.47 (19)					
Subplot (plot)		$5.24e^{-06}$	0.04					
Plot		$1.18e^{+00}$	0.00					
(2) Nitrogen gradient								
N/A								
***(F) Ratibida columnifera** (Asteraceae)**								
(1) Nitrogen x herbivory								
Nitrogen		17.46 ***	0.03	non-est	non-est	non-est	non-est	μ = 0.50%
Herbivory	1	2.62	2.19					
Site	1	11.11 ***	5.42 *					
Nitrogen x herbivory	1	0.05	0.20					
Residual		0.00	0.00					
(df)		(1175)	(117)					
Subplot (plot, site)		$1.68e^{-02}$	0.00					
Plot (site)		$2.56e^{-01}$	0.00					
Site		$1.15e^{-10}$	0.00					
(2) Nitrogen gradient								
Nitrogen	1	1.32	0.39	1.53	non-est	non-est	non-est	
$Nitrogen^2$	1	0.00	1.82	1.30	μ = 1.25	μ = 178		
Residual		0.00	97.07	0.00				
(df)		(360)	(263)	(262)				
Plot		0.23	0.06	0.00				
***(G) Ratibida pinnata* (Asteraceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	33.48 ***	4.32 *	non-est	non-est	non-est	non-est	μ = 2.05%
Herbivory	1	1.01	0.12	μ = 0.007	μ = 18	μ = 59		
Site	1	14.14 ***	13.76 ***					
Nitrogen x herbivory	1	2.87	0.02					
Residual		0.00	139.8					
(df)		(1183)	(304)					
Subplot (plot, site)		$9.81e^{-02}$	0.00					
Plot (site)		$6.04e^{-01}$	0.00					
Site		$1.59e^{-09}$	0.00					
(2) Nitrogen gradient								
Nitrogen	1	0.02	3.22 ·	non-est	non-est	non-est	non-est	
$Nitrogen^2$	1	0.08	3.80 *	μ = 0.003	μ = 11	μ = 142		

Table S4.4. (cont'd)

Residual		0.00	118.7					
(df)		(360)	(263)					
Plot		0.78	0.00					
(H) Monarda fistulosa **(Lamiaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	36.00 ***	20.85 ***	8.83 **	9.35 **	0.25	2.58 ·	μ = 15.6%
Herbivory	1	0.51	0.01	0.25	0.43	0.00	2.47	
Site	1	63.75 ***	2.12	0.16	0.12	0.02	0.08	
Nitrogen x herbivory	1	0.67	0.17	0.23	4.60 *	0.06	2.13	
Residual		0.00	$1.15e^{+03}$	0.00	0.00	7.07^{+03}	$1.26e^{+07}$	
(df)		(1065)	(580)	(575)	(86)	(74)	(74)	
Subplot (plot, site)		$9.59e^{-02}$	0.00	$0.00e^{+00}$	299.5	$3.09e^{+03}$	$1.85e^{+07}$	
Plot (site)		$2.15e^{-10}$	18.27	$4.25e^{-01}$	354.8	$7.03e^{+03}$	$3.23e^{+06}$	
Site		$0.00e^{+00}$	0.00	$2.28e^{-10}$	1816.0	$7.48e^{+03}$	$9.18e^{+06}$	
(2) Nitrogen gradient								
Nitrogen	1	0.21	8.54 **	1.86	9.24 **	0.66	1.88	
$Nitrogen^2$	1	0.74	5.56 *	0.81	5.78 *	0.77	1.43	
Residual		0.00	366.90	0.00	0.00	5900.3	$4.09e^{+06}$	
(df)		(360)	(335)	(335)	(62)	(43)	(43)	
Plot		0.72	24.97	0.06	0.22	961.5	0.00	
(I) Pycnanthemum tenuifolium* **(Lamiaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	21.37 ***	0.54	0.11	11.92 ***	0.63	8.28 **	μ = 10.85%
Herbivory	1	0.02	1.08	0.00	0.02	2.52	0.05	
Site	1	14.63 ***	10.60 ***	0.85	0.81	0.18	0.15	
Nitrogen x herbivory	1	0.31	0.24	0.05	4.55 *	0.20	0.45	
Residual		0.00	$3.86e^{+02}$	0.00	0.00	113.09	$3.62e^{+06}$	
(df)		(1188)	(671)	(669)	(63)	(48)	(48)	
Subplot (plot, site)		0.07	$0.00e^{+00}$	$1.12e^{-15}$	0.00	46.69	0.00	
Plot (site)		0.02	$1.22e^{+01}$	0.00	1087.0	42.82	0.00	
Site		0.00	$7.17e^{-06}$	0.00	7593.0	90.12	$4.58e^{+06}$	
(2) Nitrogen gradient								
Nitrogen	1	0.20	7.07 **	1.21	6.52 *	2.53	3.97	
$Nitrogen^2$	1	1.68	6.16 *	0.61	4.18 *	3.35 ·	4.34	
Residual		0.00	248.8	0.00	0.00	184.3	$3.50e^{+04}$	
(df)		(360)	(330)	(330)	(51)	(31)	(31)	
Plot		0.24	0.00	0.55	0.40	0.00	$7.96e^{+05}$	
(J) Penstemon digitalis **(Scrophulariaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	60.73 ***	1.76	1.09	0.42	1.14	1.29	μ = 11.05%
Herbivory	1	0.22	0.19	0.18	0.35	0.23	13.86 ***	
Site	1	9.28 **	4.7 *	8.16 **	0.00	0.04	0.02	
Nitrogen x herbivory	1	0.47	1.88	0.45	0.01	1.66	4.08 *	
Residual		0.00	345.56	0.00	$10.87e^{+02}$	$1.69e^{+03}$	$1.39e^{+07}$	
(df)		(1187)	(669)	(667)	(35)	(28)	(28)	
Subplot (plot, site)		0.06	2.80	$1.70e^{-07}$	365.16	0.00	0.00	
Plot (site)		0.20	10.12	$3.13e^{-01}$	0.00	$2.05e^{+03}$	0.00	
Site		0.00	0.00	$2.36e^{-06}$	1.15	$1.91e^{+03}$	$1.39e^{+07}$	
(2) Nitrogen gradient								
Nitrogen	1	1.00	6.38 *	19685.7 ***	0.45	0.26	1.36	
$Nitrogen^2$	1	2.53	5.12 *	165.5 ***	0.32	0.09	1.43	
Residual		0.00	600.26	0.00	0.00	938.7	$1.64e^{+07}$	
(df)		(360)	(337)	(336)	(44)	(43)	(43)	
Plot		0.28	11.78	0.33	0.11	402.8	$6.09e^{+04}$	
(K) Penstemon hirsutus* **(Scrophulariaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	14.77 ***	3.81 *	non-est	non-est	non-est	non-est	μ = 9.21%
Herbivory	1	0.39	0.22	μ = 0.01	μ = 34.2	μ = 76.67		
Site	1	6.25 *	0.48					
Nitrogen x herbivory	1	1.69	0.98					
Residual		0.00	74.84					
(df)		(1192)	(479)					
Subplot (plot, site)		$2.34e^{-01}$	0.81					
Plot (site)		0.00	0.00					

Table S4.4. (cont'd)

Site		$3.51e^{-10}$	0.00					
(2) Nitrogen gradient								
Nitrogen	1	0.05	2.10	1.94	0.01	non-est	non-est	
$Nitrogen^2$	1	0.66	2.38	1.97	0.04	$\mu = 6$		
Residual		0.00	56.74	0.00	0.00			
(df)		(360)	(290)	(290)	(7)			
Plot		1.06	6.07	$5.72e^{-14}$	0.40			
***(L) Penstemon pallidus** (Scrophulariaceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	50.31 ***	0.38	non-est	non-est	non-est	non-est	$\mu = 3.55\%$
Herbivory	1	0.00	0.24	$\mu = 0.01$	$\mu = 53.5$	$\mu = 52$		
Site	1	3.17 ·	0.00					
Nitrogen x herbivory	1	4.38 *	0.86					
Residual		0.00	$7.70e^{+01}$					
(df)		(1180)	(359)					
Subplot (plot, site)		0.11	$2.55e^{+00}$					
Plot (site)		0.13	$0.00e^{+00}$					
Site		0.00	$2.48e^{-04}$					
(2) Nitrogen gradient								
Nitrogen	1	0.39	0.03	0.13	4.43 *	non-est	non-est	
$Nitrogen^2$	1	0.02	0.28	0.97	1.71	$\mu = 40.1$		
Residual		0.00	166.18	0.00	0.00			
(df)		(360)	(268)	(267)	(24)			
Plot		0.38	6.10	0.00	0.07			
***(M) Silphium terebinthinaceum** (Asteraceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	0.70	0.59	non-est	non-est	non-est	non-est	$\mu = 0.05\%$
Herbivory	1	1.03	2.81					
Nitrogen x herbivory	1	0.04	3.06					
Residual (df)		0.00 (155)	87.65 (54)					
Subplot (plot)		$4.80e^{-01}$	0.00					
Plot		$1.70e^{-10}$	0.00					
(2) Nitrogen gradient								
N/A								
***(N) Silphium perfoliatum* (Asteraceae)**								
(1) Nitrogen x herbivory								
Nitrogen	1	5.30 *	1.10	non-est	non-est	non-est	non-est	$\mu = 0.1\%$
Herbivory	1	4.93 *	0.44					
Site	1	15.40 ***	4.01 *					
Nitrogen x herbivory	1	3.52 ·	1.41					
Residual		0.00	347.09					
(df)		(1185)	(369)					
Subplot (plot, site)		$2.29e^{-08}$	15.85					
Plot (site)		$1.25e^{+00}$	8.56					
Site		2.35^{-09}	0.00					
(2) Nitrogen gradient								
N/A								

Table S4.5. Species-specific, size-dependent vital rates in the nitrogen x herbivory and nitrogen gradient experiments. Results of generalized linear mixed models for (A) survival (binomial distribution), (B) growth (Gaussian distribution), (C) probability of flowering (Pr[flower]; binomial distribution), (D) reproductive effort (number of flowers produced; Poisson distribution), and (E) reproductive output (Poisson distribution) for each species (lettered tables, with plant family provided in parentheses; asterisks indicates a locally extinct species). Reproductive output is estimated as seeds/fruit (E.1) and seeds/plant (seeds/fruit x number of flowers) (E.2). (F) Recruitment (proportion of seeds germinated) did not vary across treatments, status, or species, so I provide mean germination for each species. (1) Results from the nitrogen x herbivory experiment. Height, nitrogen (control vs. addition [10 g N m^{-2} yr^{-1}]), herbivory (deer present vs. absent), and their interactions were included as predictor variables. Site (BCP vs. KHP) was included as a fixed effect, and subplot (nested in plot and site) was included as a random factor. (2) Results from the nitrogen gradient experiment. Height, nitrogen (continuous: 0-12 g N m^{-2} yr^{-1}), nitrogen^2, and interactions of height x N and height x N^2 were included as predictor variables, and plot was included as a random factor. Species that were not included in the nitrogen gradient experiment are marked as "N/A". "Non-est" indicates that there was not sufficient data to fit a model. When possible, I provide the mean (μ) value used in Integral Projection Models (if no mean is provided, no plants flowered). ***p<0.0001, **p<0.01, *p<0.5, ·p<0.1.

Source	df	(A) Survival χ^2	(B) Growth χ^2	(C) Pr(flower) χ^2	(D) Effort χ^2	(E.1) Output (per fruit) χ^2	(E.2) Output (per plant) χ^2	(F) Recruit-ment χ^2
***(A) Aster ericoides* (Asteraceae)**								
(1) Nitrogen x herbivory								
Height	1	4.35 *	8.45 **	non-est	non-est	non-est	non-est	μ = 0.00%
Nitrogen	1	0.03	3.63 *					
Herbivory	1	0.03	2.68					
Site	1	17.74 ***	12.78 ***					
Height x nitrogen	1	4.26 *	4.09 *					
Height x herbivory	1	0.07	13.64 ***					
Nitrogen x herbivory	1	0.20	2.02					
Height x nitrogen x herbivory	1	0.04	3.30 ·					
Residual (df)		0.00 (583)	97.85 (191)					
Subplot (plot, site)		$1.77e^{-01}$	0.00					
Plot (site)		$1.71e^{-01}$	0.00					
Site		$1.18e^{-07}$	0.00					
(2) Nitrogen gradient								
N/A								
***(B) Baptisia bracteata** (Fabaceae)**								
(1) Nitrogen x herbivory								
Height	1	0.78	5.34 *	non-est	non-est	non-est	non-est	μ = 0.47%
Nitrogen	1	4.16 *	3.18 ·					
Herbivory	1	0.19	3.51 ·					
Site	1	5.73 *	0.30					
Height x nitrogen	1	0.30	0.11					
Height x herbivory	1	0.20	3.13					
Nitrogen x herbivory	1	1.78	4.69 *					
Height x nitrogen x herbivory	1	1.68	4.10 *					
Residual (df)		0.00 (621)	77.85 (214)					
Subplot (plot, site)		$4.58e^{-02}$	0.00					
Plot (site)		$1.01e^{-06}$	0.00					
Site		0.00	0.00					
(2) Nitrogen gradient								
Height	1	5.71 *	5.45 *	non-est	non-est	non-est	non-est	

Table S4.5. (cont'd)

Nitrogen	1	0.02	4.99 *					
Nitrogen^2	1	0.06	7.18 **					
Height x nitrogen	1	5.03 *	4.76 *					
Height x nitrogen^2	1	3.59 ·	9.46 **					
Residual (df)		0.00 (211)	15.58 (43)					
Plot		3.67	0.00					
***(C) Baptisia tinctoria* (Fabaceae)**								
(1) Nitrogen x herbivory								
Height	1	0.01	2.32	non-est	non-est	non-est	non-est	μ = 0.33%
Nitrogen	1	3.31 *	0.14					
Herbivory	1	0.37	2.75					
Site	1	16.44 ***	0.08					
Height x nitrogen	1	0.39	1.39					
Height x herbivory	1	1.12	1.41					
Nitrogen x herbivory	1	0.87	0.81					
Height x nitrogen x herbivory	1	0.55	0.11					
Residual (df)		0.00 (564)	83.54 (154)					
Subplot (plot, site)		$7.56e^{-10}$	0.00					
Plot (site)		$9.55e^{-02}$	0.00					
Site		$3.89e^{-10}$	0.00					
(2) Nitrogen gradient								
Height	1	2.23	1.11	non-est	non-est	non-est	non-est	
Nitrogen	1	0.31	0.14					
Nitrogen^2	1	0.27	0.52					
Height x nitrogen	1	1.77	0.42					
Height x nitrogen^2	1	1.84	2.89 ·					
Residual (df)		0.00 (215)	5.98 (43)					
Plot		2.34	0.00					
***(D) Eryngium yuccifolium* (Apiaceae)**								
(1) Nitrogen x herbivory								
Height	1	2.78 ·	17.48 ***	non-est	non-est	non-est	non-est	μ = 0.05%
Nitrogen	1	0.49	3.69 *					
Herbivory	1	4.76 *	0.39					
Site	1	54.26 ***	23.32 ***					
Height x nitrogen	1	0.00	7.73 **					
Height x herbivory	1	3.89 *	0.80					
Nitrogen x herbivory	1	0.26	3.29 ·					
Height x nitrogen x herbivory	1	2.68	3.00					
Residual		0.00	141.30					
(df)		(705)	(331)					
Subplot (plot, site)		$8.91e^{-10}$	0.00					
Plot (site)		$4.24e^{-02}$	0.41					
Site		$2.87e^{-10}$	0.00					
(2) Nitrogen gradient								
N/A								
***(E) Liatris aspera* (Asteraceae)**								
(1) Nitrogen x herbivory								
Height	1	1.23	1.39	non-est	non-est	non-est	non-est	μ = 0.24%
Nitrogen	1	0.04	0.01					
Herbivory	1	2.44	1.85					
Height x nitrogen	1	0.44	0.01					
Height x herbivory	1	1.41	1.78					
Nitrogen x herbivory	1	non-est	non-est					
Height x nitrogen x herbivory	1	non-est	non-est					
Residual (df)		0.00 (178)	7.63 (19)					
Subplot (plot)		$7.83e^{-05}$	0.00					
Plot		$1.13e^{+00}$	0.00					
(2) Nitrogen gradient								
N/A								
***(F) Ratibida columnifera** (Asteraceae)**								
(1) Nitrogen x herbivory								
Height	1	0.00	0.53	non-est	non-est	non-est	non-est	μ = 0.50%
Nitrogen	1	5.22 *	0.01					

Table S4.5. (cont'd)

Herbivory	1	1.80	0.06					
Site	1	9.92 **	5.75 *					
Height x nitrogen	1	0.28	0.00					
Height x herbivory	1	0.18	0.90					
Nitrogen x herbivory	1	1.02	0.01					
Height x nitrogen x herbivory	1	0.81	0.01					
Residual		0.00	$2.26e^{+01}$					
(df)		(503)	(113)					
Subplot (plot, site)		$8.44e^{-08}$	$1.19e^{-01}$					
Plot (site)		$8.90e^{-02}$	$1.19e^{-06}$					
Site		$5.72e^{-09}$	$5.74e^{-07}$					
(2) Nitrogen gradient								
Height	1	2.21	3.73 *	non-est	non-est	non-est	non-est	
Nitrogen	1	0.48	0.39	$\mu = 0.05$	$\mu = 1.25$	$\mu = 178$		
$Nitrogen^2$	1	0.05	1.82					
Height x nitrogen	1	0.07	0.11					
Height x $nitrogen^2$	1	0.36	0.24					
Residual (df)		0.00 (347)	97.07 (263)					
Plot		0.23	0.06					
***(G) Ratibida pinnata* (Asteraceae)**								
(1) Nitrogen x herbivory								
Height	1	2.44	59.41 ***	non-est	non-est	non-est	non-est	$\mu = 2.05\%$
Nitrogen	1	23.92 ***	0.08	$\mu = 0.007$	$\mu = 18$	$\mu = 59$		
Herbivory	1	1.61	2.80 ·					
Site	1	17.07 ***	14.93 ***					
Height x nitrogen	1	3.97 *	0.08					
Height x herbivory	1	0.13	2.72 ·					
Nitrogen x herbivory	1	2.14	1.54					
Height x nitrogen x herbivory	1	0.38	0.62					
Residual (df)		0.00 (654)	66.59 (293)					
Subplot (plot, site)		$3.02e^{-08}$	0.00					
Plot (site)		$4.58e^{-01}$	0.00					
Site		$4.27e^{-10}$	0.00					
(2) Nitrogen gradient								
Height	1	0.17	22.29 ***	non-est	non-est	non-est	non-est	
Nitrogen	1	0.45	1.64	$\mu = 0.003$	$\mu = 11$	$\mu = 142$		
$Nitrogen^2$	1	0.69	1.58					
Height x nitrogen	1	0.74	1.37					
Height x $nitrogen^2$	1	0.75	1.28					
Residual		0.00	106.50					
(df)		(354)	(331)					
Plot		0.46	0.00					
***(H) Monarda fistulosa* (Lamiaceae)**								
(1) Nitrogen x herbivory								
Height	1	0.32	64.89 ***	14.50 ***	0.01	2.96 ·	0.92	$\mu = 15.6\%$
Nitrogen	1	22.90 ***	11.95 ***	0.15	1.18	7.40 **	0.48	
Herbivory	1	0.49	0.68	0.15	0.00	1.05	0.23	
Site	1	48.68 ***	5.77 *	0.59	0.00	0.28	0.00	
Height x nitrogen	1	1.81	2.98 ·	0.82	2.64	8.02 **	1.04	
Height x herbivory	1	0.05	2.61	0.02	0.00	1.26	0.46	
Nitrogen x herbivory	1	1.32	0.18	0.72	0.15	4.84 *	0.30	
Height x nitrogen x herbivory	1	0.12	0.02	1.14	0.38	5.44 *	0.58	
Residual		0.00	536.8	0.00	$1.38e^{+03}$	$4.99e^{+03}$	$4.80e^{+07}$	
(df)		(766)	(530)	(525)	(54)	(43)	(43)	
Subplot (plot, site)		$1.68e^{-14}$	0.00	$4.75e^{-05}$	$1.40e^{-05}$	0.00	$1.89e^{+06}$	
Plot (site)		0.00	0.00	$7.31e^{-01}$	$1.85e^{+01}$	$3.96e^{+03}$	$1.47e^{+06}$	
Site		0.00	0.00	$2.51e^{-05}$	$2.04e^{+02}$	$2.75e^{+03}$	$6.91e^{+06}$	
(2) Nitrogen gradient								
Height	1	0.32	60.17 ***	18.15 ***	3.26 ·	0.08	0.05	
Nitrogen	1	0.00	1.73	1.64	1.81	2.02	0.22	
$Nitrogen^2$	1	0.34	0.62	1.16	0.19	2.61	0.25	
Height x nitrogen	1	0.09	0.39	2.62	0.14	1.10	1.05	
Height x $nitrogen^2$	1	0.09	0.79	2.34	1.49	1.65	1.04	

Table S4.5. (cont'd)

Residual		0.00	175.12	0.00	0.00	6211.9	$4.18e^{+06}$	
(df)		(352)	(335)	(335)	(62)	(43)	(43)	
Plot		0.48	14.64	0.22	0.16	690.3	0.00	
(I) Pycnanthemum tenuifolium* **(Lamiaceae)**								
(1) Nitrogen x herbivory								
Height	1	2.91 ·	182.66 ***	16.80 ***	6.43 *	0.02	1.73	μ = 10.85%
Nitrogen	1	7.63 **	0.58	0.60	3.36 ·	0.09	3.76	
Herbivory	1	0.00	0.34	0.59	1.03	0.83	0.25	
Site	1	14.37 ***	2.77 ·	2.35	0.23	0.38	0.13	
Height x nitrogen	1	0.00	0.16	0.67	4.86 *	0.17	4.91 *	
Height x herbivory	1	0.12	0.20	0.77	1.57	0.66	0.24	
Nitrogen x herbivory	1	0.22	0.59	0.03	0.78	0.10	0.03	
Height x nitrogen x herbivory	1	0.91	2.64	0.04	1.30	0.08	0.06	
Residual (df)		0.00	$1.96e^{+02}$	0.00	$65.89e^{+02}$	51.90	$4.80e^{+07}$	
		(918)	(649)	(647)	(57)	(42)	(42)	
Subplot (plot, site)		$7.38e^{-07}$	$3.29e^{-08}$	0.00	0.00	105.40	$1.89e^{+06}$	
Plot (site)		$4.16e^{-02}$	$2.71e^{+00}$	0.00	0.00	48.17	$1.47e^{+06}$	
Site		$1.43e^{-08}$	$5.90e^{-06}$	0.00	$1.61e^{+02}$	48.63	$6.91e^{+07}$	
(2) Nitrogen gradient								
Height	1	0.11	49.80 ***	12.42 ***	8.29 **	1.56	1.78	
Nitrogen	1	0.01	23.30 ***	6.24 *	3.06 ·	2.08	0.69	
$Nitrogen^2$	1	0.59	15.56 ***	4.29 *	3.75 *	2.05	0.47	
Height x nitrogen	1	0.08	52.53 ***	12.67 ***	22.61 ***	1.42	1.86	
Height x $nitrogen^2$	1	0.24	37.38 ***	7.20 **	29.46 ***	1.24	1.76	
Residual		0.00	198.90	0.00	0.00	189.4	$7.56e^{+05}$	
(df)		(350)	(330)	(330)	(51)	(30)	(30)	
Plot		0.08	0.00	1.08	0.33	0.00	$1.45e^{+05}$	
(J) Penstemon digitalis **(Scrophulariaceae)**								
(1) Nitrogen x herbivory								
Height	1	1.89	142.52 ***	12.84 ***	0.21	0.02	0.09	μ = 11.05%
Nitrogen	1	22.09 ***	4.66 *	0.00	0.40	0.01	0.16	
Herbivory	1	0.09	11.04 ***	1.93	0.31	0.42	0.12	
Site	1	7.40 **	3.41 ·	7.63 **	0.02	0.00	0.11	
Height x nitrogen	1	0.63	1.92	0.03	0.25	0.03	0.09	
Height x herbivory	1	0.20	4.17 ·	2.58	0.39	0.50	0.01	
Nitrogen x herbivory	1	0.01	10.30 **	0.12	0.03	0.39	1.46	
Height x nitrogen x herbivory	1	0.18	15.73 ***	0.23	0.01	0.00	0.00	
Residual		0.00	208.58	0.00	$1.17e^{+03}$	$1.38e^{+03}$	$1.82e^{+08}$	
(df)		(918)	(661)	(659)	(32)	(25)	(25)	
Subplot (plot, site)		$3.68e^{-07}$	0.00	$1.26e^{-05}$	$5.03e^{+02}$	0.00	0.00	
Plot (site)		$2.14e^{-01}$	4.97	$5.02e^{-01}$	$0.00e^{+00}$	$1.12e^{+03}$	0.00	
Site		$1.01e^{-06}$	0.00	$1.64e^{-05}$	$1.59e^{+02}$	$1.68e^{+03}$	$3.12e^{+07}$	
(2) Nitrogen gradient								
Height	1	740.35 ***	4.92 *	8.57 **	43.90 ***	0.00	7.49 **	
Nitrogen	1	65882.92 ***	2.43	0.06	11.09 ***	0.73	2.56	
$Nitrogen^2$	1	404.68 ***	2.97 ·	0.07	15.40 ***	0.80	2.79 ·	
Height x nitrogen	1	225.90 ***	4.34 *	0.04	24.79 ***	0.48	4.19 *	
Height x $nitrogen^2$	1	64.86 ***	4.60 *	0.37	33.01 ***	0.59	4.72 *	
Residual		0.00	510.83	0.00	0.00	1007.5	$1.31e^{+07}$	
(df)		(356)	(336)	(335)	(44)	(43)	(43)	
Plot		0.19	21.58	0.58	0.10	266.9	0.00	
(K) Penstemon hirsutus* **(Scrophulariaceae)**								
(1) Nitrogen x herbivory								
Height	1	16.92 ***	13.63 ***	non-est	non-est	non-est	non-est	μ = 9.21%
Nitrogen	1	8.15 **	0.02	μ = 0.01	μ = 34.2	μ = 76.67		
Herbivory	1	2.64	0.18					
Site	1	4.04 *	0.07					
Height x nitrogen	1	1.38	1.53					
Height x herbivory	1	3.67 ·	0.19					
Nitrogen x herbivory	1	0.02	0.07					

Table S4.5. (cont'd)

Height x nitrogen x herbivory	1	0.73	0.00					
Residual		0.00	$6.80e^{+01}$					
(df)		(814)	(470)					
Subplot (plot, site)		$1.09e^{-01}$	$2.17e^{-01}$					
Plot (site)		0.00	$2.16e^{-06}$					
Site		$4.24e^{-06}$	0.00					
(2) Nitrogen gradient								
Height	1	0.25	7.67 **	0.99	non-est	non-est	non-est	
Nitrogen	1	0.01	0.73	0.07	$\mu = 32.57$	$\mu = 6$		
$Nitrogen^2$	1	0.10	1.53	0.05				
Height x nitrogen	1	0.00	2.08	0.00				
Height x $nitrogen^2$	1	0.02	3.66 ·	0.00				
Residual (df)		0.00 (348)	50.62 (290)	0.00 (290)				
Plot		0.84	4.61	$2.12e^{-15}$				
***(L) Penstemon pallidus** (Scrophulariaceae)**								
(1) Nitrogen x herbivory								
Height	1	7.01 **	106.31 ***	non-est	non-est	non-est	non-est	$\mu = 3.55\%$
Nitrogen	1	34.23 ***	1.74	$\mu = 0.01$	$\mu = 53.5$	$\mu = 52$		
Herbivory	1	1.27	0.67					
Site	1	0.71	1.63					
Height x nitrogen	1	7.60 **	6.57 *					
Height x herbivory	1	3.01 ·	0.60					
Nitrogen x herbivory	1	0.24	8.10 **					
Height x nitrogen x herbivory	1	0.75	10.66 **					
Residual (df)		0.00 (707)	41.01 (354)					
Subplot (plot, site)		$4.29e^{-03}$	0.00					
Plot (site)		$9.89e^{-02}$	0.34					
Site		$7.75e^{-09}$	0.00					
(2) Nitrogen gradient								
Height	1	0.52	2.29	0.34	5.91 *	non-est	non-est	
Nitrogen	1	0.02	4.82 *	6.01 *	6.46 *	$\mu = 40.1$		
$Nitrogen^2$	1	0.18	4.24 *	3.79	13.54 ***			
Height x nitrogen	1	0.02	4.49 *	6.51 *	2.49			
Height x $nitrogen^2$	1	0.24	4.72 *	4.66 *	11.51 ***			
Residual		0.00	155.22	0.00	0.00			
(df)		(344)	(265)	(264)	(24)			
Plot		0.43	1.89	0.00	0.22			
***(M) Silphium terebinthinaceum** (Asteraceae)**								
(1) Nitrogen x herbivory								
Height	1	2.35	9.46 **	non-est	non-est	non-est	non-est	$\mu = 0.05\%$
Nitrogen	1	0.01	0.05					
Herbivory	1	5.33 *	0.20					
Height x nitrogen	1	0.26	0.42					
Height x herbivory	1	5.96 *	0.11					
Nitrogen x herbivory	1	0.34	0.31					
Height x nitrogen x herbivory	1	1.27	0.05					
Residual		0.00 (98)	62.62 (52)					
Subplot (plot)		0.00	0.00					
Plot		0.00	0.00					
(2) Nitrogen gradient experiment								
N/A								
***(N) Silphium perfoliatum* (Asteraceae)**								
(1) Nitrogen x herbivory								
Height	1	0.01	6.90 **	non-est	non-est	non-est	non-est	$\mu = 0.1\%$
Nitrogen	1	1.09	0.66					
Herbivory	1	0.60	0.76					
Site	1	15.35 ***	1.55					
Height x nitrogen	1	0.00	0.14					
Height x herbivory	1	1.66	1.41					
Nitrogen x herbivory	1	4.04 *	0.33					
Height x nitrogen x herbivory	1	1.84	2.96 ·					

Table S4.5. (cont'd)

Residual	0.00	$2.17e^{+02}$
(df)	(693)	(358)
Subplot (plot, site)	$3.89e^{-09}$	$8.91e^{-03}$
Plot (site)	$7.87e^{-01}$	$2.94e^{+00}$
Site	$1.67e^{-09}$	0.00
(2) Nitrogen gradient		
N/A		

Table S4.6. Effects of nitrogen addition, deer herbivory, and a nitrogen gradient on lambda. (A) Effect of status (locally extinct vs. extant), nitrogen (control vs. addition [10 g N m^{-2} yr^{-1}]), and herbivory (deer present vs. absent) on population growth rates λ in the nitrogen x herbivory experiment. (B) Effect of status, nitrogen (continuous: 0-12 g N m^{-2} yr^{-1}), nitrogen^2, and their two-way interactions on λ in the nitrogen gradient experiment. Species was included as a random factor in both models. **p<0.01.

Source	df	χ^2
(A) Nitrogen x herbivory experiment		
Status	1	0.08
Nitrogen	1	8.21 **
Herbivory	1	0.20
Status x nitrogen	1	0.16
Status x herbivory	1	2.15
Nitrogen x herbivory	1	0.09
Status x nitrogen x herbivory	1	0.01
Residual	20	0.007
Species		0.017
(B) Nitrogen gradient		
Status	1	1.89
Nitrogen	1	0.14
Nitrogen^2	1	1.13
Status x nitrogen	1	1.28
Status x nitrogen^2	1	2.20
Residual	39	0.04
Species		0.05

Table S4.7. Life Table Response Experiment (LTRE). Values represent how proportional changes in each vital rate (survival, growth, probability of flowering, reproductive effort [flowers produced], and reproductive output [seed produced]) contribute to differences in λ between control conditions and each treatment ($\Delta\lambda=\lambda^{treatment}-\lambda^{control}$), where treatments include nitrogen addition, deer absent (note that the main text presents this as its inverse, or the effects of deer herbivory), or their combination ["Nitrogen x Deer absent")]). The LTRE contribution of each vital rate to Δλ is estimated as the difference in a vital rate, α_{ij}, between the treatment and control multiplied by the sensitivity of λ to changes in α_{ij} (here, a perturbation of 0.01). A negative LTRE contribution indicates that the value of α_{ij} is lower under the treatment than the control. I also provide population growth rates λ in each treatment, Δλ, and the total contribution of each vital rate to Δλ. The sum of contributions of each treatment should be approximately equal to Δλ. (Note that this is not the case for *Penstemon hirsutus* and *P. pallidus*, likely because differences in λ were also influenced by lower mean reproduction and recruitment in these locally extinct species.)

Species	MF	PT*	PD	PH*	PP*
λ estimates					
Control	0.310	0.362	0.459	0.677	0.305
Nitrogen	0.204	0.235	0.311	0.485	0.090
Deer absent	0.342	0.486	0.511	0.341	0.179
Nitrogen x Deer absent	0.295	0.357	0.335	0.198	0.041
Difference in λ (Δλ)					
Nitrogen – control	-0.105	-0.127	-0.149	-0.192	-0.216
Deer absent – control	0.032	0.124	0.051	-0.336	-0.126
Nitrogen x Deer absent – control	-0.015	-0.004	-0.124	-0.479	-0.265
LTRE contributions of survival					
Nitrogen	-0.076	-0.047	-0.117	-0.175	-0.288
Deer absent	-0.0003	0.003	-0.028	0.140	-0.047
Nitrogen x Deer absent	-0.072	-0.031	-0.131	-0.014	-0.320
LTRE contributions of growth					
Nitrogen	-0.106	-0.051	-0.0008	0.0005	-0.042
Deer absent	0.042	0.019	-0.0004	-0.0004	-0.081
Nitrogen x Deer absent	-0.044	-0.011	0.000	-0.0002	-0.108
LTRE contributions of Pr(flowering)					
Nitrogen	-0.007	-0.060	0.018	0.002	-0.234
Deer absent	0.009	0.056	0.059	-0.014	-0.241
Nitrogen x Deer absent	0.031	0.003	0.062	-0.011	-0.235
LTRE contributions of reproductive effort (flowers)					
Nitrogen	0.0007	0.005	0.0001	-	-
Deer absent	-0.0003	-0.0002	-0.0001	-	-
Nitrogen x Deer absent	0.0003	-0.001	0.0001	-	-
LTRE contributions of reproductive output (seeds)					
Nitrogen	0.008	0.001	-0.0002	-	-
Deer absent	0.003	0.006	-0.0002	-	-
Nitrogen x Deer absent	0.022	0.008	-0.0003	-	-
Total contributions to Δλ					
Nitrogen	-0.18	-0.15	-0.10	-0.17	-0.56
Deer absent	0.05	0.08	0.03	0.13	-0.37
Nitrogen x deer absent	-0.06	-0.03	-0.07	-0.03	-0.66

Table S4.8. Trait differences between locally extinct vs. extant species. (A) MANOVA results, with all traits included as a single multivariate response variable and status included as a predictor. Thereafter, differences in (B) mean specific leaf area (SLA; mm/mg), (C) mean flowering time, (D) leaf nitrogen content, and (E) leaf carbon to nitrogen (C:N) ratio (all species mean trait values) between locally extinct vs. extant species (status). Each model differs based on trait sampling methods (see Materials & Methods). I provide trait estimates are least square means (± standard error) for extant and locally extinct species. ***p<0.0001,**p<0.01, *p<0.05, · p<0.1.

Source	df	χ^2	Trait estimates
(A) MANOVA			
Status	1	4.49 *	
Residual	12		
(B) SLA			
Status	1	7.26 **	Extant = 211.0 ± 38.2 mm/mg
Nitrogen	1	7.57 **	Extinct = 155.0 ± 39.0 mm/mg
Herbivory	1	0.38	
Status x nitrogen	1	0.74	
Status x herbivory	1	0.99	
Nitrogen x herbivory	1	1.54	
Status x nitrogen x herbivory	1	1.05 *	
Species (status)		$1.14e^{+03}$ ($1.26e^{+03}$)	
Subplot (plot) (site)		$4.37e^{+00}$ ($4.46e^{+02}$) ($4.91e^{-01}$)	
Residual	1913	$2.17e^{+04}$	
(C) Flowering time			
Status	1	3.63 ·	Extant = 216.0 ± 10.9 (Aug 4)
Residual	12	30.71	Extinct = 184.0 ± 12.5 (July 3)
(D) Leaf N content			
Status	1	2.08 ·	Extant = 2.53 ± 0.18 % N
Nitrogen	1	103.52 ***	Extinct = 2.16 ± 0.20 % N
Herbivory	1	2.56 ·	
Status x nitrogen	1	2.59 ·	
Status x herbivory	1	0.04	
Nitrogen x herbivory	1	0.00	
Status x nitrogen x herbivory	1	0.08	
Species (status)		0.19 (0.04)	
Subplot (plot)		0.05 (0.004)	
Residual	230	0.49	
(E) C:N			
Status	1	0.38	Extant = 22.0 ± 2.28
Nitrogen	1	108.28 ***	Extinct = 24.0 ± 2.45
Herbivory	1	2.27 ·	
Status x nitrogen	1	0.39	
Status x herbivory	1	0.11	
Nitrogen x herbivory	1	1.23	
Status x nitrogen x herbivory	1	0.06	
Species (status)		19.10 (1.97)	
Subplot (plot)		3.05 (2.15)	
Residual	230	45.25	

Figure S4.1. Effects of nitrogen treatment (control vs. nitrogen added [10 g N m^{-2} yr^{-1}]) and deer herbivory (dark grey = deer present; light grey = fenced) on (A) proportion of Photosynthetic Active Radiation reaching ground level and (B) biomass (g) of surrounding vegetation (*Andropogon gerardii, Sorghastrum nutans, Solidago canadensis*) in the nitrogen x herbivory experiment. Bars represent least square means ± standard error. Letters represent differences between treatments at the α=0.05 level. I provide model output for linear models testing the effects of nitrogen and herbivory on (A) PAR or (B) biomass. ***p<0.0001; *p<0.05.

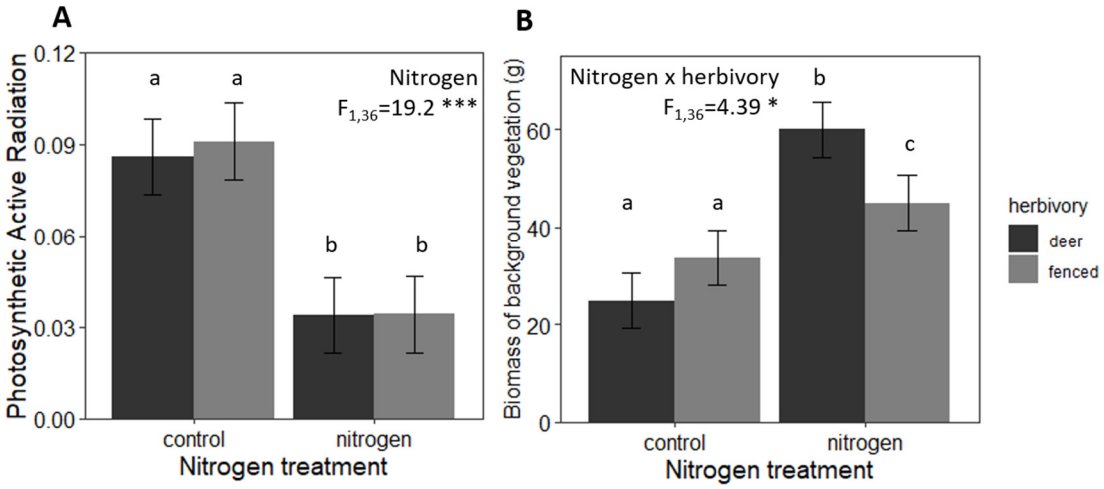


Figure S4.2. Effect of nitrogen (0-12 g N m^{-2} yr^{-1}) on the proportion of Photosynthetic Active Radiation reaching ground level in the nitrogen gradient experiment. Shaded areas represent 95% confidence intervals. I provide model output for a linear model testing the effect of nitrogen on PAR. ***p<0.0001.

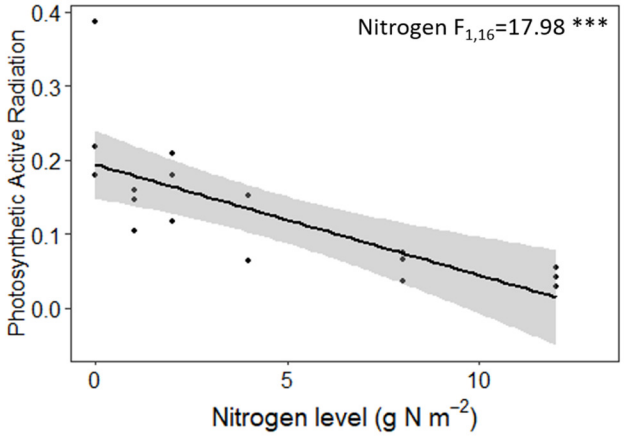


Figure S4.3. Effect of a gradient of nitrogen addition (0-12 g N m^{-2} yr^{-1}) on species' vital rates: (A) probability of survival (Pr[survival], %) from year *t* to year *t+1*, (B) growth (cm) from year *t* to *t+1*, (C) probability of flowering (Pr[flowering], %) in year *t+1,* (D) reproductive effort in year *t+1* (number of flowers produced), and (E) reproductive output in year *t+1* (number of seeds produced per fruit). In D-E, I only show species that flowered. * indicates a locally extinct species. Dots are jittered values. Lines show linear (C,E) or quadratic fit (A,B,D), depending on model fit (Table S4.4). Shaded areas represent 95% confidence intervals.

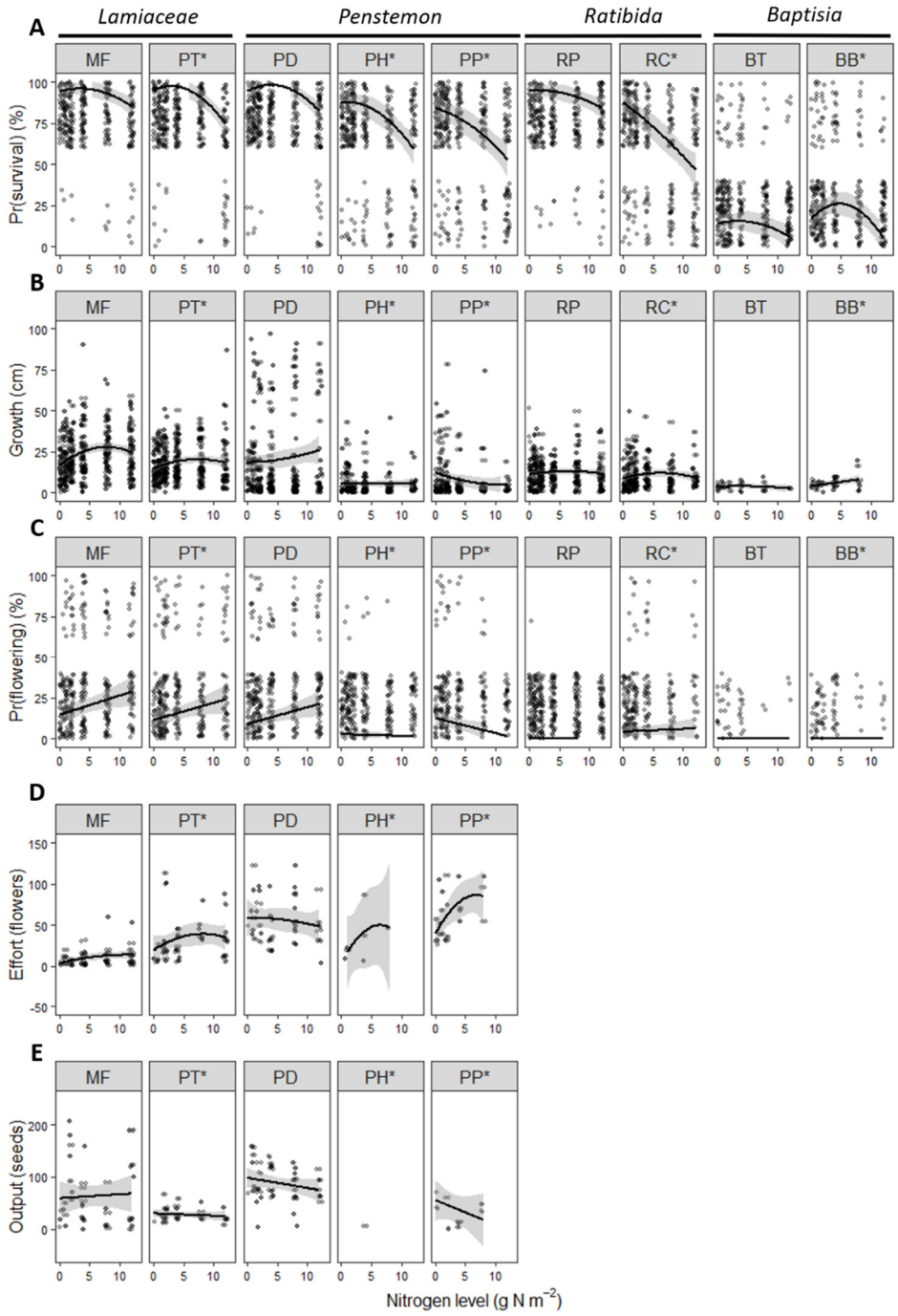


Figure S4.4. Effect of nitrogen, deer presence, and their combination on (A) probability of flowering, (B) reproductive effort (flower production) and (C) reproductive output (number of seeds produced per fruit) in extant *Monarda fistulosa* (MF), locally extinct *Pycnanthemum tenuifolium* (PT), and extant *Penstemon digitalis* (PD). Dot size represents the effect size of each treatment, and blue and orange circles indicate positive and negative responses, respectively.

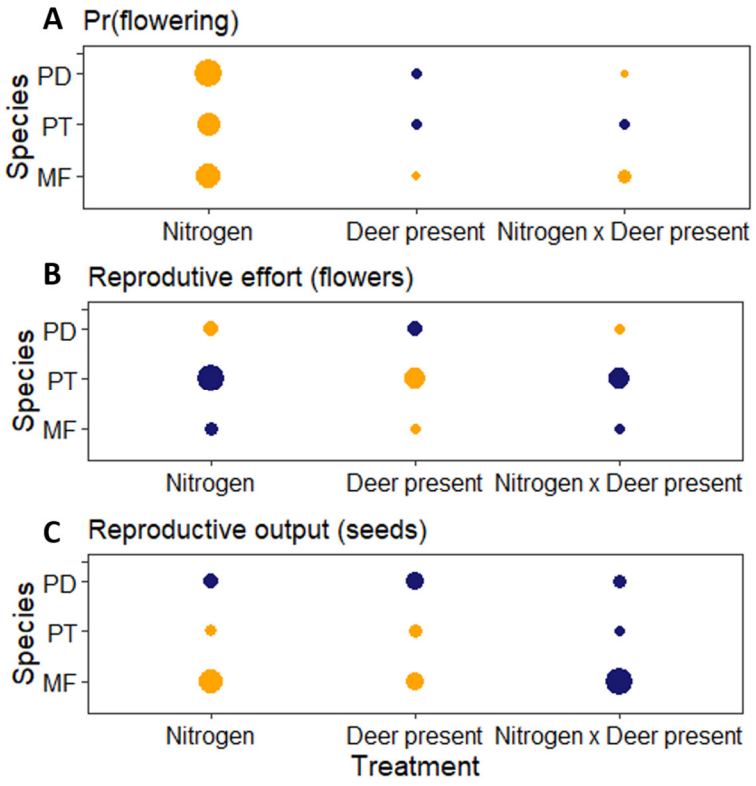


Figure S4.5. Reproductive effort and output in *Monarda fistulosa.* Effect of nitrogen treatment (black = control vs. blue = nitrogen addition [10 g N m^{-2} yr^{-1}]) and deer herbivory (left = deer herbivory vs. right = fenced) on reproductive output (number of seeds produced per fruit) as a function of plant size (height, cm) in *Monarda fistulosa.* Shaded areas represent 95% confidence intervals.

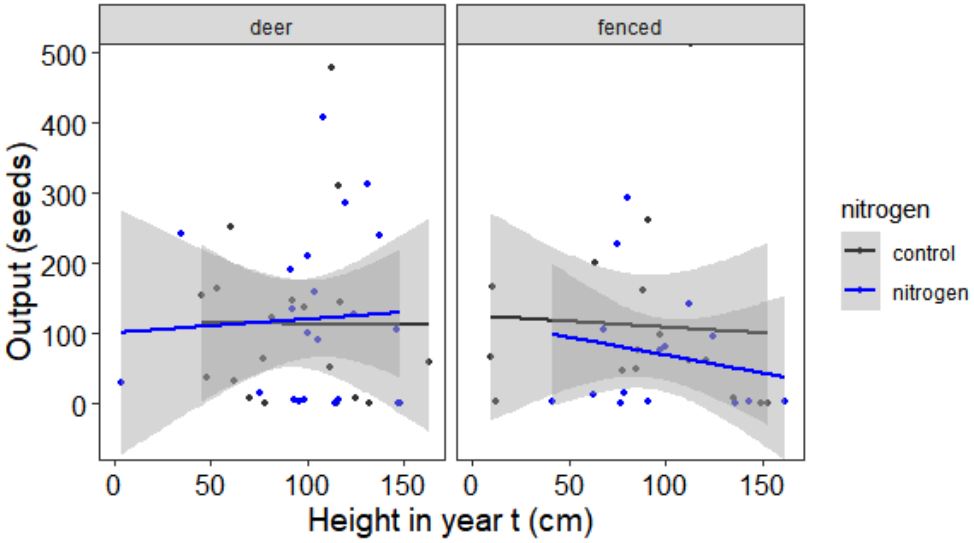
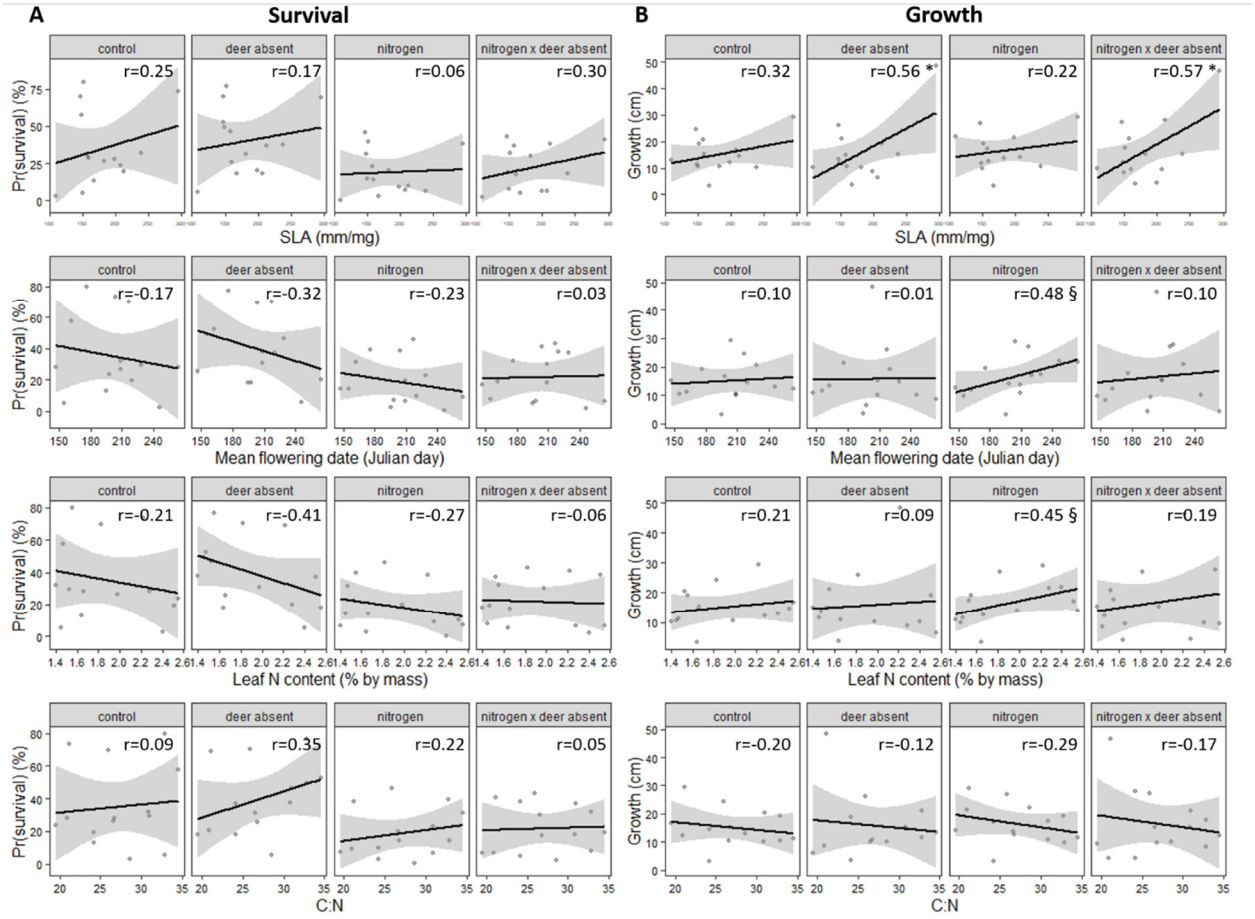


Figure S4.6. Correlations between specie mean traits and vital rates. Correlations between species mean traits (specific leaf area [SLA, mm/mg]; mean flowering date [Julian day]; leaf nitrogen content (% by mass); and leaf carbon to nitrogen (C:N) ratio) and (A) survival and (B) growth under each experimental treatment (control, deer absence [fenced plots], nitrogen addition, and nitrogen x deer absence). *p<0.05; §p<0.1.



LITERATURE CITED

LITERATURE CITED

Aerts, R., and F.S. Chapin. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of process and patterns. *Advances in Ecological Research* 30: 1-67.

Barnosky, A.D., N. Matke, S. Tomiya, G.O.U. Wogan, B. Swartz, T.B. Quental, C. Marshall, et al. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51.57.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: https://doi.org/10.18637/jss.v067.i01

Bermingham, L.H. 2010. Deer herbivory and habitat type influence long-term population dynamics of a rare wetland plant. *Plant Ecology* 210: 359-378.

Bernardo, H.L., P.I. Vitt, R. Goad, S. Masi, and T.M. Knight. 2018. Using long-term population monitoring data to prioritize conservation action among rare plant species. *Natural Areas Journal* 39: 169-181.

Bernardo, H.L., R. Goad, P. Vitt, and T.M. Knight. 2019. Nonadditive effects among threats on rare plant species. *Conservation Biology:* https://doi.org/10.1111/cobi.13441.

Bialic-Murphy, L., N.L. Brouwer, and S. Kalisz. 2019. Direct effects of a non-native invader erode native plant fitness in the forest understory. *Journal of Ecology* 108: 189-198.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, N. Bustamante, et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20: 30-59.

Bobbink, R.K., and K. Hicks. 2014. Factors affecting N deposition impacts on biodiversity: an overview. doi: 10.1007/978-94-007-7939-6_14.

Borer, E.T., E.W. Seabloom, D.S. Gruner, W.S. Harpole, H. Hillebrand, E.M. Lind, P.B. Adler, et al. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508: 517-530.

Buckley, L.B., M.C. Urban, M.J. Angilletta, L.G. Crozier, L.J. Rissler, and M.W. Sears. 2010. Can mechanism inform species' distribution models? *Ecology Letters* 13: 1041-1054.

Campbell, D. 2019. Early snowmelt projected to cause population decline in a subalpine plant. *Proceedings of the National Academy of Sciences* 116: 12901-12906.

Caswell, H. 2001. *Matrix population models: construction, analysis, and interpretation*. Sinauer Associates, Sunderland, Massachusetts, USA.

Ceballos, G., and P.R. Ehrlich. 2002. Mammal population losses and the extinction crisis. *Science* 296: 904-907.

Chapman, K.A., and R. Brewer. 2008. Prairie and savanna in southern lower Michigan: history, classification, ecology. *The Michigan Botanist* 47: 1-48.

Chazal, J., and M.D.A. Rounsevell. 2009. Land-use and climate change within assessments of biodiversity change: A review. *Global Environmental Change* 19: 306-315.

Clark, C.M., E.E. Cleland, S.L. Collins, J.E. Fargione, L. Gough, K.L. Gross, S.C. Pennings, K.N. Suding, and J.B. Grace. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters* 10: 596-607.

Clark, C.M., and D. Tilman. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451: 712-715.

Clark, C.M., B. Yongfei, W.D. Bowman, J.M. Cowles, M.E. Fenn, F.S. Gilliam, G.K. Phoenix, et al. 2013. Nitrogen deposition and terrestrial biodiversity. In: Levin, S.A. (ed.) *Encyclopedia of Biodiversity*, 2nd ed. Vol. 5, pp. 519- 536. Academic Press, Waltham, MA.

Collen, B., A Purvis, and G.M. Mace. 2010. When is a species really extinct? Testing extinction inference from a sighting record to inform conservation assessment. *Diversity and Distributions* 16: 755-764.

Collins, S.L., A.K. Knapp, J.M. Briggs, J.M. Blair, and E.L. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280: 745-747.

Craine, J.M., D. Tilman, D. Wedin, P. Reich, M. Tloelker, and J. Knops. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Functional Ecology* 16: 563-574.

Cronk, Q. 2016. Plant extinctions take time. *Science* 353: 336-337.

Davies, T.J. 2019. The macroecology and macroevolution of plant species at risk. *New Phytologist* 222: 708-713.

Dirnböck, T., F. Essl, and W. Rabitsch. 2011. Disproportional risk for habitat loss of high-altitude endemic species under climate change. *Global Change Biology* 17: 99-996.

Doak, D.F., and W. Morris. 1999. Detecting population-level consequences of ongoing environmental change without long-term monitoring. *Ecology* 80: 1537-1551.

Easterling, M.R., S.P. Ellner, and P.M. Dixon. 2000. Size-specific sensitivity: applying a new

structured population model. *Ecology* 81: 694-708.

Ellner, S.P., and M. Rees. 2006. Integral projection models for species with complex demography. *American Naturalist* 167: 410-428.

Falster, D.S., R.A. Duursma, and R.G. FitzJohn. 2018. How functional traits influence plant growth and shade tolerance across the life cycle. *Proceedings of the National Academy of Sciences* 115: 6789-6798.

Fenn, M.E., R. Haeuber, G.S. Tonnesen, J.S. Baron, S. Grossman-Clarke, D. Hope, D.A. Jaffe, et al. 2003. Nitrogen emissions, deposition, and monitoring in the western United States. *BioScience* 53: 391-403.

Fiedler, P.L. 1987. Life history and population dynamics of rare and common mariposa lilies (*Calochortus* Pursh: Liliaceae). *Journal of Ecology* 75, 977-995.

Flaherty, K.L., W.N. Grafton, and J.T. Anderson. 2017. White-tailed deer florivory influences the population demography of *Polemonium vanbruntiae. Plant Biosystems* 152: 453-463.

Fritz, S.A., O.R.P., Bininda-Emonds, and A. Purvis. 2009. Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. *Ecology Letters* 12: 538-549.

Garcia, M.B., and J. Ehlrén. 2002. Reproductive effort and herbivory timing in a perennial herb: fitness components at the individual and population levels. *American Journal of Botany* 89: 1295-1302.

González-Suárez, M., P.M. Lucas, and E. Revilla. 2012. Biases in comparative analyses of extinction risk: mind the gap. *Journal of Animal Ecology* 81: 1211-1222.

Gotelli, N.J., and A.M. Ellison. 2002. Nitrogen deposition and extinction risk in the northern pitcher plant, *Sarracenia purpurea. Ecology* 83: 2758-2765.

Grotkopp, E., and M. Rejmánek. 2007. High seedling relative growth rate and specific leaf area are traits of invasive species: phylogenetically independent contrasts of woody angiosperms. *American Journal of Botany* 94: 526-532.

Hanes, C.R., and F.N. Hanes. 1947. *Flora of Kalamazoo County, Michigan: Vascular Plants.* Anthoensen Press, Portland, ME, USA.

Hanski, I., and O. Ovaskainen. 2002. Extinction debt at extinction threshold. *Conservation Biology* 16: 666-673.

Hautier, Y., P.A. NiKlaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324: 636-638.

Heil, G.W., and W.H. Diemont. 1983. Raised nutrient levels change heathland into grassland. *Vegetatio* 53: 113-120.

Hodapp, D., E.T. Borer, W.S. Harpole, E.M. Lind, E.W. Seabloom, P.B. Adler, J. Alberti, et al. 2018. Spatial heterogeneity in species composition constrains plant community responses to herbivory and fertilisation. *Ecology Letters* 21: 1364-1371.

Horvitz, C., D.W. Schemske, and H. Caswell. 1996. The relative "importance" of life history stages to population growth: prospective and retrospective analyses. Pp. 247-271 *in* S. Tuljapurkar and H. Caswell, eds. *Structured population models in marine, terrestrial, and freshwater systems.* Chapman and Hall, New York, New York, USA.

Kalisz, S., R.B. Spigler, and C.C. Horvitz. 2014. In a long-term experimental demography study, excluding ungulates reversed invader's explosive population growth rate and restored natives. *Proceedings of the National Academy of Sciences* 111: 4501-4506.

Knight, T.M., H. Caswell, and S. Kalisz. 2009. Population growth rate of a common understory herb decreases non-linearly across a gradient of deer herbivory. *Forest Ecology and Management* 257: 1095-1103.

Kuussaari, M., R. Bommarco, R.K. Heikkinen, A. Helm, J. Krauss, R. Lindborg, E. Ockinger, et al. 2009. Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology and Evolution* 24: 564-571.

Kyle, G., and M.R. Leishman. 2009. Functional trait differences between extant exotic, native, and extinct native plants in the Hunter River, NSW: a potential tool in riparian rehabilitation. *River Research and Applications* 25: 892-903.

MacDowell, S.C.L. 2002. Photosynthetic characteristics of invasive and noninvasive species of *Rubus* (Rosaceae). *American Journal of Botany* 89: 1431-1438.

Mack, R.N. 1996. Predicting the identity and fate of plant invaders: emergent and emerging approaches. *Biological Conservation* 78: 107-121.

Maschinski, J., J.E. Baggs, P.F. Quintana-Ascencio, and E.S. Menges. 2005. Using population viability analysis to predict the effects of climate change on the extinction risk of an endangered limestone endemic shrub, Arizona cliffrose. *Conservation Biology* 20, 218-228.

McGill, B.J., B.J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21: 178-185.

McKenna, D.D. 2004. Flora and vegetation of Kalamazoo County, Michigan. *Michigan Botanist* 43: 137-359.

Menges, E.S. 2000. Population viability analyses in plants: challenges and opportunities. *Trends*

in Ecology and Evolution 15: 51-56.

Merow, C., A.M. Latimer, A.M. Wilson, S.M. McMahon, A.G. REbelo, and J.A. Silander Jr. 2014. On using integral projection models to generate demographically driven predictions of species' distributions: development and validation using sparse data. *Ecography* 37: 1167-1183.

MI DNR. Michigan Deer Management Plan. https://tinyurl.com/yc2le8fo. Web. 2019.

Mondanaro, A., M. Di Febbraro, M. Melchionna, F. Carotenuto, S. Castiglione, C. Serio, S. Danisi, et al. 2019. Additive effects of climate change and human hunting explain population decline and extinction in cave bears. *Boreas* 48: 607-615.

Morris, W.F., J. Ehrlén, J.P. Dahlgren, A.K. Loomis, and A.M. Louthan. 2020. Biotic and anthropogenic forces rival climatic/abiotic factors in determining global plant population growth and fitness. *Proceedings of the National Academy of Sciences* 117: 1107-1112.

Mouillot, D., N.A.J. Graham, S. Villéger, N.W.H. Mason, and D.R. Bellwood. 2013. A functional approach reveals community responses to disturbance. *Trends in Ecology and Evolution* 28: 167-177.

Nutrient Network. Nutrient Application. https://nutnet.org/nutrients. Web. 2017.

Murray, K.S., L.D. Verde Arregoitia, A. Davidson, M. di Marco, and M.M.I. di Fonzo. 2014. Threat to the point: improving the value of comparative extinction risk analysis for conservation action. *Global Change Biology* 20: 483-494.

Pagel, J., and F.M. Schurr. 2011. Forecasting species ranges by statistical estimation of ecological niches and spatial population dynamics. *Global Ecology and Biogeography* 21: 293-304.

Paine, C.E.T, L. Amissah, H. Auge, C. Baraloto, M. Baruffol, N. Bourland, H. Bruelheide, et al. 2015. Globally, functional traits are week predictors of juvenile tree growth, and we do not know why. *Journal of Ecology* 103: 978-989.

Pardo, L.H., M.J. Robin-Abbott, C.T. Driscoll. 2010. Assessment of nitrogen deposition and empirical critical loads of nitrogen for ecoregions of the United States. United States Forest Service, Newtown Square, PA.

Pennings, S.C., C.M. Clark, E.E. Cleland, S.L. Collins, L. Gough, K.L. Gross, D.G. Milchunas, and K.N. Suding. 2005. Do individual plant species show predictable responses to nitrogen addition across multiple experiments? *Oikos* 110: 547-555.

Phillips, T., and M.N. Maun. 1996. Population ecology of *Cirsium pitcheri* on Lake Huron sand dunes: I. Impact of white-tailed deer. *Canadian Journal of Botany* 74: 1439-1444.

Pimm, S.L., C.N. Jenkin, R. Abell, T.M. Brooks, J.L. Gittleman, L.N. Goppa, P.H. Raven, et al. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344: 987.

Press, M.C., S.J. Woodin, and J.A. Lee. 1986. The potential importance of an increased atmospheric nitrogen supply to the growth of ombrotrophic *Sphagnum* species. *New Phytologist* 103: 45-55.

Purvis, A., J.L. Gittleman, G. Cowlishaw, G.M. Mace. 2000. Predicting extinction risk in declining species. *Proceedings of the Royal Society B* 26: 1947-1952.

Quétier, F., A. Thébault, and S. Lavorel. 2007. Plant traits in a state and transition framework as markers of ecosystem response to land-use change. *Ecological Monographs* 77: 33-52.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rooney, T.P., and D.M. Waller. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management* 181: 165-176.

Seastedt, T.R., J.M. Briggs, and D.J. Gibson. 1991. Controls of nitrogen limitation in tallgrass prairie. *Oecologia* 87, 72-79.

Sheth, S.N., and A.L. Angert. 2018. Demographic compensation does not rescue populations at a trailing range edge. *Proceedings of the National Academy of Sciences* 115: 2413-2418.

Simkin, S.M., E.B. Allen, W.D. Bowman, C.M. Clark, J. Belnap, M.L. Brooks, B.S. Cade, et al. 2016. Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. *Proceedings of the National Academy of Sciences* 113: 4086-4091.

Stearns, S.C. 1989. Trade-offs in life-history evolution. *Functional Ecology* 3: 259-268.

Suding, K.N., S.L. Collins, L. Gough, C. Clark, E.E. Cleland, K.L. Gross, D.G. Milchunas, and S. Pennings. 2005. Functional- and abundance-based mechanisms to explain diversity loss due to N fertilization. *Proceedings of the National Academy of Sciences* 102: 4387-4392.

Tye, M.R., E.S. Menges, C. Weekley, P.F. Quintana-Ascencio, and R. Sakguero-Gómez. 2016. A demographic ménage à trois: interactions between disturbances both amplify and dampen population dynamics of an endemic plant. *Journal of Ecology* 104: 1778-1788.

Urban, M.C., G. Bocedi, A.P. Hendry, J-B. Mihoub, G. Pe'er, A. Singer, J.R. Bridle, et al. 2016. Improving the forecast for biodiversity under climate change. *Science* 353: 1114-1122.

van Kleunen, M., and D.M. Richardson. 2007. Invasion biology and conservation biology: time to join forces to explore the link between species traits and extinction risk and invasiveness. *Progress in Physical Geography* 31: 447-450.

Vellend, M. K. Verheyen, J. Jacquemyn, A. Kolb, H. Van Calster, G. Peterken, and M. Hermy. 2006. Extinction debt of forest plants persists for more than a century following habitat fragmentation. *Ecology* 87: 542-548.

Violle, C., M. Navas, D. VIle, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! *Oikos* 116: 882-892.

Visser, M.D., M. Bruijning, S.J. Wright, H.C. Muller-Landau, E. Jongejans, L.S. Comita, and H. de Kroon. 2016. Functional traits as predictors of vital rates across the life cycle of tropical trees. *Functional Ecology* 30: 168-180.

Wang, C., and Y. Tang. 2019. Responses of plant phenology to nitrogen addition: a meta-analysis. *Oikos* 128: 1243-1253.

Warncke, D., J. Dahl, and L. Jacobs. 2009. Nutrient recommendations for field crops in Michigan. Michigan State University Extension Bulletin E2904.

Willis, C.G., B. Ruhfel, R.B. Primack, A.J. Miller-Rushing, J.B. Losos, and C.C. Davis. 2010. Favorable climate change response explains non-native species' success in Thoreau's woods. *PLoS One* 5(1): e8878.

Wright, I.J., and M. Westoby. 2000. Cross-species relationships between seedling relative growth rate, nitrogen productivity and root vs leaf function in 28 Australian woody species. *Functional Ecology* 14, 97-107.

Zettlemoyer, M., and N. Srodes. 2019. To Bloom Again: Can prairie restoration overcome habitat loss? *Conservation Notes* November/December 2019. https://tinyurl.com/yddhroq8. Web. 2019.

Zettlemoyer, M.A., D.D. McKenna, and J.A. Lau. 2019a. Species characteristics affect local extinctions. *American Journal of Botany* 106: 1-13.

Zettlemoyer, M.A., E.H. Schultheis, and J.A. Lau. 2019b. Phenology in a warming world: differences between native and non-native plant species. *Ecology Letters* 22: 1253-1263.