TEMPERATE DECIDUOUS TREES IN A CHANGING WORLD: THEIR PHENOLOGY AND INTERACTIONS

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Abstract

This thesis examines the ecological interactions within temperate deciduous forests, focusing on wild bees, trees, and understorey herbs, and how these species respond to climate change. In my first data chapter, using pan traps, I sampled bees in both the canopy and understorey of a temperate deciduous forest bi-weekly from early spring to mid-summer of 2021. My findings reveal that forest canopies are crucial yet often overlooked habitats for wild bees. A significant number of ground-nesting bees were found foraging in the canopy, with arboreal pollen, particularly from *Quercus* and *Acer* species, forming a substantial part of their diet. This suggests that excluding the canopy from bee community studies could lead to underestimating bee populations and overlooking key aspects of their foraging behaviour.

In my second data chapter, I analyzed 13 years of phenological data for 10 tree species and 11 spring-flowering forest herbs across 965 sites in northeastern North America, collected by citizen scientists under the USA-National Phenology Network. Contrary to expectations, the data showed that spring-flowering herbs advance their phenology more rapidly than trees in response to warming, particularly at middle and higher latitudes. This faster response may allow these herbs to extend their growing season and increase carbon uptake as temperatures rise. However, the variation in responses across latitudes and species highlights the complexities involved in predicting long-term ecological impacts.

Additionally, in my final data chapter, I investigated the temperature sensitivity of phenology in temperate tree species across Europe using leaf-out data from the Pan-European Phenology Project (PEP-725) and climate data from the European Climate Assessment & Dataset (E-OBS). My analysis focused on the relationship between chilling accumulation and heating accumulations (CA-HR response rate) and how this relationship varies with local environmental conditions. The results indicate that populations in warmer, drier, and lower-elevation areas have weaker phenological responses to warming, while those in colder, wetter, and higher-elevation areas have stronger phenological responses to warming. This variation suggests that local adaptation significantly influences how different populations respond to climate change.

Co-Authorship

The research ideas, written content, analyses, and figures presented in the following chapters of this thesis are my own work, with valuable guidance and contributions from my co-authors.

Chapter 2 was developed from an idea I devised. I collected and analyzed the data and wrote the manuscript, incorporating input and revisions from the other authors, Dr. Forrest and Dr. Sargent.

Chapter 3 is based on an original manuscript idea devised by Dr. Jessica Forrest, which was published in the Journal of Ecology in 2022. The idea was further refined through discussions involving Dr. Risa Sargent and myself. I, Evelyn F. Alecrim, performed the data preparation and analysis and wrote the manuscript with input and revisions from Dr. Forrest and Dr. Sargent. In the text, *we* refers to myself and the co-authors, Dr. Forrest and Dr. Sargent.

Chapter 4 was similarly based on an idea I devised. I collected and analyzed the data and wrote the manuscript, with valuable input and revisions from the other authors, Dr. Forrest and Dr. Sargent.

To Sophie and Ivane

When I see you, I see hope.

“We have to get acquainted to this idea that there is no real harmony as we have conceived it.
But when I say this, I say this all full of admiration for the ***jungle****. It is not that I hate it,* ***I love it.***
I love it very much. But ***I love it against my better judgment****.”*
Werner Herzog

“And darling, our disease is the same one as the trees
Unaware that they've been living in a forest”
Vampire Weekend

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Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

Trees play a crucial role in maintaining biodiversity, regulating climate, and supporting various ecosystem functions. They significantly contribute to nutrient cycling, carbon sequestration, and soil stabilization, which are vital for ecosystem stability (Binder et al., 2017). Additionally, trees provide habitats for a diverse array of organisms, fostering biodiversity by supporting various plant and animal species (Horák, 2017). Their importance also extends to preventing soil erosion and maintaining water cycles, which are essential for life on Earth (Polle & Rennenberg, 2019).

Temperate deciduous forests, predominantly found in the Northern Hemisphere, exemplify the ecological significance of trees. These forests span regions such as eastern North America, western Eurasia, and eastern Asia, featuring key tree genera like *Quercus* (oaks), *Fagus* (beeches), *Betula* (birches), and *Acer* (maples) (Fang & Lechowicz, 2006). During autumn, trees lose their leaves and enter dormancy to survive the winter (Powell, 1987). In spring, environmental cues like temperature and daylight trigger key phenological events such as budburst and leaf expansion (Ettinger et al., 2020a; Laube et al., 2014; Linkosalo et al., 2006; Zohner et al., 2016, 2017).

A unique characteristic of these forests is the changing light regime due to seasonal variations in canopy cover. In early spring, the forest floor receives abundant direct sunlight, accounting for over 90% of the total energy in the understorey (Hutchison & Matt, 1977). However, after leaf-out in summer, solar radiation in the understorey dramatically decreases to 1–5% of that available above the canopy (Hicks & Chabot, 1985). In North American forests, spring-blooming forest herbs like *Trillium grandiflorum*, *Claytonia virginica*, and *Erythronium americanum* take advantage of the high light levels before canopy closure to grow their leaves, accumulate carbon, flower, and produce fruit.

Trees and their interaction with arthropods - The distinct layers within temperate forests—the understorey, home to young trees, shrubs, and spring ephemerals (Lapointe, 2001), and the canopy, formed by the crowns of mature trees—interact to support a rich biodiversity (Cavard et al., 2011). Although temperate deciduous hardwood forests are ecologically significant, canopy-dwelling organisms have been relatively understudied. In these forests, the limited research generally reports greater arthropod diversity on the forest floor compared to the canopy (Corff & Marquis, 1999; Hirao et al., 2009; Preisser et al., 1998; Ulyshen & Hanula, 2007). However, recent studies have begun to shed light on canopy-dwelling bees, with certain species showing greater abundance in the canopy compared to the understorey (Allen & Davies, 2022; Campbell et al., 2018; Harrison et al., 2018; Saunders, 2018; Smith et al., 2021; Ulyshen et al., 2010;

Urban-Mead et al., 2021). For instance, Ulyshen et al. (2010) found that *Lasioglossum* species, despite being ground-nesters, were significantly associated with the canopy, and *Augochlora pura* is often more common in the canopy than in the understorey (Campbell et al., 2018; Ulyshen et al., 2010). A recent study also reported similar species richness across forest strata but found greater diversity in the canopy, without identifying any bee species exclusive to a particular stratum (Urban-Mead et al., 2021).

In temperate deciduous forests, while entomophilous (insect-pollinated) plants are typically seen as the main pollen source for bees, anemophilous (wind-pollinated) trees also provide important pollen sources. Several studies have shown that insect pollinators do forage on anemophilous trees (Kraemer & Favi, 2005; Kratschmer et al., 2020; MacIvor et al., 2014; Persson et al., 2018; Saunders, 2018; Urban-Mead et al., 2021; Yourstone et al., 2021), and an emerging hypothesis suggests that this pollen is particularly valuable before the peak spring flowering of understorey wildflowers (Wood et al., 2018). Most knowledge about pollen use by wild bees comes from nest provisions, mainly from eusocial species like *Bombus* and *Apis*, leaving significant gaps in understanding adult bee pollen consumption. While adult bees primarily consume nectar for energy, pollen is crucial for their protein and lipid intake, especially for females needing nutrients for egg production. Beyond pollen and nectar, bees also utilize canopy resources like honeydew and sap, particularly when floral resources are scarce, with these alternative food sources often being more abundant in the canopy than at the forest floor (Moran & Southwood, 1982; Ulyshen, 2011; Wardhaugh et al., 2006).

Phenological interactions in temperate forests and the impact of climate change - Phenological events, which are seasonal changes in the life cycles of organisms such as flowering, leaf-out, and leaf senescence, play a significant role in the interactions between trees and understorey habitat. Spring ephemerals, herbaceous perennial plants that flower and complete most of their above-ground life cycle before trees leaf out, take advantage of the high levels of sunlight reaching the forest floor before the canopy closes. These plants develop their vegetative and reproductive parts in early spring, attracting pollinators who gather pollen and nectar. As spring progresses, trees begin to leaf-out and bloom, offering pollinators access to both canopy and understorey resources (Lapointe, 2001; Routhier & Lapointe, 2002; Vezina & Grandtner, 1965). This synchronization between trees and spring ephemerals is closely aligned with the seasonal cycle; therefore, understanding the environmental cues behind it is paramount to comprehending this interaction.

The timing of these phenological events is governed by different environmental cues. For temperate perennial species, three major cues underlie the timing of budburst and flowering: spring warmth (forcing), increasing daylength (photoperiod), and the length and intensity of winter chilling. However, the role of each cue can vary among species, and often more than one cue acts simultaneously. For example, temperate woody species primarily respond to air temperature, while perennial herbs tend to respond to soil temperature and snow depth (Laube et al., 2014; Thomas et al., 2003; Zohner et al., 2016; Zohner & Renner, 2014). These differing responses can influence the dynamics of canopy closure and shading, potentially affecting the growth and reproductive success of understorey plants, including spring ephemerals (Ge et al., 2015; Heberling, Cassidy, et al., 2019; Heberling, Fridley, et al., 2019).

As climate change continues to alter environmental conditions, the phenology of both trees and spring ephemerals is expected to shift, potentially disrupting their interactions. Earlier leaf-out and flowering due to warming temperatures can lead to changes in the timing of canopy closure relative to the growing season of spring ephemerals. For instance, if canopy leaf-out advances more than the growth and flowering of spring ephemerals, the period in which understorey plants receive intense light could be reduced, threatening their photosynthetic rates, reproductive success, and survival (Chuine, 2010; Ettinger et al., 2018; Heberling, Cassidy, et al., 2019; Lee, Yancy, et al., 2024).

Geographical variation in phenological responses adds another layer of complexity to understanding these interactions between trees and spring ephemerals. Plant species often occupy broad geographic areas with varying climates, altitudes, and ecological contexts, which can influence the timing of key phenological events. Intraspecific differences in phenology, driven by adaptation to local environmental cues, are common across these gradients. For example, populations of the same species may exhibit earlier leaf-out in warmer, lower-elevation sites compared to colder, higher-elevation areas (Ellwood et al., 2019). This variation reflects species' adaptability to their local environments but also poses challenges for predicting phenological shifts under climate change. As global warming continues, these regional differences become increasingly important for forecasting changes in growing seasons, species distributions, and ecosystem dynamics (Fu, Zhao, et al., 2015; Zohner et al., 2017).

As stated above, the timing of leaf-out in temperate woody species is primarily governed by the interplay between chilling, the accumulation of cold temperatures needed to break dormancy, and heating, the accumulation of warmth required to trigger budburst, both of which vary geographically. Chilling, which occurs during exposure to low temperatures (as low as −2°C and up to 10°C; Baumgarten et al., 2021), is essential for breaking endodormancy and allowing plants to transition to ecodormancy. This process ensures that trees do not leaf out prematurely during winter warm spells, which could lead to frost damage (Lenz et al., 2016). The specific amount of chilling required to break dormancy varies among species and populations, reflecting local adaptation to climatic conditions (Clark et al., 2014; Leites & Benito Garzón, 2023; Polgar & Primack, 2011; J. Prevéy, Vellend, Rüger, Hollister, Bjorkman, Myers-Smith, et al., 2017; J. S. Prevéy et al., 2018; Vitasse, 2013).

Once the chilling requirement is fulfilled, the plant's response shifts to the accumulation of heat, or "forcing," which drives budburst and leaf-out. The amount of heat required also varies, with some species or populations needing only a few warm days while others require prolonged warmth. Interestingly, a greater accumulation of chilling generally reduces the amount of subsequent heat needed for budburst, creating a negative relationship between chilling accumulations and heating requirements within a population or even within an individual plant (Fu et al., 2019).

Geographical variation plays a significant role in shaping these chilling and heating requirements. For instance, populations at higher latitudes, where temperatures are lower, tend to have lower heat requirements, whereas populations at lower latitudes may have higher heat requirements, which prevent premature leaf-out during warm spells and reduce the risk of frost damage (Peaucelle et al., 2019). Additionally, areas with high interannual temperature variability often exhibit weaker phenological responses to warming, suggesting that plants in these regions are less likely to track climatic changes closely (T. Wang et al., 2014). Precipitation patterns also influence these requirements, affecting the timing of leaf-out and the necessary growing degree days (Peaucelle et al., 2019).

Hence, although many studies have documented how chilling and heating requirements vary in response to local environmental conditions (Osada et al., 2018; Peaucelle et al., 2019), there has been less focus on how these factors interact—i.e., the relationship between chilling accumulation (CA) and the heating requirement (HR). Some research has begun to explore this relationship, such as studies by Wang et al. (2020) and Lin et al. (2022), which analyzed the CA-HR relationship to validate chilling models, and Osada et al. (2018), which explored this relationship in *Fagus crenata* across different latitudes in Japan. These studies suggest that local adaptation significantly influences the CA-HR relationship, impacting the timing of budburst and leaf-out. However, a deeper understanding of the environmental drivers behind this relationship is needed, as it offers a mechanistic explanation for the variation in temperature sensitivity observed across geographical ranges.

Structure and contributions of this thesis - In this thesis, I address several gaps in the literature. Among them, key areas of knowledge still needing exploration include bee foraging behaviour in canopy and understorey environments. We lack a clear understanding of the degree to which bees consume arboreal versus understory pollen, and how this consumption varies among bee species. Additionally, the differences in pollen use between the canopy and understorey remain uncertain, with the specific pollen types used by bees in each layer yet to be fully identified. Furthermore, the seasonal variation in bees' consumption of arboreal versus understorey pollen is not well understood. I investigate and address these gaps in Chapter 2.

In my third chapter, I investigate the phenological responses of eastern North American deciduous trees and spring-flowering forest herbs to climate change. Previous studies have yielded conflicting results on whether trees or understorey herbs are advancing their phenologies more rapidly in response to warming, with some suggesting that trees are outpacing herbs and increasing shading, while others indicate the opposite trend. These studies have often been limited in geographic scope or focused on specific species groups, leading to difficulties in drawing broader conclusions. Additionally, there has been a notable lack of comprehensive analyses that examine phenological shifts across a wide latitudinal range and among co-occurring species within the same ecosystems. My research in this chapter fills these gaps by providing a large-scale analysis of phenological responses across multiple species of trees and spring-flowering forest herbs in northeastern North America, offering new insights into how these groups respond to warming temperatures and how these responses vary geographically.

Lastly, in my fourth chapter, I address the relationship between chilling accumulation and heating requirements, and the impact of this relationship on the temperature sensitivity of leaf-out phenology across different geographical locations. While many studies have documented the geographical variation in chilling and heating requirements in response to local environmental conditions, there has been less attention paid to how these environmental factors shape the relationship between chilling and heating itself (the CA-HR response rate). Existing research has often inferred the influence of climatic variables on the CA-HR relationship without explicitly testing these influences, leaving a gap in our understanding of how local adaptation drives phenological responses to climate change. My work fills this gap by investigating how the CA-HR response rate varies across locations and how it correlates with temperature sensitivity and local environmental factors. This approach provides a mechanistic explanation for observed variations in temperature sensitivity across geographical ranges and offers insights into how different populations may respond to climate change, thereby contributing to the improvement of the accuracy of phenological predictions.

Chapter 2
Look up: the importance of trees as a pollen source for wild bees in temperate deciduous forests

Abstract

In temperate forests, trees provide potentially important, yet often overlooked, pollen sources for diverse bee populations, especially in early spring when other floral resources are scarce. However, we know relatively little about the extent to which wild bees exploit the floral resources and habitat provided by temperate tree canopies. To investigate pollen usage and habitat stratification of forest-dwelling bees, I used pan traps to sample bees in the canopy and understorey of a temperate deciduous forest bi-weekly from early spring to mid-summer of 2021. Out of 1,413 captured bees, 940 were found in the tree canopy and 473 in the understorey, with the focal trees exhibiting similar bee abundance. The most abundant bee genus was *Lasioglossum* (734 individuals), followed by *Andrena* (497 individuals). *Lasioglossum gotham* and *Andrena milwaukeensis*, the two most abundant bee species, were primarily collected in the canopy.

Bee communities did not differ significantly between canopy and understorey in terms of species richness (q0) or Shannon diversity (q1), but Simpson diversity (q2) was higher in the understorey. The most consumed pollen across both strata and all bee genera was that of canopy-forming *Acer* species. Among bees collected in the canopy, the second-most consumed pollen was sub-canopy *Acer* (mountain maple), while among understorey bees, it was *Quercus*. Most bees carried pure arboreal pollen loads, with a higher proportion of purely arboreal pollen loads observed in the canopy (60.6%) compared to the understorey (49.3%). Bees utilized arboreal pollen throughout the season in both strata, contrary to previous reports suggesting early-season use only. My results demonstrate that arboreal pollen is prevalent in the diets of forest-dwelling bees, despite the availability of herbaceous plants in the understorey, and highlight the significant role of arboreal pollen for wild bees in temperate forests throughout the season. The abundance of bees in the canopy and the extent of tree pollen consumption together show that forest canopies are a key, yet often neglected, dietary resource for wild bees.

2.1 Introduction

Temperate deciduous forests comprise 16% of the world's forested areas (Hansen et al., 2010), supporting a diversity of plants and animals within their two primary layers: the understorey and the canopy. Located beneath the leafy canopy of dominant deciduous hardwoods, the understorey is home to young trees, shrubs, and various herbaceous plants, including spring ephemerals that emerge on the forest floor before canopy leaf-out (Lapointe, 2001). In contrast, the canopy—the uppermost layer—consists of the crowns of mature trees, which in the Northern Hemisphere are typically species of maple (*Acer*), oak (*Quercus*), ash (*Fraxinus*), birch (*Betula*), beech (*Fagus*), and poplar (*Populus*), among others. Once they leaf out, these canopy trees capture most of the sunlight and provide shelter, food, and nesting sites for a diverse fauna (Ishii et al., 2004). The combination of these two layers contributes to biodiversity by providing distinct ecological niches and various resources for the forest's flora and fauna (Cavard et al., 2011).

Despite its significance, there is limited research on the organisms inhabiting the temperate forest canopy. There have been substantial efforts to study the vertical distribution (or stratification) of arthropods in the tropical forest canopy, where findings suggest that the canopy hosts a community at least as diverse as the understorey (Erwin, 1982; Intachat & Holloway, n.d.; Stork & Grimbacher, 2006). In contrast, studies conducted in temperate deciduous forests suggest greater arthropod diversity on the forest floor (Corff & Marquis, 1999; Hirao et al., 2009; Ulyshen & Hanula, 2007). Although wild bees, a subset of arthropods commonly found in forests, may often go unnoticed foraging in the canopy, growing evidence emphasizes their association with this habitat and their use of canopy resources (Allen & Davies, 2022; Harrison et al., 2018; Saunders, 2018; Smith et al., 2021; Splitt et al., 2021; Ulyshen, 2011; Urban-Mead et al., 2021). In the Northern Hemisphere, specifically, some halictid ("sweat") bees may be more common in the canopy than the understorey (Campbell et al., 2018; Ulyshen et al., 2010). Furthermore, a recent investigation found similar bee richness across forest strata but greater diversity in the canopy of deciduous forests, although no bee species was exclusively found in a single stratum (Urban-Mead et al., 2021).

In temperate deciduous forests, most studies focus on entomophilous (insect-pollinated) plants as primary resources for pollinators, even though it is increasingly evident that anemophilous (wind-pollinated) and ambophilous (wind- and insect-pollinated) trees also serve as vital pollen sources for bees (Saunders, 2018). Recent studies confirm that insect pollinators forage on anemophilous trees (Kraemer & Favi, 2005; Kratschmer et al., 2020; MacIvor et al., 2014; Persson et al., 2018; Saunders, 2018; Urban-Mead et al., 2021; Yourstone et al., 2021), and there is an emerging hypothesis that this pollen offers essential macronutrients to pollinators in temperate forests, particularly before the peak of spring flowering of understorey wildflowers (Wood et al., 2018). The nutritional value of anemophilous pollen appears to be high; on average, it contains no less protein than zoophilous pollen (Roulston et al., 2000). While most empirical work surrounding the utilization of anemophilous pollen by bees during early spring has been centred on *Apis mellifera* (honeybees)

(Pearson & Braiden, 1990; Severson & Parry, 1981), there is evidence that some *Bombus* (bumblebees) and solitary bee species can develop successfully on anemophilous pollen (MacIvor et al., 2014; Radmacher & Strohm, 2010). For instance, Radmacher & Strohm (2010) found that larval fitness (measured as cocoon weight) of the solitary bee *Osmia bicornis* did not depend on whether they were provided with anemophilous or zoophilous pollen.

To date, most knowledge about pollen use by wild bees comes from samples taken from nest provisions (Roulston et al., 2000, but see: Russo, 2017; Urban-Mead, 2022), which represent the pollen collected for larval consumption. Additionally, our insights into pollen use are predominantly from cavity-nesting megachilids (*e.g.*, *Osmia*) and eusocial apids (*Bombus* and *Apis*), since nest pollen is more challenging to sample in other taxa (Müller et al., 2006). Yet for adult wild bees of all taxa, pollen is an essential resource. While the sugar obtained from nectar consumption provides the energy adult bees need for foraging, flight, reproduction, and thermoregulation pollen is also ingested for protein and lipids (Vaudo et al., 2016, 2017, 2018, 2020). The nutrients provided by pollen are particularly critical for female bees, supporting egg production and reproductive health. In some species of solitary bees, adult females consume large quantities of pollen shortly after emerging, ensuring they have sufficient egg-laying reserves (Müller et al., 2006). Even though adult bees do not rely on pollen as heavily as their developing larvae, it is nevertheless an integral part of their diet, influencing their health, longevity, and reproductive success (Filipiak, 2018). Furthermore, samples taken from nest provisions or adult female bees' pollen-carrying structures (scopae or corbiculae), which represent external pollen loads, typically reflect the pollen types that female bees collect specifically for larval nutrition. This contrasts with the internal pollen load, which is consumed by female bees for their own nutrition (Crone et al., 2022). For instance, in a study investigating the pollen preferences of bees collected in apple orchards, Russo and Danforth (2017) found that the internal and external pollen loads of *Ceratina calcarata* had different compositions, with *Acer* pollen being found in higher proportions in internal loads compared to external ones. Therefore, more studies comparing internal and external pollen loads are essential to better understand the comprehensive nutritional needs of bees. Yet, we know little about the foraging behaviour and resource use of adult bees, specifically how they navigate the choice between arboreal and understorey pollen sources as these vary seasonally in temperate forests.

Bees' use of different forest strata throughout the season may also be influenced by factors other than the presence or abundance of preferred floral resources. Indeed, bees are known to frequent the canopy layer during mid-summer, even when trees are not in flower (Ulyshen, 2011). Bees have also been observed feeding on alternative food sources like honeydew, sap, and other sugary exudates when floral resources are scarce (Koch et al., 2011; Konrad et al., 2009; Requier & Leonhardt, 2020). These alternative food sources are often more abundant in the canopy than on the forest floor (Moran & Southwood, 1982; Wardhaugh et al., 2006).

Considering the growing appreciation of tree canopies as habitat for bees and our limited knowledge of wild bee diets, in this chapter I address questions about temperate forest canopy use by bees. Specifically, I address the following two main topics:

1. Regarding the bee community:
    a. How does bee abundance vary among bee taxa, between canopy and understorey strata, and among different tree species?
    b. Are there sex-based differences in bee distribution between the canopy and understorey?
    c. Does bee diversity differ between forest strata?
2. About pollen consumption, I ask:
    a. Which pollen taxa are consumed most frequently?
    b. How does consumption of arboreal versus non-arboreal pollen differ among bee taxa, between bees captured in the canopy and the understorey, and throughout the season?

2.2 Material and Methods

2.2.1 Study system

My study was conducted in the eastern temperate deciduous hardwood forests of Gatineau Park, Quebec, Canada (N 45°27’01” W 75°46’58”, 160 m elevation). Gatineau Park is dominated by deciduous trees that lose their leaves in fall, such that maximum amounts of direct solar radiation reach the forest floor in early spring, accounting for over 90% of the total energy received in the understorey (Hutchison & Matt, 1977). In the summer, following tree leaf-out, solar radiation in the understorey decreases to 1–5% of that available above the canopy (Hicks & Chabot, 1985). Spring ephemerals are common in the understorey of deciduous forests, taking advantage of the high irradiance before canopy closure to develop vegetative and reproductive parts each spring.

Table 2-1 Dominant canopy and understorey plant species at Gatineau Park – Trail 66. An asterisk (*) indicates species that flowered in the sampled plots along Trail 66.

Species	Common name	Pollination syndrome	Habit
*Acer saccharum**	sugar maple	Anemophilous (Roussy, 2014)	canopy tree
*Quercus rubra**	red oak	Anemophilous (Manos et al., 2001)	canopy tree
Fraxinus americana	white ash	Anemophilous (Wallander, 2001)	canopy tree
Ostrya virginiana	ironwood	Anemophilous (Kubitzki, 1993)	canopy tree
Betula papyrifera	paper birch	Anemophilous (Kubitzki, 1993)	canopy tree
*Populus deltoides**	eastern cottonwood	Anemophilous (Nagaraj, 1952)	canopy tree
*Acer spicatum**	mountain maple	Zoophilous (Sullivan, 1983)	sub-canopy tree
*Trillium grandiflorum**	white trillium	Zoophilous (Irwin, 2000)	herbaceous
*Anemone americana**	round-lobed hepatica	Zoophilous (Motten, 1982)	herbaceous
*Anemone acutiloba**	sharp-lobed hepatica	Zoophilous (Bernhardt, 1976)	herbaceous
*Erythronium americanum**	trout lily	Zoophilous (Bernhardt, 1977)	herbaceous
*Thalictrum dioicum**	early meadow-rue	Anemophilous (Kaplan & Mulcahy, 1971)	herbaceous

2.2.2 Sampling methods

I focussed on the four most common tree species in our study area: sugar maple, red oak, ironwood, and white ash (Table 2-1). I selected eight plot locations around Trail 66 in Gatineau Park (Figure 2-1), with a minimum distance of 100 m between sites and from any forest edge. These locations have been used for phenological studies involving bees and wildflowers since 2013, with the results published by Sevenello et al. (2020).

Within each plot, I selected one individual of each of the selected tree species, always an adult tree with a height of at least 20 m. The selection was based on individual tree height and the ease of passing a rope between branches. I did not sample individuals where bird nests were present. I sampled eight individuals of each species and 32 individuals in total. Following Urban-Mead et al. (2021), I placed two sets of three coloured insect-collecting pan traps in each tree: one set in the understorey directly beneath the target tree and another in the canopy. In the understorey, the top of the uppermost trap was positioned approximately 1 m above the ground, while the bottom of the lowest trap was positioned 20-30 cm off the ground. All canopy traps were positioned at least 20 m above the ground. Each set of traps consisted of a blue, a yellow, and a white 473 mL Solo ® cup in a randomly established order. Traps placed in the canopy were not visible from the ground, and to prevent visibility from one tree to another, target trees were spaced at least 25 m apart. To place the traps, I launched a rope over an accessible branch using a slingshot; then, the traps were raised using a pulley system (Figure 2-2). Cups were filled with soapy water and left in place for 48 hours. All bees were then strained, placed in plastic bags with 70% ethanol, and stored in a refrigerator until they were pinned and labelled. Sampling started right after snowmelt, occurring twice per week from April 1 to July 10, 2021, when no further bees were caught in either stratum. Bees were identified to species or genus level using available keys and online resources (*Discover Life*, n.d.; Mitchell, 1960; Gibbs, 2011). Specimens that were not dissected were kept as vouchers in the Forrest lab insect collection, and a subset will be deposited at the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC) upon manuscript submission.

Figure 2-1 Map of study sites along Trail 66 in Gatineau Park, indicating the locations of nine sampling sites.

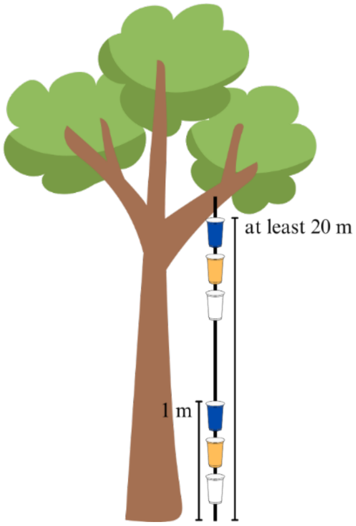


Figure 2-2 Placement of coloured pan traps (blue, yellow, and white) for bee sampling in the canopy and understorey. Canopy traps were set at least 20 m above the ground, while understorey traps were positioned closer to the ground. The order of the trap colours was randomized. Note that the figure is not to scale.

2.2.3 Phenological data

To quantify the temporal availability of floral resources in each stratum, I recorded the phenology of focal trees and surveyed nearby understorey wildflowers. For the trees selected for the pan-trap survey, I documented the first and last day of flowering for each tree during daily site visits. However, because the traps were installed prior to flowering, I did not know whether each tree would flower. As it happened, none of the selected ironwood or ash trees flowered during the study. For the understorey wildflowers, I visited the sites daily until the first flower was recorded for all identified wildflower species. Afterward, I recorded the number of flowering individuals weekly until no more flowers were present. Wildflowers were recorded within the 5 × 5 m plots used by Sevenello et al. (2020).

2.2.4 Bee rehydration and dissection

To determine whether bees are collecting pollen from the tree canopy, whether bees collected in different strata have different pollen consumption patterns, and to detect temporal trends in pollen usage, I dissected and extracted pollen from the guts of 424 female bees, which corresponded to around 25% of the total captured bees. I acknowledge the significance of pollen for male bees as well (Urban-Mead et al., 2022), but since males consume less pollen than females, my study focuses exclusively on female pollen consumption. To ensure a consistent sampling rate of bee specimens for dissection across different weeks, and to preserve as many voucher specimens as possible, I consistently sampled 25% of each bee species collected each week in each stratum using the *dplyr* R package (Wickham et al., 2023) for drawing random samples. Due to some bees lacking sufficient gut pollen or having pollen too degraded for accurate identification, additional bees from certain taxa were randomly chosen to maintain the 25% sample size.

Since bees were dried and pinned to facilitate identification, I rehydrated them before dissection. The rehydration method is described in Ungureanu (1972). Briefly, bees were first immersed in one part liquid dish soap and nine parts distilled water for 10-60 minutes, depending on the size of the bee, with larger bees being immersed for longer. Afterward, bees were immersed in a container with tap water for 20-60 minutes, depending on their size. Bees were then transferred to a Petri dish, where the terga were cut open using microscissors to expose the entire gut. Guts were then isolated, transferred to a 1.5 ml microcentrifuge tube with 70% ethanol, and kept in a refrigerator to preserve the gut until pollen extraction.

2.2.5 Pollen extraction and identification

To dislodge pollen grains from the gut, I used a metal pick to tear the gut in the microcentrifuge tube until the contents appeared dusty. Then, the tubes were centrifuged for 10 minutes at 4000 rpm. Two drops of the suspension were then transferred to a microscope slide using a pipette. After alcohol and water evaporation, I stained the pollen grains using one or two drops of Safranin-O (Jones, 2012).

For each bee, I examined ten random locations on the microscope slide at 200× magnification, identifying a total of 300 pollen grains per slide to the genus level when possible, or otherwise to the family level. Although rarefaction curves suggested that 100 grains would be sufficient for most samples, I chose to analyze 300 grains to ensure that no pollen types were missed, and to facilitate comparison with existing literature (Russo & Danforth, 2017). However, because some bees had little pollen in their guts, it was sometimes impossible to identify 300 pollen grains in just ten fields of view. For those individuals, I selected additional random locations in the slide until 300 pollen grains were identified. Only individuals with at least 100 pollen grains were included in the analysis. Therefore, I excluded two individuals that did not meet this requirement.

Pollen identification was conducted by comparison to a pollen library developed by collecting pollen from all the flowering tree and understorey plant taxa in the area from April 10 to July 15, 2021. Identified pollen was categorized as either "arboreal" or "non-arboreal" based on the plant's growth form, to facilitate ecological interpretation. Pollen grains identified as *Acer* after May 19 were considered to belong to the sub-canopy species *A. spicatum* (mountain maple), as larger *Acer* trees were no longer flowering after that date, while sub-canopy *Acer* began flowering in late May; therefore, they were categorized as "non-arboreal".

2.2.6 Statistical analysis

2.2.6.1 Bee abundance by stratum, tree species, and sex

To evaluate whether the abundance of bees and the presence of specific taxa differ between the canopy and understorey strata, as well as across tree species, I fitted Bayesian models with abundance as the response variable, and stratum and tree species as the predictors. Abundance was determined by tallying the number of bees captured in each tree over the course of the season. The analysis was structured into three main categories of models (Table 2-2):

1. Strata and tree species comparison: This model compares the differences in bee abundance between strata, among tree species, and between strata within tree species.
2. Bee species comparison: This model compares the differences in bee abundance among bee species, and between strata within bee species. Given the small sample size of some bee species, this analysis was conducted only for species with a total number of individuals higher than 15.
3. Sex stratification: Given the small sample size for other taxa, I applied this model only to *Andrena* (the most abundant genus), to assess sex differences in distribution between canopy and understorey.

Models included group-level effects (analogous to random effects) to capture variability across plots and tree species, and within specific trees in particular plots. Details of all model structures and their distributions are provided in Table 2-2 and Tables S16-S20.

2.2.6.2 Bee diversity by stratum

To compare species diversity between the canopy and understorey (over the entire sampling season), I used the iNEXT package in R (Chao et al., 2014; Hsieh et al., 2023) to calculate species richness (q0; i.e., Hill number q = 0), the exponential of Shannon diversity (q1), and Simpson diversity (q2). The q0 index provides an unweighted count of the number of species. The q1 index weights more heavily towards common species while still accounting for rare species, while the q2 index weights the most abundant species even more heavily, thus reducing the influence of rare species in the diversity calculation. After calculating all indices for each stratum within each plot, I fitted Bayesian models where q0, q1, and q2 served as response variables, with stratum as the explanatory variable. Given the variation in the number of bees collected across samples, I standardized the comparison by fixing the endpoint at 26 individuals for rarefaction. This endpoint, based on the minimum observed sample size, ensured that the extrapolations remained within a reasonable and comparable range across all plots. Differences between strata were then evaluated using the 95% Bayesian Credible Interval (BCI) of the difference between estimates, where a 95% BCI overlapping zero indicates insufficient evidence to support a difference different than zero.

2.2.6.3 Pollen consumption

I fitted hierarchical Bayesian models to explore variation in arboreal pollen consumption by bees, considering factors such as stratum, bee genus, and time period. Specifically, I fitted five main types of models: 1) Pollen consumption by plant taxon: to assess whether certain pollen taxa were consumed more frequently than others; 2) Pollen load type by stratum: to evaluate whether the proportions of bees carrying pure-arboreal, non-arboreal, or mixed pollen loads differed between the canopy and understorey; 3) Seasonal changes in pollen load type: to examine how the proportions of bees carrying different pollen load types changed throughout the season. All models are detailed in Table 2-2 and Tables S16-S20.

For all models, I used flat priors and obtained the parameters' posterior distributions using the Hamiltonian MC method (Betancourt & Girolami, 2013) by running four different chains with different start values for a minimum of 3,000 iterations, adopting a warm-up of 1,000 iterations. I monitored convergence by calculating Rubin's R (Gelman & Rubin, 1992), where convergence is assumed when R is close to 1. By sampling from the posterior distribution, I computed the mean and Bayesian credible intervals (BCI) for all parameters using 3,000 draws from the posterior. All analyses were performed using the package brms (Bürkner, 2017), which allows Stan to be run in R (R Core Team, 2023).

Table 2-2 Model structures used in the analysis. Group-level effects vary by model and capture different sources of variability: ~plot captures variability across different plots; ~tree species captures variability across different tree species; ~tree*plot captures variability due to individual trees within particular plots; ~bee species captures variability due to specific bee species within the same genus.

Model structure	Distribution	Response variable	Data	Goal
Bee community				
y ~ stratum of capture	Gaussian	q0, q1, and q2.	Diversity metrics	To assess the differences in diversity between canopy and understorey
y ~ stratum of capture * tree species + (1| tree*plot)	Zero Inflated Negative Binomial	Bee abundance	All bee species included	To estimate the difference in bee abundance among tree species and between strata
y ~ bee species + bee species:stratum of capture + (1| tree*plot)	Negative Binomial	Bee abundance	Only bee species with more than 15 individuals	To assess the differences in abundance among bee species
y ~ sex + (1|tree*plot)	Bernoulli	Binary (1 for canopy, 0 for understorey)	*Andrena* bees	To assess the differences in distribution between sexes in relation to the canopy and understorey strata
Pollen consumption				
y ~ pollen taxon + stratum of capture:pollen taxon + (1|tree*plot)	Zero Inflated Negative Binomial	Counts of pollen grains for each pollen taxon	*Andrena*, *Lasioglossum*, and *Augochlora pura*	To assess whether certain pollen taxa were consumed more frequently than others, and whether this differs between bees caught in the canopy vs. understorey
y ~ stratum of capture + s(day of the year)	Multinomial Bayesian GAM: smooth(day of year)	Categorical – pollen load type: pure-arboreal, non-arboreal, and mixed (arboreal and non-arboreal)	1) All bee species combined 2) Separate models for *Andrena*, *Lasioglossum*, and *Augochlora pura*	To assess differences between the canopy and the understorey in the proportion of captured bees carrying a certain pollen load type, and to assess whether the proportions of bees carrying a certain type of pollen load changed throughout the season

2.3 Results

2.3.1 Bee abundance and diversity by stratum, tree species, and sex

During the study period, I captured a total of 1,413 bees, with 940 (66.5%) found in the canopy and 473 (33.5%) in the understorey (Table 2-2). The bees were predominantly generalists, with the exception of a few cleptoparasitic species. Individuals from most of the commonly captured taxa were predominantly found in the canopy (Table 2-2). *Lasioglossum* was the most abundant bee genus, with 734 individuals captured, followed by *Andrena* with 497 individuals. At the species level, *L. gotham* was the most common with 299 individuals, followed by *A. milwaukeensis* with 195 individuals. All were primarily caught in the canopy.

Total bee abundance was greater in the canopy than in the understorey, with an average of 30.3 ± 24.3 bees per tree in the canopy compared to 16.0 ± 13.9 bees per tree in the understorey. The 95% BCI of the difference between these estimates indicates a stronger positive effect of the canopy on bee abundance compared to the understorey (Table 2-3, Table 2-3, Figure 2-3).

No *Andrena* species were more abundant in the understorey, with *A. mandibularis*, *A. milwaukeensis*, and *A. rugosa* being more abundant in the canopy (Figure 2-2). For the other *Andrena* species, there was not enough evidence to support a difference in abundance between the strata. *Augochlora pura* and *Lasioglossum gotham* were also more abundant in the canopy, while *Lasioglossum admirandum* was more abundant in the understorey. For the remaining *Lasioglossum* species, there was not enough evidence to support a difference between the strata. Overall, when analysing bee abundance per tree, *Lasioglossum gotham* was the most abundant species, with *L. gotham* being the most abundant in the canopy and *Lasioglossum versans* being the most abundant in the understorey (Tables S2–S4).

Bee diversity did not differ between the canopy and the understorey in terms of species richness (q0) or Shannon diversity (q1), as indicated by 95% BCIs of the differences overlapping zero ([−0.3, 3.6] and [−0.0, 3.5], respectively). However, Simpson's diversity (q2) was higher in the understorey (95% BCI of the difference [0.1, 3.0]).

There were no differences in bee abundance among the different tree species, whether considering canopy and understorey strata together or examining the canopy or understorey separately. The 95% BCIs of the differences in bee abundance all overlapped with zero, indicating insufficient evidence to suggest a difference (Table 2-4, Table S1). Finally, *Andrena* males and females exhibited similar probabilities of being associated with the canopy (females: 83.2% [75.0%, 89.1%], males: 78.6% [69.0%, 87.1%], 95% BCI of the difference in logit: −0.2 [−0.7, 0.3]).

Table 2-4 Bee abundance by genus and species in the canopy and understorey, with totals for each genus and each stratum; followed by the 95% BCIs of the difference in the estimated effect of stratum on bee abundance, expressed as log-count differences between the canopy and understorey. Positive values indicate that bee counts are higher in the canopy compared to the understorey, while negative values suggest lower bee abundance in the understorey relative to the canopy. Asterisks (*) denote estimates where the credible interval does not overlap zero, indicating a significant difference in abundance between strata for that taxon.

	Canopy	**Understorey**	**Total**	**95% BCI of the difference**
Andrena	**383**	**114**	**497**	**-**
carlini	11	7	18	0.5 [-0.6, 1.6]
crataegi	4	2	6	-
dunningi	64	40	104	0.5 [-0.0, 1.1]
imitatrix	1	0	1	-
mandibularis	100	35	135	1.2 [0.6, 1.7]*
milwaukeensis	172	23	195	2.1 [1.5, 2.7]*
nasonii	1	0	1	-
robertsonii	1	0	1	-
rugosa	29	7	36	1.6 [0.7, 2.6]*
Augochlora pura	**101**	**33**	**134**	1.0 [0.4, 1.5]*
Bombus	**6**	**4**	**10**	**-**
borealis	1	0	1	-
impatiens	4	4	8	-
rufocinctus	1	0	1	-
Ceratina	**3**	**3**	**6**	**-**
Colletes	**0**	**2**	**2**	**-**
Hylaeus	**3**	**0**	**3**	**-**
Lasioglossum	**430**	**304**	**734**	**-**
admirandum	25	63	88	-0.9 [-1.5, -0.3]*
coriaceum	26	33	59	-0.2 [-0.9, 0.4]
gotham	225	74	299	1.1 [0.6, 1.6]*
nigroviride	45	29	74	0.3 [-0.3, 0.9]
versans	71	91	162	-0.1 [-0.6, 0.4]
zonulum	11	7	18	0.5 [-0.5, 1.6]
sp.	27	7	34	-
Nomada	**10**	**12**	**22**	**-**
Osmia	**2**	**1**	**3**	**-**
Sphecodes	**2**	**0**	**2**	**-**
Total	**940**	**473**	**1413**	**-**

Table 2-5 Mean bee abundance and standard deviation by tree species for canopy and understorey, followed by the 95% BCIs of the difference between the estimates for canopy and understorey. Positive values indicate a canopy's favorable influence, where bee counts exceed those in the understorey. Sample size (number of trees) is shown in parentheses.

	Mean± SD		
	Canopy	**Understorey**	**95% BCI of the difference**
Overall (32)	30.3±24.3	16.0±13.9	20.1 [7.5, 53.5]
Ironwood (8)	25.3±22.7	14.5±14.9	1.8 [1.1, 3.2]
Oak (8)	35.7±28.4	18.5±14.2	1.7 [1.1, 2.8]
Sugar maple (8)	24.2±18.8	13.9±17.8	2.0 [1.2, 3.5]
White ash (8)	36.8±27.5	15.8±5.5	2.7 [1.5, 4.8]

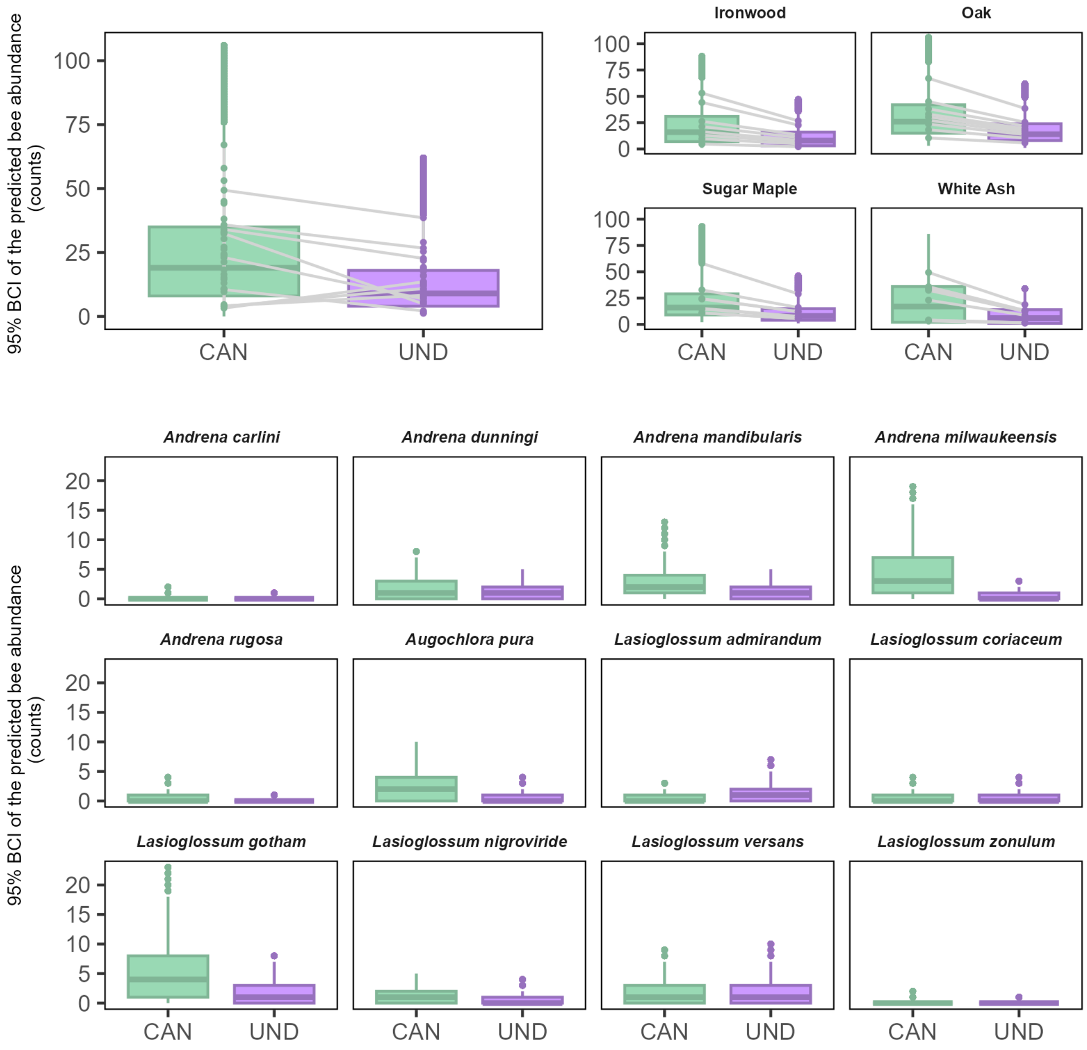


Figure 2-3 Boxplots representing the 95% posterior distribution of the predictions for bee abundance in the canopy and understorey for the overall data, each tree species, and each bee species. Bee species not represented in the graph were excluded due to low sample size. Grey lines and points on the top panels represent the mean predicted values for each site.

2.3.2 Pollen consumption

I dissected the gut region of a total of 424 bees. Of those, 73 (17.2%) were removed from analysis because they had pollen that was too degraded for reliable identification, and 32 (7.5%) had too few pollen grains. Therefore, 319 (75.2%) bees remained for analysis. I identified seven different pollen types in the bee gut dissections: *Acer,* Betulaceae (most likely *Ostrya virginiana* or *Betula papyrifera*), *Quercus*, *Anemone*, Asteraceae, *Thalictrum*, and *Trillium.* Most bees contained only a single pollen type in their digestive tracts, with none having more than three types (Table S6).

The most-consumed type of pollen overall was canopy-forming *Acer*. Among bees collected in the canopy, the most-consumed pollen came from canopy-forming *Acer*, and the second most consumed pollen was understorey *Acer*. For bees collected in the understorey, the most consumed pollen was again canopy-forming *Acer*, and the next most common pollen type was *Quercus*. This pattern held true for all bees considered as a group, and for *Andrena* and *Lasioglossum* bees considered separately (Tables 2-S7 to 2-S18).

Proportion of bees carrying different pollen load types compared between strata – Across all bee species, the proportion of bees carrying pure arboreal pollen loads was higher in the canopy (60.6%, 95% BCI: [47.0, 71.0]) compared to the understorey (49.3%, 95% BCI: [33.4, 71.1]). No differences in the proportion of bees carrying pure arboreal pollen loads were observed between the canopy and understorey for individual bee taxa (*Andrena*, *Lasioglossum*, or *Augochlora pura*). Similarly, there were no differences in the proportions of bees carrying mixed or pure non-arboreal pollen loads between strata for all bee species combined, or for any of the genera or species separately (Figure 2-4).

Difference between arboreal vs. non-arboreal pollen grain counts per bee - For all bee species combined, arboreal pollen counts per bee were higher than non-arboreal pollen counts across both strata (Table 2-5). In the canopy, bees carried an average of 81.3% arboreal pollen grains compared to 18.7% non-arboreal grains. In the understorey, the averages were 73% arboreal and 27% non-arboreal pollen grains. *Lasioglossum* and *Andrena* bees consistently contained more arboreal than non-arboreal pollen, regardless of the stratum in which they were captured; however, for understorey *Andrena*, this trend was weak (Table 2-5).

Pollen loads over time – There is strong evidence that the proportions of bees carrying specific types of pollen loads shifted significantly throughout the season, with the effect of the smooth function of Day of Year (s(DOY)) not overlapping zero. Specifically, the proportion of bees carrying purely arboreal pollen loads changed throughout the season and varied among strata and bee genera (Figure 2-5). For all *Andrena* bees combined, the proportion of those carrying a pure arboreal load increased from the beginning of the season until around May 7, then decreased until around May 20, and then increased again around May 30. Canopy and understorey *Andrena* displayed different patterns: in the canopy, pure arboreal pollen loads increased until around May 10 and then declined, while in the understorey, the proportion of bees carrying pure arboreal pollen loads rose continuously from the beginning of the season until the end. For both strata combined, non-arboreal pollen loads peaked at the beginning of the season and again around May 23. Canopy and understorey bees exhibited this same pattern for pure non-arboreal pollen loads. For mixed pollen loads, the proportion of bees increased as the season progressed in the canopy but decreased in the understorey.

For *Lasioglossum* bees, considering both strata combined, purely arboreal pollen loads peaked around May 15. Non-arboreal pollen loads showed three peaks: around April 25, May 23, and the end of the season. For mixed pollen loads, the proportion increased towards the end of the season, with the same pattern observed in both canopy and understorey bees. For *Augochlora pura*, both arboreal and mixed pollen loads increased towards the end of the season, while non-arboreal pollen loads decreased. There was not enough data to analyze the pattern in the understorey for *Augochlora* bees.

The observed peaks in pollen types closely relate to the flowering times of the local plant species. Arboreal pollen peaks during the flowering periods of *Acer* (mid-April to late May) and *Quercus* (early May to mid-June). Non-arboreal pollen peaks align with the flowering periods of herbaceous species like *Trillium* and *Thalictrum*, with peaks in late April to early May and from late May to July. Mixed pollen types show peaks corresponding to the overlapping flowering periods of multiple species, with peaks in late April to early May and late May to July.

Table 2-6 95% BCIs of the estimated pollen percentages calculated from the counts for each pollen type (arboreal vs. non-arboreal), followed by the mean and 95% BCI of the difference between estimates. Separate models were fit for the combined strata, the canopy, and the understorey. Positive values for the difference indicate higher values for arboreal pollen percentages. Asterisks (*) represent estimates where the credible interval does not overlap zero, indicating a significant difference between pollen types in pollen percentages per bee.

	Arboreal	**Non-Arboreal**	**Difference**
		All bees	
Both strata	71.2 [52.1, 82.2]	28.8 [17.8, 47.9]	42.4 [24.3, 64.5]*
Canopy	75.7 [50.1, 82.3]	24.3 [17.7, 49.9]	51.4 [32.3, 74.1]*
Understorey	63.7 [36.2, 82.2]	36.3 [17.8, 63.8]	27.3 [0.9, 56.5]*
		Andrena	
Both strata	69.3 [44.4, 82.3]	30.7 [17.7, 55.6]	38.6 [14.5, 67.4]*
Canopy	73.5 [34.2, 83.6]	26.5 [16.4, 65.8]	47.0 [13.4, 72.8]*
Understorey	56.8 [8.2, 79.1]	43.2 [20.9, 91.8]	13.6 [-62.0, 64.4]
		Lasioglossum	
Both strata	69.2 [50.7, 79.1]	30.8 [20.9, 49.3]	38.4 [19.7, 57.3]*
Canopy	73.4 [39.7, 81.6]	26.6 [18.4, 60.3]	46.8 [21.3, 66.1]*
Understorey	64.7 [38.5, 74.8]	35.3 [25.2, 61.5]	29.4 [0.1, 49.5]*

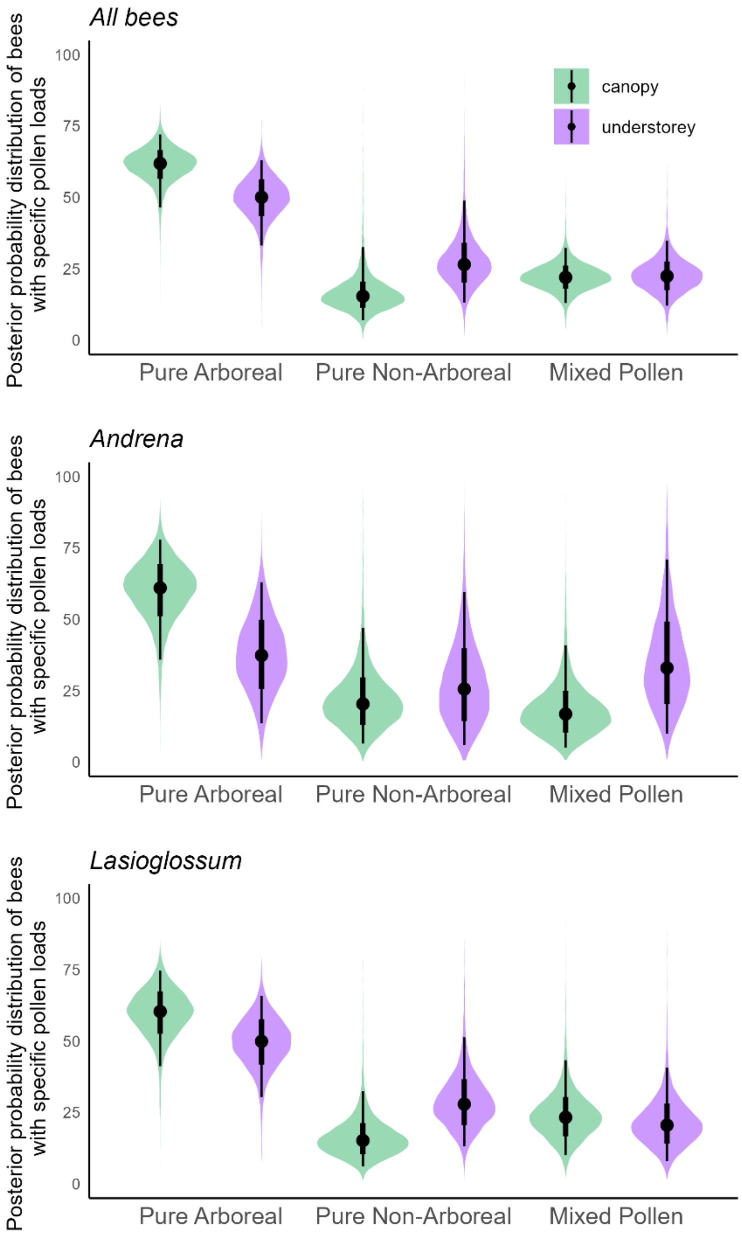


Figure 2-4 Posterior probability distributions for the proportions of bees carrying a pure arboreal, a pure non-arboreal, or a mixed pollen load, for bees collected in the canopy (green) and understorey (purple). There was not enough data to produce accurate estimates for *Augochlora pura*.

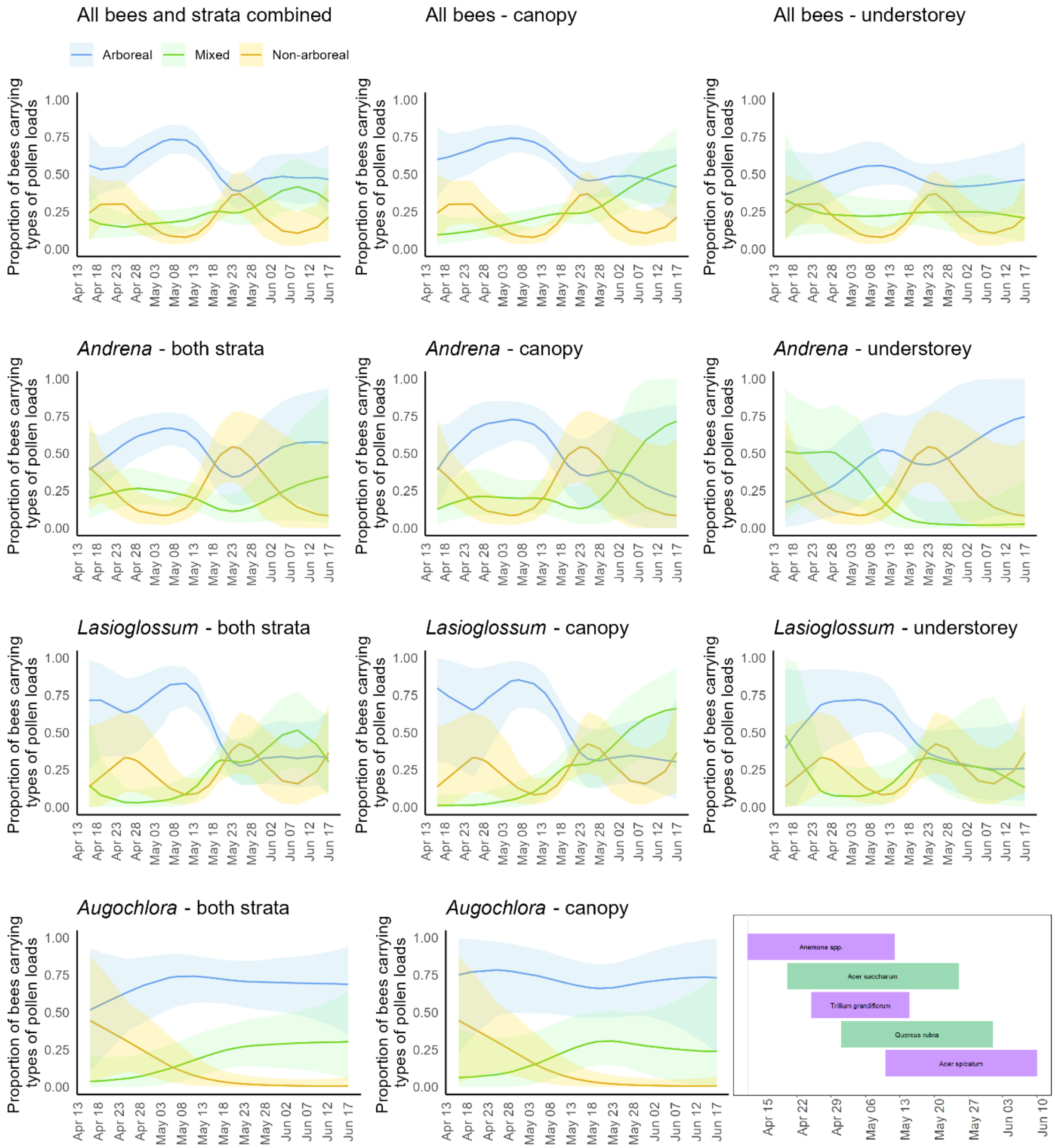


Figure 2-5 Proportion of bees carrying different types of pollen loads (arboreal, mixed, and non-arboreal) over the day of the year (DOY) for different data subsets. The top row shows results for all bees combined, with separate plots for both strata, canopy, and understorey. The second row presents the same for the genus *Andrena*, and the third for the genus *Lasioglossum*. The fourth row shows the results for the species *Augochlora pura*, with data for both strata and canopy alone. Each line represents the fitted model results with 95% Bayesian Credible Intervals (BCIs) shaded around them. The models were fitted using Bayesian regression with the smooth function of DOY (s(DOY)) as the predictor variable, indicating the proportion of bees carrying each type of pollen load over time. The bars represent the duration of flowering for each species.

2.4 Discussion

My study shows that the deciduous forest canopy hosts an abundant and diverse community of bees in spring. In fact, I found almost twice as many bees in the canopy as the understorey, and a similar diversity and richness, suggesting that studies that do not include this stratum are underestimating the size of the forest-dwelling bee community. Furthermore, my study makes clear that trees are an important pollen source for wild bees, and that spring-flying bees of this temperate deciduous forest study area—even bees found predominantly in the understorey—consistently consume arboreal pollen. Arboreal pollen forms a substantial part of bee diets, and a high proportion of bees in both strata have a purely arboreal pollen load. Furthermore, arboreal pollen consumption remains high throughout the entire season in which bees are active in this habitat, reinforcing the critical role trees play as a pollen source for wild bees in temperate deciduous-forest ecosystems.

Bee community – My study extends previous work by focusing on the deciduous forest ecosystem at more northern latitudes, currently underrepresented in the existing body of research. While there is some overlap with previous findings, there are also distinct differences.

First, I observed a greater abundance of bees in the canopy than in the understorey, consistent with findings from a bottomland hardwood forest in Georgia, USA (Ulyshen et al., 2010). This contrasts with observations from deciduous forests in New York, USA, where similar bee abundances were found in both strata (Urban-Mead et al., 2021). Additionally, higher overall bee abundance was found in the understorey of woodlands within an English agricultural landscape (Allen & Davies, 2022), and temporal changes in bee communities were noted in forest fragments in Massachusetts, USA, where abundance and richness were initially higher in the understorey but increased in the canopy as the season progressed (Cunningham-Minnick et al., 2022).

Second, I found that bee diversity in terms of species richness (q0) and Shannon diversity (q1) was similar between strata. However, Simpson's diversity (q2) was higher in the understorey, aligning with the results from Georgia (Ulyshen et al., 2010). On the other hand, Urban-Mead et al. (2021) observed higher diversity in the canopy than in the understorey in New York, USA. Third, to my knowledge, no other study has evaluated how tree identity influences the abundance of bees caught in deciduous temperate forests. I found that no tree was associated with higher bee abundance, suggesting that it is possible that the bees were primarily foraging on *Acer* and *Quercus* trees. However, because the trees were closely intermingled, well within the typical flight distance of bees, I was able to sample similar numbers of bees in association with all tree species. Additionally, it is important to note that two species, *Fraxinus* and *Ostrya*, were not flowering during the sampling period.

Generalizing the interactions between the bee community and forest strata is challenging, as evidenced by varying results from other studies comparing bee abundance and diversity between canopy and understorey in deciduous temperate forest ecosystems. Differences in bee communities between forest strata can be influenced by a variety of factors, including the forest's ecological characteristics, geographical setting, available floral resources, presence of invasive species, and forest management practices. For example, Cunningham-Minnick & Crist, 2020 observed that the presence of the invasive shrub *Lonicera maackii* boosts understorey bee abundance during and after bloom by providing floral resources. Additionally, forest management practices such as prescribed fire and mechanical thinning have been shown to influence bee diversity and abundance in the understorey by promoting more diverse and abundant herbaceous plant growth and better nesting conditions (Campbell et al., 2018). Furthermore, the removal of another invasive, Chinese privet (*Ligustrum sinense*) in hardwoods in northeastern Georgia, U.S.A., was associated with a significant increase in bee abundance, richness, and diversity in the understorey, but did not affect bee populations in the canopy, suggesting that canopy bee communities may be relatively unaffected by understorey vegetation management (Ulyshen et al., 2020).

Moreover, forest types can differ from one another in bee community composition and abundance in both the canopy and the understorey. For example, a study compared a pine forest that was characterized by continuous blooming of understorey plants like *Vaccinium* and *Calluna vulgaris*, with oak and oak-hornbeam forests within the Wielkopolski National Park, which lack these plants in their understorey. The authors found that pine forests support diverse bee populations over a more extended period due to the prolonged availability of floral resources, leading to multiple peaks in bee activity throughout the season. In contrast, the oak and oak-hornbeam forests, which typically offer a single major peak of floral resource availability in spring, show a corresponding single peak in bee activity (Bąk-Badowska, 2012; Banaszak & Cierzniak, 1994). Although these studies did not directly assess differences between bee communities in the canopy versus the understorey, they help us identify factors that could influence spatial and seasonal variation in forest bee communities.

Why do bees visit the canopy? - Interestingly, while previous studies report *Augochlora pura* as a species associated with the canopy of eastern North American deciduous forests (Urban-Mead et al., 2021; Ulyshen et al., 2010), I found that its abundance in the canopy in relation to the understorey, although still high, was not as high as that of other species, such as *Andrena milwaukeensis*, or *Lasioglossum gotham*. The presence of *Augochlora pura* in the canopy might be attributed to its preference for nesting in dead wood (Stockhammer, 1966). In contrast, both *Andrena* and *Lasioglossum* are soil nesters. Therefore, my finding of *Andrena* exhibiting their greatest abundance in the canopy is surprising. In my study, this genus carried high amounts of arboreal pollen, mainly *Acer* and *Quercus*; greater abundance of pollen in the canopy could explain its presence in the canopy. The same explanation could be applied to the presence of ground-nesting

Lasioglossum in the canopy, as all *Lasioglossum* species sampled in my study had high consumption of arboreal pollen. Furthermore, given the high proportion of pure arboreal pollen loads, even among bees caught in the understory, it is possible that bees, regardless of their nesting mode, move between collecting pollen in the canopy and pollen and nectar in the understory. I also captured cleptoparasites in the canopy, even though these species do not collect pollen for their larvae. Because there is a lack of studies assessing the use of pollen by cleptoparasitic bees for their own nutrition, it is possible that they were collecting pollen for this purpose. As the season progresses and canopy flowering ceases, bees would need to forage in the subcanopy—e.g., on *Acer spicatum*, which flowers later and also provides nectar (Sullivan, 1983)—or in the understorey where nectar-producing herbs are available.

Contrary to earlier reports, my findings did not reveal differences in vertical stratification by sex; I observed similarly high proportions of *Andrena* males and females within the canopy. While Urban-Mead et al. (2021) reported a higher presence of *Andrena* males in the canopy, Allen and Davis (2022) found that the sex ratio of the most abundant species in their surveys, *Bombus pratorum*, skewed towards more females in the canopy than in the understorey. Male bees do consume pollen, albeit in smaller quantities than females (Urban-Mead et al., 2022). Therefore, it is possible that these males were visiting the canopy in search of pollen. However, more studies examining the contents of male guts are needed to address this question, as I did not dissect the guts of male bees.

Differences in pollen consumption among bee taxa - I found no bees with pollen from more than three plant taxa in their guts, despite sampling 300 pollen grains per bee; most bees contained only a single pollen type. In one of the few other studies examining pollen in the guts of wild bees, the authors found that most female *Andrena* bees had five or more pollen types in their guts (Urban-Mead et al., 2022). In the Russo & Danforth (2017) study cited above, the authors found that *Ceratina calcarata* bees carried, on average, 2.9 ±0.99 different types of pollen (genus or family level) in their guts. However, although the latter study reported a higher mean number of pollen types per individual than what I observed, the total number of pollen types identified across all bees combined was similar to my findings: they recorded only five different plant taxa in the internal pollen loads of *Ceratina calcarata*, with *Acer* being predominant. In the Urban-Mead et al. study, bees were sampled in forest edges and orchards, both in the canopy and understorey. In the Russo & Danforth study, bees were sampled in flowering apple orchards surrounded by forest patches. Thus, both previous studies were conducted in a more diverse ecosystem than the enclosed forest interior, which could have contributed to the higher pollen richness in bee guts. Furthermore, my study site is located farther north than the previous studies, in an ecosystem that is, on average, less diverse. Despite the lower diversity per individual in this study, bees in both strata collectively consumed pollen from almost all available flowering plants during the spring flowering period in Gatineau Park. Given that the bee species I collected are dietary generalists, it is unsurprising that they opportunistically utilize the pollen sources that are most available in their environment.

Most studies on bees' use of arboreal pollen focus on the pollen collected for larval consumption, often showing that bee taxa—including *Osmia* and *Andrena*—collect pollen from trees like *Acer* and *Quercus* (Kraemer & Favi, 2005; Kratschmer et al., 2020; MacIvor et al., 2014; Persson et al., 2018; Saunders, 2018; Urban-Mead et al., 2021; Yourstone et al., 2021). However, since internal pollen loads serve different nutritional purposes than external ones, arboreal pollen may be consumed more by bees than what external pollen loads or nest provisions suggest as found by Russo & Danforth (2017), highlighting the need for further studies comparing internal and external pollen loads.

My results reinforce the idea that bees rely heavily on arboreal pollen in temperate forests in spring. On average, 60.6% of the bees in the canopy and 49.3% in the understorey carried a purely arboreal pollen load. These findings strongly suggest that trees are a critical pollen source for bees, even in the understorey, where one might expect herbaceous plants to dominate. Arboreal pollen was the most commonly consumed pollen type across the entire season, remaining a major resource for bees from early to late spring, perhaps because of the large quantities of pollen that can be provided by a single flowering canopy tree (Donkersley, 2019). The proportion of bees carrying purely arboreal pollen was consistently high in both canopy and understorey strata, with peaks early in the season and sustained high levels through mid-season. In the canopy, bees predominantly carried purely arboreal pollen loads, with proportions peaking around April 30 and remaining high throughout the season. In the understorey, the proportion of bees carrying purely arboreal pollen loads steadily increased throughout the season, reaching a peak towards the end. This trend reveals a strong connection between bees and trees in temperate forests and indicates the need for more study to understand when and how much arboreal pollen is available and how nutritionally suitable it is for bees—something I did not quantify in this study. This information could greatly impact how we work to conserve bees in these forests, and other environments.

Study limitations - One caveat of this study is that the use of pan traps may bias the types and numbers of bees captured: certain species are more susceptible to capture by these traps, and bees may be more attracted to traps during periods of floral scarcity, when they may mistake the traps for flowers (Portman et al., 2020; Westerberg et al., 2021). Moreover, sampling conducted over a single year might not capture typical ecological conditions, with factors like unusual weather or climatic anomalies potentially skewing results by affecting plant phenology and bee activity. For example, in the year bees were sampled, *Ostrya* and *Fraxinus* did not flower. Also, common understorey wildflowers such as *Erythronium* and *Sanguinaria* flowered in low numbers. Despite these limitations, my study provides new insight into the pollen consumption and foraging patterns of bees in temperate forests, highlighting the significant role of arboreal pollen, which has been less documented in the literature.

Future directions – The fact that bees collect large quantities of pollen from plants typically considered wind-pollinated could have implications for plant reproduction and pollination ecology. Insect visitation to anemophilous or ambophilous plants could enhance pollen dispersal to conspecific stigmas, potentially increasing the chances of successful pollination compared to wind pollination alone (Saunders, 2018). However, more species-specific studies are needed to determine whether foraging bees in fact move between male and female flowers of trees with unisexual flowers (like most of those in my study), and whether they move between trees, to determine their role in pollination (Whitehead, 1969). If bees are visiting canopy trees primarily for pollen (rather than nectar), visits to female inflorescences, which do not produce pollen, may be unlikely. Additionally*,* some insects might act as pollen thieves, consuming pollen without effectively transferring it, thereby reducing pollen availability for wind pollination (Larson et al., 2001). These factors highlight the need for more studies to better understand the link between anemophilous pollen consumption and the reproduction of ostensibly wind-pollinated species.

Additionally, climate change could threaten the synchronization between bees and the trees they rely on for pollen. As temperatures rise and seasons shift, bees and trees may track different environmental triggers, such as temperature and photoperiod, or respond to these triggers at different rates, leading to potential mismatches (Sevenello et al., 2020). Moreover, it is well-documented that wildflowers and trees respond to temperature changes at different rates (Alecrim et al., 2023; Heberling, Fridley, et al., 2019; Miller et al., 2023). This highlights the need for continued research to elucidate how climate change will influence the complex relationships between trees, wildflowers, and their pollinators.

Moreover, understanding the importance of trees for bee conservation can guide several practical applications. In simplified landscapes, such as agro-ecosystems, recognizing the role of trees can lead to more effective conservation strategies, as trees and hedgerows provide a stable and dense source of pollen and nectar, supporting efficient foraging by bees (Donkersley, 2019). Additionally, these woody habitats offer essential shelter and nesting sites, making them crucial for pollinator conservation and overall ecosystem health. Conversely, in urbanized landscapes, flowering trees are particularly crucial for urban wild bees in early spring when few herbaceous plants are blooming, with much of the early-season pollen collected by bees coming from wind-pollinated trees like *Quercus* and *Acer* (Casanelles-Abella et al., 2021; Splitt et al., 2021, Ballare et al., 2019; Birdshire et al., 2020; Fischer et al., 2016; Fortel et al., 2014; Sivakoff et al., 2018).

Conclusion - In this study of wild bees in temperate deciduous forests, I show that bees make heavy use of arboreal pollen during the whole spring season. I found a surprising number of ground-nesting bees foraging in the forest canopy and confirmed *Quercus* and *Acer* as important pollen sources for wild bees. The abundance of bees in the canopy indicates that omitting this stratum in bee community studies could overlook a significant portion of forest-dwelling bees, and future studies on wild bees in forest ecosystems should not ignore the canopy. Furthermore, the differences between my study and others show that we need more studies comparing bee communities between canopy and understorey—across tree species, forest types and geographical ranges—and testing the influence of varied sampling techniques to facilitate more consistent and comparable studies in the future.

2.5 Supplementary Information

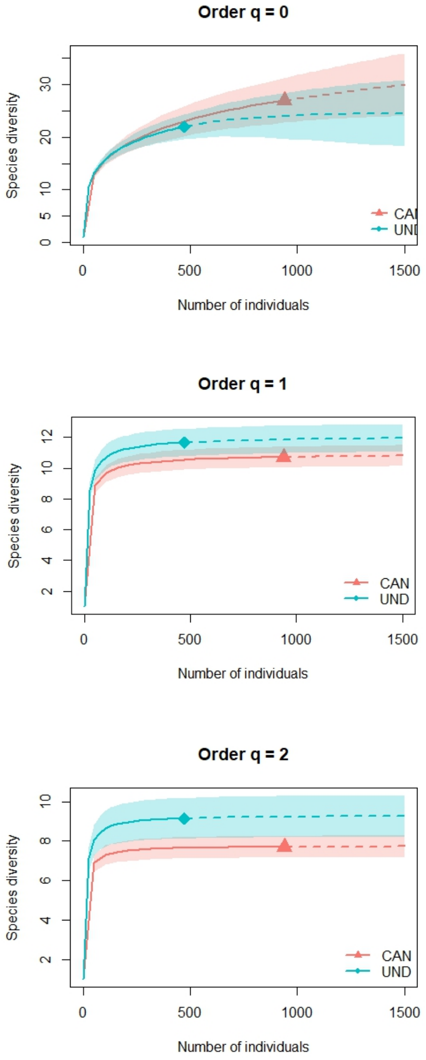


Figure 2-S1: Bee diversity curves for species richness (q0), the exponential of Shannon entropy (q1), and the inverse of Simpson's concentration index (q2), respectively. Each curve shows species diversity relative to the number of individuals sampled in the canopy (CAN) and understorey (UND), and the shaded areas represent the 95% confidence intervals for the diversity estimates.

Table 2-S1: 95% Bayesian Credible Intervals (BCIs) for the estimated differences in bee abundance among tree species. The estimated differences were determined by subtracting the log-abundance of bees associated with one tree species from another, using 3,000 samples from the posterior distribution for each species. A positive difference indicates a higher abundance in association with the row species, while a negative difference indicates lower abundance. Values overlapping zero indicate insufficient evidence to support a significant difference between species. Here, all 95% BCIs overlap zero, suggesting little difference in abundance between these tree species.

		Oak	**Sugar maple**	**White ash**
Overall	**Ironwood**	-0.4 [-1.4, 0.5]	0.2 [-0.9, 1.1]	0.7 [-0.4, 1.9]
	Oak	-	0.6 [-0.4, 1.5]	1.1 [-0.0, 2.2]
	Sugar maple	-	-	0.6 [-0.5, 1.7]
Canopy	**Ironwood**	-0.3 [-1.4, 0.6]	0.1 [-0.9, 1.2]	0.6 [-0.6, 1.8]
	Oak	-	0.5 [-0.5, 1.5]	0.9 [-0.3, 2.1]
	Sugar maple	-	-	0.4 [-0.7, 1.6]
Understory	**Ironwood**	-0.4 [-1.5, 0.6]	0.2 [-1.0, 1.2]	0.9 [-0.3, 2.0]
	Oak	-	0.6 [-0.4, 1.6]	1.3 [0.1, 2.5]
	Sugar maple	-	-	0.7 [-0.4, 1.9]

Table 2-S2: Estimated differences in bee abundance including **both canopy and understorey** bees. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of bee abundance per tree. The difference was determined by subtracting 3,000 samples from the posterior distribution of one bee species from another. A positive mean difference indicates a higher abundance of the bee species in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two species.

	A. dunningi	*A. mandibularis*	*A. milwaukeensis*	*A. rugosa*	*Au. pura*	*L. admirandum*	*L. coriaceum*	*L. gotham*	*L. nigroviride*	*L. versans*	*L. zonulum*
A. carlini	-1.8 [-2.4, -1.2]	-2.0 [-2.6, -1.4]	-2.0 [-2.6, -1.3]	-0.5 [-1.2, 0.2]	-1.8 [-2.4, -1.2]	-1.5 [-2.1, -0.9]	-1.2 [-1.9, -0.6]	-2.6 [-3.2, -2.1]	-1.4 [-2.0, -0.8]	-2.2 [-2.8, -1.7]	-0.0 [-0.7, 0.7]
A. dunningi		-0.2 [-0.6, 0.1]	-0.2 [-0.6, 0.2]	1.2 [0.7, 1.8]	-0.1 [-0.5, 0.3]	0.3 [-0.1, 0.7]	0.5 [0.1, 1.0]	-0.9 [-1.2, -0.5]	0.4 [-0.1, 0.7]	-0.4 [-0.8, -0.1]	1.7 [1.2, 2.4]
A. mandibularis			0.0 [-0.4, 0.4]	1.4 [0.9, 2.0]	0.2 [-0.2, 0.6]	0.5 [0.1, 0.9]	0.8 [0.3, 1.2]	-0.7 [-1.0, -0.3]	0.6 [0.2, 1.0]	-0.2 [-0.6, 0.2]	2.0 [1.4, 2.6]
A. milwaukeensis				1.4 [0.9, 2.0]	0.1 [-0.3, 0.6]	0.5 [0.1, 0.9]	0.7 [0.3, 1.2]	-0.7 [-1.1, -0.3]	0.6 [0.1, 1.0]	-0.2 [-0.6, 0.1]	1.9 [1.4, 2.6]
A. rugosa					-1.3 [-1.9, -0.7]	-0.9 [-1.5, -0.3]	-0.7 [-1.3, -0.1]	-2.1 [-2.7, -1.6]	-0.9 [-1.4, -0.3]	-1.6 [-2.2, -1.1]	0.5 [-0.2, 1.2]
Augochlora pura						0.4 [-0.1, 0.8]	0.6 [0.2, 1.0]	-0.8 [-1.2, -0.5]	0.4 [0.0, 0.8]	-0.4 [-0.8, -0.0]	1.8 [1.2, 2.4]
L. admirandum							0.3 [-0.2, 0.7]	-1.2 [-1.6, -0.8]	0.1 [-0.4, 0.5]	-0.7 [-1.1, -0.4]	1.5 [0.9, 2.1]
L. coriaceum								-1.4 [-1.8, -1.0]	-0.2 [-0.6, 0.3]	-1.0 [-1.4, -0.6]	1.2 [0.6, 1.8]
L. gotham									1.2 [0.8, 1.6]	0.4 [0.1, 0.8]	2.6 [2.1, 3.2]
L. nigroviride										-0.8 [-1.2, -0.4]	1.4 [0.8, 2.0]
L. versans											2.2 [1.6, 2.8]

Table 2-S3: Estimated differences in bee abundance in the **canopy**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of bee abundance per tree. The difference was determined by subtracting 3,000 samples from the posterior distribution of one bee species from another. A positive mean difference indicates a higher abundance of the bee species in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two species.

	A. dunningi	*A. mandibularis*	*A. milwaukeensis*	*A. rugosa*	*Au. pura*	*L. admirandum*	*L. coriaceum*	*L. gotham*	*L. nigroviride*	*L. versans*	*L. zonulum*
A. carlini	-1.8 [-2.5, -1.0]	-2.3 [-3.1, -1.6]	-2.7 [-3.5, -2.1]	-1.1 [-1.9, -0.3]	-2.1 [-2.8, -1.3]	-0.7 [-1.6, 0.0]	-0.8 [-1.7, -0.1]	-2.9 [-3.6, -2.2]	-1.3 [-2.1, -0.5]	-1.9 [-2.6, -1.1]	-0.0 [-1.0, 0.9]
A. dunningi		-0.5 [-1.0, 0.0]	-1.0 [-1.5, -0.5]	0.7 [0.1, 1.3]	-0.3 [-0.8, 0.2]	1.0 [0.4, 1.6]	0.9 [0.3, 1.5]	-1.2 [-1.6, -0.7]	0.5 [-0.1, 1.0]	-0.1 [-0.6, 0.4]	1.7 [0.9, 2.5]
A. mandibularis			-0.4 [-0.9, 0.0]	1.2 [0.6, 1.8]	0.2 [-0.2, 0.7]	1.6 [1.0, 2.1]	1.4 [0.9, 2.0]	-0.6 [-1.1, -0.2]	1.0 [0.5, 1.5]	0.4 [-0.1, 0.9]	2.3 [1.5, 3.1]
A. milwaukeensis				1.7 [1.1, 2.3]	0.7 [0.2, 1.1]	2.0 [1.4, 2.6]	1.9 [1.3, 2.5]	-0.2 [-0.6, 0.2]	1.4 [0.9, 1.9]	0.9 [0.4, 1.4]	2.7 [2.0, 3.5]
A. rugosa					-1.0 [-1.6, -0.4]	0.3 [-0.4, 1.0]	0.2 [-0.5, 0.9]	-1.8 [-2.4, -1.3]	-0.2 [-0.9, 0.4]	-0.8 [-1.4, -0.2]	1.0 [0.2, 1.9]
Augochlora pura						1.3 [0.7, 1.9]	1.2 [0.6, 1.8]	-0.9 [-1.3, -0.4]	0.8 [0.2, 1.3]	0.2 [-0.3, 0.7]	2.0 [1.3, 2.8]
L. admirandum							-0.1 [-0.8, 0.6]	-2.2 [-2.7, -1.6]	-0.6 [-1.2, 0.1]	-1.1 [-1.7, -0.5]	0.7 [-0.1, 1.6]
L. coriaceum								-2.1 [-2.6, -1.5]	-0.5 [-1.1, 0.2]	-1.0 [-1.6, -0.4]	0.8 [0.0, 1.7]
L. gotham									1.6 [1.1, 2.1]	1.1 [0.6, 1.5]	2.9 [2.1, 3.7]
L. nigroviride										-0.6 [-1.1, 0.0]	1.3 [0.5, 2.1]
L. versans											1.8 [1.1, 2.7]

Table 2-S4: Estimated differences in bee abundance in the **understorey**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of bee abundance per tree. The difference was determined by subtracting 3,000 samples from the posterior distribution of one bee species from another. A positive mean difference indicates a higher abundance of the bee species in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two species.

	A. dunningi	***A. mandibularis***	***A. milwaukeensis***	***A. rugosa***	***Au. pura***	***L. admirandum***	***L. coriaceum***	***L. gotham***	***L. nigroviride***	***L. versans***	***L. zonulum***
A. carlini	-1.7 [-2.7, -0.8]	-1.6 [-2.6, -0.7]	-1.1 [-2.1, -0.2]	0.0 [-1.1, 1.2]	-1.6 [-2.6, -0.7]	-2.2 [-3.1, -1.3]	-1.5 [-2.5, -0.7]	-2.3 [-3.3, -1.4]	-1.5 [-2.4, -0.5]	-2.5 [-3.4, -1.6]	0.0 [-1.1, 1.2]
A. dunningi		0.1 [-0.6, 0.7]	0.6 [-0.0, 1.3]	1.7 [0.9, 2.7]	0.2 [-0.5, 0.7]	-0.4 [-1.0, 0.1]	0.2 [-0.4, 0.7]	-0.6 [-1.2, -0.1]	0.3 [-0.4, 0.9]	-0.7 [-1.3, -0.1]	1.7 [0.9, 2.7]
A. mandibularis			0.5 [-0.2, 1.1]	1.7 [0.8, 2.6]	0.1 [-0.5, 0.7]	-0.5 [-1.1, 0.1]	0.1 [-0.5, 0.7]	-0.7 [-1.3, -0.1]	0.2 [-0.5, 0.8]	-0.8 [-1.4, -0.2]	2.3 [1.5, 3.1]
A. milwaukeensis				1.1 [0.2, 2.1]	-0.4 [-1.1, 0.3]	-1.0 [-1.7, -0.4]	-0.4 [-1.1, 0.2]	-1.2 [-1.8, -0.6]	-0.3 [-1.0, 0.3]	-1.4 [-2.1, -0.7]	1.1 [0.2, 2.1]
A. rugosa					-1.0 [-1.6, -0.4]	0.3 [-0.4, 1.0]	0.2 [-0.5, 0.9]	-1.8 [-2.4, -1.3]	-0.2 [-0.9, 0.4]	-0.8 [-1.4, -0.2]	1.0 [0.2, 1.9]
Augochlora pura						1.3 [0.7, 1.9]	1.2 [0.6, 1.8]	-0.9 [-1.3, -0.4]	0.8 [0.2, 1.3]	0.2 [-0.3, 0.7]	2.0 [1.3, 2.8]
L. admirandum							-0.1 [-0.8, 0.6]	-2.2 [-2.7, -1.6]	-0.6 [-1.2, 0.1]	-1.1 [-1.7, -0.5]	0.7 [-0.1, 1.6]
L. coriaceum								-2.1 [-2.6, -1.5]	-0.5 [-1.1, 0.2]	-1.0 [-1.6, -0.4]	0.8 [0.0, 1.7]
L. gotham									1.6 [1.1, 2.1]	1.1 [0.6, 1.5]	2.9 [2.1, 3.7]
L. nigroviride										-0.6 [-1.1, 0.0]	1.3 [0.5, 2.1]
L. versans											1.8 [1.1, 2.7]

Table 2-S5: Numbers of dissected bees in each stratum by genus and species

Taxa	Canopy	Understorey	Total
Andrena	**100**	**29**	**129**
carlini	8	4	12
dunningi	21	10	31
mandibularis	36	7	43
milwaukeensis	33	8	41
nasonii	1		1
Augochlora pura	**46**	**16**	**62**
Lasioglossum	**126**	**103**	**229**
admirandum	3	2	5
coriaceum	12	16	28
gotham	70	36	106
nigroviride	11	10	21
versans	28	37	65
zonulum	3	3	6
Total	**272**	**149**	**421**

Table 2-S6: Mean numbers of consumed pollen types per individual bee followed by the standard deviation for each bee genus and species. Pollen richness was estimated by identifying 300 pollen grains for each dissected bee. The majority of bees had only consumed a single type of pollen.

	Mean	SD
Andrena	**1.4**	**0.6**
carlini	1.3	0.7
dunningi	1.3	0.5
mandibularis	1.3	0.5
milwaukeensis	1.6	0.8
Augochlora pura	**1.3**	**0.5**
Lasioglossum	**1.4**	**0.6**
admirandum	1.2	0.4
coriaceum	1.3	0.4
gotham	1.2	0.5
nigroviride	1.3	0.6
versans	1.7	0.7
zonulum	1.2	0.4
Total	**1.4**	**0.6**

Table 2-S7: Estimated differences in pollen counts between pollen types for the overall model (including **both canopy and understorey** bees). Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance per bee. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI of the difference between the estimates of pollen abundance per bee that does not overlap zero.

	Acer (non-arboreal)	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
Acer	0.1 [-0.0, 0.3]	**0.4 [0.1, 0.6]**	**1.6 [1.1, 2.1]**	**85.3 [14.3, 154.8]**	-0.0 [-0.2, 0.1]	**0.4 [0.3, 0.6]**	0.3 [-0.0, 0.5]
Acer (non-arboreal)	-	0.2 [-0.0, 0.4]	**1.5 [0.9, 2.0]**	**85.1 [11.3, 151.7]**	**-0.2 [-0.3, -0.0]**	**0.3 [0.1, 0.5]**	0.1 [-0.2, 0.4]
Anemone	-	-	**1.2 [0.7, 1.8]**	**84.9 [13.9, 154.4]**	**-0.4 [-0.6, -0.2]**	0.1 [-0.2, 0.3]	-0.1 [-0.4, 0.2]
Asteraceae	-	-	-	**83.7 [12.9, 153.3]**	**-1.6 [-2.1, -1.1]**	**-1.2 [-1.7, -0.6]**	**-1.3 [-1.9, -0.8]**
Betulaceae	-	-	-	-	**-85.3 [-154.8, -14.3]**	**-84.9 [-154.3, -13.8]**	**-84.9 [-154.5, -14.1]**
Quercus	-	-	-	-	-	**0.5 [0.3, 0.6]**	**0.3 [0.0, 0.6]**
Thalictrum	-	-	-	-	-	-	-0.2 [-0.5, 0.1]
Trillium	-	-	-	-	-	-	-

Table 2-S8: Estimated differences in pollen counts between pollen types for the including only bees caught in the **canopy**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	Acer	*Acer* (non-arboreal)	*Anemone*	Asteraceae	Betulaceae	*Quercus*	*Thalictrum*
***Acer* (non-arboreal)**	0.1 [-0.1, 0.3]	-	-	-	-	-	-
Anemone	0.2 [-0.0, 0.5]	0.2 [-0.1, 0.4]	-	-	-	-	-
Asteraceae	**2.4 [1.5, 3.3]**	**2.3 [1.4, 3.2]**	**2.1 [1.2, 3.0]**	-	-	-	-
Betulaceae	-0.3 [-1.1, 0.4]	-0.4 [-1.2, 0.3]	-0.6 [-1.4, 0.2]	**-2.7 [-3.9, -1.5]**	-	-	-
Quercus	-0.1 [-0.2, 0.1]	-0.2 [-0.3, 0.0]	-0.3 [-0.5, -0.1]	**-2.5 [-3.3, -1.5]**	0.2 [-0.5, 1.0]	-	-
Thalictrum	**0.8 [0.6, 1.0]**	**0.7 [0.5, 0.9]**	**0.6 [0.3, 0.9]**	**-1.6 [-2.5, -0.7]**	**1.1 [0.4, 1.9]**	**0.9 [0.7, 1.0]**	-
Trillium	-0.2 [-0.6, 0.3]	-0.3 [-0.7, 0.2]	-0.4 [-0.9, 0.1]	**-2.6 [-3.5, -1.6]**	0.1 [-0.7, 1.0]	-0.1 [-0.6, 0.3]	**-1.0 [-1.5, -0.5]**

Table 2-S9: Estimated differences in pollen counts between pollen types for the including only bees caught in the **understorey**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	Acer	*Acer* (non-arboreal)	*Anemone*	Asteraceae	Betulaceae	*Quercus*	*Thalictrum*
***Acer* (non-arboreal)**	0.2 [-0.0, 0.4]	-	-	-	-	-	-
Anemone	**0.5 [0.1, 0.8]**	0.3 [-0.1, 0.7]	-	-	-	-	-
Asteraceae	**0.8 [0.2, 1.4]**	0.6 [-0.0, 1.3]	0.3 [-0.4, 1.0]	-	-	-	-
Betulaceae	**171.0 [22.6, 303.6]**	**171.0 [22.4, 303.5]**	**170.0 [21.4, 302.5]**	**170.0 [18.9, 300.0]**	-	-	-
Quercus	-0.0 [-0.2, 0.2]	-0.2 [-0.4, 0.0]	**-0.5 [-0.8, -0.1]**	**-0.8 [-1.4, -0.1]**	**-171.0 [-300.8, -19.8]**	-	-
Thalictrum	0.1 [-0.2, 0.3]	-0.1 [-0.4, 0.1]	**-0.4 [-0.8, -0.1]**	**-0.8 [-1.4, -0.1]**	**-171.0 [-303.7, -22.8]**	0.1 [-0.2, 0.3]	-
Trillium	**0.7 [0.4, 1.0]**	**0.5 [0.2, 0.8]**	0.2 [-0.2, 0.6]	-0.1 [-0.8, 0.6]	**-171.0 [-300.3, -19.1]**	**0.7 [0.4, 1.0]**	**0.6 [0.3, 1.0]**

Table 2-S10: Estimated differences in pollen counts between pollen types for the ***Andrena*** bees caught in the **canopy** and the **understorey**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	***Acer* (non-arboreal)**	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
***Acer* (arboreal)**	**5.9 [3.5, 8.6]**	**6.1 [3.8, 8.7]**	**9.8 [7.2, 12.8]**	**55.4 [15.1, 224.6]**	**4.6 [2.3, 7.3]**	**6.3 [4.0, 9.1]**	**7.1 [4.8, 9.9]**
***Acer* (non-arboreal)**	-	0.2 [-1.3, 1.7]	**3.9 [2.1, 5.8]**	**49.5 [9.5, 217.7]**	-1.3 [-2.7, 0.1]	0.4 [-1.1, 1.8]	1.2 [-0.3, 2.7]
Anemone	-		**3.7 [2.0, 5.5]**	**49.3 [9.4, 217.7]**	**-1.5 [-2.9, -0.1]**	0.2 [-1.3, 1.7]	1.0 [-0.4, 2.4]
Asteraceae	-	-	-	**45.6 [5.7, 214.5]**	**-5.2 [-7.1, -3.4]**	**-3.5 [-5.3, -1.8]**	**-2.7 [-4.4, -1.0]**
Betulaceae	-	-	-	-	**-50.8 [-219.0, -10.8]**	**-49.1 [-217.6, -9.1]**	**-48.3 [-216.6, -8.4]**
Quercus	-	-	-	-	-	**1.7 [0.3, 3.1]**	**2.5 [1.1, 3.9]**
Thalictrum	-	-	-	-	-	-	0.8 [-0.6, 2.2]

Table 2-S11: Estimated differences in pollen counts between pollen types for the ***Andrena*** bees caught in the **canopy**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	***Acer* (non-arboreal)**	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
***Acer* (arboreal)**	**6.6 [3.7, 10.2]**	**6.8 [4.1, 10.3]**	**12.4 [8.9, 16.6]**	**68.9 [15.7, 306.3]**	**5.2 [2.3, 8.7]**	**7.8 [4.7, 11.5]**	**9.6 [6.2, 14.0]**
***Acer* (non-arboreal)**	-	0.2 [-1.5, 2.0]	**5.8 [3.6, 8.2]**	**62.3 [9.7, 300.2]**	-1.3 [-3.1, 0.3]	1.2 [-0.7, 3.0]	**3.0 [0.9, 5.5]**
Anemone	-	-	**5.6 [3.4, 8.0]**	**62.0 [9.5, 300.8]**	-1.6 [-3.2, 0.0]	0.9 [-0.9, 2.8]	**2.8 [0.7, 5.2]**
Asteraceae	-	-	-	**56.5 [3.9, 294.9]**	**-7.2 [-9.6, -4.9]**	**-4.6 [-6.9, -2.6]**	**-2.8 [-5.0, -0.6]**
Betulaceae	-	-	-	-	**-63.6 [-301.7, -11.0]**	**-61.1 [-299.9, -8.6]**	**-59.2 [-298.3, -6.7]**
Quercus	-	-	-	-	-	**2.5 [0.8, 4.4]**	**4.4 [2.2, 6.9]**
Thalictrum	-	-	-	-	-	-	1.9 [-0.1, 4.1]

Table 2-S12: Estimated differences in pollen counts between pollen types for the ***Andrena*** bees caught in the **understorey**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	Acer (non-arboreal)	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
***Acer* (arboreal)**	**7.6 [1.4, 16.1]**	**7.4 [1.4, 16.0]**	**9.8 [3.5, 18.6]**	**63.6 [13.8, 280.3]**	5.6 [-0.3, 13.7]	**6.3 [0.3, 14.4]**	**7.2 [1.1, 15.6]**
***Acer* (non-arboreal)**	-	-0.1 [-4.8, 4.5]	2.2 [-2.3, 7.2]	**56.0 [7.3, 273.6]**	-1.9 [-6.7, 2.5]	-1.3 [-6.0, 3.4]	-0.4 [-5.1, 4.3]
Anemone	-	-	2.4 [-2.3, 7.4]	**56.1 [7.5, 273.9]**	-1.8 [-6.5, 2.5]	-1.1 [-5.8, 3.2]	-0.2 [-4.4, 4.1]
Asteraceae	-	-	-	**53.7 [5.0, 271.7]**	-4.2 [-9.3, 0.6]	-3.5 [-8.6, 1.0]	-2.6 [-7.7, 2.2]
Betulaceae	-	-	-	-	**-57.9 [-274.0, -9.3]**	**-57.2 [-275.2, -8.7]**	**-56.3 [-275.0, -7.7]**
Quercus	-	-	-	-	-	0.7 [-3.4, 4.7]	1.6 [-2.8, 6.2]
Thalictrum	-	-	-	-	-	-	0.9 [-3.7, 5.6]

Table 2-S13: Estimated differences in pollen counts between pollen types for the ***Lasioglossum*** bees caught in the **canopy and the understorey**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	***Acer* (non-arboreal)**	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
***Acer* (arboreal)**	**4.4 [2.5, 6.5]**	**7.1 [5.0, 9.3]**	**10.4 [8.3, 12.6]**	**12.1 [9.6, 14.8]**	**3.4 [1.4, 5.5]**	**5.6 [3.6, 7.7]**	**7.5 [5.4, 9.6]**
***Acer* (non-arboreal)**	-	**2.7 [1.5, 3.9]**	**5.9 [4.6, 7.3]**	**7.7 [5.8, 9.6]**	-1.0 [-2.1, 0.0]	**1.2 [0.1, 2.3]**	**3.0 [1.8, 4.2]**
Anemone	-	-	**3.3 [1.9, 4.7]**	**5.0 [3.1, 6.9]**	**-3.7 [-4.9, -2.5]**	**-1.5 [-2.7, -0.3]**	0.4 [-0.8, 1.5]
Asteraceae	-	-	-	1.7 [-0.1, 3.6]	**-7.0 [-8.4, -5.7]**	**-4.8 [-6.2, -3.4]**	**-2.9 [-4.3, -1.6]**
Betulaceae	-	-	-	-	**-8.7 [-10.6, -6.8]**	**-6.5 [-8.4, -4.6]**	**-4.6 [-6.4, -2.9]**
Quercus	-	-	-	-	-	**2.2 [1.2, 3.3]**	**4.1 [2.9, 5.3]**
Thalictrum	-	-	-	-	-	-	**1.9 [0.7, 3.0]**

Table 2-S14: Estimated differences in pollen abundance between pollen types for the ***Lasioglossum*** bees caught in the **canopy**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	***Acer* (non-arboreal)**	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
***Acer* (arboreal)**	**4.8 [2.3, 7.6]**	**8.1 [5.2, 11.4]**	**62.5 [15.5, 310.0]**	**11.8 [8.0, 15.7]**	**3.5 [1.1, 6.3]**	**6.6 [4.1, 9.4]**	**8.5 [5.6, 11.9]**
***Acer* (non-arboreal)**	-	**3.3 [1.4, 5.3]**	**57.7 [11.1, 305.0]**	**7.0 [4.2, 9.7]**	-1.3 [-2.6, 0.1]	**1.8 [0.4, 3.3]**	**3.7 [1.8, 6.0]**
Anemone	-	-	**54.4 [7.9, 302.3]**	**3.7 [1.1, 6.2]**	**-4.6 [-6.7, -2.7]**	-1.5 [-3.6, 0.4]	0.4 [-1.8, 2.7]
Asteraceae	-	-	-	**-50.7 [-298.6, -4.3]**	**-59.0 [-306.3, -12.3]**	**-55.9 [-303.6, -9.3]**	**-54.0 [-301.7, -7.4]**
Betulaceae	-	-	-	-	**-8.3 [-11.0, -5.3]**	**-5.2 [-7.8, -2.3]**	**-3.3 [-5.9, -0.6]**
Quercus	-	-	-	-	-	**3.1 [1.7, 4.5]**	**5.0 [3.0, 7.3]**
Thalictrum	-	-	-	-	-	-	1.9 [-0.1, 4.2]

Table 2-S15: Estimated differences in pollen abundance between pollen types for the ***Lasioglossum*** bees caught in the **understorey**. Values represent the mean difference in log counts and 95% BCI of the difference between the estimates of pollen abundance. The difference was determined by subtracting 3,000 samples from the posterior distribution of one pollen type from another. A positive mean difference indicates a higher abundance of the pollen type in the row compared to the column, while a negative mean difference indicates the opposite. If the 95% BCI of the difference between the estimates overlaps zero, there is insufficient evidence to conclude a credible difference in abundance between the two pollen types. **Bold values** indicate a 95% BCI that does not overlap zero.

	***Acer* (non-arboreal)**	***Anemone***	**Asteraceae**	**Betulaceae**	***Quercus***	***Thalictrum***	***Trillium***
***Acer* (arboreal)**	**4.6 [1.9, 7.6]**	**7.4 [4.5, 10.7]**	**9.1 [6.4, 12.2]**	**56.9 [14.6, 236.5]**	**3.8 [1.2, 6.8]**	**5.3 [2.6, 8.3]**	**7.2 [4.5, 10.2]**
***Acer* (non-arboreal)**	-	**2.8 [0.7, 5.0]**	**4.5 [2.6, 6.5]**	**52.3 [10.3, 231.2]**	-0.8 [-2.4, 0.8]	0.7 [-1.0, 2.4]	**2.6 [0.8, 4.5]**
Anemone	-	-	1.8 [-0.2, 3.7]	**49.5 [7.5, 228.3]**	**-3.6 [-5.6, -1.6]**	**-2.0 [-4.2, -0.1]**	-0.2 [-2.0, 1.6]
Asteraceae	-	-	-	**47.8 [5.7, 226.1]**	**-5.3 [-7.2, -3.5]**	**-3.8 [-5.8, -1.9]**	**-2.0 [-3.7, -0.2]**
Betulaceae	-	-	-	-	**-53.1 [-232.7, -11.1]**	**51.6 [-230.9, -9.5]**	**-49.7 [-228.7, -7.7]**
Quercus	-	-	-	-	-	1.5 [-0.1, 3.2]	**3.4 [1.6, 5.1]**
Thalictrum	-	-	-	-	-	-	**1.9 [0.2, 3.6]**

Table 2-S16: Priors for Bayesian Model: q0,q1 or q2 ~ stratum of capture

Prior	Parameter Type	Predictor or Term	Random Effect Grouping
Flat	Slope	-	-
Flat	Slope	xUND	-
Student_t(3, 9.7, 2.5)	Intercept	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	-

Table 2-S17: Priors for Bayesian Model: Bee abundance ~ stratum of capture * tree species + (1\| tree*plot)

Prior	Parameter Type	Predictor or Term	Random Effect Grouping
Flat	Slope	-	-
Flat	Slope	stratumUND	-
Flat	Slope	stratumUND:treeOak	-
Flat	Slope	stratumUND:treeSugarMaple	-
Flat	Slope	stratumUND:treeWhiteAsh	-
Flat	Slope	treeOak	-
Flat	Slope	treeSugarMaple	-
Flat	Slope	treeWhiteAsh	-
Student_t(3, 2.6, 2.5)	Intercept	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	plot
Student_t(3, 0, 2.5)	Standard Deviation	Intercept	plot
Student_t(3, 0, 2.5)	Standard Deviation	-	plot:ind
Student_t(3, 0, 2.5)	Standard Deviation	Intercept	plot:ind
Gamma(0.01, 0.01)	Shape	-	-
Beta(1, 1)	Zero-Inflation	-	-

Table 2-S18: Priors for Bayesian Model: Bee counts ~ bee species + bee species:stratum of capture + (1| tree*plot)

Prior	Parameter Type	Predictor or Term	Random Effect Grouping
Flat	Slope	-	-
Flat	Slope	speciesAndrenacarlini:stratumUND	-
Flat	Slope	speciesAndrenadunningi	-
Flat	Slope	speciesAndrenadunningi:stratumUND	-
Flat	Slope	speciesAndrenamandibularis	-
Flat	Slope	speciesAndrenamandibularis:stratumUND	-
Flat	Slope	speciesAndrenamilwaukeensis	-
Flat	Slope	speciesAndrenamilwaukeensis:stratumUND	-
Flat	Slope	speciesAndrenarugosa	-
Flat	Slope	speciesAndrenarugosa:stratumUND	-
Flat	Slope	speciesAugochlorapura	-
Flat	Slope	speciesAugochlorapura:stratumUND	-
Flat	Slope	speciesLasioglossumadmirandum	-
Flat	Slope	speciesLasioglossumadmirandum:stratumUND	-
Flat	Slope	speciesLasioglossumcoriaceum	-
Flat	Slope	speciesLasioglossumcoriaceum:stratumUND	-
Flat	Slope	speciesLasioglossumgotham	-
Flat	Slope	speciesLasioglossumgotham:stratumUND	-
Flat	Slope	speciesLasioglossumnigroviride	-
Flat	Slope	speciesLasioglossumnigroviride:stratumUND	-
Flat	Slope	speciesLasioglossumversans	-
Flat	Slope	speciesLasioglossumversans:stratumUND	-
Flat	Slope	speciesLasioglossumzonulum	-
Flat	Slope	speciesLasioglossumzonulum:stratumUND	-
Student_t(3, 0, 3.4)	Intercept	-	-
Student_t(3, 0, 3.4)	Standard Deviation	-	-
Student_t(3, 0, 3.4)	Standard Deviation	-	plot
Student_t(3, 0, 3.4)	Standard Deviation	Intercept	plot
Student_t(3, 0, 3.4)	Standard Deviation	-	plot:ind
Student_t(3, 0, 3.4)	Standard Deviation	Intercept	plot:ind
Gamma(0.01, 0.01)	Shape	-	-
Beta(1, 1)	Zero-Inflation	-	-

Table 2-S19: Priors for Bayesian Model: y ~ sex + (1|tree*plot)

Prior	Parameter Type	Predictor or Term	Random Effect Grouping
Flat	Slope	-	-
Flat	Slope	sexmale	-
Student_t(3, 0, 2.5)	Intercept	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	tree:plot
Student_t(3, 0, 2.5)	Standard Deviation	Intercept	tree:plot

Table 2-S20: Priors for Bayesian Model: Pollen counts ~ pollen taxon + stratum of capture:pollen taxon + (1|tree*plot)

Prior	Parameter Type	Predictor or Term	Random Effect Grouping
Flat	Slope	-	-
Flat	Slope	nameAcer:strataUND	-
Flat	Slope	nameAcer_shrub	-
Flat	Slope	nameAcer_shrub:strataUND	-
Flat	Slope	nameAnemone	-
Flat	Slope	nameAnemone:strataUND	-
Flat	Slope	nameAsteraceae	-
Flat	Slope	nameAsteraceae:strataUND	-
Flat	Slope	nameBetulaceae	-
Flat	Slope	nameBetulaceae:strataUND	-
Flat	Slope	nameQuercus	-
Flat	Slope	nameQuercus:strataUND	-
Flat	Slope	nameThalictrum	-
Flat	Slope	nameThalictrum:strataUND	-
Flat	Slope	nameTrillium	-
Flat	Slope	nameTrillium:strataUND	-
Student_t(3, -2.3, 2.5)	Intercept	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	plot
Student_t(3, 0, 2.5)	Standard Deviation	Intercept	plot
Student_t(3, 0, 2.5)	Standard Deviation	-	plot:ind
Student_t(3, 0, 2.5)	Standard Deviation	Intercept	plot:ind
Gamma(0.01, 0.01)	Shape	-	-
Beta(1, 1)	Zero-Inflation	-	-

Table 2-S21: Priors for Bayesian Model: pollen load type ~ s(day of the year)

Prior	Parameter Type	Predictor or Term	Random Effect Grouping
Flat	Slope	-	-
Flat	Slope	sJDay_1	-
Student_t(3, 5.7, 2.5)	Intercept	-	-
Student_t(3, 0, 2.5)	Standard Deviation	-	-
Student_t(3, 0, 2.5)	Standard Deviation	s(JDay)	-
Gamma(0.01, 0.01)	Shape	-	-
Beta(1, 1)	Zero-Inflation	-	-

Chapter 3
Higher latitude spring-flowering herbs advance their phenology more than trees with warming temperatures

Abstract

The phenologies of co-occurring trees and spring-blooming understorey herbs in northeastern North American hardwood forests appear to be regulated by different environmental drivers—air temperature and soil temperature/snowpack, respectively. Accordingly, it has been hypothesized that climate change-driven asymmetry in the advancement of canopy leaf-out relative to the timing of understorey growth could reduce photosynthetic rates and reproductive success of understorey herbs through greater early-season shading. To determine whether trees and spring-flowering forest herbs are advancing their phenologies at different rates with respect to increasing global temperatures, we examined the phenological responses to warming of 10 species of trees and 11 species of spring-flowering forest herbs (8,045 observations from 965 sites) in northeastern North America using 13 years of data collected by citizen scientists under the auspices of the USA-National Phenology Network. Contrary to expectation, the degree of advancement of leaf-out as a function of temperature was greater in spring-flowering forest herbs than in trees, with a mean response rate of −4.9 days/˚C (95% BCI [−5.2, −4.6]) for spring-flowering forest herbs *vs.* −3.3 days/°C (95% BCI [−3.5, −3.1]) for trees. However, the response to temperature was not consistent across the latitudinal range, with spring-flowering forest herbs responding more strongly to warming than trees at middle (40–44˚N) and higher (45–48˚N) latitudes but not at lower latitudes (35–39˚ N). In contrast to previous suggestions, our study shows spring-flowering forest herbs advancing their phenology at a higher rate than trees with respect to warming through most of the latitudinal range investigated, which could translate into a longer growing season and increased carbon uptake for spring-flowering forest herbs as spring temperatures rise.

3.1 Introduction

The timing of phenological events, such as flowering and leaf-out, affects the growth, competitiveness, reproductive success, and, ultimately, the fitness of plants (Chuine, 2010). Leaf-out date (defined as the timing of leaf emergence) determines the availability of daylight for photosynthetic carbon uptake and also affects the risk of frost damage (Kramer et al., 2010; Lenz et al., 2016) and herbivory (Pilson, 2000). Likewise, flowering time can influence plant reproductive success by affecting synchrony with pollinators (Rafferty & Ives, 2011), the time available for fruit development and seed dispersal (Chuine, 2010; Ettinger et al., 2018), rates of florivory and seed predation (Lacey et al., 2003; Pilson, 2000), and the risk of frost damage to flowers and fruits (Inouye, 2008). Consequently, the timing of emergence of leaf and flower buds is controlled by precise physiological mechanisms that respond to environmental triggers such as temperature, daylength, and precipitation (Flynn & Wolkovich, 2018; Jánosi et al., 2020; Korner & Basler, 2010; Linkosalo et al., 2006).

Several studies have demonstrated that different functional groups of plants have different environmental triggers for bud break (i.e., the timing of bud unfolding and start of leaf emergence), with temperate-zone woody species responding mainly to air temperature (Laube et al., 2014; Thomas et al., 2003; Zohner et al., 2016; Zohner & Renner, 2014), while perennial herbs appear to respond primarily to soil temperature and snow depth (Jánosi et al., 2020). If the phenologies of co-occurring trees and perennial herbs are indeed regulated by different environmental triggers, they could respond differently to global climate change. For example, canopy closure could advance at a faster rate than growth and development of perennial herbs, leading to increased shading, reduced photosynthetic rates and lower reproductive success for understorey plants (Ge et al., 2015; Heberling, Cassidy, et al., 2019; Heberling, Fridley, et al., 2019).

A recent study of a woodland ecosystem in Massachusetts, USA, concluded that canopy tree species, including *Acer saccharum, Quercus alba,* and *Fagus grandifolia,* advanced their leaf-out timing by an average of nearly 2 weeks over a 160-year period, while spring-flowering herbs (i.e., understorey perennial herbs that grow and bloom for a short period before canopy closure) advanced their first leaf-out date by less than a week, on average (Heberling, Fridley, et al., 2019). The authors predicted that the resulting change in temporal overlap between understorey and canopy plants could reduce the carbon budget of the former by 12–26% by 2080, due to increased canopy shading. This loss of photosynthetic opportunity could be critical for spring-flowering herbs, which are a common and charismatic component of the understorey of deciduous temperate forests, and which require high levels of sunlight reaching the forest floor to develop vegetative and reproductive parts before canopy closure. Reductions in carbon budgets via earlier canopy shading of these species could lead to population declines and eventual species replacement (Heberling, Fridley, et al., 2019; Lapointe, 2001).

Conversely, in a meta-analysis of phenological shifts among 112 species (including trees, shrubs, herbs, birds, amphibians, and insects) across various ecosystems in China, Ge et al. (2015) reached the opposite conclusion, finding that herbaceous species were exhibiting greater phenological advances than trees (5.7 days/decade, *vs.* 2.3 days/decade for trees). Clearly, there is no consensus yet on whether trees or perennial herbs are advancing their phenologies more strongly in response to climate change (Du et al., 2020; Ge et al., 2015; Heberling, Fridley, et al., 2019; Parmesan, 2007). One of the major challenges in answering this question is that phenological responses to warming vary interspecifically and geographically, making it difficult to generalize about entire functional groups (Buonaiuto et al., 2021; Nagahama & Yahara, 2019; Parmesan, 2007; Primack et al., 2009; Zhang et al., 2007).

Geography is likely to play a role in species' responses to climate change, for three largely separate reasons. First, certain areas, such as higher-latitude regions, are warming faster than others (2 to 3 times faster, depending on latitude; IPCC, 2018), which could lead to greater magnitudes of phenological change over time in those locations if species' phenologies are responding largely to temperature. Second, species or populations occurring in cooler regions may be more responsive to a given amount of warming, either because a given amount of warming represents a larger relative change (J. Prevéy, Vellend, Rüger, Hollister, Bjorkman, Myers-Smith, et al., 2017), or because in cooler climates, a given amount of warming still allows chilling requirements to be met. For example, Zhang et al. (2007), using North American satellite and climate data from 1982 to 2005, found that species whose mid-range latitude is north of 40˚N showed greater advances in phenology over time than species with more southerly distributions. They hypothesized that this pattern was driven by the fact that chilling requirements were still being met at higher latitudes, where even a reduced number of chilling days owing to warming winter temperatures would still be sufficient to break winter dormancy, unlike at lower latitudes. Third, the unique environmental variables at a single site, such as precipitation, elevation, and edaphic factors (Du et al., 2020; Wielgolaski, 2001), all affect how species track a changing climate. For all these reasons, extrapolation from one location to another is problematic.

Overall, despite a large number of studies on the effects of warming temperatures on phenology, we still have little ability to predict where mismatches among functional groups are most likely—or even the direction of mismatches—because we lack studies that examine phenological responses across different sets of species that co-occur over wide geographical areas. We therefore asked whether: (1) eastern North American deciduous trees and spring-flowering forest herbs shift their leaf-out phenology at different rates with respect to temperature; (2) there is latitudinal variation in the response to temperature; and (3) leaf-out of trees and spring-flowering forest herbs has advanced (i.e., become earlier) over time. To answer these questions, we used data from the USA National Phenology Network to examine phenological responses to warming in 21 species (10 deciduous trees and 11 spring-flowering understorey herbaceous perennials), over a broad latitudinal range (35˚ to 48˚ N) in this region.

3.2 Material and Methods

3.2.1 Study system

The temperate deciduous hardwood forest is an ecosystem of major ecological and economic importance in eastern Canada and the northeastern US, dominated by tree genera such as *Acer*, *Quercus*, *Betula*, and *Fagus*. A key characteristic of this ecosystem is the changing light regime caused by seasonal variation in canopy cover as trees produce their leaves in spring and shed them in autumn. Maximum amounts of direct solar radiation reach the forest floor in early spring, accounting for over 90% of the total energy received in the understorey during this period (Hutchison & Matt, 1977). In summer, following tree leaf-out, solar radiation in the understorey decreases to 1–5% of that available above the canopy (Hicks & Chabot, 1985). Spring-blooming forest herbs (e.g., *Trillium grandiflorum, Claytonia virginica, Erythronium americanum*) are common in the understorey of northeastern deciduous forests, taking advantage of the high luminosity before canopy closure to develop vegetative and reproductive parts.

3.2.2 Phenology data

Data for this study were provided by the USA National Phenology Network (USA–NPN 2021, https://data.usanpn.org/observations/get-started; henceforth "NPN"). NPN data are collected by professional and citizen scientists. To ensure data quality, several protocols are applied, and volunteers go through extensive training. All protocols are standardized within plant functional types (e.g., deciduous trees, herbs). The accuracy of the volunteer-collected NPN plant phenology data was evaluated by Fuccillo et al., (2015), who found that professionals and volunteers had a 91% concordance rate, indicating that volunteers provide reliable observations.

We selected 10 species of trees and 11 species of spring-flowering forest herbs based on those with the greatest numbers of observations in the NPN dataset (Table S1). Spring-flowering forest herbs are defined here as early-flowering herbaceous species that emerge at the beginning of spring, taking advantage of the high levels of light in the understorey to accumulate carbon reserves and reproduce (Lapointe, 2001). Their epigeous growth normally occurs from late March to late June; they then enter a period of hypogeous growth following canopy closure and seasonal (summer) temperature increases (Lapointe, 2001). We included only those species that the literature reports as developing their vegetative and reproductive parts before canopy closure (Augspurger & Salk, 2017; Augspurger & Zaya, 2020; R. L. Brown et al., 1985; Hall & Sibley, 1976; Heberling, Cassidy, et al., 2019; Jacques et al., 2015; Kricsfalusy & Ponomarenko, 2013; Muller, 1978; Reader & Bricker, 1992).

Species observations had to meet three criteria for inclusion: their geographical distribution had to encompass eastern North America, observations had to go back at least 10 years from 2021, and species needed to have a minimum of 50 observations (Table S1). For each selected species, we extracted individual phenometrics reports for the first day of the year (DOY) for which a phenophase (i.e., an observable stage or phase in the annual life cycle that can be defined by a start and end point) had a positive observation, focusing on the phenophases defined as "leaves" for both functional groups. The phenophase "leaves" for forbs includes both "above ground buds with green tips" and "fully unfolded leaves". Since forest herb leaf emergence occurs rapidly, we included both phenophase descriptions in our data, keeping only the earliest observation when both phenophases were available for the same individual. For trees, the phenophase "leaves" refers to "fully unfolded leaves". For both groups, only the first entry for "leaves" was kept, *i.e.*, the earliest positive observation occuring after a "0" observation in the same calendar year, and it is possible that the true start of the phenophase occurred at some point before these first positive observations. However, it seems unlikely that any resulting bias in our estimates of first dates would be greater for one functional group than the other. Note that individual plants are defined differently for the two functional groups in this study: For trees, a unique identifier is provided for each individual, whereas for forbs, the unique identifier can represent a patch of individuals or a single plant (see Appendix for details).

We extracted all North American data available in the NPN portal for the 21 focal species. We filtered the data to retain observations from January 1, 2009, to July 31, 2021, and from latitudes 35˚ to 48.2˚ N and longitudes east of 91˚ W. These choices reflect the fact that there were few observations for spring-flowering forest herbs prior to 2009, below 35˚, and above 48.2˚, and to maintain observations within the geographical range of the eastern temperate forests (as described by Dyer 2006). We excluded records occurring after DOY 172 (June 21) for all species, and for trees, we also excluded records where budbreak occurred later than leaf-out, or where the interval between budbreak and leaf-out was greater than 30 days, reasoning that such observations were likely errors. After those restrictions were applied, the number of remaining observations was 8,045 (Figure 3-1 and Table S1).

3.2.3 Spring phenology and temperature

To determine whether trees and spring-flowering forest herbs shift their leaf-out phenology at different rates with respect to temperature, we fitted a hierarchical Bayesian model in which the DOY was a function of temperature. DOY refers to the first day of the year at a given site in a given year on which the phenophase "leaves" was reported. Monthly mean temperatures for winter and spring were extracted for each site for the 2009–2021 period from the PRISM database (http://www.prism.oregonstate.edu). Mean spring temperatures were calculated by averaging the monthly mean temperatures for each location for the period from March 1 to May 31; mean winter temperatures were calculated for the period from December 1 to February 28.

We fitted separate models for trees and spring-flowering forest herbs, and then to assess whether trees and spring-flowering forest herbs differed in their response to warming, we calculated the estimate of the difference between the distributions of the slope for each functional group using 3,000 samples from the posterior distributions.

The models take the following form:

$$Day\ of\ the\ year_{[i]} = \alpha_{jk[i]} + \beta_{j[i]} \times temperature_{[i]} \quad (1)$$

where α is the intercept and β is the slope, both including a group-level interaction (*j*) between species and site (analogous to random effects in the frequentist literature). Since species are expected to vary in their mean leaf-out day and in their response to temperature, and because this response also varies with geographical location, we allowed the intercept and the slope to vary, meaning the effect of temperature could vary by the interaction between species and site. We also allowed the intercept to vary by year (k) to account for the non-independence of observations taken in the same year. We did not include plant individual as a group-level term since most observations are single observations rather than repeated measures across the years. For population-level effects (analogous to fixed effects in the frequentist literature), the prior distributions were: $\alpha \sim Normal(100,33)$; $\beta \sim Normal(0, 1000)$. The correlations between varying intercepts and slopes were also estimated using $lkj(2)$ (Lewandowski-Kurowicka-Joe distribution, Lewandowski et al., 2009) as a prior for the correlation matrix. The intercept priors were weakly informative to speed convergence by preventing the Markov chain from sampling highly unlikely values, such as negative values for mean leaf-out date. Since the data were centred (to speed convergence), the choice of $\mu_\alpha = 100$ is equivalent to a mean DOY of 100 (April 10), which is a reasonable value given the latitudinal range of the data and the values normally reported in the literature for the leaf-out day of trees and spring-flowering forest herbs (Diez et al., 2012; Morin et al., 2009a; Park et al., 2019; Willems et al., 2021). For the slope, we applied a zero-centred non-informative prior. Since models using mean spring and mean winter temperatures produced similar results, we focus on the results using spring temperatures; however, we also present results based on other time-windows for temperature calculation (see section 2.5 and Table S3).

To assess whether individual species advanced their phenology with warming, we also fitted separate hierarchical Bayesian models of the same form as model (1) for each species. To account for variation in the mean and the rate of change due to geographical location, we allowed the intercept and slope to vary for each site. The priors were chosen as described for the previous model.

3.2.4 Changes in spring phenology throughout the latitudinal range

To assess whether spring-flowering forest herbs and trees differ in their response to temperature as a function of latitude, we fitted a multilevel Bayesian model where latitude was treated as categorical, such that separate models were fitted for each of three categories: equal or greater than 35˚ and lower than 40˚, greater or equal to 40˚ and lower than 45˚, and greater or equal to 45˚ and up to 48.2˚ (for simplicity, henceforth, 35–39˚, 40–44˚, and 45–48˚ N). The model takes the same form as model (1), and prior distributions were as described above. Similar to model (1), we allowed the intercept to vary for each year and for the interaction between each species and site, and the slope to vary for the interaction between each species and site.

To test whether these results were driven by our choice of the March 1 to May 31 time-window for the temperature data (which might be later than the period of peak temperature sensitivity for low-latitude populations and earlier for high-latitude populations), we repeated all the temperature models using different time-windows for the calculation of mean spring temperature—an earlier time-window, from February to April; and a later time-window, from April to June.

3.2.5 Change in spring phenology with time

To determine whether leaf-out dates of trees and spring-flowering forest herbs have advanced since 2009, we fitted a multilevel Bayesian model, in which the first day of the year on which an observation occurred (DOY) was a function of year, treated as continuous. A separate model was fitted for each functional group (trees and spring-flowering forest herbs), and since species are expected to have different spring phenologies, and because their phenologies also vary with geographical location, we allowed the intercept and the slope to vary, allowing the effect of year to vary by the interaction between species and site:

$$Day\ of\ the\ year_{[i]} = \alpha_{j[i]} + \beta_{j[i]} \times year_{[i]} \quad (2)$$

Here, α is the intercept, and β is a random slope, both including a group-level interaction (*j*) between species and site. Because the magnitude of directional change in temperature in our study region over the study period (2009–2021) has been modest compared to among-year variation (see Results 3.3), we also ran a similar set of models with year treated as categorical to assess year-to-year variation in leaf-out dates This model takes the following form:

$$Day\ of\ the\ year_{[i]} = \alpha_{[i]} + \beta_{jyear[i]} \quad (3)$$

where α is the intercept, and β is a second intercept including a group-level interaction (*j*) between species and site.

The prior distributions were the same as described previously. For both models, we fitted separate models for each functional group and for species.

For all models, we obtained the parameters' posterior distributions using the **Hamiltonian MC** method (Betancourt & Girolami, 2013). We ran all chains for a minimum of 4,000 iterations, adopting a warm-up of 1,000 iterations. We monitored convergence by running four different chains with different start values and calculating Rubin's R (Gelman & Rubin, 1992), where convergence is assumed when R is close to 1. By sampling from the posterior distribution, we computed the mean and Bayesian credible intervals (BCI) for all parameters using 3,000 draws from the posterior. As a measure of model fit, we also computed the Bayes-R^2 for each (Gelman et al., 2019). All analyses were performed using the package brms (Bürkner, 2017), which allows the implementation of Stan in R (R Core Team, 2023).

3.3 Results

3.3.1 Spring phenology and temperature

Overall, the spring phenology of both trees and spring-flowering forest herbs tended to be earlier when temperatures were warmer. Slopes describing each functional group were negative and did not overlap zero (trees: μ_β = −3.3 days/˚C, 95% BCI [−3.5, −3.1]; spring-flowering forest herbs: μ_β = −4.9 days/˚C, 95% BCI [−5.2, −4.6]; Table 3-1 and Figure 3-2). Contrary to expectation, the 95% BCI of the estimated difference between posterior distributions of the slopes for spring-flowering forest herbs and trees was negative and did not overlap zero, indicating that spring-flowering forest herbs responded more strongly to warmer temperatures than did trees (μ_β= −1.6 days/˚C, 95% BCI [−1.9, −1.2]). Therefore, on average, spring-flowering forest herbs advanced their spring phenology by 1.6 days more than trees for each degree increase in temperature. This pattern was similar for the model using winter temperature data, with spring-flowering forest herbs also advancing their spring flowering phenology more than trees. Using different time-windows for calculation of spring temperature did not change the results: spring-flowering forest herbs still exhibited greater responsiveness to temperature than trees (Table S3).

3.3.2 Changes in spring phenology throughout the latitudinal range

For lower latitudes (35–39˚ N), there was no detectable difference between the functional groups in responsiveness of spring phenology to temperature, regardless of the time-window over which mean spring temperature was calculated. At middle (40–44˚ N) and higher latitudes (45–48˚ N), spring-flowering forest herbs advanced leaf-out more strongly than trees with respect to temperature, regardless of time-window (Figure 3-3, Table 3-2). To address the possibility that the observed phenological trends across the latitudinal range could have been driven by an outlier species, we re-ran the latitudinal analysis removing the most abundant species within each latitudinal band, and also restricting the analysis to species that occur throughout the latitudinal range, and, in both cases, our results were unchanged (see R code provided).

The phenological response of spring-flowering forest herbs to temperature differed among the latitudinal bands, with these species advancing their phenology more strongly with warmer temperatures at middle latitudes than at lower or higher latitudes (Figure 3-3, Tables 3-2 and S4). Conversely, the magnitude of the trees' phenological response decreased from the lowest- to the highest-latitude band, except when mean spring temperature was calculated over the period of April to June, i.e., the later time-window (Figure 3-3, Tables 3-2 and S4). We found no pattern of greater responsiveness to temperature at higher latitudes (45–48˚ N), for spring-flowering forest herbs or trees, independent of the time-window used to calculate mean spring temperatures.

3.3.3 Change in spring phenology with time

Contrary to our expectation, mean spring temperatures in our dataset decreased slightly over the timespan analysed (Figure 3-4A; see Figure S1 for separate temperature trends for each latitudinal band). This trend was largely due to an exceptionally warm spring in 2012 (mean spring temperature of 11.9 ˚C; in comparison, the coolest year, 2014, had a mean spring temperature of 7.8 ˚C), which leveraged the early end of the regression line upward (Figure 3-4A and S1). In accordance with the modest cooling trend observed in the dataset as a whole, the model revealed slight overall delay in spring phenology of trees and spring-flowering forest herbs between 2009 and 2021 (Table 3-3). There is no evidence that the functional groups differed in their rate of phenological shift with time, since the 95% BCI of the difference of the slope estimates overlapped zero (Table 3-3; mean and 95% BCI of the difference between the posterior distributions of the slope: 0.3 days/year [−0.2, 0.9]). For several species, there was no detectable directional change in phenology over the timespan analysed, and therefore no evidence that their spring phenology has advanced or delayed in our study region over the last 13 years (Table 3-3).

In 2012, the warmest year of the study period (Figure 3-4A), spring-flowering forest herbs exhibited an earlier spring phenology than trees—to the extent that leaf-out of spring-flowering forest herbs occurred 10 days earlier than tree leaf-out (mean and 95% BCI of the difference between posterior distributions of means for trees and spring-flowering forest herbs: −10.0 [−17.2, −3.0]). Conversely, both trees and spring-flowering forest herbs exhibited delayed spring phenology in 2014 (Figure 3-4B), the coolest year in the time-series analysed, resulting in overlapping leaf-out dates for the two functional groups in that year (mean and 95% BCI of the difference between posterior distributions of means for trees and spring-flowering forest herbs: 2.2 [−4.3, 8.6], Figure 3-4B).

3.4 Discussion

Using a plant phenology dataset that spans 13 years and 21 species, with 8,045 data points from 965 locations, we show that, overall, spring leaf-out is advancing more strongly with temperature for spring-blooming forest herb species than it is for trees in North America's eastern hardwood forest ecosystems. However, this trend varies with latitude, with no detectable difference between functional groups for lower latitudes (35–39° N), and spring-flowering forest herbs responding more strongly to temperature than trees at middle and higher latitudes (40–44° N and 45–48° N). Furthermore, both functional groups vary in their phenological response to warmer temperatures across the latitudinal range, with spring-flowering forest herbs at middle latitudes advancing their leaf-out date more than those at lower and higher latitudes, and trees at lower latitudes advancing their spring phenology more than those at middle and higher latitudes. In contrast to what has previously been reported in the literature, our study shows spring-flowering forest herbs advancing their phenology more strongly with temperature than deciduous trees throughout most of our North American study region.

3.4.1 Phenological responses of trees and spring-flowering forest herbs

Our finding that both trees and spring-flowering forest herbs advanced their spring phenology in response to warming temperatures is consistent with previous studies and suggests that the response of plants to rising temperatures could increase the length of the growing season in deciduous temperate forests (Jacques et al., 2015; Menzel et al., 2006; Zohner & Renner, 2014; although see Zani et al., 2020). For spring-flowering forest herbs, earlier emergence relative to tree leaf-out could increase fitness since they produce most of their photosynthates in spring before canopy closure (Dion et al., 2017; Heberling, Cassidy, et al., 2019; Jacques et al., 2015). Those photosynthates are used not only for vegetative growth and reproduction but also for rhizomal resource storage (Ida & Kudo, 2008). Thus, an earlier spring growing season could influence fitness both by increasing seed production and by increasing resource storage for future growth. Spring-flowering forest herbs are known for being slow colonizers, and there is concern that these species will not shift their geographic ranges poleward fast enough to respond to climate change (Augspurger & Salk, 2017; De Frenne et al., 2011). If spring-flowering forest herbs can respond to temperature changes through temporal shifts, they may adjust well to climate change over the short term, i.e., via phenotypic plasticity. Additional studies are needed to evaluate whether the phenotypic plasticity we observed would serve as an adaptive response to a warming climate (Duputié et al., 2015).

The different responses of trees and spring-flowering forest herbs to temperature in our study may occur because co-occurring trees and spring-flowering forest herbs in northeastern temperate deciduous forests sense temperature in different ways, even if the phenologies of both functional groups are mainly regulated by temperature. The main trigger for tree leaf-out is thought to be the accumulation of warm air temperatures (forcing), but tree phenology also responds to photoperiod and chilling (Basler & Korner, 2014; Laube et al., 2014; Zohner et al., 2016, 2017; Zohner & Renner, 2015), while spring-flowering herbs respond largely to soil temperature and snow depth (Jánosi et al., 2020; Pardee et al., 2019). However, the relationship between air temperatures, soil temperature, and snow depth is not straightforward because higher air temperatures are linked to decreased snow depth, and snow acts to insulate soil from temperature oscillations during winter. Thus, warmer air temperatures can lead, paradoxically, to colder soil temperatures, if soil remains frozen but not snow-covered (P. J. Brown & DeGaetano, 2011; Zhu et al., 2019). Therefore, higher temperatures could allow spring-flowering forest herbs to fulfill their chilling requirements (Augspurger & Salk, 2017) due to still-cold below-ground temperatures through the winter, and to experience warm temperatures in early spring due to the lack of insulating snow (P. J. Brown & DeGaetano, 2011; Zhu et al., 2019), which could explain their stronger response to warmer temperatures.

Despite general agreement that phenological responses to temperature are likely to vary across latitudes, there is still no consensus as to whether populations at higher latitudes are indeed more sensitive to temperature. Previous studies of herbaceous plants have reported more responsiveness to temperature for populations at higher latitudes or lower latitudes (Park et al., 2019; Parmesan, 2007; J. Prevéy, Vellend, Rüger, Hollister, Bjorkman, Myers-Smith, et al., 2017; Routhier & Lapointe, 2002; Zhang et al., 2007). For trees, there is no consensus either way, with studies reporting higher latitudes responding more than middle and lower latitudes (Morin et al., 2009a), or a similar response across latitudes (Phillimore et al., 2012). However, the studies mentioned above vary widely in terms of methodologies, geographical location, and latitudinal range analysed, which could contribute to the differences reported. Here, we found that spring-flowering forest herbs at middle latitudes (40–44˚ N) were more responsive to warmer temperatures than those at lower (35–39˚ N) or higher latitudes (45–48˚ N). On the other hand, trees' response to temperature was higher at lower latitudes (35–39˚ N) relative to middle and high latitudes.

We hypothesize that the divergent results of our study across the latitudinal range could lie in factors other than temperature, such as snowpack, precipitation, or even solar radiation. As mentioned above, temperature is regarded as a crucial driver of spring phenology. However, phenological responses are mechanistically complex, and temperature, photoperiod, and other variables correlated with latitude interact with each other. Changes in air temperature and precipitation with warming likely vary from one location to another, with mid-latitudes experiencing a larger number of days when the ground is not covered by snow during the winter relative to higher latitudes, and, consequently, earlier snowmelt (Zhu et al., 2019). As discussed, reduced snow cover when the soil is still frozen could allow spring-flowering forest herbs to fulfill their chilling requirements and respond rapidly to forcing, perhaps explaining the stronger response of spring-flowering forest herbs at these locations. As for trees, their response to warming temperatures is mediated by interactions with chilling and photoperiod (Basler & Korner, 2014; Laube et al., 2014; Zohner et al., 2016; Zohner & Renner, 2015). In particular, the shorter day lengths in late winter may prevent leafing out in response to warmer temperatures, and this photoperiodic constraint may be particularly acute at high latitudes (Flynn & Wolkovich, 2018; Fu et al., 2012; Fu, Zhao, et al., 2015; Fu et al., 2019; Zohner et al., 2017; Zohner & Renner, 2014, 2015). Our results reinforce the need for further research into how environmental variables and local adaptation interact to produce different phenological responses across geographic and environmental gradients.

Our finding that spring-flowering forest herbs advanced their phenology more than trees with warmer temperatures at middle latitudes (40–44˚) directly contradicts the findings of an earlier study, conducted within the same latitudinal range, but in a single location (Heberling, Fridley, et al., 2019). Specifically, Heberling et al. (2019) reported that trees were advancing their phenology more strongly in response to warming than spring-flowering forest herbs, resulting in a shorter period for the latter to accumulate carbon before canopy closure, which the authors suggested could jeopardize fitness. To ensure that differences between the two studies were not due to differences in the time-window used to define spring, we re-analyzed our data using temperatures from the same date range used by Heberling et al. (2019), i.e., from March 1 to April 30. This change did not qualitatively affect our results, which still showed spring-flowering forest herbs advancing their phenology more than trees by –1.5 days/˚C on average (or by −0.9 days/˚C if we include only observations coming from the latitudinal range of 40–44˚N; see Table S2). Therefore, the discrepancy between the two studies could be a consequence of three non-mutually exclusive factors. First, the two studies, despite having some overlap in the tree species selection (six species out of the 15 in the Heberling's study), have no overlap in the set of herbaceous species studied. Species-specific responses to temperature variation could therefore be driving the different results. Secondly, the studies differ in their geographical scopes. Heberling et al. (2019) studied a single geographical location, while our dataset spans 965 locations across northeastern North America. The specific environmental conditions at a single site, such as precipitation, elevation, and edaphic factors, can affect phenology at a small scale (Ge et al., 2015; Wielgolaski, 2001), making it unwise to extrapolate from a single site to larger areas. Third, despite encompassing a broader geographical range, our study covered a relatively short time-period (2009–2021), whereas Heberling et al. (2019) analyzed contemporary data (2004–2018) and historical observations (1850s) together. We re-analyzed their data for the contemporary period only and found no difference in the rate of change between trees and spring-flowering forest herbs, suggesting that the findings of Heberling et al. (2019) were largely driven by the historical observations, which encompassed cooler temperatures than those observed today. It is therefore possible that differences in the results were driven by the differences in the time-period analysed (see Dose & Menzel, 2004; Ge et al., 2015; Rutishauser et al., 2007). Past and future temperatures beyond the range of conditions observed in our dataset could yield phenological shifts that would not be predicted by our model results, if, for example, phenological responses to temperature are actually non-linear—as is expected if factors other than forcing temperatures also influence phenology, as discussed by Wolkovich et al. (2021), Flynn & Wolkovich (2018) and Iler et al. (2013). The differences between the studies reinforce the need for more large-scale phenological syntheses encompassing long-term observations of multiple species (Gallinat et al., 2021).

3.4.2 Implications of warmer temperatures for the hardwood forest ecosystem

There are still several unanswered question about the implications of warmer temperatures for the fitness of spring-flowering forest herbs and trees. For instance, warmer temperatures during spring can hinder bulb growth (Lapointe, 2001; Nault & Gagnon, 1993), but we lack studies investigating how such changes to hypogeous growth caused by warming affect plant fitness. Also, earlier leaf senescence under warmer temperatures has been reported in spring-flowering forest herbs and has been attributed to reduced bulb growth (Heberling, Cassidy, et al., 2019; Lapointe, 2001; McKenna & Houle, 2000); however, this response varies widely among individuals and species (Jacques et al., 2015; McKenna & Houle, 2000). Furthermore, species could be affected differently by warmer temperatures given their different carbon gain strategies (Augspurger & Salk, 2017; Heberling, Cassidy, et al., 2019; Jacques et al., 2015). Again, more studies are needed to address how warmer temperatures influence rhizome growth and forest herb fitness over the long term.

For trees, earlier leaf-out and longer growing season could also be beneficial if they increase the time available for carbon uptake. In contrast to spring-flowering forest herbs, most of the energy used by trees in temperate deciduous forests for the development of flowers and fruits comes from previously stored nutrients, since most flower before leaf-out (Augspurger & Zaya, 2020). Thus, consistently earlier leaf-out could translate into more stored resources for reproduction in subsequent years. However, increased photosynthetic productivity by trees at the beginning of the growing season can also cause earlier leaf senescence, which in turn shortens the growth period (Zani et al., 2020). More studies are needed to address how changes in the length of the growing season due to higher temperatures will affect carbon uptake by trees in the future.

Earlier emergence and leaf-out could expose both spring ephemerals and trees to unpredictable frost events, since warmer springs may not be accompanied by parallel increases in winter temperatures. Climate change is also expected to increase the frequency of extreme weather events (Marino et al., 2011; Solomon, 2007). Late frost can cause significant damage and fitness loss to plants (Augspurger, 2009; Gu et al., 2008; Inouye, 2008; Lenz et al., 2013; Pardee et al., 2019). For instance, in 2007, a major frost event took place in Illinois, USA, and as a result, the leaf expansion period was extended by 16–34 days for refoliating species (Augspurger, 2009). Leaf or leaf-bud loss to frost represents a significant cost for deciduous trees, affecting growth, reproduction, canopy expansion and nutrient reserves, as refoliation to compensate for damage demands extra resources, despite a shorter growing season in which to acquire them. However, spring-flowering forest herbs might benefit from such frost events if trees suffer the brunt of the damage from exposure to the cold night sky while sheltering the understorey plants (Man et al., 2009), which can then profit from the decreased shading.

Furthermore, to fully assess the consequences of warmer temperatures for plant fitness it is necessary to consider their interactions with herbivores and pollinators. For instance, shifts in timing of herbivore emergence relative to plant phenology, as well as changes in the frequency or severity of herbivore outbreaks (independent of phenology), could have major impacts on understorey shading and carbon budgets of trees and understorey plants (Fabina et al., 2010; Pureswaran et al., 2018; Ren et al., 2020). Also, if plants and insects do not respond at the same rate to warming, mismatches between flowers and flower-visitors could occur (Kharouba et al., 2018; Kudo & Ida, 2013). Most tree species in temperate deciduous forests are wind-pollinated; warmer temperatures would therefore not affect their fitness through interactions with insect visitors. However, bees are known to visit and collect pollen from wind-pollinated trees in early spring when other pollen sources are scarce (Saunders, 2018); how earlier leaf-out of wind-pollinated trees would affect the fitness of these species is unknown. Spring-flowering forest herbs are generally insect-pollinated, and given the short flowering period of these species, phenological mismatches between these plants and their pollinators are possible (Kudo & Cooper, 2019). Also, since different phenophases can respond to warming at different rates (Buonaiuto et al., 2021), wind-pollinated trees could suffer reductions in pollination through mechanical interference if leaf-out shifts more dramatically than flowering.

3.5 Conclusions

Our study demonstrates that spring-flowering forest herbs advance their phenology more strongly than trees in response to higher temperatures throughout much of the range of deciduous temperate forests in North America. This greater responsiveness to warming could translate into a longer growing season for these understorey wildflowers. However, predicting how warmer temperatures will affect individual species over the long term is challenging. As we demonstrated, the phenological response to temperature varies with latitude, with functional groups differing in how their responses vary throughout the latitudinal range. We are still a long way from a comprehensive understanding of the complex mechanisms regulating the phenologies of trees and spring-flowering forest herbs, and how those drivers differ not only between functional groups, but also among species within those functional groups. Furthermore, the short-term and presumably plastic responses to temperature observed in this study do not tell us about the capacity for these long-lived plant species to adapt to climate change over evolutionary time scales. Finally, it will be important to investigate the generality of our results by testing whether the observed pattern holds for deciduous temperate forests in Europe and Asia.

Table 3-1 Relationships between spring phenology and temperature for 10 tree species and 11 forest herb species at 965 temperate deciduous forest sites in eastern North America. Mean spring temperatures were calculated by averaging the monthly mean temperatures from March 1 to May 31. Sample sizes for each species are shown in Table S1. Values shown are slope estimates (in days per °C) and Bayes-R^2 followed by their 95% BCI for the following model: $\boldsymbol{DOY = \alpha + \beta * temperature}$. Separate models were fitted for each species and functional group. Overall estimates for trees and spring-flowering forest herbs are shown at the top of the table. A negative BCI that does not overlap zero indicates an earlier spring phenology with higher temperatures.

	Species	**95% BCI**	**Bayes-R^2**
	Spring-flowering forest herbs	-4.9 [-5.2, -4.6]*	87.1 [86.4, 87.6]
	Trees	-3.3 [-3.5, -3.1]*	87.3 [86.9, 87.6]
Spring-flowering forest herbs	*Arisaema triphyllum*	-4.8 [-6.1, -3.5]*	69.3 [60.4, 75.9]
	Claytonia virginica	-4.6 [-6.7, -2.5]*	55.0 [44.3, 63.7]
	Clintonia borealis	-4.2 [-6.0, -2.6]*	82.4 [79.4, 84.7]
	Cornus canadensis	-3.4 [-4.4, -2.2]*	78.3 [72.1, 82.9]
	Erythronium americanum	-4.7 [-5.3, -4.0]*	71.7 [66.7, 75.6]
	Maianthemum canadense	-4.0 [-4.9, -3.0]*	90.1 [88.9, 91.2]
	Mertensia virginica	-4.9 [-6.4, -3.3]*	70.6 [63.2, 76.3]
	Podophyllum peltatum	-3.4 [-4.8, -2.1]*	63.9 [54.9, 70.9]
	Sanguinaria canadensis	-5.4 [-6.7, -4.1]*	64.8 [53.8, 73.5]
	Trillium erectum	-3.6 [-4.3, -2.9]*	73.8 [66.5,79.2]
	Trillium grandiflorum	-3.0 [-5.5, -0.1]*	70.8 [48.6, 88.4]
Trees	*Acer rubrum*	-3.8 [-4.1, -3.4]*	87.7 [86.8, 88.6]
	Acer saccharum	-3.2 [-3.8, -2.6]*	85.2 [83.1, 87.6]
	Betula alleghaniensis	-1.3 [-2.9, 0.7]	83.8 [81.0, 85.9]
	Fagus grandifolia	-2.8 [-3.2, -2.4]*	89.5 [88.4, 90.4]
	Fraxinus americana	-3.4 [-5.5, -1.3]*	94.7 [91.3, 96.5]
	Juglans nigra	-2.6 [-3.9, -1.1]*	73.8 [66.8, 78.6]
	Liriodendron tulipifera	-3.3 [-4.0, -2.7]*	64.9 [61.1, 67.9]
	Populus tremuloides	-3.3 [-4.4, -2.3]*	59.0 [48.0, 66.9]
	Prunus serotina	-3.9 [-4.8, -2.9]*	85.4 [83.2, 87.0]
	Quercus rubra	-3.5 [-4.4, -2.6]*	83.3 [79.5, 86.0]

*95% BCI does not overlap zero

Table 3-2 Relationships between spring phenology and temperature for 10 tree species and 11 forest herb species at 965 temperate deciduous forest sites in eastern North America. Mean spring temperatures were calculated by averaging the monthly mean temperatures from March 1 to May 31. Values shown are slope estimates (in days per °C) and 95% BCI for the following model: $\boldsymbol{DOY = \alpha + \beta * temperature}$. The difference among models is the time period over which the mean spring temperature was calculated. Separate models were fitted for each functional group. Overall estimates for trees and spring-flowering forest herbs are shown followed by the 95% BCI, and by the mean and 95% BCI of the difference between the estimates of the slopes. A negative BCI that does not overlap zero indicates an earlier spring phenology with higher temperatures. A negative value for the difference of the slope estimates indicates that spring-flowering forest herbs are advancing their phenology more than trees in response to temperature, and a positive value indicates that trees are advancing their phenology more in response to temperature.

Model	**Mean and 95% BCI**		**Mean and 95% BCI of the difference between the slopes**
	Spring-flowering forest herbs	**Trees**	
	February - April		
Overall	-4.5 [-4.8, -4.2]*	-2.6 [-2.8, -2.4]*	-1.9 [-2.2, -1.6]*
35°- 39°	-3.1 [-4.5, -1.6]*	-3.4 [-3.9, -2.8]*	0.3 [-1.3, 1.8]
40°- 44°	-3.9 [-4.7, -3.1]*	-2.3 [-2.7, -2.0]*	-1.5 [-2.4, -0.7]*
45°- 48°	-1.1 [-2.2, 0.0]	0.4 [-0.2, 1.0]	-1.6 [-2.8, -0.3]*
	March - May		
Overall	-4.9 [-5.2, -4.6]*	-3.3 [-3.5, -3.1]*	-1.6 [-1.9, -1.3]*
35°- 39°	-2.7 [-4.4, -1.0]*	-3.7 [-4.3, -3.1]*	1.0 [-0.8, 2.9]
40°- 44°	-4.9 [-5.7, -4.0]*	-3.5 [-4.0, -3.0]*	-1.4 [-2.4, -0.4]*
45°- 48°	-3.4 [-4.5, -2.2]*	0.4 [-0.6, 1.5]	-3.8 [-5.3, -2.3]*
	April - June		
Overall	-5.3 [-5.6, -5.0]*	-3.5 [-3.8, -3.2]*	-1.8 [-2.2, -1.3]*
35°- 39°	-2.1 [-4.0, -0.2]*	-2.5 [-3.5, -1.4]*	1.7 [-0.4, 3.7]
40°- 44°	-5.0 [-6.0, -4.0]*	-2.7 [-3.5, -1.9]*	-2.2 [-3.5, -1.0]*
45°- 48°	-4.3 [-5.6, -3.0]*	-2.3 [-3.1, -1.3]*	-2.0 [-3.6, -0.5]*

*95% BCI does not overlap zero

Table 3-3 Relationships between spring phenology and year for 10 tree species and 11 spring-flowering forest herb species at 965 temperate deciduous forest sites in eastern North America. Sample sizes for each species are shown in Table S1. Values shown are slope estimates and Bayes-R^2 followed by their 95% BCI for the following model: $\boldsymbol{DOY = \alpha + \beta * year}$. Separate models were fitted for each species and functional group. Overall estimates for trees and spring-flowering forest herbs are shown at the top of the table. A positive BCI that does not overlap zero indicates that spring phenology became later over the time period analysed here; a negative BCI indicates that spring phenology became earlier.

	Species	**95% BCI**	**Bayes-R^2**
	Spring-flowering forest herbs	0.6 [0.1, 1.0]	83.3 [82.5, 84.2]
	Trees	0.2 [0.0, 0.5]	83.5 [83.0, 84.0]
Spring-flowering forest herbs	*Arisaema triphyllum*	1.1 [-0.3, 2.6]	54.4 [42.0, 63.3]
	Claytonia virginica	1.0 [-1.3, 3.5]	49.4 [38.8, 57.9]
	Clintonia borealis	-0.1 [-1.0, 0.8]	72.4 [67.5, 76.1]
	Cornus canadensis	0.6 [-0.9, 2.0]	63.9 [54.7, 70.6]
	Erythronium americanum	0.2 [-0.7, 1.0]	58.8 [50.7, 65.1]
	Maianthemum canadense	0.6 [-0.3, 1.5]	84.6 [82.9, 86.0]
	Mertensia virginica	-0.3 [-1.3, 0.8]	62.0 [54.0, 58.0]
	Podophyllum peltatum	0.1 [-1.2, 1.3]	51.4 [41.2, 59.7]
	Sanguinaria canadensis	-2.2 [-4.5, -0.1]	56.1 [41.2, 67.0]
	Trillium erectum	0.7 [-0.2, 1.7]	63.2 [53.2, 70.5]
	Trillium grandiflorum	-0.8 [-2.3, 1.2]	64.3 [45.6, 75.2]
Trees	*Acer rubrum*	0.2 [-0.2, 0.7]	79.4 [78.4, 80.3]
	Acer saccharum	-0.8 [-1.4, -0.2]	82.0 [80.8, 83.1]
	Betula alleghaniensis	1.9 [0.3, 3.6]	82.2 [80.0, 84.0]
	Fagus grandifolia	1.3 [0.6, 2.0]	85.7 [84.4, 86.7]
	Fraxinus americana	2.8 [-0.9, 6.7]	87.4 [84.4, 89.4]
	Juglans nigra	0.4 [-0.5, 1.3]	52.4 [43.5, 59.6]
	Liriodendron tulipifera	-0.2 [-0.7, 0.3]	49.0 [45.3, 52.3]
	Populus tremuloides	1.2 [-0.1, 2.7]	60.4 [52.0, 66.8]
	Prunus serotina	-0.2 [-1.2, 0.8]	79.0 [77.7, 80.8]
	Quercus rubra	-0.4 [-1.3, 0.3]	63.5 [59.6, 66.8]

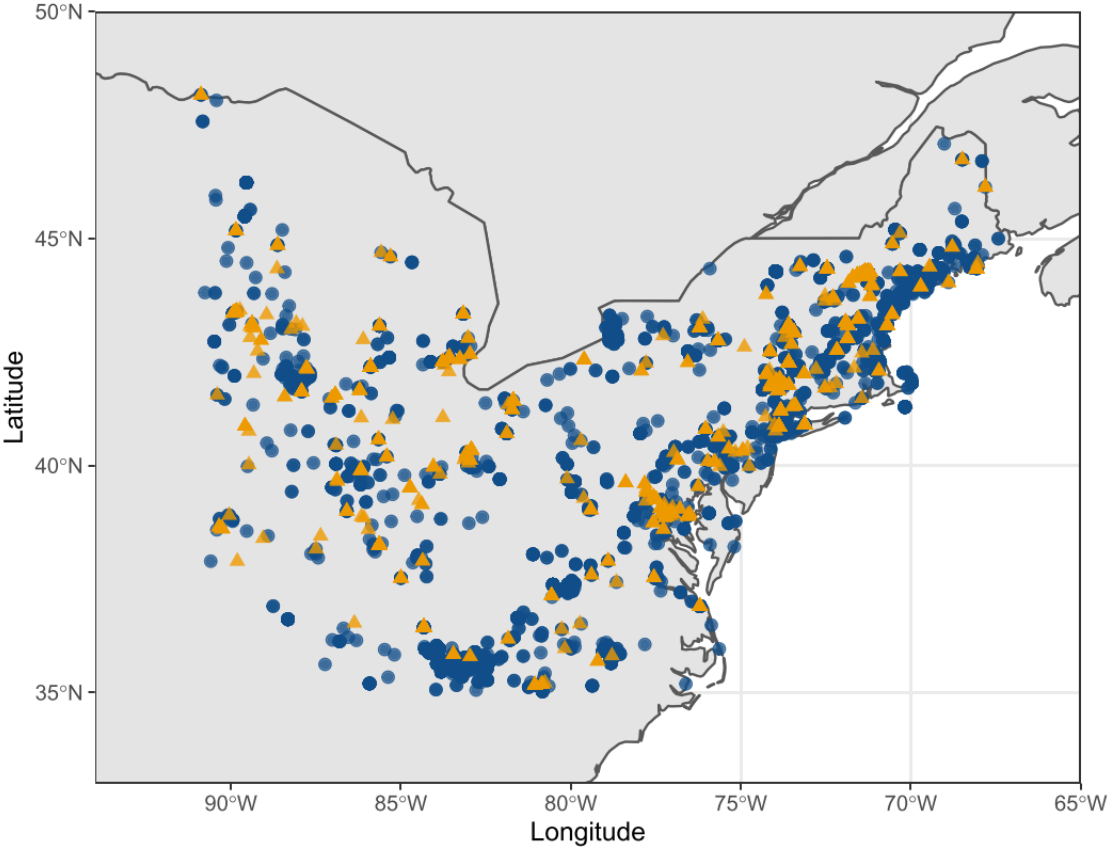


Figure 3-1 Map of eastern North America showing the locations of the 965 temperate deciduous forest sites for 10 tree species (blue dots) and 11 forest herb species (yellow triangles).

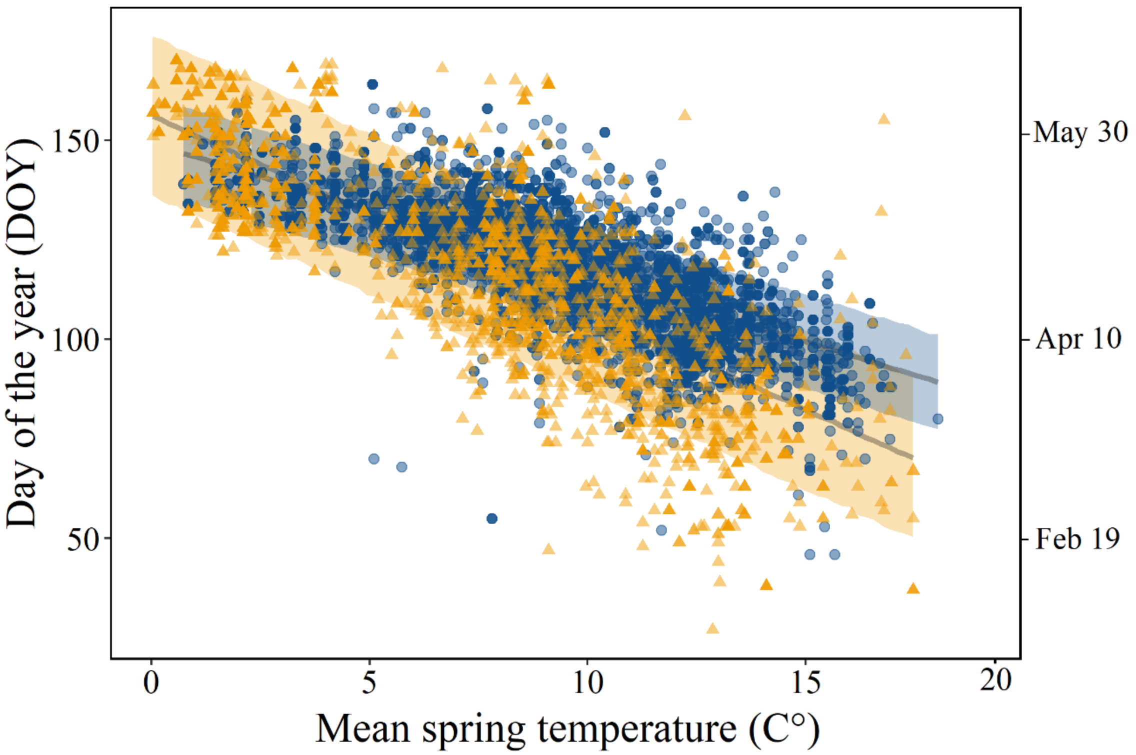


Figure 3-2 Relationship between spring phenology (day of year of leaf-out) and temperature for 10 tree species (blue dots) and 11 forest herb species (yellow triangles) (N = 6,040 tree observations, 2,005 forest herb observations) at 965 temperate deciduous forest sites in eastern North America. Separate models were fitted for each functional group. Solid lines show predicted relationships ($\boldsymbol{DOY = \alpha + \beta * temperature}$), blue and yellow shading indicate the 95% BCI. Mean spring temperatures were calculated by averaging the monthly mean temperatures from March 1 to May 31.

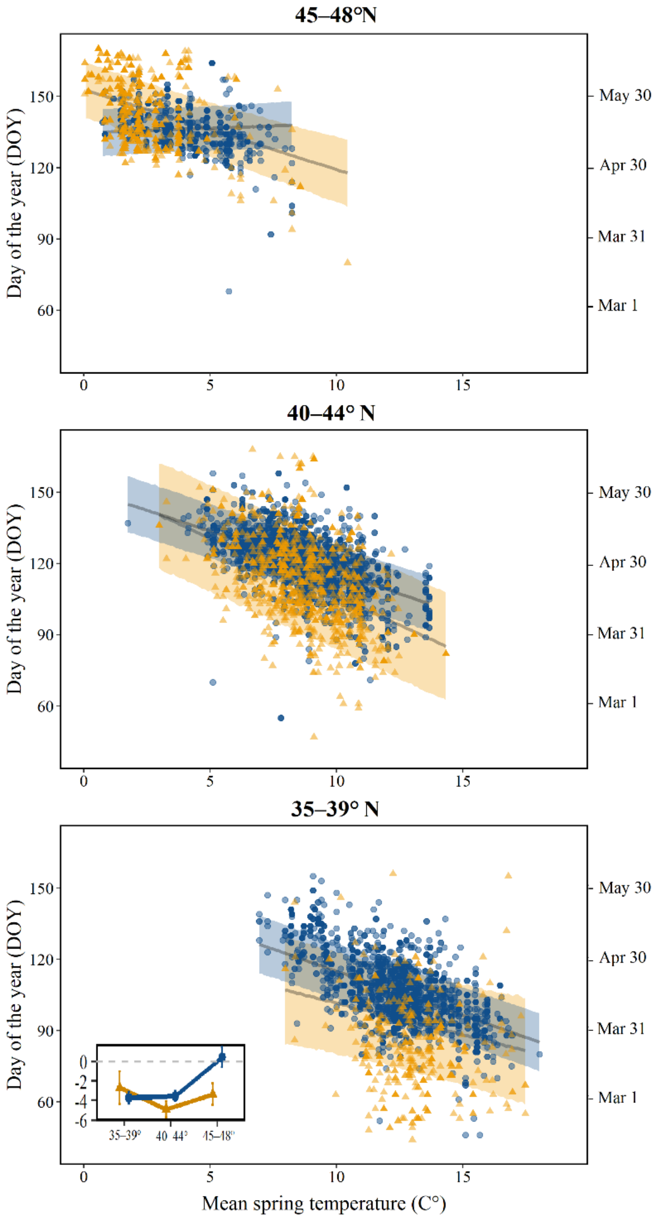


Figure 3-3 Predicted relationships between leaf-out day and temperature (solid lines) for different latitudinal bands, from the model $\boldsymbol{DOY = \alpha + \beta \times temperature}$, for trees (blue dots) and spring-flowering forest herbs (yellow triangles). Blue and yellow shading indicate the 95% BCI. Inset figure shows the response to temperature (days/˚C) of both functional groups for each of the latitude categories (35–39˚, 40–44˚, 45–48˚ N). Mean spring temperatures were calculated by averaging the monthly mean temperatures from March 1 to May 31.

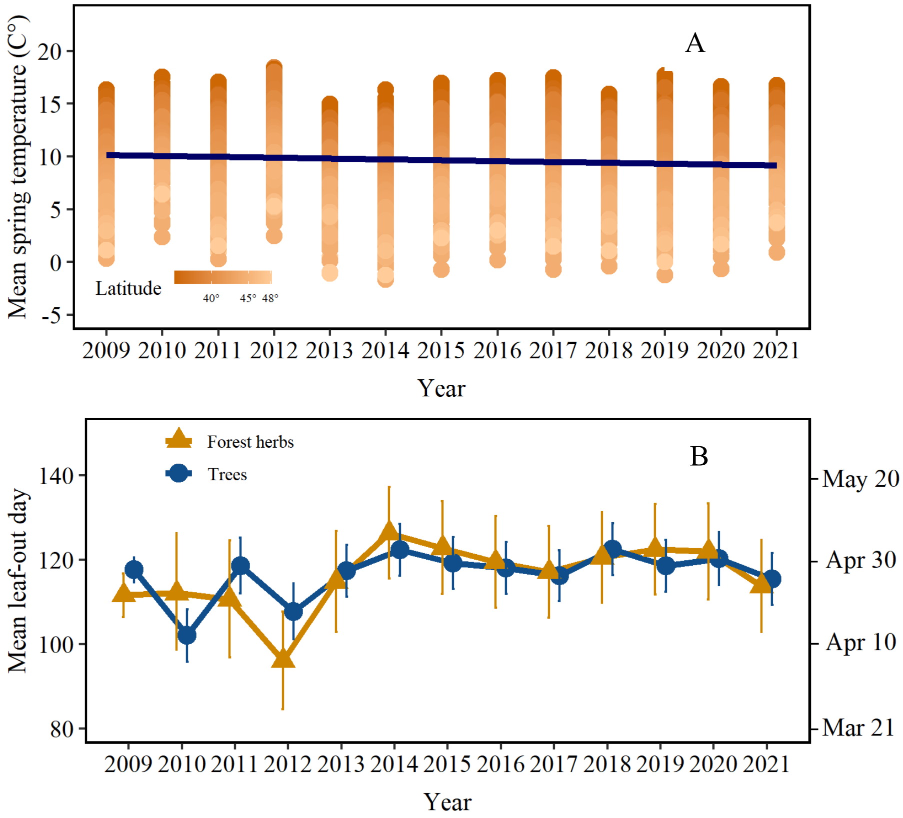


Figure 3-4 Mean spring temperature (March 1 to May 31, calculated by averaging the monthly mean temperatures) from 2009 to 2021 for 965 temperate deciduous forest sites in eastern North America. The decrease in mean spring temperature (shown by the dark blue line, representing the linear trend over time across all sites) was caused by a warm 2012. B) Estimates and 95% BCI for the mean leaf-out dates for 10 tree species and 11 spring-flowering forest herb species at 965 temperate deciduous forest sites in eastern North America ($\boldsymbol{DOY = \alpha + \beta * year}$, where year is categorical).

3.6 Appendix

USA–NPN – phenological data format and individual definition

USA–NPN offers four primary data formats: Individual phenometrics, site phenometrics, status and intensity data, and magnitude phenometrics. Phenometrics are defined by the USA–NPN documentation as “derived metrics that characterize the expression of a phenophase at multiple temporal and spatial scales”. For this study, we used the individual phenometrics data format, which provides information about the onset and end of a specific phenophase on an individual plant. Individual plants are defined differently for the two functional groups in this study (trees and forbs). For trees, an individual ID, i.e., a unique identifier, is provided for every single individual. However, for forbs, the individual ID can be linked to a patch of individuals, or to a single plant. A patch is a permanently marked area and the unit of observation is all the stems within the patch instead of a single individual plant. In our study, most of the observations came from patches, and not from single individuals (1,146 observations from patches, and 859 observations for which no information was available on whether the individual ID referred to a single individual or a patch).

3.7 Supplementary information

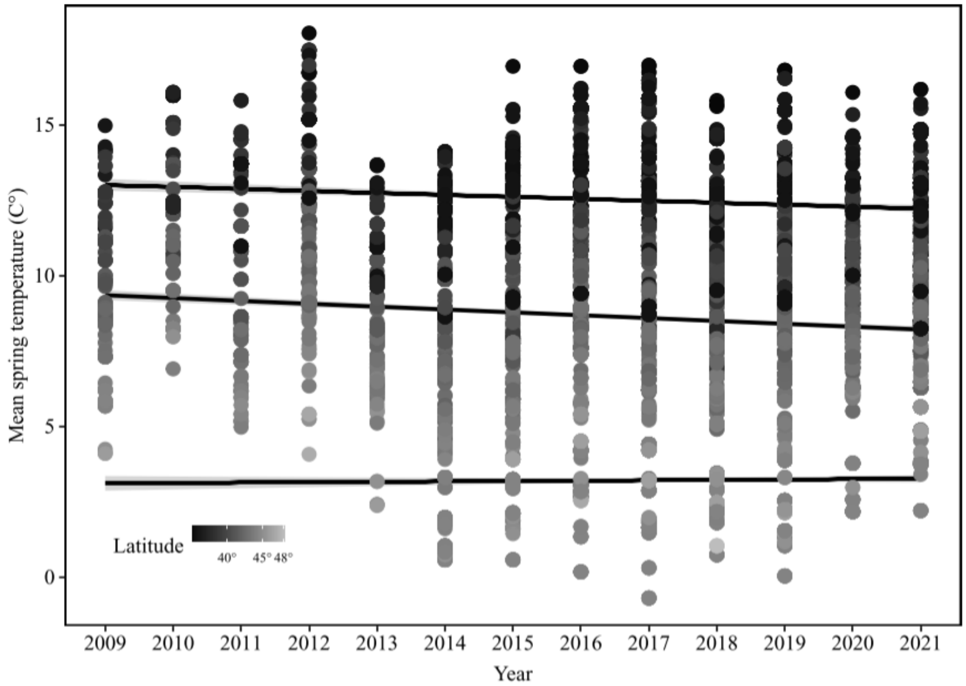


Figure 3-S1: Mean spring temperature (March 1 to May 31, calculated by averaging the monthly mean temperatures) from 2009 to 2021 for 965 temperate deciduous forest sites in eastern North America. The regression lines from top to bottom represent the temperature trend for each one of the latitudinal ranges: 35–39˚N, 40–44˚N, 45–48˚N.

Table 3-S1: Total number of observations for 10 trees species and 11 forest herbs, followed by the number of observations for each one of the latitude categories (35–39˚, 40–44˚, 45–48˚N), at 965 temperate deciduous forest sites in eastern North America. The leaf‒out date and its standard deviation (SD) are also shown for trees and for forest herbs.

Species	**Number of observations**				**Mean leaf-out**	**SD leaf-out**
	Total	**35–39˚N**	**40–44˚N**	**45–48˚N**		
Arisaema triphyllum	159	21	129	9	127	17
Claytonia virginica	154	115	34	5	78	23
Clintonia borealis	210	0	15	195	146	12
Cornus canadensis	158	1	30	127	149	15
Erythronium americanum	219	23	165	31	104	18
Maianthemum canadense	408	1	146	261	133	19
Mertensia virginica	203	120	81	2	88	20
Podophyllum peltatum	186	45	141	0	116	17
Sanguinaria canadensis	113	33	78	2	102	21
Trillium erectum	139	4	51	84	121	15
Trillium grandiflorum	56	18	36	2	104	16
Forest Herbs	**2005**	**381**	**906**	**718**	**118**	**28**
Acer rubrum	1857	563	812	482	122	16
Acer saccharum	994	141	512	341	124	14
Betula alleghaniensis	278	104	46	128	136	13
Fagus grandifolia	575	195	171	209	125	15
Fraxinus americana	112	25	80	7	121	18
Juglans nigra	201	150	51	0	119	9
Liriodendron tulipifera	852	723	129	0	100	10
Populus tremuloides	154	3	37	114	130	10
Prunus serotina	420	138	264	18	115	16
Quercus rubra	569	125	433	11	123	14
Trees	**6040**	**2172**	**2558**	**1310**	**120**	**17**
Grand total	**8045**	**2553**	**3464**	**2028**	**119**	**20**

Table 3-S2: Relationships between spring phenology and temperature for 10 tree species and 11 forest herb species at 965 temperate deciduous forest sites in eastern North America. Mean winter temperatures were calculated by averaging the monthly mean temperatures from December 1 to February 28. Values shown are slope estimates (in days per °C) and followed by the 95% BCI for the following model: $DOY = \alpha + \beta * temperature$. Separate models were fitted for each functional group. A negative BCI that does not overlap zero indicates an earlier spring phenology with higher temperatures.

Forest herbs	**Trees**	**Mean and 95% BCI of the difference between slope estimates**
Winter - December 1 to February 28		
-4.5 [-4.9, -4.1]*	-1.9 [-2.1, -1.7]*	-2.6 [-3.0, -2.1]*
March 1 to April 30		
-4.5 [-4.7, -4.2]*	-2.3 [-3.1, -2.8]*	-1.5 [-1.9, -1.2]*

*95% BCI does not overlap zero

Table 3-S3: Mean and 95% BCI of the difference between slope estimates (response to mean spring temperature, in days per °C) for the model: $DOY = \alpha + \beta * temperature$. Separate models were fitted for each latitudinal range and mean spring temperature was calculated using three different time-windows: from February to April, from March to May, and from April to June. A negative value for the difference between slope estimates indicates that plants at lower latitudes are advancing their phenology at a higher rate than plants at higher latitudes in response to temperature, and a positive value indicates that plants at higher latitude are advancing their phenology at a higher rate.

	Mean and 95% BCI of the difference between slope estimates	
	Forest herbs	**Trees**
	February - April	
35-39 & 40-44° N	0.9 [-0.8, 2.5]	-1.2 [-2.1, -0.2]*
40-44 & 45-48° N	-2.7 [-4.2, -1.4]*	-1.9 [-3.0, -0.9]*
35-39 & 45-48° N	-1.9 [-3.7, 0]	-3.1 [-4.3, -1.9]*
	March – May	
35-39 & 40-44° N	2.2 [0.3, 4.1]*	0.2 [-1.0, 1.4]
40-44 & 45-48° N	-1.6 [-3.0, -0.2]*	-3.3 [-4.8, -1.9]*
35-39 & 45-48° N	0.7 [-1.4, 2.7]	-3.1 [-4.7, -1.6]*
	April – June	
35-39 & 40-44° N	2.8 [0.7, 5.0]*	0.2 [-1.1, 1.5]
40-44 & 45-48° N	-0.7 [-2.3, 1.0]	-1.2 [-2.6, 0.1]
35-39 & 45-48° N	2.2 [-0.1, 4.5]	-1.0 [-2.5, 0.5]

* 95% BCI does not overlap zero

Table 3-S4: Mean and 95% BCI of the difference between slope estimates (response to mean spring temperature, in days per °C) for the model: $DOY = \alpha + \beta * temperature$. Separate models were fitted for each of the time-windows used to calculate the mean spring temperature, and the differences between slope estimates were calculated for each pair of time-windows within a latitudinal band. A negative value for the difference between slope estimates indicates that plants are more responsive to the earlier time-window of the pair being compared, and a positive value indicates that the plants are more responsive to the later time-window.

	Mean and 95% BCI of the difference between slope estimates	
	Forest herbs	**Trees**
35 - 39° N		
Feb - Apr/ Apr - Jun	-0.3 [-2.6, 2.0]	-0.6 [-1.9, 0.6]
Feb - Apr/ Mar - May	2.1 [0.5, 3.8]*	-0.6 [-1.8, 0.6]
Mar - May/ Apr - Jun	-0.9 [-3.4, 1.5]	-0.6 [-1.9, 0.7]
40 - 44° N		
Feb - Apr/ Apr - Jun	1.0 [-0.1, 2.1]	0.7 [-0.2, 1.5]
Feb - Apr/ Mar - May	-0.6 [-3.2, 2.0]	0.1 [-1.4, 1.5]
Mar - May/ Apr - Jun	1.1 [-0.2, 2.3]	0.8 [-0.2, 1.7]
45 - 48° N		
Feb - Apr/ Apr - Jun	2.2 [0.7, 3.9]*	-0.7 [-2.3, 0.8]
Feb - Apr/ Mar - May	0.1 [-1.2, 1.4]	0.1 [-1.0, 1.2]
Mar - May/ Apr - Jun	3.2 [1.5, 4.9]*	1.4 [0.0, 2.8]

* 95% BCI does not overlap zero

Chapter 4
Local environmental variables predict temperature sensitivities of phenology through chilling and heating dynamics in temperate deciduous trees

Abstract

Spring phenology, marked by events such as perennial plant leaf-out, is a crucial indicator of climate change due to its sensitivity to variation in temperature and precipitation. Yet, this sensitivity is itself variable, as each population adapts to its historical local climate conditions to maximize the growing season while minimizing costs (*e.g.*, from frost damage). Therefore, events such as leaf-out typically occur only after a specific duration of cold (chilling) temperatures and sufficient warming (forcing). A plant that experiences more chilling temperatures requires less forcing to trigger these events. Here, I examine whether variation across locations in the relationship between chilling accumulation and heat requirement (i.e., the CA-HR response rate) can be explained by local environmental factors, such as temperature and precipitation. Additionally, I examine whether these differences in CA-HR response rates are associated with the temperature sensitivities of phenology among locations. I tested these assumptions using leaf-out data from three temperate deciduous tree species (*Aesculus hippocastanum*, *Fagus sylvatica*, and *Quercus robur*) across various locations in Europe. Using phenological data from the Pan-European Phenological Project (PEP-725) and climatic data from the European Climate Assessment & Dataset (E-OBS) dataset, I applied Bayesian regression methods to estimate the CA-HR response rate for each location. I then correlated the CA-HR response rate with temperature sensitivities of phenology (days/°C) and regressed it against historical local environmental variables. Greater phenological sensitivity was associated with a flatter CA-HR response rate, meaning that populations exhibiting stronger phenological responses to temperature require less heating for each decrease in chilling accumulation. My analysis also revealed that historical temperature, precipitation, and elevation are all significantly associated with the CA-HR response rate, with warmer, drier, and lower-elevation locations exhibiting steeper CA-HR slopes, indicating a weaker phenological response to warming. Conversely, colder, wetter, and higher-elevation areas showed flatter CA-HR slopes, suggesting a stronger phenological response to warming. I also found that interannual variability in temperature and precipitation is related to the CA-HR response rate, with higher temperature variability linked to flatter CA-HR slopes and higher precipitation variability to steeper slopes. This temperature relationship varied by species, with *Q. robur* and *F. sylvatica* showing the pattern, while *A. hippocastanum* did not. These findings emphasize the importance of considering local adaptation in order to accurately predict how climate change will impact the phenology of temperate tree species.

4.1 Introduction

Phenology is recognized as a key indicator of climate change, given that changes in environmental variables such as temperature and precipitation can directly impact the timing of life cycle events in many species (Inouye, 2022; Piao et al., 2019). In temperate-zone woody plants, the timing of events such as bud-break is critical for maximizing growth and reproductive success and minimizing the risk of late frost damage in the face of variable weather conditions (Bennie et al., 2010). Precise physiological mechanisms control these spring phenological events, with air temperature being identified as the principal environmental trigger for bud-break (Ettinger et al., 2020b; Laube et al., 2014; Linkosalo et al., 2006; Zohner et al., 2017). Thus, understanding the impact of winter and spring temperatures on phenological events is paramount for predicting how climate change will reshape plant populations and communities.

Woody species enter dormancy during the fall, enabling the plant to withstand freezing temperatures (Powell, 1987). During the dormant period, exposure to chilling temperatures (as low as −2˚C and up to 10˚C; Baumgarten et al., 2021) is required for breaking endodormancy (the first stage of dormancy; Lang, 1987) and entering ecodormancy (the second dormancy stage; Lang, 1987). Endodormancy is broken when plants have experienced a sufficient duration of cold temperatures over autumn and winter. This physiological process prevents trees from mistaking a warm spell in winter for the onset of spring, which could lead to premature leaf-out and exposure to frost or other costly tissue damage (Lenz et al., 2016). The exact chilling requirement varies among species and even among populations of the same species, reflecting adaptation to local climatic conditions (Clark et al., 2014; Leites & Benito Garzón, 2023; Polgar & Primack, 2011; J. Prevéy, Vellend, Rüger, Hollister, Bjorkman, Myers-Smith, et al., 2017; J. S. Prevéy et al., 2018; Vitasse et al., 2013). Following the fulfillment of the chilling requirement, plants enter the ecodormancy phase (Chuine & Régnière, 2017; Hänninen, 2016), and meristem cells begin to grow, a process that is accelerated by warm temperatures (Hänninen, 2016). Finally, prolonged exposure to spring warmth (*i.e.*, forcing temperatures higher than 0˚C) leads to leaf-out (Perry, 1971). As with chilling, the amount of heat required varies, with some species or populations requiring only a few warm days while others need prolonged warmth.

Within a population or even within a single individual, chilling accumulation and heat requirements are not independent of one another: a greater accumulation of chilling normally reduces the amount of subsequent forcing required for budburst to occur (Fu et al., 2019). In other words, the within-individual or within-population relationship between a plant's chilling accumulation (CA) and its heat requirement (HR) is typically negative. The CA-HR relationship describes a species' or population's phenological response to winter and spring temperatures, allowing characterization of interspecific and intraspecific variation in temperature response. This variation could reflect adaptation to local environmental conditions, including not only temperature but also precipitation (Kramer et al., 2010; Osada et al., 2018; Peaucelle et al., 2019).

Two scenarios describe how within-population relationships between chilling accumulation and heat requirement might vary (Figure 4-1). Differences in the CA-HR relationship are characterized by differences in the intercept, α, and/or by differences in the slope (*β*) of the equation $HR = \alpha + \beta \times CA$, which measures the rate at which heat requirements vary with respect to changes in chilling accumulation. (For simplicity, I treat the CA-HR relationship as linear here, although a curvilinear relationship is also possible; see Section 4.2.3.) In the first scenario, two hypothetical locations, represented by the blue and red lines in Figure 1A, have different heating requirements for a given amount of chilling, *i.e.*, different intercepts; however, the rate of change in heat requirement in response to changes in chilling is the same. In the second scenario (Figure 4-1B), the rate of change in heat requirement in response to changes in chilling, *i.e.*, the slope, varies between locations. These slopes can then be used to investigate the environmental drivers of intraspecific geographic variation in the heating:chilling relationship. A steeper CA-HR slope means that a reduction in chilling corresponds to a larger increase in heat requirement than a flatter slope; perhaps counterintuitively, a steeper slope reflects a more risk-averse strategy in response to temperature changes, suggesting that these plants exhibit weaker phenological responses to warming and are less likely to track climatic changes closely.

As described above, historical environmental variables affect local adaptation in chilling and heating requirements, shaping a species' phenological response due to differing optimal strategies for survival. For instance, Peaucelle et al. (2019) found significant variation in temperature sensitivity and heat requirements for leaf-out among eight dominant European deciduous tree species using data from the Pan-European Phenology network (PEP-725). Populations at higher latitudes, with typically lower temperatures, had lower heat requirements, while trees at lower latitudes had higher heat requirements. The authors hypothesized that this was an adaptation preventing premature leaf-out during warm spells, thereby reducing the risk of frost damage. Interannual variation in environmental variables also affects temperature sensitivities; locations with higher interannual temperature variability exhibit weaker phenological responses to warmer temperatures, suggesting that plants are less likely to track climatic warming at these locations (T. Wang et al., 2014). Similarly, interannual variability in precipitation appears to drive local adaptation in chilling and heating requirements, influencing the timing of leaf-out and the required growing degree days for different populations (Peaucelle et al., 2019). Because the relationship between chilling accumulation and heat requirement varies geographically (Osada et al., 2018), this variation may influence a population's temperature sensitivity across a species' range and along latitudinal gradients (reported in Alecrim et al., 2023; Delgado et al., 2020; Miller et al., 2023; Park et al., 2019; Wolkovich et al., 2021)

Conversely, while numerous studies have documented geographical variation in chilling and heating requirements in response to local environmental conditions (Peaucelle et al., 2019 add others), there has been less attention to how the environment shapes the heating:chilling relationship itself. Wang et al. (2020)

and Lin et al. (2022) analyzed the CA-HR relationship to explore the validity of some chilling models, and a study conducted by Osada et al. (2018) explored the CA-HR relationship for *Fagus crenata* in Japan using a common garden experiment. The authors of the latter study concluded that trees from different latitudes of origin differed in their budburst timing due to local adaptation in the CA-HR relationship. However, while the study inferred the influence of climatic variables on the CA-HR relationship, it did not explicitly test these influences, focusing instead on observing patterns and making inferences based on geographical and climatic contexts. Thus, given the limited number of studies, a better understanding of the various drivers of the CA-HR relationship is needed, as the CA-HR response rate offers a mechanistic explanation for the observed variation in temperature sensitivity across geographical ranges by encapsulating how different populations adjust their heat requirements in response to chilling. Such an understanding is crucial for predicting phenological events and understanding how different populations may respond to climate change in terms of timing and growth cycles. Failure to account for intraspecific variation in temperature sensitivity when predicting how climate change will affect spring phenology could lead to unreliable predictions (Lin et al., 2022).

In this study, I test the hypothesis that geographical differences in how a plant species' heating requirements respond to changes in chilling, *i.e.*, geographic variation in the CA-HR response rate, can be explained by local environmental variables such as temperature and precipitation. Furthermore, given that chilling and heating determine leaf-out dates of woody species, I expect that intraspecific differences in the sensitivity of leaf-out timing to temperature would be related to how different populations modify their heat requirements in response to reductions in chilling. For this, I applied Bayesian regression methods to calculate the CA-HR response rates for three species across more than 2,000 locations in Europe. First, I regressed the heat requirement (HR) against chilling accumulation (CA) and obtained a unique CA-HR response rate for each location. Then, I tested whether geographical differences in the CA-HR response rate are consistent with an evolutionary influence of local environmental variables by analyzing how the CA-HR response rate changes with site characteristics through a regression analysis. Finally, I calculated the spring temperature sensitivity of leaf-out phenology (expressed in days/°C) for each location and tested for a correlation with the CA-HR response rate. The expectation was that populations with a relatively steep CA-HR response rate would show weak responsiveness to temperature changes, or lower temperature sensitivity, as a steep CA-HR response indicates that decreased chilling triggers a compensatory increase in the heat requirement for leaf-out.

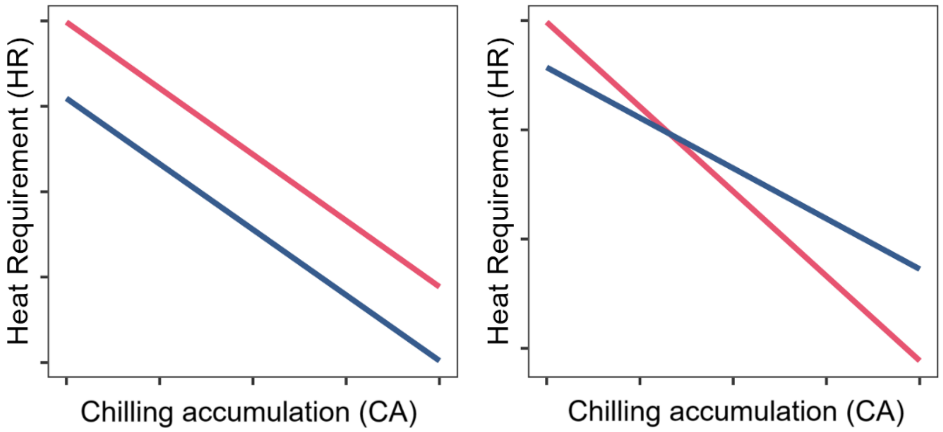


Figure 4-1 Representation of the theoretical CA-HR relationship for a species across different locations (or populations), represented by red and blue lines, under two different scenarios (A and B). A) Different locations have distinct heat requirements but respond to a decrease in chilling at a uniform rate (constant slope). B) Different locations have distinct baseline heat requirements and respond to decreases in chilling at different rates.

4.2 Methods

4.2.1 Phenological data and environmental variables

Phenological data - Phenological data were obtained from the Pan-European Phenological Project (PEP-725, http://www.pep725.eu/, Temple et al., 2018). PEP-725 was developed as a collaborative effort among experts in phenology to provide a standardized approach to capturing phenological data across a wide range of European plant species, ensuring that observers collect consistent and high-quality data. PEP-725 incorporates diverse phenological stages, such as bud break, leaf-out, flowering, fruiting, and senescence. The database contains almost 12 million records from plant species in 31 European countries, with data collected from 1868 to the present (Temple et al., 2018). The project started in Germany, and most of the observations come from there (Fig. 4-2).

I selected three temperate forest tree species: *Aesculus hippocastanum* (horse chestnut; Sapindaceae), *Fagus sylvatica* (European beech; Fagaceae), and *Quercus robur* (English oak; Fagaceae). Species were selected based on two key criteria: a broad geographical distribution across a latitudinal gradient spanning approximately 10°, and at least 20 years of consistent observations per location. I downloaded the phenological data from the PEP-725 website for each species for the phenophase 'new leaves' (BBCH=11), which is defined as when 'the first true leaf of a plant is fully expanded, and its entire leaf lamina is completely unfolded' (Meier, 2018).

Environmental variables - For each location, I also obtained daily records of precipitation and minimum, maximum, and mean air temperature from the E-OBS dataset (version v28.0e with spatial resolutions of 0.1°; https://www.ecad.eu). This dataset, provided by the European Climate Assessment & Dataset project, offers daily gridded observational data for various climatic variables across Europe, covering the period from 1950 to 2023.

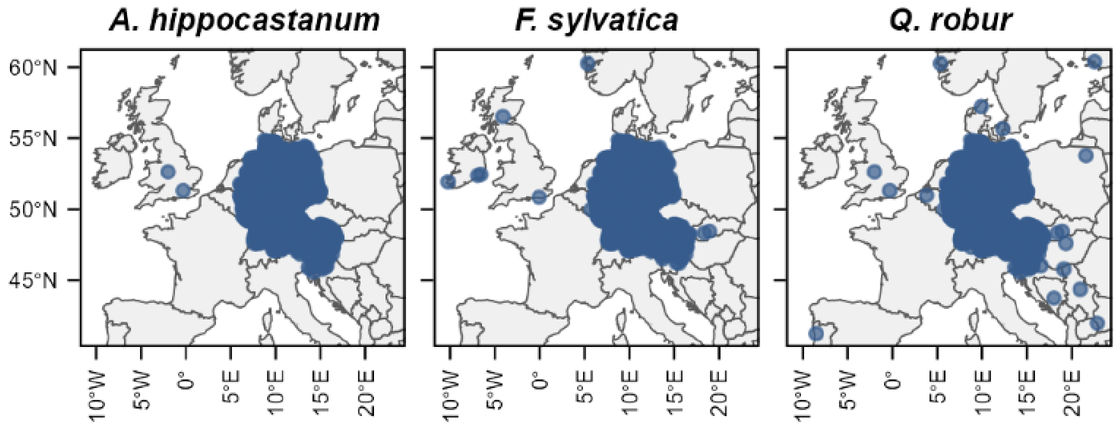


Figure 4-2 Locations of phenological stations for the species *A. hippocastanum* (n =3,703 locations), *F. sylvatica* (n = 2,914), and *Q. robur* (n = 2,785).

4.2.2 Chilling accumulation and heat requirement

4.2.2.1 Validation of different methods to define chilling and heating windows

There has long been debate about the best approach for detecting the start dates for chilling and heating phases (Guo et al., 2017; Luedeling & Gassner, 2012) and the possible variation in these dates across locations. To address this, I tested two methods—Partial Least Squares (PLS) regression and a fixed-window approach (Luedeling, Kunz, et al., 2013)—to examine their impact on predicting leaf-out dates. The process consisted of three steps. First, I used the PLS regression method to detect the beginning and end of the heating and chilling phases for each location. Second, I calculated chilling and heating for each location using the PLS regression-detected dates and using fixed dates applied across all locations. Finally, I compared which method best explained the leaf-out day. Each step is explained in detail below.

Step 1: Identification of location-specific chilling and heating windows using PLS regression - I used the *PLS_pheno* function from R's chillR package (Luedeling & Fernandez, 2022), which identifies critical days when temperature affects leaf-out date. For each day, the PLS regression provides two statistics: the Variable Importance in the Projection (VIP) and the standardized coefficient. The VIP assesses the relevance of daily temperature in predicting leaf-out, with high VIPs (greater than or equal to 0.8; Luedeling and Gassner, 2012) indicating a strong correlation between daily temperature and leaf-out.

The coefficient indicates the direction and intensity of the relationship between daily temperature and leaf-out date, with positive coefficients suggesting that higher temperatures delay budburst (chilling periods), while negative coefficients indicate that higher temperatures hasten budburst (forcing periods).

Following Luedeling & Gassner (2012), I identified the start of chilling by the presence of five consecutive days of positive coefficients and VIP ≥ 0.8 (Luedeling & Gassner, 2012). The chilling phase was defined as the period between the first and last occurrence of these 5-day patterns. The heating phase started with five consecutive days of negative coefficients and VIP ≥ 0.8, and the end of heating was marked by budburst. Following previous studies (Caffarra & Donnelly, 2011; Zhao et al., 2021), the heating phase was only allowed to start after December 1, ensuring that heating did not begin before chilling but allowing chilling and heating to accumulate in parallel (Kramer, 1994; Landsberg, 1974).

Step 2: Calculation of chilling and heating - I quantified chilling accumulation each year at each location using five commonly used chill models (Table 4-1). These models have previously been demonstrated to accurately represent the negative relationship between heat requirement and chilling accumulation (H. Wang et al., 2020). Since chilling accumulation and heat requirement calculations required hourly temperatures, which were unavailable, I used the chillR package to estimate hourly maximum and minimum temperatures from daily data. Briefly, chillR uses a sine wave model that assumes temperature follows a predictable, sinusoidal pattern, peaking at the daily maximum and reaching the lowest point at the daily minimum (Almorox et al., 2005; Luedeling, Guo, et al., 2013). I obtained each location's daily minimum and maximum air temperature records based on their coordinates from the abovementioned E-OBS project.

For the fixed-window approach, chilling accumulation was assumed to begin on November 1 (Cannell & Smith, 1984; Zhao et al., 2021) and end with budburst. For the PLS approach, I used location-specific dates derived from the PLS regression analysis. For the fixed-window approach, I calculated heat accumulation starting on January 1, and ending with budburst, following previous studies (Dantec et al., 2014; Zhao et al., 2021). For the PLS approach, as with the chilling calculations, I used location-specific dates derived from the PLS regression analysis. For both approaches, the heat requirement was quantified using the Growing Degree Hour Model (GDH; Anderson et al., 1986), calculated from hourly temperatures, as a function of a base (T_b=4°C), an optimum (T_μ=25°C) and a critical (T_c=35°C) temperature, meaning that hourly temperatures above the base threshold of 4°C were counted towards heat accumulation, with the contribution to growth reaching a maximum at 25°C and halting at the critical temperature of 35°C. For temperatures within the base to optimum range, the GDH contribution is calculated using a cosine function that smoothly transitions from zero at T_b to a maximum at T_μ, mirroring temperature's increasing effect on growth within this range. For temperatures between T_μ and T_c, the model uses a different cosine function to gradually reduce the GDH contribution, reflecting the diminished growth efficiency or potential thermal stress as temperatures approach T_c. The cumulative GDH value, representing total heat accumulation favourable to plant development, is obtained by summing these weighted contributions across the temperature series.

Additionally, I validated each chilling model based on the percentage of positive slopes obtained from the regression between GDH and chilling accumulation. Since positive slopes indicate a deviation from the expected physiological relationship, where a rise in chilling accumulation typically reduces forcing requirements, a model was considered validated only where fewer than 30% of the locations had positive slopes (H. Wang et al., 2020). I also tested how effectively different chilling models predicted the leaf-out day. For this purpose, I fit a linear regression with leaf-out day of year (DOY) as the response variable and chilling accumulation as the explanatory variable. I fit one regression for each location and chilling model and then compared the set of R^2 values across models using ANOVA, followed by a Tukey's HSD post-hoc test using the "stats" package in R (R Core Team, 2023).

Step 3: Comparison between the location-specific PLS regression and the fixed-window approaches - After calculating chilling and heating, I compared how much of the variation in leaf-out day was explained by each approach. This was done using separate regressions of leaf-out day as a linear function of chilling and heating for each location. Then, I used t-tests (one for each species) to compare the sets of R^2 values (one for each location and method) between the two methods to determine which one better explained the variation in leaf-out day.

4.2.3 CA-HR relationship

I then investigated the CA-HR relationship for each species in each location by fitting two models: a linear and a non-linear one (*i.e.,* curvilinear negative ($HR = a + b * exp(c * CR)$; where a is the asymptote, b is the initial value, and c is coefficient representing the rate of exponential change, which can be negative, indicating a decreasing relationship). The latter has been shown to be a good approximation for the relationship between chilling accumulation and heat requirement (H. Wang et al., 2020). However, since most non-linear models did not converge, and the ones that did converge exhibited an almost linear relationship, I present only the results for the linear models, which imply a constant rate of change in heat requirement for every unit increase in chilling accumulation ($HR = a + b * CH$; where a is the intercept and b is the slope).

Linear models were fit using a Bayesian linear regression, where heat requirement was the response variable and chilling accumulation was the explanatory variable. I fit separate regressions for each chilling model in Table 4-1. The Bayesian linear regression approach provided 95% credible intervals for the CA-HR response rates, indicating measurement uncertainty. This uncertainty was integrated as probabilistic distributions in subsequent models, where the CA-HR response rate was regressed against climate variables (section 2.4), thus incorporating the uncertainty in the CA-HR response rate into the regression.

This first regression (Figure 4-3, left panel) has the form:

$$HR_i = \alpha + \beta \times CR_i + \epsilon_i \tag{1}$$

Where:

- HR_i is the heat requirement for the i^{th} year,
- CR_i is the chilling accumulation for the i^{th} year,
- α is the intercept,
- β is the slope (CA-HR response rate),
- ϵ_i is the error term for the i^{th} year.

4.2.4 Environmental predictors of the CA-HR response rate

To explore the drivers of intraspecific geographic variation in the relationship between heat requirement and chilling accumulation, I used the slopes (CA-HR response rates) from the linear models described in section 4.2.2.2, in which heat requirement was the response variable and chilling accumulation was the explanatory variable. Here, the CA-HR response rate was used as the response variable, and 8 site-specific environmental variables were the predictors. These environmental variables included: (1-2) the historical average maximum and minimum temperatures; (3-4) the historical interannual variation in maximum and minimum temperatures; (5) the historical average precipitation; (6) the historical interannual variation in precipitation; (7) elevation (obtained using the elevatr R package (Hollister et al., 2023), and (8) latitude. The historical averages were calculated as the mean of all annual minimum/maximum daily records for each location from 1950 to 2023. The interannual variation in temperature was computed as the standard deviation, and the interannual variation in precipitation was computed as the coefficient of variation over the same period. I included only the years for which data were available for both phenological and environmental variables (1950 to 2023) and only sites where all eight variables were available. To further investigate how environmental variables covaried, I calculated a table of correlations between the environmental variables using Pearson's correlation coefficient with the *cor* function from the *stats* package in R (R Core Team, 2023).

For each species, I fit Bayesian models of CA-HR response rate vs. the explanatory variables listed above. Since these explanatory variables are highly correlated (see Results), I fit a separate model for each one. The likelihood for each model was structured as follows (Figure 4-3, right panel):

$$\beta_j \sim N(\gamma_0 + \gamma_1 \times Env.Var._j \;, \sigma^2_{\beta j}) \tag{2}$$

Where:

- β_j is the slope (CA-HR response rate) obtained from the CA-HR regression for location j,
- γ_0 is the intercept,

- γ_1 is the slope for the environmental variable,
- $\sigma^2_{\beta j}$ is the variance of β_j, representing the uncertainty in the slope of the CA-HR regression for location j,
- N is the distribution for β_j**,** indicating that it follows a normal distribution with mean $\gamma_0 + \gamma_1 \times Env.Var._j$ and variance $\sigma^2_{\beta j}$.

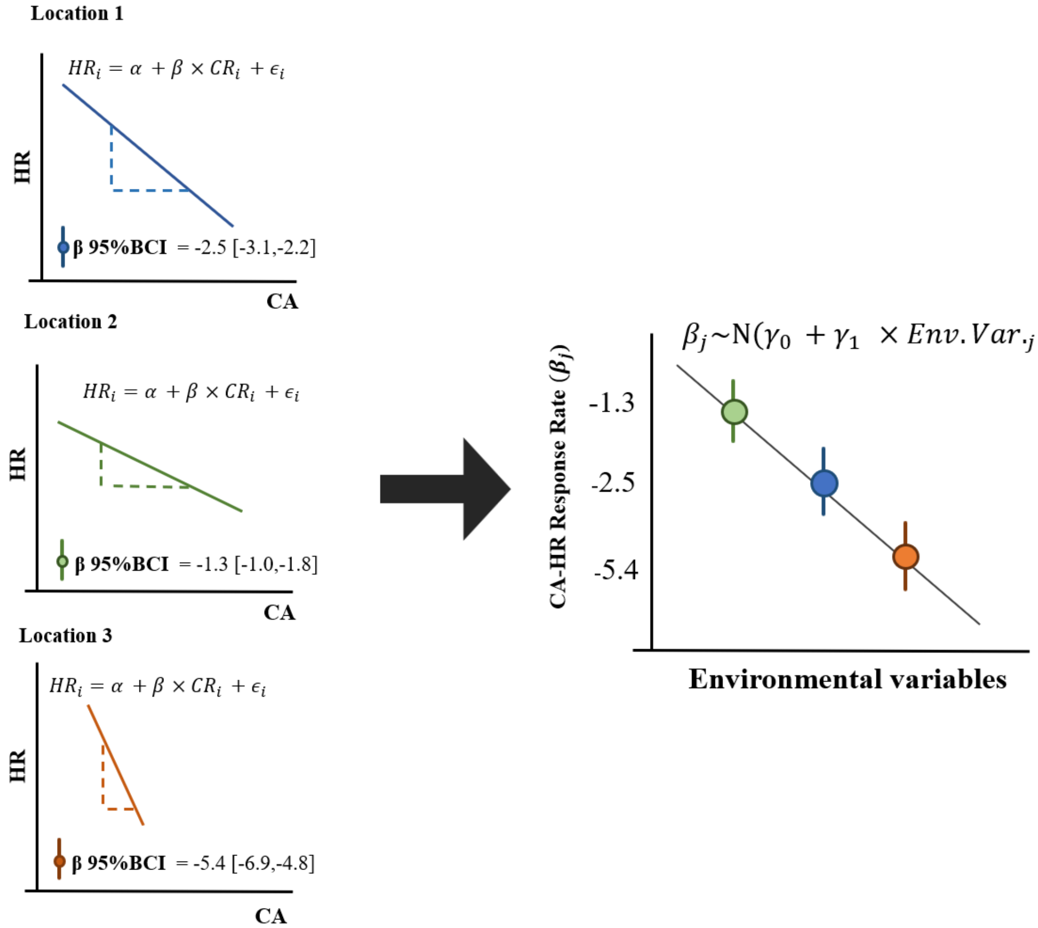


Figure 4-3 The CA-HR response rate (β) was obtained across multiple locations, here represented by three illustrative locations. The left three panels represent the first-stage regression models (Eqn. 1, section 2.2) for the heat requirement (HR) as a function of the chilling accumulation (CA) at those locations, each resulting in a specific CA-HR response rate (β) with a 95% Bayesian Credible Interval. The right panel illustrates the second-stage regression (Eqn. 2, section 4.2.5), where the CA-HR response rate from each location is regressed against environmental variables, incorporating the uncertainty around the CA-HR response.

4.2.5 Geographic variation in phenological sensitivity and the CA-HR response rate

To assess the extent to which differences in the CA-HR response rate can account for geographic variation in phenological sensitivity, I calculated the correlation between phenological sensitivity and CA-

HR at each location. Here, phenological sensitivity is defined as the change in timing of phenological events for each degree of variation in springtime mean temperature, expressed in days/°C. As explained in the introduction, I expect locations with flatter CA-HR response rates to exhibit greater phenological sensitivity.

This analysis was done in two phases: initially, I fit a linear regression where the leaf-out day (DOY) was a function of temperature, where DOY represents the day of the year when the leaf-out phenophase, indicated by the appearance of new leaves, was first observed, as detailed in section 4.2.1. The linear regression was produced using the *lm* function in (R Core Team, 2023). Temperature data for each location were obtained from the E-OBS project, as described in section 4.2.1. For this analysis, spring temperatures were calculated for each location and year from February 1 to April 30. This timeframe was chosen because the average leaf-out day occurs around April 10, based on analysis of the data. This first step produced a phenological sensitivity estimate (*i.e.*, the slope for each regression) for each location. Then, I correlated this estimate with the CA-HR response rate obtained for each location as described above, using the R cor.test function (R Core Team, 2023).

All Bayesian models were fit using the *brms* package (Bürkner, 2017), which allows stan to be run from R (R Core Team, 2023). I obtained the parameters' posterior distributions using Hamiltonian MC (Betancourt & Girolami, 2013). To monitor convergence, I ran three chains with different start values for a minimum of 3,000 iterations. I adopted a warm-up of 1,000 iterations and calculated Rubin's R (Gelman & Rubin, 1992), where convergence is assumed when R is close to 1. By sampling from the posterior distribution, I computed the mean and the 95% Bayesian credible intervals (BCIs) for all parameters using 3,000 draws from the posterior. All priors were flat.

4.3 Results

4.3.1 Chilling and heating windows

Compared to the fixed-window method for determining the start and end of chilling and heating periods, PLS regression did not enhance the ability of chilling and heat accumulation to predict leaf-out day (across all sites combined). For all three species (*A. hippocastanum*, *F. sylvatica*, and *Q. robur*), the mean R^2 was higher using the fixed-window method (0.3–0.4) compared to the PLS regression method (0.1–0.2). Since the fixed-window approach had better performance, and given that, for several locations, it was not possible to determine the beginning of chilling and heating phases using the PLS regression method due to the large number of records required by this analysis, all subsequent analyses were done using the fixed-window method.

4.3.2 Performance of chilling models

All five chilling models were validated across species, *i.e.*, had fewer than 30% of the total locations exhibiting positive relationships between chilling accumulation and heat requirement (Table 4-2). For all models, the slope estimates for many locations overlapped zero (Table 4-2). Instead of removing locations with positive or near-zero slopes from the analysis, I propagated this uncertainty to the subsequent analysis, where the CA-HR response rate (slopes) was regressed against climate variables, as explained in the methods (section 4.2.3).

For all three tree species, the chilling models differed in their ability to explain interannual variance in leaf-out day (Table 4-3). On average, across all locations, the Chill 3 model exhibited the highest R^2 values for each species, indicating it was the most effective at explaining variation in leaf-out timing. Specifically, the Chill 3 model showed mean R^2 values of 60.5% for *A. hippocastanum*, 43.2% for *Q. robur*, and 39.1% for *F. sylvatica*.

4.3.3 Environmental predictors of the CA-HR response rate

The CA-HR response rate varied with environmental variables for the three temperate tree species analyzed (Figures 4-4–4-7). Higher historical mean maximum and minimum temperatures were consistently associated with steeper, more negative CA-HR slopes across all models and species, suggesting that in warmer locations, a reduction in chilling is followed by a more pronounced increase in heat requirements than in colder climates. A similar pattern was observed for elevation across species and models: at higher elevations, the CA-HR response rate becomes flatter, indicating a diminished need for heat accumulation following a decrease in chilling relative to lower elevations. The results for elevation thus support the results for temperature: at higher elevations (where temperatures are lower; Figures 4-4–4-8), and at lower temperatures, the CA-HR response rate becomes flatter.

The relationship between the CA-HR response rate and interannual temperature variability differed across species. For *Q. robur* and *F. sylvatica*, greater variability in historical minimum and maximum temperatures was associated with a flatter CA-HR response rate, suggesting that trees from areas with more substantial temperature variability experience a smaller increase in heat requirements following a reduction in chilling, compared to more stable climates (Figures 4-4, 4-6, and 4-7). For *A. hippocastanum*, the CA-HR response rate did not exhibit any clear associations with interannual variability of minimum or maximum temperatures (Figure 4-5).

For all species, the CA-HR response rate also varied across locations with respect to precipitation, where locations with more precipitation exhibited flatter CA-HR response rates, indicating a reduced need for heat following a decrease in chilling compared to drier locations. Areas with greater variability in precipitation required more heat in response to reductions in chilling (Figures 4-4, 4-6, and 4-7).

Lastly, regarding latitude, for *Q. robur* and *A. hippocastanum*, higher latitudes were associated with a steeper CA-HR response rate (Figure 4-4, 4-5). On the other hand, for *F. sylvatica*, there was a positive association between latitude and the CA-HR response rate, indicating that locations at higher latitudes have a flatter CA-HR response rate (Figure 4-4, 4-6). These results might appear to contradict the temperature findings for *Q. robur* and *A. hippocastanum*, as an increase in latitude typically leads to a decrease in temperature. However, latitude serves as a proxy for various other environmental variables (Figure 4-8), meaning these results reflect the variation of all those variables (not only temperature) across the latitudinal gradient. For all species (Figure 4-8), elevation decreased with increasing latitude, indicating that the higher-latitude locations tended to be at lower elevations. Additionally, precipitation tended to decrease with increasing latitude for each species. These observed patterns are likely due to the presence of the Alps in the southern part of the latitudinal range of the data.

4.3.4 Geographic variation in phenological sensitivity and the CA-HR response rate

Across all species, higher phenological sensitivity was associated with a flatter CA-HR response rate. *Quercus robur* exhibited the strongest correlation with phenological sensitivity at −0.60 (95% CI [−0.60, −0.50], t = −36.9, df = 2783, p < 0.001), followed by *F. sylvatica* with a correlation of −0.56 (95% CI [−0.59, −0.54], t = −35.1, df = 2656, p < 0.001). *Aesculus hippocastanum* showed the weakest correlation at −0.28 (95% CI [−0.31, −0.25], t = −17.6, df = 3701, p < 0.001).

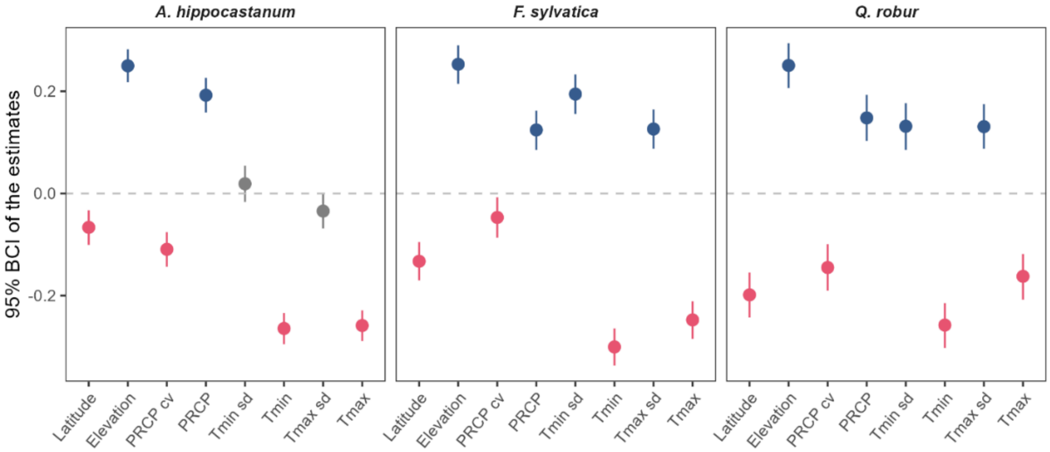


Figure 4-4 95% BCI for the slopes of the model $\boldsymbol{\beta_j \sim N(\gamma_0 + \gamma_1 \times Env.Var._{\cdot j}, \sigma^2_{\beta j})}$ for *A. hippocastanum*, *F. sylvatica*, and *Q. robur*. The CA-HR response rate (β) was regressed against the environmental variables: Tmax (historical maximum temperature), Tmin (historical minimum temperature), Tmax sd (interannual variability in Tmax), Tmin sd (interannual variability in Tmin), PRCP (historical precipitation), PRCP cv (interannual variability in PRCP), elevation, and latitude. All climate values

averaged from 1950-2023. Blue circles represent positive estimates that do not overlap zero, indicating that locations with a higher historical average of the environmental variable in question have a higher CA-HR response rate (less negative, flatter slopes). Red circles represent negative estimates that do not overlap zero, indicating that locations with a higher historical average for the environmental variable in question have a lower CA-HR response rate (more negative, steeper slopes), meaning that for each unit decrease in chilling, the local population needs more heat to achieve leaf-out. Grey circles represent estimates that overlap zero. All environmental variables were standardized.

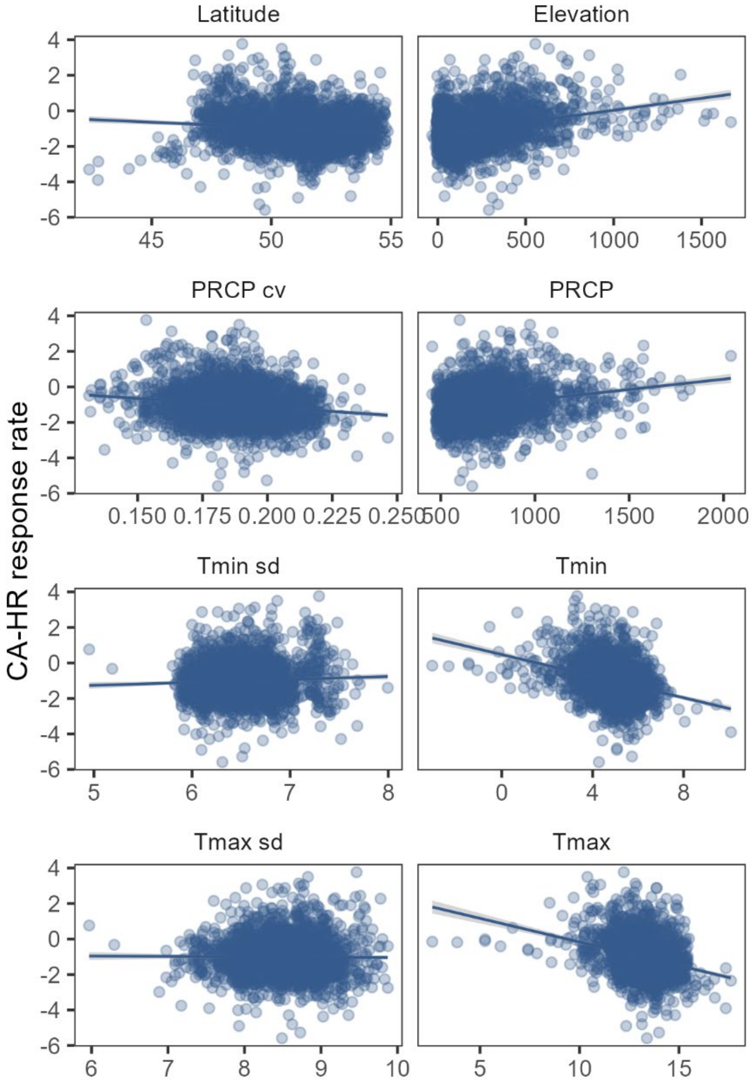


Figure 4-5 Individual regression lines for the model $\boldsymbol{\beta_j \sim N(\gamma_0 + \gamma_1 \times Env.Var._j, \sigma^2_{\beta j})}$ for ***A. hippocastanum*** (n= 2,437). The CA-HR response rate (β) was regressed against the environmental variables: Tmax (historical maximum temperature), Tmin (historical minimum temperature), Tmax sd (interannual variability in Tmax), Tmin sd (interannual variability in Tmin), PRCP (historical precipitation), PRCP cv (interannual variability in PRCP), elevation, and latitude. All climate values averaged from 1950-2023. Individual points represent single locations. Grey shaded areas around the lines represent the 95% BCI.

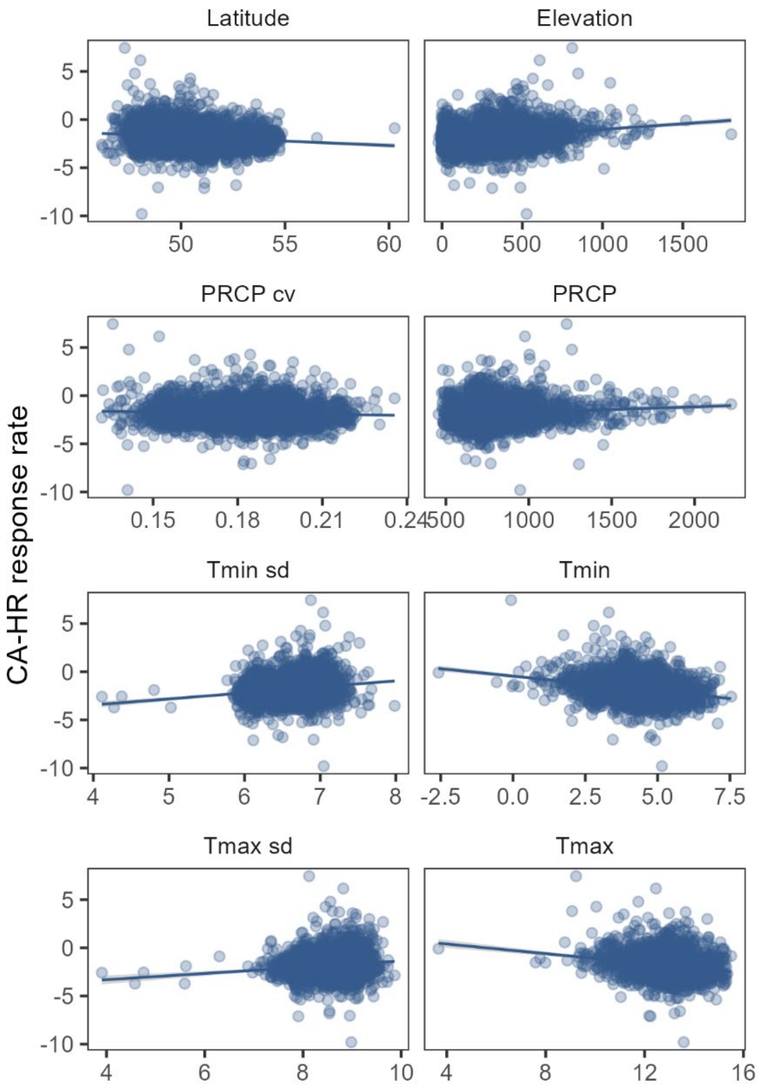


Figure 4-6 Individual regression lines for the model $\beta_j \sim N(\gamma_0 + \gamma_1 \times Env.Var._j \ , \sigma^2_{\beta j})$ for ***F. sylvatica*** (n= 2,658). The CA-HR response rate (β) was regressed against the environmental variables: Tmax (historical maximum temperature), Tmin (historical minimum temperature), Tmax sd (interannual variability in Tmax), Tmin sd (interannual variability in Tmin), PRCP (historical precipitation), PRCP cv (interannual variability in PRCP), elevation, and latitude. All climate values averaged from 1950-2023. Individual points represent single locations. Grey shaded areas around the lines represent the 95% BCI.

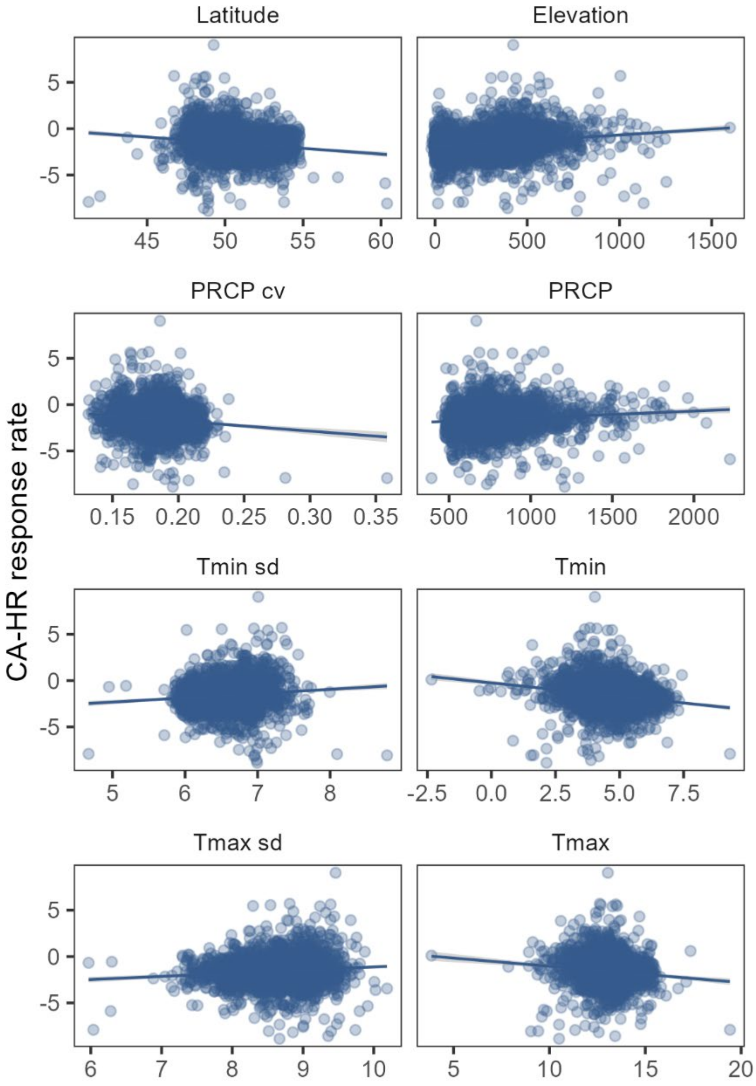
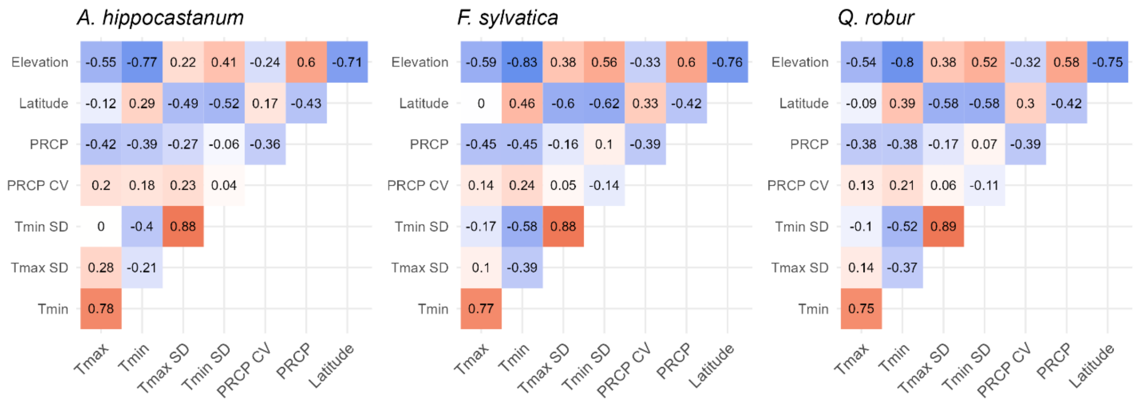


Figure 4-7 Individual regression lines for the model $\boldsymbol{\beta_j \sim N(\gamma_0 + \gamma_1 \times Env.Var._j , \sigma^2_{\beta j})}$ for ***Q. robur*** (n= 2,785). The CA-HR response rate (β) was regressed against the environmental variables: Tmax (historical maximum temperature), Tmin (historical minimum temperature), Tmax sd (interannual variability in Tmax), Tmin sd (interannual variability in Tmin), PRCP (historical precipitation), PRCP cv (interannual variability in PRCP), elevation, and latitude. All climate values averaged from 1950-2023. Individual points represent single locations. Grey shaded areas around the lines represent the 95% BCI.



A. hippocastanum

	Tmax	Tmin	Tmax SD	Tmin SD	PRCP CV	PRCP	Latitude
Elevation	-0.55	-0.77	0.22	0.41	-0.24	0.6	-0.71
Latitude	-0.12	0.29	-0.49	-0.52	0.17	-0.43	
PRCP	-0.42	-0.39	-0.27	-0.06	-0.36		
PRCP CV	0.2	0.18	0.23	0.04			
Tmin SD	0	-0.4	0.88				
Tmax SD	0.28	-0.21					
Tmin	0.78						

F. sylvatica

	Tmax	Tmin	Tmax SD	Tmin SD	PRCP CV	PRCP	Latitude
Elevation	-0.59	-0.83	0.38	0.56	-0.33	0.6	-0.76
Latitude	0	0.46	-0.6	-0.62	0.33	-0.42	
PRCP	-0.45	-0.45	-0.16	0.1	-0.39		
PRCP CV	0.14	0.24	0.05	-0.14			
Tmin SD	-0.17	-0.58	0.88				
Tmax SD	0.1	-0.39					
Tmin	0.77						

Q. robur

	Tmax	Tmin	Tmax SD	Tmin SD	PRCP CV	PRCP	Latitude
Elevation	-0.54	-0.8	0.38	0.52	-0.32	0.58	-0.75
Latitude	-0.09	0.39	-0.58	-0.58	0.3	-0.42	
PRCP	-0.38	-0.38	-0.17	0.07	-0.39		
PRCP CV	0.13	0.21	0.06	-0.11			
Tmin SD	-0.1	-0.52	0.89				
Tmax SD	0.14	-0.37					
Tmin	0.75						

Figure 4-8 Correlation matrices of environmental variables for three species: *Aesculus hippocastanum*, *Fagus sylvatica*, and *Quercus robur*. Positive correlations are indicated by red, and negative correlations by blue. The magnitude of the correlation is represented by the intensity of the colour and the numerical value in each cell.

Table 4-1 Chilling models considered for this study and their corresponding R^2 values for each focal species. The five models (Chill 1–5) were selected from Wang et al. (2020) because they adhere to the physiological assumption that increased chilling accumulation leads to decreased heating requirements. Mean R^2 ±S.D. values from regression analyses of leaf-out day vs. heating and chilling (the latter calculated under six different chilling models) are presented for the three tree species, averaged across all locations. For all species, the Chill 3 model better explained leaf-out day across locations. Models are ordered by decreasing R^2.

Model	**Equation**	***A. hippocastanum***	***F. sylvatica***	***Q. robur***
Chill 1	chill = Σ (0 if temp. > 5°C, 1 if temp. ≤ 5°C)	60.5 ±0.2	39.1 ±0.2	43.2 ±0.2
Chill 2	chill = Σ (1 if -10°C ≤ temp. ≤ 5°C, 0 otherwise)	48.5 ±0.2	26.9 ±0.1	33.2 ±0.2
Chill 3	chill = Σ (1 if temp. ≤ 7°C, 0 if temp. > 7°C)	42.5±0.2	26.8 ±0.2	27.6 ±0.1
Chill 4	chill = Σ (1 if −10°C ≤ temp. ≤ 7°C, 0 otherwise)	36.6 ±0.1	20.3 ±0.1	22.9 ±0.1
Chill 5	chill = Σ (0 if temp. ≥ − 6.5°C or temp. ≥ 6.9°C, (temp. + 6.5)/(6.9 − 0.2) if 6.5 < temp. ≤ 0.2, (6.9 − temp.)/(6.9 − 0.2) if 0.2 < temp. < 6.9)	21.6 ±0.1	10.6 ±0.1	14.7 ±0.1

4.4 Discussion

Previous studies have explored the idea that the phenological differences observed among populations are due to local adaptation and that different populations require different amounts of chilling and heating to achieve key stages in their seasonal developmental cycles (Bennie et al., 2010; Gao et al., 2020; Peaucelle et al., 2019; J. Prevéy, Vellend, Rüger, Hollister, Bjorkman, Myers-Smith, et al., 2017). My findings reveal that the CA-HR response rate reflects local environmental conditions, with warmer, drier, and lower-elevation locations exhibiting steeper CA-HR slopes, while colder, wetter, and higher-elevation areas have flatter CA-HR slopes. Additionally, greater interannual variability in temperature and precipitation is associated with flatter CA-HR slopes for *Q. robur* and *F. sylvatica*. In light of the observation that phenological temperature sensitivity varies across the geographical range of species, I investigated how this spatial variation manifests in the context of the relationship between chilling accumulation and heat requirement (CA-HR) for different species across different locations. I found that greater phenological sensitivity is linked to a flatter CA-HR response rate, meaning less compensatory heating is required following decreased chilling accumulation. My study is the first to show that the variation in temperature sensitivity across locations is explained by the variation in the CA-HR response rate. This offers a mechanistic explanation for phenological sensitivity differences across locations and emphasizes the interaction between climate, geography, and species-specific adaptations in shaping temperate tree spring phenology. While numerous studies have documented geographical variation in chilling and heating requirements in response to local environmental conditions (Fu et al., 2014; Fu, Piao, et al., 2015; Peaucelle et al., 2019; J. Wang et al., 2022; T. Wang et al., 2014; Xu et al., 2021), no study has investigated how the relationship between the two is affected by local environmental variables. My results suggest that environmental variables such as temperature, elevation, precipitation, and latitude have significantly influenced the CA-HR response rate across different species and locations, showing the importance of considering a broad range of environmental factors to understand the evolution of phenological sensitivities and highlighting the importance of accounting for local variation in the CA-HR relationship to make accurate predictions regarding future climate scenarios.

I found that locations with steeper, more negative CA-HR slopes have higher historical mean maximum and minimum temperatures (*i.e.*, are warmer) and are at lower elevations. These locations also exhibit smaller temperature sensitivity of phenology (compared to colder, higher-elevation climates), thanks to a pronounced increase in heat requirements following a reduction in chilling, a more risk-averse strategy. On the other hand, locations with flatter CA-HR slopes are in colder or higher-elevation areas, indicating a less risk-averse response to increased temperatures and reduced chilling. These locations require a smaller increase in heat requirements following a reduction in chilling, resulting in a higher temperature sensitivity of phenology.

Species' spring budburst reflects a trade-off between maximizing the growing season (earlier budburst) and minimizing frost damage (later budburst) (Bennie et al., 2010).. In warmer locations and at lower elevations, a risk-averse strategy dominates, likely because the benefits of an early start are less critical compared to cooler regions. This helps populations avoid flowering too early after a warm winter, reducing frost exposure. For instance, Bennie et al. (2010) found that species protect themselves from premature flowering by requiring more subsequent heat accumulation when winter chilling is reduced. Similarly, Lenz et al. (2015) demonstrated that leaf-out timing aligns with minimizing freezing risks, with species leafing out precisely at the start of the safe period, optimizing survival and growth across varying climatic conditions.

In years when winter is warmer than usual and chilling is reduced, these populations protect themselves from premature flowering by requiring more subsequent heat accumulation. For instance, Bennie et al. (2010) found that the timing of spring budburst in temperate, boreal, and Arctic trees and shrubs involves a trade-off between capacity adaptation (earlier budburst to maximize the growing season) and survival adaptation (later budburst to minimize frost damage). Selection maximizes long-term ecological fitness under this trade-off, given any particular set of climatic conditions. Similarly, Lenz et al. (2015) demonstrated that leaf-out timing in temperate trees aligns with minimizing the risk of freezing damage, with species leafing out precisely at the beginning of the safe period, regardless of elevation, thereby optimizing survival and growth across varying climatic conditions.

These results imply that in warmer locations and at lower elevations, populations will require significantly more heat to break dormancy if chilling is reduced due to higher-than-usual temperatures during winter and spring. Although a year with warmer temperatures allows heat requirements to be reached faster (Wenden et al., 2020), an increase in heat requirements due to reduced chilling could still mean that the net effect of a warmer year is a delay in phenological events such as leaf-out and flowering (Cook et al., 2012; Morin et al., 2009b). However, a meta-analysis using data from Central Europe (with considerable overlap in geographical locations and species with my study) showed that phenological delays are only probable under severe warming (generally above 4°C warming; Ettinger et al., 2020a).

Surprisingly, I found that locations with higher interannual temperature variability adopt a less risk-averse approach, showing greater phenological sensitivity to temperature changes. These locations exhibit a flatter CA-HR response rate, indicating a smaller increase in heat requirements following a reduction in chilling. This pattern differs from what has been found in earlier studies. For instance, Wang et al. (2013), in a study also using the PEP-725 database and with some overlapping species, found that in regions with higher interannual spring temperature variance, plants exhibit reduced temperature sensitivity in their phenological responses, likely an adaptation to minimize the risk of frost damage. Two possible explanations, which are not mutually exclusive, could account for these differences: First, the locations with higher interannual temperature variability in my study are also colder (Figure 4-8), so the trend observed here might be influenced by the mean temperature profile of the location. Second, Wang et al. (2013) considered only spring temperature variation, while I used year-round interannual temperature variation.

Compared to the effects of temperature on plant phenology, the effects of precipitation are less studied. My results show that locations with a flatter CA-HR response rate are in areas with higher precipitation and lower interannual variability in precipitation. This indicates a smaller increase in heat requirements following a reduction in chilling, demonstrating a less risk-averse response to temperature increases compared to drier areas. Similarly, Gao et al. (2020) found that wetter areas tend to have higher temperature sensitivity, expressed as days/°C, for both spring and autumn phenology compared to drier areas. In drier regions, the lack of sufficient moisture and higher temperature variance reduce the plants' responsiveness to temperature changes, resulting in lower temperature sensitivity (fewer days/°C). Conversely, wetter areas, with more stable moisture conditions, support stronger phenological responses to temperature variations (more days/°C).

However, given the negative correlation between temperature and precipitation in the areas of my study, where higher temperatures are generally associated with lower precipitation (Figures 4-8), it is possible that the observed patterns are primarily driven by temperature responses, with precipitation acting as a confounding variable. Furthermore, caution is needed when interpreting and extrapolating these results beyond Europe, as even the lowest recorded precipitation levels in my study sites (Figures 4-5–4-7) surpass global averages. The frequency of precipitation, exceeding 160 days per year on average, further highlights the distinct rainfall patterns observed.

Although my results cannot conclusively prove local adaptation, which involves genetic changes that enhance survival and reproduction in specific environments, the observed patterns of temperature sensitivity and CA-HR response in my study align with the expected outcomes of local adaptation, at least in a general sense. Species in colder, more variable environments exhibit different phenological strategies than those in more stable, warmer climates, with populations from colder climates evolving higher sensitivities (likely to exploit shorter growing seasons) and those from warmer climates evolving less sensitivity (likely to avoid premature development during warm spells in winter) (Bennie et al., 2010; Lenz et al., 2016). Furthermore, it is important to note that both temperature sensitivity and the CA-HR slope include budburst timing in their calculations, which could introduce circularity. While the correlation was expected and the analysis aimed to confirm it, a permutation test randomizing budburst dates across years would help ensure the result is not a mathematical artifact.

“All models are wrong, but some are useful.” (George Box) - Despite the significant findings, this study demonstrates the limitations inherent in the use of chilling models to predict phenological responses.

For instance, the chilling models used were not specifically calibrated to the species studied, and they often oversimplify the complexity of plant responses to winter temperatures (Fu et al., 2014; Lin et al., 2022; Zhao et al., 2021). Although it was not the primary objective of this study to compare chilling models, it was important to validate them to ensure the reliability of the chilling and heating calculations used in subsequent analyses.

There is significant uncertainty about when trees start to accumulate chilling and what temperatures contribute to this process. For instance, in the fixed-window approach, the start of chilling accumulation was set to November 1 based on previous work, but this arbitrary date lacks strong evidence. Furthermore, there is still uncertainty about whether chilling and forcing occur in parallel or sequentially. Some experimental studies suggest that forcing temperatures do not start to accumulate until chilling accumulation is complete, whereas other studies indicate that chilling and forcing temperatures accumulate in parallel (Hanninen, 1990; Kramer, 1994). Additionally, unusual weather patterns, such as the unseasonable heat waves and cold snaps that are becoming more common as the climate changes, can disrupt normal chill accumulation (Xu et al., 2021). Alternating warm and cold days during endodormancy might be less effective than continuous cold, and we often overlook the potential role of warm spells during this phase.

Finally, previous studies have demonstrated that PLS regression outperforms the fixed-window approach in locally detecting periods of chilling accumulation and heating requirement (Luedeling, Guo, et al., 2013; Luedeling & Gassner, 2012). However, I found the fixed-window method to be more effective across multiple locations. Although PLS regression effectively identifies critical periods when temperature affects phenological events, it requires long-term phenological and climate data, which were not available at every location. This sensitivity to variations in data and local climate specificities can introduce high variability in identifying critical periods for chilling and heating. This variability results in a lower average R^2, as the method produces a disproportionate number of low R^2 values. In contrast, the fixed-window approach uses the same predefined periods for all locations, leading to more consistent R^2 values and higher average R^2 values in an aggregated analysis.

Conclusions - This study highlights that different tree populations track temperature differently due to their unique responses to local environmental variables. Specifically, trees growing in warmer, drier, and lower-elevation locations exhibit lower temperature sensitivity compared to those growing in colder, higher-elevation. The temperature response of these populations is mediated by the rate of change in heating requirements in response to changes in chilling, the CA-HR response rate. This rate of change is correlated with local environmental variables, suggesting that intra-specific temperature sensitivity variation could reflect local adaptation. The CA-HR response rate provides a valuable mechanism for understanding observed spatial differences in the temperature sensitivity of phenology. By recognizing how local environmental factors shape phenological responses, we can better predict and manage the impacts of climate change on different populations and species.

Chapter 5

General Discussion

Throughout human history, changes in climate have played a significant role in the collapse of civilizations, acting as catalysts for societal stress and decline. For example, the Sumerian civilization in Mesopotamia experienced prolonged droughts and increased aridity around 2200 BC, exacerbating existing social and political tensions and contributing to its decline. Similarly, the Bronze Age collapse around 1200 BC, affecting various civilizations across the Mediterranean and Near East, has been linked to a period of abrupt climate change characterized by droughts and shifts in precipitation patterns. These environmental changes, combined with other factors such as overpopulation, internal conflicts, and invasions, disrupted agricultural systems, caused widespread famine, and triggered mass migrations, contributing to the political instability and collapse of these once prosperous societies.

These historical examples act as a cautionary tale, highlighting the vulnerability of human societies to climate change and demonstrating the potential for even gradual environmental shifts to compound existing challenges and have far-reaching social and political consequences. Today, human activities have caused approximately 1.2°C of global warming above pre-industrial levels (1850–1900), and the average global temperature in 2023 was 1.36°C above the pre-industrial average. The current rate of warming is 0.20°C per decade, and the past decade (2014–2023) was the warmest on record. If greenhouse gas emissions continue unchanged, global warming is likely to exceed 1.5°C in the early 2030s, and possibly reach 2°C by mid-century (IPCC, 2021). The potential consequences of our current trajectory serve as a modern parallel to the historical collapses, emphasizing the urgent need for climate action to mitigate similar outcomes.

In this context, studying phenology, the timing of seasonal events in plants and animals, offers invaluable insights into how ecosystems are responding to climate change. Phenological shifts, such as earlier leaf-out in trees and changes in flowering times, are direct and observable indicators of changing environmental conditions. By monitoring and understanding these shifts, phenology studies play a crucial role in predicting the future impacts of climate change and informing strategies to mitigate its effects.

My thesis advances our knowledge of the phenology and interactions of temperate deciduous trees. I demonstrate that they serve as an important pollen source for wild bees and that their response to temperature differs from that of the spring-flowering herbs in their understorey. Furthermore, I examine how spatial variation in phenological temperature sensitivity manifests in the context of chilling accumulation and heat requirements (CA-HR) across different species and locations.

In the second chapter of this thesis, I revealed that the deciduous forest canopy supports a rich and diverse community of bees during spring, with nearly twice as many bees observed in the canopy compared to the understorey. This suggests that studies excluding the canopy may significantly underestimate the overall size of the forest-dwelling bee population. Additionally, trees play a critical role as a pollen source for wild bees in these temperate forests. Spring-flying bees, even those predominantly found in the understorey, consistently consume arboreal pollen, with a high percentage of bees in both the canopy and understorey carrying exclusively arboreal pollen loads throughout the entire bee activity season, emphasizing the importance of trees as a primary pollen source in these ecosystems.

This study, conducted in deciduous forests at northern latitudes, extends previous research and offers new insights into bee communities in these regions. While there is some overlap with prior studies, my findings differ in several respects. For example, I found higher bee abundance in the canopy, consistent with findings from a bottomland hardwood forest in Georgia, USA (Ulyshen et al., 2010), but contrasting with studies from New York forests, where similar bee abundances were reported in both canopy and understorey (Urban-Mead et al., 2021).

Chapter 2 emphasizes the significant reliance of bees on arboreal pollen in these temperate forests. On average, 60.6% of bees in the canopy and 49.3% in the understorey carried purely arboreal pollen loads, indicating that trees are a vital pollen source even in the understorey, where one might expect herbaceous plants to dominate. Arboreal pollen was the most frequently consumed pollen type throughout the bee activity season, likely due to the large quantities of pollen produced by a single flowering canopy tree (Donkersley, 2019).

In chapter 3, based on a 13-year plant phenology dataset covering 21 species and 8,045 data points from 965 locations, I revealed differential responses to rising temperatures among plant species in North America's eastern hardwood forest ecosystems. I found that spring leaf-out is advancing more rapidly for spring-blooming forest herb species compared to trees, particularly at middle and higher latitudes (40–48° N). However, this trend lessens at lower latitudes (35–39° N), where no significant difference is observed between these functional groups. This suggests that latitudinal variation plays a critical role in how different species respond to climate change.

While my findings align with the broader understanding that phenological responses to warming vary by latitude, they challenge a previous study that suggested trees might be more responsive to temperature changes than forest herbs within similar latitudinal ranges (Heberling, Fridley, et al., 2019), demonstrating the importance of broad geographic sampling for accurately understanding these dynamics.

My results also indicate that at middle latitudes (40–44° N), spring-flowering herbs advance their leaf-out date more strongly than those at lower or higher latitudes. Conversely, trees at lower latitudes (35–39° N) exhibit a stronger phenological response than those at middle and higher latitudes. The differing responses likely result from the various ways in which these species perceive and respond to environmental cues like temperature, photoperiod, and snow cover. For instance, spring-flowering forest herbs are heavily influenced by soil temperature and snow depth (Jánosi et al., 2020; Pardee et al., 2019), while trees respond primarily to air temperature, photoperiod, and chilling accumulation (Basler & Korner, 2014; Laube et al., 2014). Overall, this study indicates the need for a better understanding of geographical variation in phenological responses, which I addressed in chapter 4.

A key focus of my fourth chapter was to explore how spatial variation in phenological temperature sensitivity manifests in the context of chilling accumulation and heat requirements (CA-HR) across distinct species and locations. My findings indicate that greater phenological sensitivity is associated with a flatter CA-HR response rate, meaning less compensatory heating is required following decreased chilling accumulation. This CA-HR response rate reflects local environmental conditions, with warmer, drier, and lower-elevation locations exhibiting steeper CA-HR slopes, while colder, wetter, and higher-elevation areas have flatter slopes. Additionally, I found that interannual variability in temperature and precipitation influences the CA-HR response rate. Higher temperature variability flattens CA-HR slopes, while greater precipitation variability steepens them. This temperature effect was evident in *Quercus robur* and *Fagus sylvatica*, but not in *Aesculus hippocastanum*., suggesting a more complex interaction between local climate and phenological responses.

The relationship between chilling accumulation and heat requirement provides a mechanistic explanation for observed differences in temperature sensitivity across locations, like those I documented in Chapter 3. In warmer areas and at lower elevations, plants appear to adopt a more risk-averse strategy, delaying flowering to avoid frost following a relatively warm winter (Bennie et al., 2010). These populations require more subsequent heat accumulation to break dormancy, which helps prevent premature flowering and may be crucial for avoiding even earlier flowering during abnormal warm spells. This approach likely reflects the reduced importance of an early start in these regions, where maximizing the growing season is less critical (Gao et al., 2020; T. Wang et al., 2014; Xu et al., 2021).

Conversely, in colder or higher-elevation regions, trees and other species have a flatter CA-HR slope, which can be seen as an adaptation to the shorter growing seasons typical of these regions, favouring an extended growing season despite the risks of frost damage. This adaptation is reflected in the flatter CA-HR slopes observed in these colder environments, where less additional heating is required after a reduction in chilling.

These findings highlight the complexity of phenological responses to climate change in temperate forest ecosystems. The variation in CA-HR response rates across different environments suggests that local adaptation plays a significant role in shaping phenological sensitivity. The mechanistic insights provided by the CA-HR analysis emphasize the need to consider a broad range of environmental factors when predicting how plant species will respond to future climate scenarios. Understanding these dynamics is crucial for accurately modeling the impacts of climate change on temperate forests and for predicting how different species might respond to ongoing environmental changes.

Taken as a whole, my thesis highlights several areas for future research. Firstly, we need a deeper understanding of the availability and nutritional suitability of arboreal pollen for bees, as well as the broader ecological implications of its use for wind-pollinated trees. Bees may enhance pollination in wind-pollinated plants, but their role, especially in unisexual flowers, needs more research. Some insects might actually reduce pollen availability by consuming it without effective transfer (Saunders, 2018; Whitehead, 1969).

Secondly, the impact of warmer temperatures on the fitness of spring-flowering forest herbs and trees remains incompletely understood. Warmer springs can hinder bulb growth and cause earlier leaf senescence in some herbs, though these effects vary by species (Heberling, Cassidy, et al., 2019; Lapointe, 2001). For trees, the benefits of an extended growing season from earlier leaf-out may be offset by earlier leaf senescence, potentially shortening the growth period and affecting carbon uptake (Zani et al., 2020). Further research is needed to understand how these changes influence long-term fitness and resource allocation across different species.

Thirdly, the potential for mismatches between plant phenology and the timing of herbivore and pollinator activity also warrants investigation, as these interactions are crucial for the survival of many species (Kharouba et al., 2018; Kudo & Ida, 2013). Furthermore, climate change may disrupt synchronization between bees and the trees they rely on for pollen, as they respond differently to temperature shifts, potentially leading to mismatches (Sevenello et al., 2020). The extent of these shifts in trees versus other taxa is still debated (Alecrim et al., 2023; Heberling, Fridley, et al., 2019; Lee, Alecrim, et al., 2024; Miller et al., 2023).

Finally, my study shows that we still have a long way to go in developing accurate species-specific chilling models, which are essential for predicting how species will respond to climate change. There is considerable uncertainty about when and how chilling accumulates in trees, particularly whether chilling and forcing occur sequentially or in parallel, with conflicting evidence from experimental studies (Hanninen, 1990; Kramer, 1994). The impact of unusual weather patterns on chilling processes also needs further exploration (Xu et al., 2021).

Conclusions - The findings from this body of work emphasize the complexity of ecological interactions and the need for more studies in temperate deciduous forests. My research on wild bees reveals the important role of arboreal pollen and the importance of considering the forest canopy when studying bee communities. In addition, my study on the phenological responses of spring-flowering forest herbs and trees to warming temperatures demonstrates that these functional groups respond differently across latitudinal gradients, with forest herbs showing generally greater responsiveness. Finally, my research shows that the phenological responses of different populations are shaped by local environmental conditions, with temperature sensitivity varying according to the specific climate and geography of each location. This variation suggests that local adaptation plays a significant role in how species respond to climate change.

Overall, this work highlights the relationship between species, environmental conditions, and climate change, calling for more species-specific research to better predict the ecological consequences of a warming world. Just as ancient civilizations like the Sumerians and those of the Bronze Age attempted to adapt to their changing environments, often with limited success, our modern society must act decisively. As has been observed, 'the selective changes that societies adopt in response to crisis are what determine their survival or collapse.' (Diamond, 2019). This calls for practical measures such as preserving and restoring temperate forests, enacting policies that protect biodiversity, and investing in research to refine species-specific models predicting phenological shifts. By taking these steps, we can better anticipate and mitigate the impacts of climate change, ensuring that our ecosystems and the societies they support remain resilient in the face of inevitable environmental shifts.

References

Alecrim, E. F., Sargent, R. D., & Forrest, J. R. K. (2023). Higher-latitude spring-flowering herbs advance their phenology more than trees with warming temperatures. *Journal of Ecology*, *111*(1), 156–169. https://doi.org/10.1111/1365-2745.14023

Allen, G., & Davies, R. G. (2022). Canopy sampling reveals hidden potential value of woodland trees for wild bee assemblages. *Insect Conservation and Diversity*, icad.12606. https://doi.org/10.1111/icad.12606

Almorox, J., Hontoria, C., & Benito, M. (2005). Statistical validation of daylength definitions for estimation of global solar radiation in Toledo, Spain. *Energy Conversion and Management*, *46*(9–10), 1465–1471.

Augspurger, C. K. (2009). Spring 2007 warmth and frost: Phenology, damage and refoliation in a temperate deciduous forest: *Warmth and frost: phenology, damage and refoliation*. *Functional Ecology*, *23*(6), 1031–1039. https://doi.org/10.1111/j.1365-2435.2009.01587.x

Augspurger, C. K., & Salk, C. F. (2017). Constraints of cold and shade on the phenology of spring ephemeral herb species. *Journal of Ecology*, *105*(1), 246–254. https://doi.org/10.1111/1365-2745.12651

Augspurger, C. K., & Zaya, D. N. (2020). Concordance of long-term shifts with climate warming varies among phenological events and herbaceous species. *Ecological Monographs*, *90*(4). https://doi.org/10.1002/ecm.1421

Bąk-Badowska, J. (2012). Spatial diversification of Bee (Hymenoptera: Apoidea: Apiformes) assemblages in forest communities of the Suchedniów-Oblęgorek Landscape park. *Journal of Apicultural Science*, *56*(2), 89–106.

Banaszak, J., & Cierzniak, T. (1994). *Spatial and temporal differentiation of bees (Apoidea) in the forests of Wielkopolski National Park, western Poland.*

Basler, D., & Korner, C. (2014). Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology*, *34*(4), 377–388. https://doi.org/10.1093/treephys/tpu021

Baumgarten, F., Zohner, C. M., Gessler, A., & Vitasse, Y. (2021). Chilled to be forced: The best dose to wake up buds from winter dormancy. *New Phytologist*, *230*(4), 1366–1377. https://doi.org/10.1111/nph.17270

Bennie, J., Kubin, E., Wiltshire, A., Huntley, B., & Baxter, R. (2010). Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: The implications of local adaptation to climate. *Global Change Biology*, *16*(5), 1503–1514. https://doi.org/10.1111/j.1365-2486.2009.02095.x

Bernhardt, P. (1976). The pollination ecology of Hepatica acutiloba DC.(Ranunculaceae). *Bulletin of the Torrey Botanical Club*, 255–258.

Bernhardt, P. (1977). The Pollination Ecology Of A Population Of Erythronium Americanum Ker. (Liliaceae). *Rhodora*, *79*(818), 278–282. JSTOR.

Betancourt, M. J., & Girolami, M. (2013). Hamiltonian Monte Carlo for Hierarchical Models. *arXiv:1312.0906 [Stat]*. http://arxiv.org/abs/1312.0906

Binder, S., Haight, R. G., Polasky, S., Warziniack, T., Mockrin, M. H., Deal, R. L., & Arthaud, G. (2017). Assessment and valuation of forest ecosystem services: State of the science review. *Gen. Tech. Rep. NRS-170. Newtown Square, PA: US Department of Agriculture, Forest Service, Northern Research Station. 47 p.*, *170*, 1–47.

Brown, P. J., & DeGaetano, A. T. (2011). A paradox of cooling winter soil surface temperatures in a warming northeastern United States. *Agricultural and Forest Meteorology*, *151*(7), 947–956. https://doi.org/10.1016/j.agrformet.2011.02.014

Brown, R. L., Ashmun, J. W., & Pitelka, L. F. (1985). Within- and Between-Species Variation in Vegetative Phenology in Two Forest Herbs. *Ecology*, *66*(1), 251–258. https://doi.org/10.2307/1941325

Buonaiuto, D. M., Morales-Castilla, I., & Wolkovich, E. M. (2021). Reconciling competing hypotheses regarding flower–leaf sequences in temperate forests for fundamental and global change biology. *New Phytologist*, *229*(3), 1206–1214. https://doi.org/10.1111/nph.16848

Bürkner, P.-C. (2017). **brms**: An *R* Package for Bayesian Multilevel Models Using *Stan*. *Journal of Statistical Software*, *80*(1). https://doi.org/10.18637/jss.v080.i01

Caffarra, A., & Donnelly, A. (2011). The ecological significance of phenology in four different tree species: Effects of light and temperature on bud burst. *International Journal of Biometeorology*, *55*(5), 711–721. https://doi.org/10.1007/s00484-010-0386-1

Campbell, J. W., Vigueira, P. A., Viguiera, C. C., & Greenberg, C. H. (2018). The Effects of Repeated Prescribed Fire and Thinning on Bees, Wasps, and Other Flower Visitors in the Understory and Midstory of a Temperate Forest in North Carolina. *Forest Science*, *64*(3), 299–306. https://doi.org/10.1093/forsci/fxx008

Cannell, M. G. R., & Smith, R. I. (1984). Spring Frost Damage on Young *Picea sitchensis* 2. Predicted Dates of Budburst and Probability of Frost Damage. *Forestry*, *57*(2), 177–197. https://doi.org/10.1093/forestry/57.2.177

Casanelles-Abella, J., Müller, S., Keller, A., Aleixo, C., Alós Orti, M., Chiron, F., Deguines, N., Hallikma, T., Laanisto, L., Pinho, P., Samson, R., Tryjanowski, P., Van Mensel, A., Pellissier, L., & Moretti, M. (2021). How wild bees find a way in European cities: Pollen metabarcoding unravels multiple feeding strategies and their effects on distribution patterns in four wild bee species. *Journal of Applied Ecology*, 1365-2664.14063. https://doi.org/10.1111/1365-2664.14063

Cavard, X., Macdonald, S. E., Bergeron, Y., & Chen, H. Y. H. (2011). Importance of mixedwoods for biodiversity conservation: Evidence for understory plants, songbirds, soil fauna, and ectomycorrhizae in northern forests. *Environmental Reviews*, *19*(NA), 142–161. https://doi.org/10.1139/a11-004

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecological Monographs*, *84*(1), 45–67. https://doi.org/10.1890/13-0133.1

Chuine, I. (2010). Why does phenology drive species distribution? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3149–3160. https://doi.org/10.1098/rstb.2010.0142

Chuine, I., & Régnière, J. (2017). Process-Based Models of Phenology for Plants and Animals. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 159–182. https://doi.org/10.1146/annurev-ecolsys-110316-022706

Clark, J. S., Salk, C., Melillo, J., & Mohan, J. (2014). Tree phenology responses to winter chilling, spring warming, at north and south range limits. *Functional Ecology*, *28*(6), 1344–1355. https://doi.org/10.1111/1365-2435.12309

Cook, B. I., Wolkovich, E. M., & Parmesan, C. (2012). Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings of the National Academy of Sciences*, *109*(23), 9000–9005. https://doi.org/10.1073/pnas.1118364109

Corff, J. LE., & Marquis, R. J. (1999). Differences between understorey and canopy in herbivore community composition and leaf quality for two oak species in Missouri. *Ecological Entomology*, *24*(1), 46–58. https://doi.org/10.1046/j.1365-2311.1999.00174.x

Crone, M. K., Biddinger, D. J., & Grozinger, C. M. (2022). Wild Bee Nutritional Ecology: Integrative Strategies to Assess Foraging Preferences and Nutritional Requirements. *Frontiers in Sustainable Food Systems*, *6*, 847003. https://doi.org/10.3389/fsufs.2022.847003

Cunningham-Minnick, M. J., & Crist, T. O. (2020). Floral resources of an invasive shrub alter native bee communities at different vertical strata in forest-edge habitat. *Biological Invasions*, *22*(7), 2283–2298. https://doi.org/10.1007/s10530-020-02248-y

Cunningham-Minnick, M. J., Roberts, H. P., Kane, B., Milam, J., & King, D. I. (2022). A cost-effective method to passively sample communities at the forest canopy–aerosphere interface. *Methods in Ecology and Evolution*, *13*(11), 2389–2396. https://doi.org/10.1111/2041-210X.13987

Dantec, C. F., Vitasse, Y., Bonhomme, M., Louvet, J.-M., Kremer, A., & Delzon, S. (2014). Chilling and heat requirements for leaf unfolding in European beech and sessile oak populations at the southern limit of their distribution range. *International Journal of Biometeorology*, *58*(9), 1853–1864. https://doi.org/10.1007/s00484-014-0787-7

De Frenne, P., Brunet, J., Shevtsova, A., Kolb, A., Graae, B. J., Chabrerie, O., Cousins, S. A., Decocq, G., De Schrijver, A., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Nilsson, C., Stanton, S., Tack, W., Willaert, J., & Verheyen, K. (2011). Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient: Forest Herbs And Climate Warming. *Global Change Biology*, *17*(10), 3240–3253. https://doi.org/10.1111/j.1365-2486.2011.02449.x

Delgado, M. del M., Roslin, T., Tikhonov, G., Meyke, E., Lo, C., Gurarie, E., Abadonova, M., Abduraimov, O., Adrianova, O., Akimova, T., Akkiev, M., Ananin, A., Andreeva, E., Andriychuk, N., Antipin, M., Arzamascev, K., Babina, S., Babushkin, M., Bakin, O., … Ovaskainen, O. (2020). Differences in spatial versus temporal reaction norms for spring and autumn phenological events. *Proceedings of the National Academy of Sciences*, *117*(49), 31249–31258. https://doi.org/10.1073/pnas.2002713117

Diamond, J. M. (2019). *Upheaval: Turning points for nations in crisis* (First edition). Little, Brown and Company.

Diez, J. M., Ibáñez, I., Miller-Rushing, A. J., Mazer, S. J., Crimmins, T. M., Crimmins, M. A., Bertelsen, C. D., & Inouye, D. W. (2012). Forecasting phenology: From species variability to community patterns: Phenological variation across scales. *Ecology Letters*, *15*(6), 545–553. https://doi.org/10.1111/j.1461-0248.2012.01765.x

Dion, P.-P., Bussières, J., & Lapointe, L. (2017). Late canopy closure delays senescence and promotes growth of the spring ephemeral wild leek (*Allium tricoccum*). *Botany*, *95*(5), 457–467. https://doi.org/10.1139/cjb-2016-0317

Discover Life. (n.d.). Retrieved August 27, 2024, from https://www.discoverlife.org/

Donkersley, P. (2019). Trees for bees. *Agriculture, Ecosystems & Environment*, *270–271*, 79–83. https://doi.org/10.1016/j.agee.2018.10.024

Dose, V., & Menzel, A. (2004). Bayesian analysis of climate change impacts in phenology: Climate Change Impacts. *Global Change Biology*, *10*(2), 259–272. https://doi.org/10.1111/j.1529-8817.2003.00731.x

Du, Y., Mao, L., Queenborough, S. A., Primack, R., Comita, L. S., Hampe, A., & Ma, K. (2020). Macro-scale variation and environmental predictors of flowering and fruiting phenology in the Chinese angiosperm flora. *Journal of Biogeography*, *47*(11), 2303–2314. https://doi.org/10.1111/jbi.13938

Duputié, A., Rutschmann, A., Ronce, O., & Chuine, I. (2015). Phenological plasticity will not help all species adapt to climate change. *Global Change Biology*, *21*(8), 3062–3073. https://doi.org/10.1111/gcb.12914

Dyer, J. M. (2006). Revisiting the Deciduous Forests of Eastern North America. *BioScience*, *56*(4), 341. https://doi.org/10.1641/0006-3568(2006)56[341:RTDFOE]2.0.CO;2

Ellwood, E. R., Primack, R. B., Willis, C. G., & HilleRisLambers, J. (2019). Phenology models using herbarium specimens are only slightly improved by using finer-scale stages of reproduction. *Applications in Plant Sciences*, *7*(3), e01225. https://doi.org/10.1002/aps3.1225

Ettinger, A. K., Chamberlain, C. J., Morales-Castilla, I., Buonaiuto, D. M., Flynn, D. F. B., Savas, T., Samaha, J. A., & Wolkovich, E. M. (2020a). Winter temperatures predominate in spring phenological responses to warming. *Nature Climate Change*, *10*(12), 1137–1142. https://doi.org/10.1038/s41558-020-00917-3

Ettinger, A. K., Chamberlain, C. J., Morales-Castilla, I., Buonaiuto, D. M., Flynn, D. F. B., Savas, T., Samaha, J. A., & Wolkovich, E. M. (2020b). Winter temperatures predominate in spring phenological responses to warming. *Nature Climate Change*, *10*(12), 1137–1142. https://doi.org/10.1038/s41558-020-00917-3

Ettinger, A. K., Gee, S., & Wolkovich, E. M. (2018). Phenological sequences: How early-season events define those that follow. *American Journal of Botany*, *105*(10), 1771–1780. https://doi.org/10.1002/ajb2.1174

Fabina, N. S., Abbott, K. C., & Gilman, R. T. (2010). Sensitivity of plant–pollinator–herbivore communities to changes in phenology. *Ecological Modelling*, *221*(3), 453–458. https://doi.org/10.1016/j.ecolmodel.2009.10.020

Fang, J., & Lechowicz, M. J. (2006). Climatic limits for the present distribution of beech (*Fagus* L.) species in the world. *Journal of Biogeography*, *33*(10), 1804–1819. https://doi.org/10.1111/j.1365-2699.2006.01533.x

Filipiak, M. (2018). A Better Understanding of Bee Nutritional Ecology Is Needed to Optimize Conservation Strategies for Wild Bees—The Application of Ecological Stoichiometry. *Insects*, *9*(3), 85. https://doi.org/10.3390/insects9030085

Flynn, D. F. B., & Wolkovich, E. M. (2018). Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist*, *219*(4), 1353–1362. https://doi.org/10.1111/nph.15232

Fu, Y. H., Campioli, M., Deckmyn, G., & Janssens, I. A. (2012). The Impact of Winter and Spring Temperatures on Temperate Tree Budburst Dates: Results from an Experimental Climate Manipulation. *PLoS ONE*, *7*(10), e47324. https://doi.org/10.1371/journal.pone.0047324

Fu, Y. H., Piao, S., Vitasse, Y., Zhao, H., De Boeck, H. J., Liu, Q., Yang, H., Weber, U., Hänninen, H., & Janssens, I. A. (2015). Increased heat requirement for leaf flushing in temperate woody species over 1980-2012: Effects of chilling, precipitation and insolation. *Global Change Biology*, *21*(7), 2687–2697. https://doi.org/10.1111/gcb.12863

Fu, Y. H., Piao, S., Zhao, H., Jeong, S., Wang, X., Vitasse, Y., Ciais, P., & Janssens, I. A. (2014). Unexpected role of winter precipitation in determining heat requirement for spring vegetation green-up at northern middle and high latitudes. *Global Change Biology*, *20*(12), 3743–3755. https://doi.org/10.1111/gcb.12610

Fu, Y. H., Zhang, X., Piao, S., Hao, F., Geng, X., Vitasse, Y., Zohner, C., Peñuelas, J., & Janssens, I. A. (2019). Daylength helps temperate deciduous trees to leaf-out at the optimal time. *Global Change Biology*, gcb.14633. https://doi.org/10.1111/gcb.14633

Fu, Y. H., Zhao, H., Piao, S., Peaucelle, M., Peng, S., Zhou, G., Ciais, P., Huang, M., Menzel, A., Peñuelas, J., Song, Y., Vitasse, Y., Zeng, Z., & Janssens, I. A. (2015). Declining global warming effects on the phenology of spring leaf unfolding. *Nature*, *526*(7571), 104–107. https://doi.org/10.1038/nature15402

Fuccillo, K. K., Crimmins, T. M., de Rivera, C. E., & Elder, T. S. (2015). Assessing accuracy in citizen science-based plant phenology monitoring. *International Journal of Biometeorology*, *59*(7), 917–926. https://doi.org/10.1007/s00484-014-0892-7

Gallinat, A. S., Ellwood, E. R., Heberling, J. M., Miller-Rushing, A. J., Pearse, W. D., & Primack, R. B. (2021). Macrophenology: Insights into the broad-scale patterns, drivers, and consequences of phenology. *American Journal of Botany*, *108*(11), 2112–2126. https://doi.org/10.1002/ajb2.1793

Gao, M., Wang, X., Meng, F., Liu, Q., Li, X., Zhang, Y., & Piao, S. (2020). Three-dimensional change in temperature sensitivity of northern vegetation phenology. *Global Change Biology*, *26*(9), 5189–5201. https://doi.org/10.1111/gcb.15200

Ge, Q., Wang, H., Rutishauser, T., & Dai, J. (2015). Phenological response to climate change in China: A meta-analysis. *Global Change Biology*, *21*(1), 265–274. https://doi.org/10.1111/gcb.12648

Gelman, A., Goodrich, B., Gabry, J., & Vehtari, A. (2019). R-squared for Bayesian Regression Models. *The American Statistician*, *73*(3), 307–309. https://doi.org/10.1080/00031305.2018.1549100

Gelman, A., & Rubin, D. B. (1992). Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science*, *7*(4). https://doi.org/10.1214/ss/1177011136

Gu, L., Hanson, P. J., Post, W. M., Kaiser, D. P., Yang, B., Nemani, R., Pallardy, S. G., & Meyers, T. (2008). The 2007 Eastern US Spring Freeze: Increased Cold Damage in a Warming World? *BioScience*, *58*(3), 253–262. https://doi.org/10.1641/B580311

Guo, J., Boe, A., Kim, D.-S., & Lee, D. K. (2017). Growth and Development of Two Perennial Grasses in Ambient Light Conditions during their Natural Dormant Period. *Crop Science*, *57*(4), 2213–2225. https://doi.org/10.2135/cropsci2016.09.0823

Hall, I. V., & Sibley, J. D. (1976). The Biology Of Canadian Weeds: 20. *Cornus canadensis* L. *Canadian Journal of Plant Science*, *56*(4), 885–892. https://doi.org/10.4141/cjps76-144

Hanninen, H. (1990). Modeling bud dormancy release in trees from cool and temperate regions. *Acta. For. Fenn.*, *213*, 1–47.

Hänninen, H. (2016). *Boreal and Temperate Trees in a Changing Climate*. Springer Netherlands. https://doi.org/10.1007/978-94-017-7549-6

Harrison, T., Gibbs, J., & Winfree, R. (2018). Forest bees are replaced in agricultural and urban landscapes by native species with different phenologies and life-history traits. *Global Change Biology*, *24*(1), 287–296. https://doi.org/10.1111/gcb.13921

Heberling, J. M., Cassidy, S. T., Fridley, J. D., & Kalisz, S. (2019). Carbon gain phenologies of spring-flowering perennials in a deciduous forest indicate a novel niche for a widespread invader. *New Phytologist*, *221*(2), 778–788. https://doi.org/10.1111/nph.15404

Heberling, J. M., Fridley, J. D., Kalisz, S., & Primack, R. B. (2019). Phenological mismatch with trees reduces wildflower carbon budgets. *Ecology Letters*, *22*(4), 616–623. https://doi.org/10.1111/ele.13224

Hicks, D. J., & Chabot, B. F. (1985). Deciduous forest. In B. F. Chabot & H. A. Mooney (Eds.), *Physiological Ecology of North American Plant Communities* (pp. 257–277). Springer Netherlands. https://doi.org/10.1007/978-94-009-4830-3_12

Hirao, T., Murakami, M., & Kashizaki, A. (2009). Importance of the understory stratum to entomofaunal diversity in a temperate deciduous forest. *Ecological Research*, *24*(2), 263–272. https://doi.org/10.1007/s11284-008-0502-4

Hollister, J., Shah, T., Nowosad, J., Robitaille, A. L., Beck, M. W., & Johnson, M. (2023). *elevatr: Access Elevation Data from Various APIs*. https://doi.org/10.5281/zenodo.8335450

Horák, J. (2017). Insect ecology and veteran trees. *Journal of Insect Conservation*, *21*(1), 1–5. https://doi.org/10.1007/s10841-017-9953-7

Hsieh, T., Ma, K. H., & Chao, A. (2023). *iNEXT: Interpolation and Extrapolation for Species Diversity* (Version R package version 3.0.1) [R]. http://chao.stat.nthu.edu.tw/wordpress/software_download/.

Hutchison, B. A., & Matt, D. R. (1977). The Distribution of Solar Radiation within a Deciduous Forest. *Ecological Monographs*, *47*(2), 185–207. https://doi.org/10.2307/1942616

Ida, T. Y., & Kudo, G. (2008). Timing of Canopy Closure Influences Carbon Translocation and Seed Production of an Understorey Herb, Trillium apetalon (Trilliaceae). *Annals of Botany*, *101*(3), 435–446. https://doi.org/10.1093/aob/mcm296

Iler, A. M., Høye, T. T., Inouye, D. W., & Schmidt, N. M. (2013). Nonlinear flowering responses to climate: Are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1624), 20120489. https://doi.org/10.1098/rstb.2012.0489

Inouye, D. W. (2008). Effects of Climate Change on Phenology, Frost Damage, and Floral Abundance of Montane Wildflowers. *Ecology*, *89*(2), 353–362.

Inouye, D. W. (2022). Climate change and phenology. *WIREs Climate Change*, *13*(3), e764. https://doi.org/10.1002/wcc.764

IPCC. (2021). *Climate Change 2021 – The Physical Science Basis: Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (1st ed.). Cambridge University Press. https://doi.org/10.1017/9781009157896

Irwin, R. E. (2000). Morphological variation and female reproductive success in two sympatric Trillium species: Evidence for phenotypic selection in Trillium erectum and Trillium grandiflorum (Liliaceae). *American Journal of Botany*, *87*(2), 205–214.

Jacques, M.-H., Lapointe, L., Rice, K., Montgomery, R. A., Stefanski, A., & Reich, P. B. (2015). Responses of two understory herbs, *Maianthemum canadense* and *Eurybia macrophylla* , to experimental forest warming: Early emergence is the key to enhanced reproductive output. *American Journal of Botany*, *102*(10), 1610–1624. https://doi.org/10.3732/ajb.1500046

Jánosi, I. M., Silhavy, D., Tamás, J., & Csontos, P. (2020). Bulbous perennials precisely detect the length of winter and adjust flowering dates. *New Phytologist*, *228*(5), 1535–1547. https://doi.org/10.1111/nph.16740

Jones, G. D. (2012). Pollen extraction from insects. *Palynology*, *36*(1), 86–109. https://doi.org/10.1080/01916122.2011.629523

Kaplan, S. M., & Mulcahy, D. L. (1971). Mode of Pollination and Floral Sexuality in Thalictrum. *Evolution*, *25*(4), 659–668. JSTOR. https://doi.org/10.2307/2406946

Kharouba, H. M., Ehrlén, J., Gelman, A., Bolmgren, K., Allen, J. M., Travers, S. E., & Wolkovich, E. M. (2018). Global shifts in the phenological synchrony of species interactions over recent decades. *Proceedings of the National Academy of Sciences*, *115*(20), 5211–5216. https://doi.org/10.1073/pnas.1714511115

Koch, H., Corcoran, C., & Jonker, M. (2011). Honeydew Collecting in Malagasy Stingless Bees (Hymenoptera: Apidae: Meliponini) and Observations on Competition with Invasive Ants. *African Entomology*, *19*(1), 36–41. https://doi.org/10.4001/003.019.0111

Konrad, R., Wäckers, F. L., Romeis, J., & Babendreier, D. (2009). Honeydew feeding in the solitary bee Osmia bicornis as affected by aphid species and nectar availability. *Journal of Insect Physiology*, *55*(12), 1158–1166. https://doi.org/10.1016/j.jinsphys.2009.08.012

Korner, C., & Basler, D. (2010). Phenology Under Global Warming. *Science*, *327*(5972), 1461–1462. https://doi.org/10.1126/science.1186473

Kraemer, M. E., & Favi, F. D. (2005). Flower Phenology and Pollen Choice of <I>Osmia lignaria</I> (Hymenoptera: Megachilidae) in Central Virginia. *Environmental Entomology*, *34*(6), 1593–1605. https://doi.org/10.1603/0046-225X-34.6.1593

Kramer, K. (1994). Selecting a Model to Predict the Onset of Growth of Fagus sylvatica. *The Journal of Applied Ecology*, *31*(1), 172. https://doi.org/10.2307/2404609

Kramer, K., Degen, B., Buschbom, J., Hickler, T., Thuiller, W., Sykes, M. T., & de Winter, W. (2010). Modelling exploration of the future of European beech (Fagus sylvatica L.) under climate change—Range, abundance, genetic diversity and adaptive response. *Forest Ecology and Management*, *259*(11), 2213–2222. https://doi.org/10.1016/j.foreco.2009.12.023

Kratschmer, S., Petrović, B., Curto, M., Meimberg, H., & Pachinger, B. (2020). Pollen availability for the Horned mason bee (*Osmia cornuta*) in regions of different land use and landscape structures. *Ecological Entomology*, *45*(3), 525–537. https://doi.org/10.1111/een.12823

Kricsfalusy, V. V., & Ponomarenko, Y. P. (2013). Notes on Biology and Ecology of the Prairie Crocus (Anemone Patens L.) and Its Current Status in Saskatchewan. *Blue Jay*, *71*(3), 135–148. https://doi.org/10.29173/bluejay207

Kubitzki, K. (1993). Betulaceae. In K. Kubitzki, J. G. Rohwer, & V. Bittrich (Eds.), *Flowering Plants · Dicotyledons* (pp. 152–157). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-02899-5_15

Kudo, G., & Cooper, E. J. (2019). When spring ephemerals fail to meet pollinators: Mechanism of phenological mismatch and its impact on plant reproduction. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1904), 20190573. https://doi.org/10.1098/rspb.2019.0573

Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, *94*(10), 2311–2320. https://doi.org/10.1890/12-2003.1

Lacey, E. P., Roach, D. A., Herr, D., Kincaid, S., & Perrott, R. (2003). MULTIGENERATIONAL EFFECTS OF FLOWERING AND FRUITING PHENOLOGY IN PLANTAGO LANCEOLATA. *Ecology*, *84*(9), 2462–2475. https://doi.org/10.1890/02-0101

Landsberg, J. J. (1974). Apple Fruit Bud Development and Growth; Analysis and an Empirical Model. *Annals of Botany*, *38*(5), 1013–1023. https://doi.org/10.1093/oxfordjournals.aob.a084891

Lapointe, L. (2001). How phenology influences physiology in deciduous forest spring ephemerals. *Physiologia Plantarum*, *113*(2), 151–157. https://doi.org/10.1034/j.1399-3054.2001.1130201.x

Laube, J., Sparks, T. H., Estrella, N., Höfler, J., Ankerst, D. P., & Menzel, A. (2014). Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology*, *20*(1), 170–182. https://doi.org/10.1111/gcb.12360

Lee, B. R., Alecrim, E. F., Miller, T. K., Forrest, J. R. K., Heberling, J. M., Primack, R. B., & Sargent, R. D. (2024). Phenological mismatch between trees and wildflowers: Reconciling divergent findings in two recent analyses. *Journal of Ecology*, 1365-2745.14317. https://doi.org/10.1111/1365-2745.14317

Lee, B. R., Yancy, A. J., & Heberling, J. M. (2024). Phenological Escape and Its Importance for Understory Plant Species in Temperate Forests. *International Journal of Plant Sciences*, *185*(4), 321–333. https://doi.org/10.1086/729439

Leites, L., & Benito Garzón, M. (2023). Forest tree species adaptation to climate across biomes: Building on the legacy of ecological genetics to anticipate responses to climate change. *Global Change Biology*, *29*(17), 4711–4730. https://doi.org/10.1111/gcb.16711

Lenz, A., Hoch, G., Körner, C., & Vitasse, Y. (2016). Convergence of leaf-out towards minimum risk of freezing damage in temperate trees. *Functional Ecology*, *30*(9), 1480–1490. https://doi.org/10.1111/1365-2435.12623

Lenz, A., Hoch, G., Vitasse, Y., & Körner, C. (2013). European deciduous trees exhibit similar safety margins against damage by spring freeze events along elevational gradients. *New Phytologist*, *200*(4), 1166–1175. https://doi.org/10.1111/nph.12452

Lewandowski, D., Kurowicka, D., & Joe, H. (2009). Generating random correlation matrices based on vines and extended onion method. *Journal of Multivariate Analysis*, *100*(9), 1989–2001. https://doi.org/10.1016/j.jmva.2009.04.008

Lin, S., Wang, H., Ge, Q., & Hu, Z. (2022). Effects of chilling on heat requirement of spring phenology vary between years. *Agricultural and Forest Meteorology*, *312*, 108718. https://doi.org/10.1016/j.agrformet.2021.108718

Linkosalo, T., Hakkinen, R., & Hanninen, H. (2006). Models of the spring phenology of boreal and temperate trees: Is there something missing? *Tree Physiology*, *26*(9), 1165–1172. https://doi.org/10.1093/treephys/26.9.1165

Luedeling, E., & Fernandez, E. (2022). *chillR: Statistical Methods for Phenology Analysis in Temperate Fruit Trees*. https://CRAN.R-project.org/package=chillR

Luedeling, E., & Gassner, A. (2012). Partial Least Squares Regression for analyzing walnut phenology in California. *Agricultural and Forest Meteorology*, *158–159*, 43–52. https://doi.org/10.1016/j.agrformet.2011.10.020

Luedeling, E., Guo, L., Dai, J., Leslie, C., & Blanke, M. M. (2013). Differential responses of trees to temperature variation during the chilling and forcing phases. *Agricultural and Forest Meteorology*, *181*, 33–42. https://doi.org/10.1016/j.agrformet.2013.06.018

Luedeling, E., Kunz, A., & Blanke, M. M. (2013). Identification of chilling and heat requirements of cherry trees—A statistical approach. *International Journal of Biometeorology*, *57*(5), 679–689. https://doi.org/10.1007/s00484-012-0594-y

MacIvor, J. S., Cabral, J. M., & Packer, L. (2014). Pollen specialization by solitary bees in an urban landscape. *Urban Ecosystems*, *17*(1), 139–147. https://doi.org/10.1007/s11252-013-0321-4

Man, R., Kayahara, G. J., Dang, Q.-L., & Rice, J. A. (2009). A case of severe frost damage prior to budbreak in young conifers in Northeastern Ontario: Consequence of climate change? *The Forestry Chronicle*, *85*(3), 453–462. https://doi.org/10.5558/tfc85453-3

Manos, P. S., Zhou, Z., & Cannon, C. H. (2001). Systematics of Fagaceae: Phylogenetic Tests of Reproductive Trait Evolution. *International Journal of Plant Sciences*, *162*(6), 1361–1379. https://doi.org/10.1086/322949

Marino, G. P., Kaiser, D. P., Gu, L., & Ricciuto, D. M. (2011). Reconstruction of false spring occurrences over the southeastern United States, 1901–2007: An increasing risk of spring freeze damage? *Environmental Research Letters*, *6*(2), 024015. https://doi.org/10.1088/1748-9326/6/2/024015

McKenna, M. F., & Houle, G. (2000). Why are annual plants rarely spring ephemerals?: RESEARCH Rarity of spring ephemeral annuals. *New Phytologist*, *148*(2), 295–302. https://doi.org/10.1046/j.1469-8137.2000.00756.x

Meier, U. (2018). *Growth stages of mono- and dicotyledonous plants: BBCH Monograph*. https://doi.org/10.5073/20180906-074619

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kübler, K., Bissolli, P., Braslavská, O., Briede, A., Chmielewski, F. M., Crepinsek, Z., Curnel, Y., Dahl, Å., Defila, C., Donnelly, A., Filella, Y., Jatczak, K., Måge, F., … Zust, A. (2006). European phenological response to climate change matches the warming pattern: EUROPEAN Phenological Response To Climate Change. *Global Change Biology*, *12*(10), 1969–1976. https://doi.org/10.1111/j.1365-2486.2006.01193.x

Miller, T. K., Heberling, J. M., Kuebbing, S. E., & Primack, R. B. (2023). Warmer temperatures are linked to widespread phenological mismatch among native and non-native forest plants. *Journal of Ecology*, *111*(2), 356–371. https://doi.org/10.1111/1365-2745.14021

Moran, V. C., & Southwood, T. R. E. (1982). The Guild Composition of Arthropod Communities in Trees. *The Journal of Animal Ecology*, *51*(1), 289. https://doi.org/10.2307/4325

Morin, X., Lechowicz, M. J., Augspurger, C., O'Keefe, J., Viner, D., & Chuine, I. (2009a). Leaf phenology in 22 North American tree species during the 21st century. *Global Change Biology*, *15*(4), 961–975. https://doi.org/10.1111/j.1365-2486.2008.01735.x

Morin, X., Lechowicz, M. J., Augspurger, C., O'Keefe, J., Viner, D., & Chuine, I. (2009b). Leaf phenology in 22 North American tree species during the 21st century. *Global Change Biology*, *15*(4), 961–975. https://doi.org/10.1111/j.1365-2486.2008.01735.x

Motten, A. F. (1982). Autogamy And Competition For Pollinators In Hepatica Americana (Ranunculaceae). *American Journal of Botany*, *69*(8), 1296–1305. https://doi.org/10.1002/j.1537-2197.1982.tb13375.x

Müller, A., Diener, S., Schnyder, S., Stutz, K., Sedivy, C., & Dorn, S. (2006). Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee–flower relationships. *Biological Conservation*, *130*(4), 604–615. https://doi.org/10.1016/j.biocon.2006.01.023

Muller, R. N. (1978). The Phenology, Growth and Ecosystem Dynamics of Erythronium americanum in the Northern Hardwood Forest. *Ecological Monographs*, *48*(1), 1–20. https://doi.org/10.2307/2937357

Nagahama, A., & Yahara, T. (2019). Quantitative comparison of flowering phenology traits among trees, perennial herbs, and annuals in a temperate plant community. *American Journal of Botany*, *106*(12), 1545–1557. https://doi.org/10.1002/ajb2.1387

Nagaraj, M. (1952). Floral Morphology of Populus deltoides and P. tremuloides. Contributions from the Hull Botanical Laboratory 639. *Botanical Gazette*, *114*(2), 222–243. JSTOR.

Nault, A., & Gagnon, D. (1993). Ramet Demography of Allium Tricoccum, A Spring Ephemeral, Perennial Forest Herb. *The Journal of Ecology*, *81*(1), 101. https://doi.org/10.2307/2261228

Osada, N., Murase, K., Tsuji, K., Sawada, H., Nunokawa, K., Tsukahara, M., & Hiura, T. (2018). Genetic differentiation in the timing of budburst in Fagus crenata in relation to temperature and photoperiod. *International Journal of Biometeorology*, *62*(9), 1763–1776. https://doi.org/10.1007/s00484-018-1579-2

Pardee, G. L., Jensen, I. O., Inouye, D. W., & Irwin, R. E. (2019). The individual and combined effects of snowmelt timing and frost exposure on the reproductive success of montane forbs. *Journal of Ecology*, *107*(4), 1970–1981. https://doi.org/10.1111/1365-2745.13152

Park, D. S., Breckheimer, I., Williams, A. C., Law, E., Ellison, A. M., & Davis, C. C. (2019). Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1763), 20170394. https://doi.org/10.1098/rstb.2017.0394

Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, *13*(9), 1860–1872. https://doi.org/10.1111/j.1365-2486.2007.01404.x

Pearson, W. D., & Braiden, V. (1990). Seasonal Pollen Collection by Honeybees from Grass/Shrub Highlands in Canterbury, New Zealand. *Journal of Apicultural Research*, *29*(4), 206–213. https://doi.org/10.1080/00218839.1990.11101221

Peaucelle, M., Janssens, I. A., Stocker, B. D., Descals Ferrando, A., Fu, Y. H., Molowny-Horas, R., Ciais, P., & Peñuelas, J. (2019). Spatial variance of spring phenology in temperate deciduous forests is constrained by background climatic conditions. *Nature Communications*, *10*(1), 5388. https://doi.org/10.1038/s41467-019-13365-1

Perry, T. O. (1971). Dormancy of Trees in Winter. *Science*, *171*(3966), 29–36. https://doi.org/10.1126/science.171.3966.29

Persson, A. S., Mazier, F., & Smith, H. G. (2018). When beggars are choosers—How nesting of a solitary bee is affected by temporal dynamics of pollen plants in the landscape. *Ecology and Evolution*, *8*(11), 5777–5791. https://doi.org/10.1002/ece3.4116

Phillimore, A. B., Stålhandske, S., Smithers, R. J., & Bernard, R. (2012). Dissecting the Contributions of Plasticity and Local Adaptation to the Phenology of a Butterfly and Its Host Plants. *The American Naturalist*, *180*(5), 655–670. https://doi.org/10.1086/667893

Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., Liu, L., Lian, X., Shen, M., & Zhu, X. (2019). Plant phenology and global climate change: Current progresses and challenges. *Global Change Biology*, *25*(6), 1922–1940. https://doi.org/10.1111/gcb.14619

Pilson, D. (2000). The evolution of plant response to herbivory: Simultaneously considering resistance and tolerance in Brassica rapa. *Evolutionary Ecology*, *14*(4–6), 457. https://doi.org/10.1023/A:1010953714344

Polgar, C. A., & Primack, R. B. (2011). Leaf-out phenology of temperate woody plants: From trees to ecosystems: Tansley review. *New Phytologist*, *191*(4), 926–941. https://doi.org/10.1111/j.1469-8137.2011.03803.x

Polle, A., & Rennenberg, H. (2019). Physiological Responses to Abiotic and Biotic Stress in Forest Trees. *Forests*, *10*(9), 711. https://doi.org/10.3390/f10090711

Portman, Z. M., Bruninga-Socolar, B., & Cariveau, D. P. (2020). The State of Bee Monitoring in the United States: A Call to Refocus Away From Bowl Traps and Towards More Effective Methods. *Annals of the Entomological Society of America*, *113*(5), 337–342. https://doi.org/10.1093/aesa/saaa010

Powell, L. E. (1987). The Hormonal Control of Bud and Seed Dormancy in Woody Plants. In P. J. Davies (Ed.), *Plant Hormones and their Role in Plant Growth and Development* (pp. 539–552). Springer Netherlands. https://doi.org/10.1007/978-94-009-3585-3_28

Preisser, E., Smith, D., & Lowman, M. (1998). *Canopy And Ground Level Insect Distribution In A Temperate Forest*. *19*(2), 141–146.

Prevéy, J. S., Harrington, C. A., & St. Clair, J. B. (2018). The timing of flowering in Douglas-fir is determined by cool-season temperatures and genetic variation. *Forest Ecology and Management*, *409*, 729–739. https://doi.org/10.1016/j.foreco.2017.11.062

Prevéy, J., Vellend, M., Rüger, N., Hollister, R. D., Bjorkman, A. D., Myers-Smith, I. H., Elmendorf, S. C., Clark, K., Cooper, E. J., Elberling, B., Fosaa, A. M., Henry, G. H. R., Høye, T. T., Jónsdóttir, I. S., Klanderud, K., Lévesque, E., Mauritz, M., Molau, U., Natali, S. M., … Rixen, C. (2017). Greater temperature sensitivity of plant phenology at colder sites: Implications for convergence across northern latitudes. *Global Change Biology*, *23*(7), 2660–2671. https://doi.org/10.1111/gcb.13619

Prevéy, J., Vellend, M., Rüger, N., Hollister, R. D., Bjorkman, A. D., Myers-Smith, I. H., Elmendorf, S. C., Clark, K., Cooper, E. J., Elberling, B., Fosaa, A. M., Henry, G. H. R., Høye, T. T., Jónsdóttir, I. S., Klanderud, K., Lévesque, E., Mauritz, M., Molau, U., Natali, S. M., … Rixen, C. (2017). Greater temperature sensitivity of plant phenology at colder sites: Implications for convergence across northern latitudes. *Global Change Biology*, *23*(7), 2660–2671. https://doi.org/10.1111/gcb.13619

Primack, R. B., Ibáñez, I., Higuchi, H., Lee, S. D., Miller-Rushing, A. J., Wilson, A. M., & Silander, J. A. (2009). Spatial and interspecific variability in phenological responses to warming temperatures. *Biological Conservation*, *142*(11), 2569–2577. https://doi.org/10.1016/j.biocon.2009.06.003

Pureswaran, D. S., Roques, A., & Battisti, A. (2018). Forest Insects and Climate Change. *Current Forestry Reports*, *4*(2), 35–50. https://doi.org/10.1007/s40725-018-0075-6

R Core Team. (2023). *R: A Language and Environment for Statistical Computing* [R]. https://www.R-project.org/

Radmacher, S., & Strohm, E. (2010). Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, *41*(2), 169–177. https://doi.org/10.1051/apido/2009064

Rafferty, N. E., & Ives, A. R. (2011). Effects of experimental shifts in flowering phenology on plant-pollinator interactions: Experimental shifts in flowering phenology. *Ecology Letters*, *14*(1), 69–74. https://doi.org/10.1111/j.1461-0248.2010.01557.x

Reader, R. J., & Bricker, B. D. (1992). Response of Five Deciduous Forest Herbs to Partial Canopy Removal and Patch Size. *American Midland Naturalist*, *127*(1), 149. https://doi.org/10.2307/2426330

Ren, P., Néron, V., Rossi, S., Liang, E., Bouchard, M., & Deslauriers, A. (2020). Warming counteracts defoliation-induced mismatch by increasing herbivore-plant phenological synchrony. *Global Change Biology*, *26*(4), 2072–2080. https://doi.org/10.1111/gcb.14991

Requier, F., & Leonhardt, S. D. (2020). Beyond flowers: Including non-floral resources in bee conservation schemes. *Journal of Insect Conservation*, *24*(1), 5–16. https://doi.org/10.1007/s10841-019-00206-1

Roulston, T. H., Cane, J. H., & Buchmann, S. L. (2000). What Governs Protein Content Of Pollen: Pollinator Preferences, Pollen–Pistil Interactions, Or Phylogeny? *Ecological Monographs*, *70*(4), 27.

Roussy, A.-M. (2014). *The Sexual and Vegetative Propagation of Sugar Maple and its Threat from Norway Maple*. University of Guelph.

Routhier, M.-C., & Lapointe, L. (2002). Impact of tree leaf phenology on growth rates and reproduction in the spring flowering species Trillium erectum (Liliaceae). *American Journal of Botany*, *89*(3), 500–505. https://doi.org/10.3732/ajb.89.3.500

Russo, L., & Danforth, B. (2017). Pollen preferences among the bee species visiting apple (Malus pumila) in New York. *Apidologie*, *48*(6), 806–820. https://doi.org/10.1007/s13592-017-0525-3

Rutishauser, T., Luterbacher, J., Jeanneret, F., Pfister, C., & Wanner, H. (2007). A phenology-based reconstruction of interannual changes in past spring seasons: RECONSTRUCTING SPRING SEASON. *Journal of Geophysical Research: Biogeosciences*, *112*(G4), n/a-n/a. https://doi.org/10.1029/2006JG000382

Saunders, M. E. (2018). Insect pollinators collect pollen from wind-pollinated plants: Implications for pollination ecology and sustainable agriculture. *Insect Conservation and Diversity*, *11*(1), 13–31. https://doi.org/10.1111/icad.12243

Sevenello, M., Sargent, R. D., & Forrest, J. R. K. (2020). Spring wildflower phenology and pollinator activity respond similarly to climatic variation in an eastern hardwood forest. *Oecologia*, *193*(2), 475–488. https://doi.org/10.1007/s00442-020-04670-4

Severson, D. W., & Parry, J. E. (1981). A Chronology of Pollen Collection by Honeybees. *Journal of Apicultural Research*, *20*(2), 97–103. https://doi.org/10.1080/00218839.1981.11100480

Smith, C., Harrison, T., Gardner, J., & Winfree, R. (2021). Forest-associated bee species persist amid forest loss and regrowth in eastern North America. *Biological Conservation*, *260*, 109202. https://doi.org/10.1016/j.biocon.2021.109202

Solomon, S. (2007). IPCC (2007): Climate Change The Physical Science Basis. *AGU Fall Meeting Abstracts*, *2007*, U43D-01.

Splitt, A., Skórka, P., Strachecka, A., Borański, M., & Teper, D. (2021). Keep trees for bees: Pollen collection by Osmia bicornis along the urbanization gradient. *Urban Forestry & Urban Greening*, *64*, 127250. https://doi.org/10.1016/j.ufug.2021.127250

Stockhammer, K. A. (1966). Nesting Habits and Life Cycle of a Sweat Bee, Augochlora pura (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society*, *39*(2), 157–192. JSTOR.

Sullivan, J. R. (1983). Comparative Reproductive Biology Of Acer Pensylvanicum And A. Spicatum (Aceraceae). *American Journal of Botany*, *70*(6), 916–924. https://doi.org/10.1002/j.1537-2197.1983.tb06431.x

Temple, B., Koch, E., Bolmgren, K., Ungersböck, M., Paul, A., Scheifinger, H., Rutishauser, T., Busto, M., Chmielewski, F.-M., Hájková, L., Hodzić, S., Kaspar, F., Pietragalla, B., Romero-Fresneda, R., Tolvanen, A., Vučetič, V., Zimmermann, K., & Zust, A. (2018). Pan European Phenological database (PEP725): A single point of access for European data. *International Journal of Biometeorology*, *62*(6), 1109–1113. https://doi.org/10.1007/s00484-018-1512-8

Thomas, B., Murphy, D. J., & Murray, B. G. (Eds.). (2003). *Encyclopedia of applied plant sciences* (1st ed). Elsevier Academic.

Ulyshen, M. D. (2011). Arthropod vertical stratification in temperate deciduous forests: Implications for conservation-oriented management. *Forest Ecology and Management*, *261*(9), 1479–1489. https://doi.org/10.1016/j.foreco.2011.01.033

Ulyshen, M. D., & Hanula, J. L. (2007). A Comparison of the Beetle (Coleoptera) Fauna Captured at Two Heights above the Ground in a North American Temperate Deciduous Forest. *American Midland Naturalist*, *158*(2), 260–278.

Ulyshen, M. D., Horn, S., & Hanula, J. L. (2020). Effects of Chinese Privet on Bees and Their Vertical Distribution in Riparian Forests. *Forest Science*, *66*(4), 416–423. https://doi.org/10.1093/forsci/fxz088

Ulyshen, M. D., Soon, V., & Hanula, J. L. (2010). On the vertical distribution of bees in a temperate deciduous forest. *Insect Conservation and Diversity*. https://doi.org/10.1111/j.1752-4598.2010.00092.x

Ungureanu, E. M. (1972). Methods for dissecting dry insects and insects preserved in fixative solutions or by refrigeration. *Bulletin of the World Health Organization*, *47*(2), 239–244.

Urban-Mead, K. R., Muñiz, P., Gillung, J., Espinoza, A., Fordyce, R., van Dyke, M., McArt, S. H., & Danforth, B. N. (2021). Bees in the trees: Diverse spring fauna in temperate forest edge canopies. *Forest Ecology and Management*, *482*, 118903. https://doi.org/10.1016/j.foreco.2020.118903

Urban-Mead, K. R., Walter, E., Mcart, S. H., & Danforth, B. N. (2022). Nearly half of spring-flying male Andrena bees consume pollen, but less than female conspecifics. *Apidologie*, *53*(4), 49. https://doi.org/10.1007/s13592-022-00951-4

Vaudo, A. D., Farrell, L. M., Patch, H. M., Grozinger, C. M., & Tooker, J. F. (2018). Consistent pollen nutritional intake drives bumble bee (*Bombus impatiens*) colony growth and reproduction across different habitats. *Ecology and Evolution*, *8*(11), 5765–5776. https://doi.org/10.1002/ece3.4115

Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F., & Grozinger, C. M. (2016). Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences*, *113*(28), E4035–E4042. https://doi.org/10.1073/pnas.1606101113

Vaudo, A. D., Stabler, D., Patch, H. M., Tooker, J. F., Grozinger, C. M., & Wright, G. A. (2017). Correction: Bumble bees regulate their intake of essential protein and lipid pollen macronutrients. *The Journal of Experimental Biology*, *220*(3), 507–507. https://doi.org/10.1242/jeb.155911

Vaudo, A. D., Tooker, J. F., Patch, H. M., Biddinger, D. J., Coccia, M., Crone, M. K., Fiely, M., Francis, J. S., Hines, H. M., Hodges, M., Jackson, S. W., Michez, D., Mu, J., Russo, L., Safari, M., Treanore, E. D., Vanderplanck, M., Yip, E., Leonard, A. S., & Grozinger, C.

M. (2020). Pollen Protein: Lipid Macronutrient Ratios May Guide Broad Patterns of Bee Species Floral Preferences. *Insects*, *11*(2), 132. https://doi.org/10.3390/insects11020132

Vezina, P. E., & Grandtner, M. M. (1965). Phenological Observations of Spring Geophytes in Quebec. *Ecology*, *46*(6), 869–872. https://doi.org/10.2307/1934022

Vitasse, Y. (2013). Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier. *New Phytologist*, *198*(1), 149–155. https://doi.org/10.1111/nph.12130

Vitasse, Y., Hoch, G., Randin, C. F., Lenz, A., Kollas, C., Scheepens, J. F., & Körner, C. (2013). Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species. *Oecologia*, *171*(3), 663–678. https://doi.org/10.1007/s00442-012-2580-9

Wallander, E. (2001). *Evolution of wind-pollination in Fraxinus (Oleaceae): An ecophylogenetic approach*. Botanical Institute (Botaniska institutionen), Univ.

Wang, H., Wu, C., Ciais, P., Peñuelas, J., Dai, J., Fu, Y., & Ge, Q. (2020). Overestimation of the effect of climatic warming on spring phenology due to misrepresentation of chilling. *Nature Communications*, *11*(1), 4945. https://doi.org/10.1038/s41467-020-18743-8

Wang, J., Liu, D., Ciais, P., & Peñuelas, J. (2022). Decreasing rainfall frequency contributes to earlier leaf onset in northern ecosystems. *Nature Climate Change*, *12*(4), 386–392. https://doi.org/10.1038/s41558-022-01285-w

Wang, T., Ottlé, C., Peng, S., Janssens, I. A., Lin, X., Poulter, B., Yue, C., & Ciais, P. (2014). The influence of local spring temperature variance on temperature sensitivity of spring phenology. *Global Change Biology*, *20*(5), 1473–1480. https://doi.org/10.1111/gcb.12509

Wardhaugh, C. W., Blakely, T. J., Greig, H., Morris, P. D., Barnden, A., Rickard, S., Atkinson, B., Fagan, L. L., Ewers, R. M., & Didham, R. K. (2006). Vertical stratification in the spatial distribution of the beech scale insect (Ultracoelostoma assimile) in Nothofagus tree canopies in New Zealand. *Ecological Entomology*, *31*(2), 185–195. https://doi.org/10.1111/j.0307-6946.2006.00778.x

Wenden, B., Mariadassou, M., Chmielewski, F., & Vitasse, Y. (2020). Shifts in the temperature-sensitive periods for spring phenology in European beech and pedunculate oak clones across latitudes and over recent decades. *Global Change Biology*, *26*(3), 1808–1819. https://doi.org/10.1111/gcb.14918

Westerberg, L., Berglund, H., Jonason, D., & Milberg, P. (2021). Color pan traps often catch less when there are more flowers around. *Ecology and Evolution*, *11*(9), 3830–3840. https://doi.org/10.1002/ece3.7252

Whitehead, D. R. (1969). Wind pollination in the angiosperms: Evolutionary and environmental considerations. *Evolution*, *23*, 28015–28035.

Wickham, H., François R, Henry L, Müller K, & Vaughan D. (2023). _*dplyr: A Grammar of Data Manipulation*_. (Version R package version 1.1.2) [R]. <https://CRAN.R-project.org/package=dplyr>

Wielgolaski, F. E. (2001). Phenological modifications in plants by various edaphic factors. *International Journal of Biometeorology*, *45*(4), 196–202. https://doi.org/10.1007/s004840100100

Willems, F. M., Scheepens, J. F., Ammer, C., Block, S., Bucharova, A., Schall, P., Sehrt, M., & Bossdorf, O. (2021). Spring understory herbs flower later in intensively managed forests. *Ecological Applications*, *31*(5). https://doi.org/10.1002/eap.2332

Wolkovich, E. M., Auerbach, J., Chamberlain, C. J., Buonaiuto, D. M., Ettinger, A. K., Morales-Castilla, I., & Gelman, A. (2021). A simple explanation for declining temperature sensitivity with warming. *Global Change Biology*, *27*(20), 4947–4949. https://doi.org/10.1111/gcb.15746

Wood, T. J., Kaplan, I., & Szendrei, Z. (2018). Wild Bee Pollen Diets Reveal Patterns of Seasonal Foraging Resources for Honey Bees. *Frontiers in Ecology and Evolution*, *6*, 210. https://doi.org/10.3389/fevo.2018.00210

Xu, Y., Dai, J., Ge, Q., Wang, H., & Tao, Z. (2021). Comparison of chilling and heat requirements for leaf unfolding in deciduous woody species in temperate and subtropical China. *International Journal of Biometeorology*, *65*(3), 393–403. https://doi.org/10.1007/s00484-020-02007-7

Yourstone, J., Karlsson, M., Klatt, B. K., Olsson, O., & Smith, H. G. (2021). Effects of crop and non-crop resources and competition: High importance of trees and oilseed rape for solitary bee reproduction. *Biological Conservation*, *261*, 109249. https://doi.org/10.1016/j.biocon.2021.109249

Zani, D., Crowther, T. W., Mo, L., Renner, S. S., & Zohner, C. M. (2020). Increased growing-season productivity drives earlier autumn leaf senescence in temperate trees. *Science*, *370*(6520), 1066–1071. https://doi.org/10.1126/science.abd8911

Zhang, X., Tarpley, D., & Sullivan, J. T. (2007). Diverse responses of vegetation phenology to a warming climate. *Geophysical Research Letters*, *34*(19), L19405. https://doi.org/10.1029/2007GL031447

Zhao, H., Fu, Y. H., Wang, X., Zhang, Y., Liu, Y., & Janssens, I. A. (2021). Diverging models introduce large uncertainty in future climate warming impact on spring phenology of temperate deciduous trees. *Science of The Total Environment*, *757*, 143903. https://doi.org/10.1016/j.scitotenv.2020.143903

Zhu, L., Ives, A. R., Zhang, C., Guo, Y., & Radeloff, V. C. (2019). Climate change causes functionally colder winters for snow cover-dependent organisms. *Nature Climate Change*, *9*(11), 886–893. https://doi.org/10.1038/s41558-019-0588-4

Zohner, C. M., Benito, B. M., Fridley, J. D., Svenning, J.-C., & Renner, S. S. (2017). Spring predictability explains different leaf-out strategies in the woody floras of North America, Europe and East Asia. *Ecology Letters*, *20*(4), 452–460. https://doi.org/10.1111/ele.12746

Zohner, C. M., Benito, B. M., Svenning, J.-C., & Renner, S. S. (2016). Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. *Nature Climate Change*, *6*(12), 1120–1123. https://doi.org/10.1038/nclimate3138

Zohner, C. M., & Renner, S. S. (2014). Common garden comparison of the leaf-out phenology of woody species from different native climates, combined with herbarium records, forecasts long-term change. *Ecology Letters*, *17*(8), 1016–1025. https://doi.org/10.1111/ele.12308

Zohner, C. M., & Renner, S. S. (2015). Perception of photoperiod in individual buds of mature trees regulates leaf-out. *New Phytologist*, *208*(4), 1023–1030. https://doi.org/10.1111/nph.13510