Effects of global change on plants: tracing the footprints of climate warming and land use from herbaria to forest understories.

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Declaration of author contributions

This thesis, "Effects of global change on plants: tracing the footprints of climate warming and land use from herbaria to forest understories", is based on the work I did during my PhD at University of Tübingen, supervised by Prof. Dr. Oliver Bossdorf and Prof. Dr. Johannes Fredericus Scheepens. **Chapters II–V** of this thesis include four independent scientific manuscripts, each containing co-authorship, and all are or will be published. The contribution of the authors for each chapter is stated as following:

Chapter II - Using herbaria to study global environmental change

Patricia L.M. Lang, **Franziska M. Willems**, J.F. Scheepens, Hernán A. Burbano and Oliver Bossdorf

Published in *The New Phytologist*, Volume 221, Issue 1 (January 2019)

All authors developed the ideas for this review; FMW and PLML undertook the literature research and PLML designed the figures and wrote the paper with input from all authors.

Chapter III – Forest wildflowers bloom earlier as Europe warms – but not everywhere equally

Franziska M. Willems, J.F. Scheepens and Oliver Bossdorf

Status in publication process: in preparation

All authors designed the study. FMW collected, compiled and analyzed all data. FMW wrote the manuscript with all coauthors contributing to revisions.

Chapter IV – Spring understory herbs flower later in intensively managed forests

Franziska M. Willems, J.F. Scheepens, Christian Ammer, Svenja Block, Anna Bucharova, Peter Schall, Melissa Sehrt and Oliver Bossdorf

Status in publication process: *Ecological Applications*, in press

OB, JFS and AB designed the study. FMW, SB and MS collected the phenology data, and CA and PS contributed the forest management data. FMW compiled and analyzed all data with input from OB and JFS. FMW wrote the manuscript with all coauthors contributing to revisions.

Chapter V – Climate warming changes synchrony of plants and pollinators in Germany

Jonas Freimuth, Oliver Bossdorf, J.F. Scheepens, **Franziska M. Willems**

Status in publication process: To be resubmitted to *Proceedings of the Royal Society B*

JF and FMW conceived the study. JF collected and analyzed the data, and wrote the first draft of the manuscript, with guidance from FMW. JFS and OB provided input to data analysis and manuscript writing.

Summary

Over the last centuries humans have drastically changed the global environment. Since the industrial revolution, they altered the climate of the Earth by emitting greenhouse gases, deteriorated its ecosystems and decimated biodiversity by intensifying land-use. One of the most apparent fingerprints of this anthropogenic global change is that all over the world the rhythms of life of plants and animals are changing. In order for species to survive and thrive in their natural environments, their life-history events (for example the leaf-out, flowering or leaf-coloring of plants, or the appearance, hatching, migration or reproduction of animals) must be timed well with particular environmental conditions. Climate warming has already caused shifts in the timing of many such phenological events. Shifts in the phenology of plants can have particularly large impacts on the natural environment, because plants are the bases of many food chains, and therefore anything that affects them also affects the organisms that (directly or indirectly) depend on them. Climate changes can particularly impact biotic interactions if the phenologies of interacting species, e.g. plants and their pollinators, do not change in the same way, causing "phenological mismatches" with fitness consequences for both partners. However, our understanding of the mechanisms – the cues and drivers – that determine many phenological responses to global change is still limited. Most studies investigating phenology have a limited temporal, geographical and taxonomic scope, because historical, long-term data are rare. And if such data exist, they have often been collected at only few locations and for few (groups of) species. In this context herbaria are extremely precious data sources that allow to study how global change has affected plants during the last centuries. Herbaria store plant specimens from across the world and from a multitude of species, thus allowing to track phenology (and other responses of plants to global change) not only over time but also over space and across many taxa. This is important since phenology of course also depends on latitude, altitude as well as other environmental factors and is species-specific. Furthermore, we know little about the influences that other global change drivers than climate warming, such as land use change, have on plant phenology. Especially in forests, management that influence tree species composition and stand structure could affect the phenology of forest understory herbs through changes in radiation, microclimate or other factors. However, knowledge about how multiple drivers of phenology interact, and how their influence varies among species, populations or geographic regions, is still limited.

With this thesis I aimed to narrow these gaps, by addressing the following questions: i) How can herbaria generally be used to study long-term global change effects on plants? And specifically, how do ii) climate change and iii) land-use change affect the flowering

phenology of forest understory plants? iv) Do phenological shifts of plants and their pollinating insects differ, resulting in ecological mismatches? **Chapter II** is a literature review that maps out how historic data from herbaria can be used to trace the effects that global change (including pollution, land-use and climate change, and the rise of invasive species) had on plants during the last centuries. In **Chapters III** and **IV**, I zoom in on the effects that global change has on the flowering phenology of spring-flowering forest understory herbs. I used herbarium data and spatial modeling to estimate how the flowering time of these wildflowers shifted during the last century due to climate change in Europe (**Chapter III**). For a subset of the same species, I analyzed field data from 100 forest plots in Germany to assess how forest management affects flowering time (**Chapter IV**). I focused on spring-flowering forest herbs, because they have a narrow and distinct flowering period, which makes them well-suited for tracking phenological shifts over time and probably particularly susceptible to management changes. Finally, **Chapter V** used data of more than 1000 plant and 80 insect species from the Global Biodiversity Information Facility (GBIF) to analyze how the phenologies of plants and insect pollinators, and their synchrony, have shifted over the last decades and with climate warming. My work shows i) that herbaria are an invaluable data treasure for studying how anthropogenic global change affects plants (**Chapter II**) – especially their phenology. Further, I found that ii) flowering time of forest herbs advanced by almost a week during the last century in Europe and that these changes were associated with climate warming (**Chapter III**). I also demonstrated that it is crucial to account for spatial correlation when analyzing herbarium data spanning a broad geographic range: When spatial correlation was ignored, models severely overestimated phenology shifts and failed to acknowledge that plant phenological responses varied significantly within Europe. My analysis of field data showed that iii) land use also affects plant phenology (**Chapter IV**). In intensively managed forests plants flowered around two weeks later than in unmanaged forests - partly, but not solely, because management altered microclimate. Finally, the GBIF study showed that iv) during the last ~50 years plant phenology advanced stronger and more consistently than that of insects (**Chapter V**). This influenced also their synchrony, with potential far-reaching ecological consequences. Understanding why and how the phenologies of plants (and animals) shift is important to estimate the vulnerability of species, populations, and ecological communities to ongoing global change.

Zusammenfassung

In den letzten Jahrhunderten haben die Menschen die Umwelt der Erde drastisch verändert. Seit der industriellen Revolution hat sich das Klima der Erde durch den Ausstoß riesiger Mengen von Treibhausgasen verändert, und durch die Intensivierung der Landnutzung hat sich der Zustand vieler Ökosysteme verschlechtert und die Artenvielfalt wurde dezimiert. Einer der markantesten Fingerabdrücke dieses anthropogenen globalen Wandels ist, dass sich überall auf der Welt die Lebensrhythmen von Pflanzen und Tieren verändern. Damit Arten in ihrer natürlichen Umgebung überleben und gedeihen können, muss der Zeitpunkt ihrer lebensgeschichtlichen Ereignisse (z. B. der Blattaustrieb, die Blüte oder die Blattfärbung von Pflanzen sowie das Erwachen, Schlüpfen, die Migration oder die Fortpflanzung von Tieren) gut auf spezifische, günstige Umweltbedingungen abgestimmt sein. Die Klimaerwärmung hat bereits zu Verschiebungen im Timing solcher phänologischen Ereignisse geführt. Verschiebungen in der Phänologie von Pflanzen können besonders große Auswirkungen auf die natürliche Umwelt haben, da Pflanzen die Basis vieler Nahrungskette sind, und sich daher alles, was sie betrifft, auch auf die Organismen auswirkt, die (direkt oder indirekt) von ihnen abhängen. Klimaveränderungen können sich besonders auf biotische Interaktionen auswirken, wenn sich die Phänologien interagierenden Arten, z. B. die von Pflanzen und ihren Bestäubern, nicht in gleicher Weise verändern, was zu "phänologischen Asynchronität" führt, die Folgen für die Fitness beider Partner hat. Allerdings ist unser Verständnis der Mechanismen, die viele phänologische Reaktionen auf den globalen Wandel bestimmen, noch begrenzt. Die meisten Studien, die Phänologie untersuchen, haben einen begrenzten zeitlichen, geografischen und taxonomischen Umfang, da historische Langzeitdaten selten sind. Und wenn solche Daten vorhanden sind, wurden sie oft nur an wenigen spezifischen Standorten und für bestimmte (Gruppen von) Arten gesammelt. In diesem Zusammenhang sind Herbarien wertvolle Datenquellen, die es ermöglichen, zu untersuchen, wie sich der globale Wandel im Laufe der letzten Jahrhunderte auf Pflanzen ausgewirkt hat. Herbarien beherbergen gepresste und konservierte Pflanzenexemplare aus aller Welt und von vielen Arten, und machen es damit möglich, die Phänologie nicht nur über die Zeit, sondern auch über den Raum und über Taxa hinweg zu verfolgen. Dies ist wichtig, da die Phänologie auch vom Breitengrad, der Höhenlage sowie von anderen Umweltfaktoren abhängt und artspezifisch ist. Darüber hinaus ist wenig über die Einflüsse bekannt, die andere Treiber des globalen Wandels neben der Klimaerwärmung, wie z. B. Landnutzungsänderungen, auf die Phänologie von Pflanzen haben. Besonders in Wäldern könnten Managementmaßnahmen, die

die Baumartenzusammensetzung und die Bestandsstruktur beeinflussen, die Phänologie von Waldunterholzkräutern beeinflussen, weil sie, unter Anderem, mit Veränderungen der Sonneneinstrahlung und des Mikroklimas einhergehen. Wir wissen jedoch immer noch nicht genau, wie die diversen Einflussfaktoren auf die Phänologie zusammenwirken und wie sehr ihr Einfluss zwischen Arten, Populationen oder geografischen Regionen variiert. Meine Arbeit ist ein Betrag dazu, diese Wissenslücken zu verringern. Ich habe mich dazu mit den folgenden Fragen beschäftigt: i) Wie können Herbarien generell genutzt werden, um langfristige Auswirkungen des globalen Wandels auf Pflanzen zu untersuchen? Und im Besonderen, wie wirken sich ii) Klimawandel und iii) Landnutzungsänderungen auf die Blühphänologie von Waldunterwuchspflanzen aus? Und iv) Unterscheiden sich phänologische Verschiebungen von Pflanzen und ihren Insektenbestäubern, sodass es zu ökologischen Asynchronität zwischen ihnen kommt?

Kapitel II ist eine Literaturübersicht, in der dargelegt wird, wie historische Daten aus Herbarien verwendet werden können, um die Auswirkungen des globalen Wandels (einschließlich Umweltverschmutzung, Landnutzungs- und Klimaveränderungen sowie das Aufkommen invasiver Arten) auf Pflanzen in den letzten Jahrhunderten zu verfolgen. In den Kapiteln III und IV gehe ich auf die Auswirkungen des globalen Wandels auf die Blütenphänologie von Waldunterwuchsplanzen ein. Mit Herbariendaten und räumlicher Modelierung habe ich ermittelt, wie sich die Blütezeit dieser Wildblumen im letzten Jahrhundert aufgrund des Klimawandels in Europa verschoben hat (Kapitel III). Für eine Untergruppe derselben Arten habe ich Felddaten von 100 Waldparzellen in Deutschland analysiert, um festzustellen, wie sich die Waldnutzung auf die Blütezeit auswirkt (Kapitel IV). Ich habe mich auf frühlingsblühende Waldunterwuchsplanzen konzentriert, da sie eine relativ kurze und abgegrenzte Blütezeit haben, und sich Veränderungen ihrer Phänologie daher gut nachverfolgen lassen. In Kapitel V wird verglichen, wie sich die Phänologien von Pflanzen und Insektenbestäubern, und ihre Synchronität, in den letzten Jahrzehnten und mit der Klimaerwärmung verändert haben, indem Daten von mehr als 1000 Pflanzen- und 80 Insektenarten aus der Global Biodiversity Information Facility (GBIF) analysiert werden.

Meine Arbeit zeigt i), dass Herbarien ein unschätzbarer Datenschatz sind, um zu untersuchen, wie sich anthropogene globale Veränderungen auf Pflanzen auswirken (Kapitel II) - insbesondere auf deren Phänologie. Meine Analysen zeigen, dass: ii) die Blütezeit von Waldunterwuchsplanzen heute fast eine Woche früher ist als vor 100 Jahren, und dass diese Veränderungen mit der Klimaerwärmung zusammenhängen (Kapitel III). Ich habe auch gezeigt, dass es entscheidend ist, räumliche Korrelationen zu berücksichtigen, wenn

Herbardaten über einen großen geografischen Bereich hinweg analysiert werden: Wenn die räumliche Korrelation ignoriert wurde, überschätzten die Modelle die phänologischen Verschiebungen stark und ließen außer Acht, dass die phänologischen Reaktionen der Pflanzen innerhalb Europas erheblich variiierten. Meine Analyse von Felddaten zeigte, dass iii) auch die Landnutzung die Pflanzenphänologie beeinflusst (Kapitel IV). In intensiv bewirtschafteten Wäldern blühten die Pflanzen etwa zwei Wochen später als in unbewirtschafteten Wäldern - teilweise, aber nicht nur, weil die Bewirtschaftung das Mikroklima veränderte. Schließlich zeigte die GBIF-Studie, dass iv) während der letzten ~50 Jahre die Pflanzenphänologie stärker und konsequenter voranschritt als die der Insekten (Kapitel V). Dies beeinflusste auch deren Synchronität, mit potenziell weitreichenden ökologischen Konsequenzen. Es ist wichtig zu verstehen, warum und wie sich die Lebensrhythmen von Pflanzen (und Tieren) verändern, um die Anfälligkeit von Arten, Populationen und ökologischen Gemeinschaften für den fortschreitende globalen Wandel abzuschätzen.

Chapter I

General Introduction

The impacts of anthropogenic global change

Natural ecosystems are essential for human existence and quality of life. However, over the course of time the relationship of humans with the Earth's environment has changed. For almost all of the time since the evolution of *Homo sapiens,* at least 200.000 years ago (Galway-Witham and Stringer 2018), interactions with the environment happened at local or regional scales (Steffen et al. 2006). During the last centuries this changed drastically. Technological developments and the turn towards mechanized work and mass production in the second half of the 18th century, known as industrialization, changed the landscape world-wide. Since then humans started to modify the environment at a global scale to fit the needs of society. Such planetary-scale changes in the Earth System, subsumed under the term "global change", have become increasingly calamitous. Among the main drivers of anthropogenic global change are land-use change, climate change, pollution, invasive species and natural resource exploitation, all of which are consequences of population growth, socio-economic trends and technological innovations (IPBES 2019).

Over the course of the last two centuries the human population soared from less than one to over 7.8 billion, average national income decoupled and since the 1950s economic activity increased almost 10-fold (Steffen et al. 2006, Piketty 2020). Within the last century the terrestrial biosphere transitioned from being mostly wild to mostly human-altered biomes (Ellis et al. 2010). Global land-use change, especially the expansion and intensification of agriculture and deforestation (mostly due to expansion of agriculture), is maybe humanity's biggest impact on the environment and probably the greatest cause of the ongoing biodiversity losses (Newbold et al. 2015, Newbold 2018). In 1700, before the industrial revolution, around half of the terrestrial biosphere was wild, and not used for human settlements or agriculture. Most of the rest (45%) was in a seminatural state, only extensively used for agriculture and settlements. Today, human use affects more than 70% of Earth's ice-free land, and only a minority of the biosphere is still in a wild (25%) or seminatural (20%) state (Ellis et al. 2010). By 2000, almost 40% of Earth's ice-free land (or ~55% of all habitable land) has been domesticated for agriculture (Ellis et al. 2010). The vast majority (~83%) of it is used to produce animal products (i.e. meat, aquaculture, eggs and dairy) while producing only 18% of the world's calories (Poore and Nemecek 2018). Moreover, most of it

is sprayed with fertilizer, herbicides, pesticides, or antibiotics. Even though humans make up only 0.01% of the Earth's biomass themselves (Bar-On et al. 2018), they use one quarter to one third of the potential net primary production on land (IPCC 2019). Almost all (terrestrial) life on Earth relies on this primary production, that is produced almost completely by plants via photosynthesis, making them the foundation for terrestrial life on Earth. However, humans have managed to reduce plant biomass by around 40% (Smil 2016), while increasing that of livestock so drastically that they outweigh all terrestrial vertebrates combined – making up 96% of all mammal biomass and 70% of the world's birds (Bar-On et al. 2018). Land use, that is both land-cover conversion and land management due to agriculture, deforestation and forestry etc., halves the amount of carbon that is potentially stored in terrestrial biomass (Erb et al. 2018).

Most of the world's remaining standing phytomass (nearly 90%) is in forests (Smil 2012). They cover around 30% of the land surface and are indispensable and invaluable for human survival. Forests provide many ecosystem services. We need them for the air we breathe, they store massive amounts of carbon, produce the wood we use, help purify water, prevent soil erosion, provide livelihoods for millions of people, host most of the worlds biodiversity and mitigate climate change (Brockerhoff et al. 2017). Over half of all forests world-wide are used in one way or another (IPPC 2019). The global forest area declined over 30% compared to the estimated pre-industrial level (IPBES 2019). Over the course of the industrial revolution, a significant part of temperate woodlands were converted into human settlements (18-25%) and croplands (23–28%) (Ellis et al. 2010), the majority of which are used as pastures and crop fields for livestock – making animal product consumption by humans the major cause of deforestation (Machovina et al. 2015). Globally, raw timber harvest has increased 45% since 1970, up to around four billion cubic meters in 2017, with the forestry industry providing about 13.2 million jobs (IPBES 2019). In Central Europe, only around 0.2% of the deciduous forests are still in a relatively natural state (Hannah et al. 1995), rendering them on of the most endangered ecosystems in the world (Bengtsson et al. 2000).

Agriculture and forestry together account for around a third (~31%) of global greenhouse gas emissions (Poore and Nemecek 2018). The other main sources of global greenhouse gas emissions, running rampant since the industrial revolution and with the growing world population, are electricity and heat (25%), industry (15%) and transportation (14%) (Herzog 2009). As a consequence, the composition of the atmosphere has changed significantly over the course of the last century. These changes are affecting the basic functioning of the Earth System, especially the climate (Steffen et al. 2006). The average

temperature over land for the period 2006–2015 increased circa 1.5°C compared to pre-industrial times (1850–1900) (IPPC 2019). The frequency and intensity of extreme weather events, and the fires, floods and droughts that they can cause, have increased significantly within the last 50 years (IPBES, 2019). Overall, climate change has adversely impacted terrestrial ecosystems, contributed to desertification and land degradation in many regions and thus threatens food security and biodiversity, causes shifts in species distributions and in their phenologies, and impacts population dynamics, community structure and ecosystem function and resilience (IPPC 2019, IPBES 2019).

The magnitude and rates of these human-driven changes to the global environment are in large part unprecedented (Steffen et al. 2006, IPBES 2019), they have inflicted great harm, and some of the changes will last for millennia (or might even be irrevocable) (Smil 2016). Therefore, it is crucial to understand how they affect the global environment, to (hopefully) be able to counteract some of their negative effects, by adapting land-use and conservation policies, management and, ultimately, politics – to at least cushion the blow. In this context my work is a piece of the puzzle, investigating how plants (and their pollinators) are affected by global change – especially how their rhythms of life (phenology) have been changed.

Herbaria as historic data-treasures

Studies investigating the effects of global change face the challenge to reconstruct how organisms or ecosystems changed over the course of the last centuries, because such long-term data are scarce. Especially experiments almost always have a local focus and a duration of a few years or, at best, decades (Leuzinger et al. 2011). Observational studies are often more large-scale and long-term, but they are usually still restricted to a time period of 50–80 years (Fitter and Fitter 2002, Thomas et al. 2004). In field observations, latitude and altitude are often used as a proxy for warming, assuming that patterns across space stand for future patterns across time – at the risk of oversimplification. Therefore, understanding global change as a long-term process, and its ecological and evolutionary impact, is only really possible with large-scale data that goes back to the beginning of large-scale industrialization and matches the scales at which global change is occurring. In this context, natural history collections are a still underused treasure chest of exactly such data – expanding across time, space and taxonomy (Pyke and Ehrlich 2010, Holmes et al. 2016, Meineke et al. 2018).

Particularly herbaria are an invaluable data source, consisting of pressed and preserved plants for which usually rich meta-information on species, collection site, date and collector is available. World-wide there are >350 million specimens in almost 3000 herbaria that date

back up to the 16th century (Thiers 2017). Herbaria have unique scientific value, they can be used to tackle a broad range of questions not only related to classical plant taxonomy and systematics, but also global change-related topics, investigating the impact of land-use, climate change and pollution on plants or tracing invasive species (Funk 2003, Willis et al. 2017, Meineke et al. 2018, Lang et al. 2019; see also Chapter II of this thesis). Since individual plants cannot simply swerve environmental change by moving or flying away they are particularly exposed to it. As a result, herbarium specimens can serve as snapshots in time of how plants responded to environmental change. Taken together they provide unique spatiotemporal data for studying global change (Lavoie 2013, Vellend et al. 2013, Willis et al. 2017, Meineke et al. 2018, Lang et al. 2019). Dense time-series of herbarium specimens make it possible to study long-term processes like recent invasions and genetic population histories as well as long-term shifts of life-history events, such as leaf-out or flowering time. These data are becoming increasingly accessible, since herbaria, other collections and observation data (also including other taxa, e.g. insects, birds and mammals) from long-term monitoring networks, are digitized. Together they create public data bases (such as the Global Biodiversity Information Facility, GBIF.org) that contain vast amounts of natural history data that cover large timespans and spatial scales (Newbold 2010, Meineke et al. 2018, Lang et al. 2019).

Phenology: species' rhythms of life are changing

One of the most sweeping and apparent consequences of global change, especially global warming, is that all over the world species' rhythms of life are changing. The study of periodic events in the life cycles of organisms that determine these rhythms is called phenology. Phenology is literally "the science of appearance", coming from the Greek words *phaino* (to show or appear) and *logos* (to study). Phenological events include the time when plants sprout or bloom, the leaves of deciduous trees change color in the fall, as well as the date that insects emerge, birds and mammals migrate, or animals reproduce (e.g. the egg-laying dates of birds and amphibia), but also annual cycles of ecosystem processes (Lieth 1974). The timing of these events is crucial because it affects whether plants (among them those that constitute our food source) and animals survive and thrive in their environments – a plant, for example, is unlikely to reproduce successfully if it would start flowering before spring is on the verge.

The phenology of organisms depends on seasonal and interannual climate variability, and habitat characteristics (like elevation), that influence microclimatic conditions (Sparks

and Menzel 2013). Especially in temperate ecosystems, organisms need to handle annual fluctuations in daylength, temperature, rainfall or humidity. To time their life cycle with the conditions that are suitable to grow, reproduce or mate organisms must synchronize their phenology with these environmental changes. To apprehend phenology, it is crucial to better understand which attributes of the environment are the triggers (cues) or proximate causes (drivers) of life cycle events. While cues and drivers are to some extent species-specific, we know that most species' life cycles are particularly dependent on temperature, other common cues are for example daylength and precipitation (Chmura et al. 2019). Over the last years evidence has accumulated that the timing of many phenological events are shifting globally – mainly as a consequence of climate warming. These alterations in the timing of phenological events are among the strongest and most consistent indicators of changing climates. Such fingerprints of global change are crucial because they demonstrate to policy makers and the general public that real changes have already happened (Tang et al. 2016, Forrest 2016, Piao et al. 2019).

How organisms react to seasonal change can have a strong impact on them and the natural environment. Phenological shifts can determine species' local persistence, (flower) abundance and community diversity (Inouye 2008, Willis et al. 2008, Wheeler et al. 2015). In the case of plants, such changes can impact other elements of ecosystems, since plants are the base of most food chains. This is especially true since phenology is synchronized across biotic interactions and food webs (Schmitz 2013). If the timing of phenological events shifts differently for the species involved in intertwined biotic interactions, this can lead to asynchrony, causing mismatches between species and their (food) resources (Schmitz 2013). Plant-pollinator systems are among the biotic interactions that would probably suffer a lot from such phenological mismatches and whose disruption would be especially serious – given that more than 75% of global food crops (and some of the most important cash crops, such as coffee, cocoa and almonds), rely on animal pollination (IPBES 2019). However, such mismatches are not only detrimental for agriculture. It has already been shown that global change has also disrupted plant-pollinator interactions in temperate forest understory communities over the course of the last ~120 years, resulting in declined quantity and quality of pollination services (Burkle et al. 2013).

Phenological responses can not only vary between species, they can also change over time and space – on continental or even local scales. One reason for this is that the direction and magnitude of global change differs geographically. For example, some regions of the world are warming faster than others, eliciting variable phenological responses. Another

cause for diverging phenological responses is that different species or populations can be adapted to geographically specific environmental conditions which may result in different phenological sensitivities (Riihimäki and Savolainen 2004, Zohner and Renner 2014, Prevéy et al. 2017, Zohner et al. 2020). As a consequence, phenological responses to global change can differ between regions, even when they experience similar global change impacts. Further, on small scales microclimatic patterns, and thus phenological responses, can differ strongly from regional climate patterns (Hwang et al., 2011; Ward et al., 2018).

Phenology in forest understories

The influences of other global change drivers besides climate change – such as land use, e.g. forest management – on plant phenology are understudied. Most previous studies focused on how organisms and ecosystems respond to macroclimatic changes (as I did in **Chapters III** and **V**), while microclimatic conditions are often neglected when investigating biotic responses to global change. However, most organisms on Earth experience microclimatic conditions that are shaped by the local topography and vegetation via interception of solar radiation, air mixing, and evapotranspiration (Geiger et al. 2003) and thus often differ profoundly from the macroclimate (Zellweger et al. 2020). Therefore, microclimates should be taken into account for understanding how organisms and ecosystems respond to macroclimatic change. This is especially true for plants since they are sessile and thus stuck with the local microclimatic conditions, and because vegetation can also directly influence microclimate. Intriguingly, here the effects of two global change drivers intertwine, because microclimatic conditions change with both climate change and land-use change (Valdés et al. 2015, Zellweger et al. 2020). Microclimates are probably nowhere more conspicuous than in forests (Geiger et al. 2003, De Frenne et al. 2019, Zellweger et al. 2020). On the one hand, forest microclimates can deviate strongly from their surroundings because they are buffered against extreme heat or cold and macroclimatic warming (De Frenne et al. 2013, 2019, Zellweger et al. 2020). On the other hand, the microclimatic conditions in forest understories depend strongly on the way how forests are used. In temperate forests, management alters tree species composition and homogenizes stand structure, and as a consequence changes light conditions and microclimate (Aussenac 2000, Hale 2003). Especially, planting of economically important evergreen coniferous trees for timber production where otherwise deciduous trees would grow is often drastically reducing light availability during spring and thus also changes microclimate. This could have a profound impact on understory vegetation, especially on its phenology (see **Chapter IV** of this thesis), which hence could have

consequences for both forest conservation and commercial productivity in the long run (Nilsson and Wardle 2005, De Frenne et al. 2013, Zellweger et al. 2020).

In this thesis, I focused on spring-flowering understory wildflowers to explore how climate change (**Chapter III**) and forest management (**Chapter IV**) impact plant phenology. Often forest-related research focuses on trees, while the forest understory vegetation (such as dwarf shrubs, herbs, mosses, and lichens) receives less attention – even though this vegetation layer encompasses most plant diversity in forests (Gilliam 2007), its productivity is comparable to that of trees, and it is of crucial ecological importance (Nilsson and Wardle 2005). The forest understory vegetation contributes up to 20% of (high nutrient) foliar litter to the forest floor and influences, for example, tree seedling regeneration, and in the long run affects belowground processes such as decomposition and nutrient cycling (Nilsson and Wardle 2005, Gilliam 2007). Early-spring flowering understory herbs are especially important in this context, because they grow and flower when nutrient uptake by (the yet leaf-less deciduous) trees is minimal and the subsequent decomposition of their foliage makes these nutrients available to trees later in the year (Muller 2003, Gilliam 2007). Most of these forest wildflowers, especially those in deciduous forests, rely on the time window during spring when the leaf-out of trees is not yet completed, since during that time they can take full advantage of the available sunlight, moisture and nutrients of the forest floor (Lapointe 2001). Because of their narrow and distinct flowering period, these spring-flowering forest herbs should be particularly susceptible to management changes and well suited for tracking phenological shifts over time.

Goals of my thesis

As outlined above, global change affects plants in multiple ways, and I address several of them in my thesis. **Chapter II** presents an overview of how historical herbarium data can be used to track global change impacts on plants: starting with pollution caused by industrialization, that coincides with increasing habitat loss, land-use changes and climate change, and the rise of invasive species caused by global trade and transport. In **Chapter III,** I used herbarium data and spatial modeling to analyze in detail how climate change affected the phenology of forest wildflowers in Europe during the last century. This study is the first analysis of long-term flowering-time trends using herbarium data that spans more than one country and that explicitly maps geographic variation in phenological responses. In **Chapter IV,** I investigated if, besides climate change, also land-use change affects plant phenology. For this I analyzed how forest management affected flowering time of forest understory

plants, and I disentangled how forest management related to changes in forest structure and microclimatic conditions and, as a consequence, flowering phenology. Finally, **Chapter V** compared the phenological shifts of plants and pollinating insects over the last decades to estimate the potential of phenological mismatches that could disrupt these crucial biotic interactions.

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Chapter II

Using herbaria to study global environmental change

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Research review

Using herbaria to study global environmental change

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Summary

During the last centuries, humans have transformed global ecosystems. With their temporal dimension, herbaria provide the otherwise scarce long-term data crucial for tracking ecological and evolutionary changes over this period of intense global change. The sheer size of herbaria, together with their increasing digitization and the possibility of sequencing DNA from the preserved plant material, makes them invaluable resources for understanding ecological and evolutionary species' responses to global environmental change. Following the chronology of global change, we highlight how herbaria can inform about long-term effects on plants of at least four of the main drivers of global change: pollution, habitat change, climate change and invasive species. We summarize how herbarium specimens so far have been used in global change research, discuss future opportunities and challenges posed by the nature of these data, and advocate for an intensified use of these 'windows into the past' for global change research and beyond.

Introduction

Global environmental change is one of the major challenges of the 20th and 21st centuries. It has been evident since the age of industrialization in the late 18th century – sometimes also referred to as the advent of the anthropocene – and has continuously gained momentum (Fig. 1a; Steffen *et al.*, 2011; Hamilton, 2016). Biologists study global change for its broad ecological impact, and its negative effects on biodiversity. Also, as it represents an unplanned, long-term and large-scale experiment, studying global change can promote understanding of fundamental processes such as rapid adaptation. Experimental approaches to study these topics are usually locally focused, and limited to a duration of a few decades (Leuzinger *et al.*, 2011). Although observational methods are often more large-scale and long-term, they are with few exceptions still restricted to a time frame of 50–80 yr (Fig. 1a; Fitter & Fitter, 2002; Thomas *et al.*, 2004). To understand both the extent of global change as a long-term process, and its full ecological and evolutionary impact, global data that go back to the onset of industrialization are crucial.

In this context, natural history collections are an underused treasure of temporally and geographically broad samples that we have just begun to dust off (Holmes *et al.*, 2016). Especially rich is the botany section of this vault: plants collected, pressed and preserved, in most cases together with meta-information on species, collection site, date and collector (Fig. 2): In terms of extent, there are > 350 million specimens in almost 3000 herbaria world-wide (Fig. 1b; Thiers, 2017; http://sweetgum.nybg.org/science/ih/), sampled from the 16th century up to today (Sprague & Nelmes, 1931), and the collections' potential uses range from classical taxonomy and systematics, to archaeobotany, archaeoecology and climate change research (Funk, 2003). Because plants are sessile, they are particularly exposed to environmental change. The time courses of many of their responses to environmental change are preserved in herbarium specimens, which therefore provide unique spatiotemporal data for studying global change (Primack & Miller-Rushing, 2009; Lavoie, 2013; Vellend *et al.*, 2013; Meineke *et al.*, 2018).

Recent studies have emphasized the scientific value of herbaria for a broad range of global change-related topics (Fig. 2; e.g. Zschau *et al.*, 2003; Miller-Rushing *et al.*, 2006; Feeley & Silman, 2011; Willis *et al.*, 2017). Dense time-series of herbarium specimens even permit studying long-term processes such as recent invasions and their genetic population history (Exposito-Alonso *et al.*, 2018a).
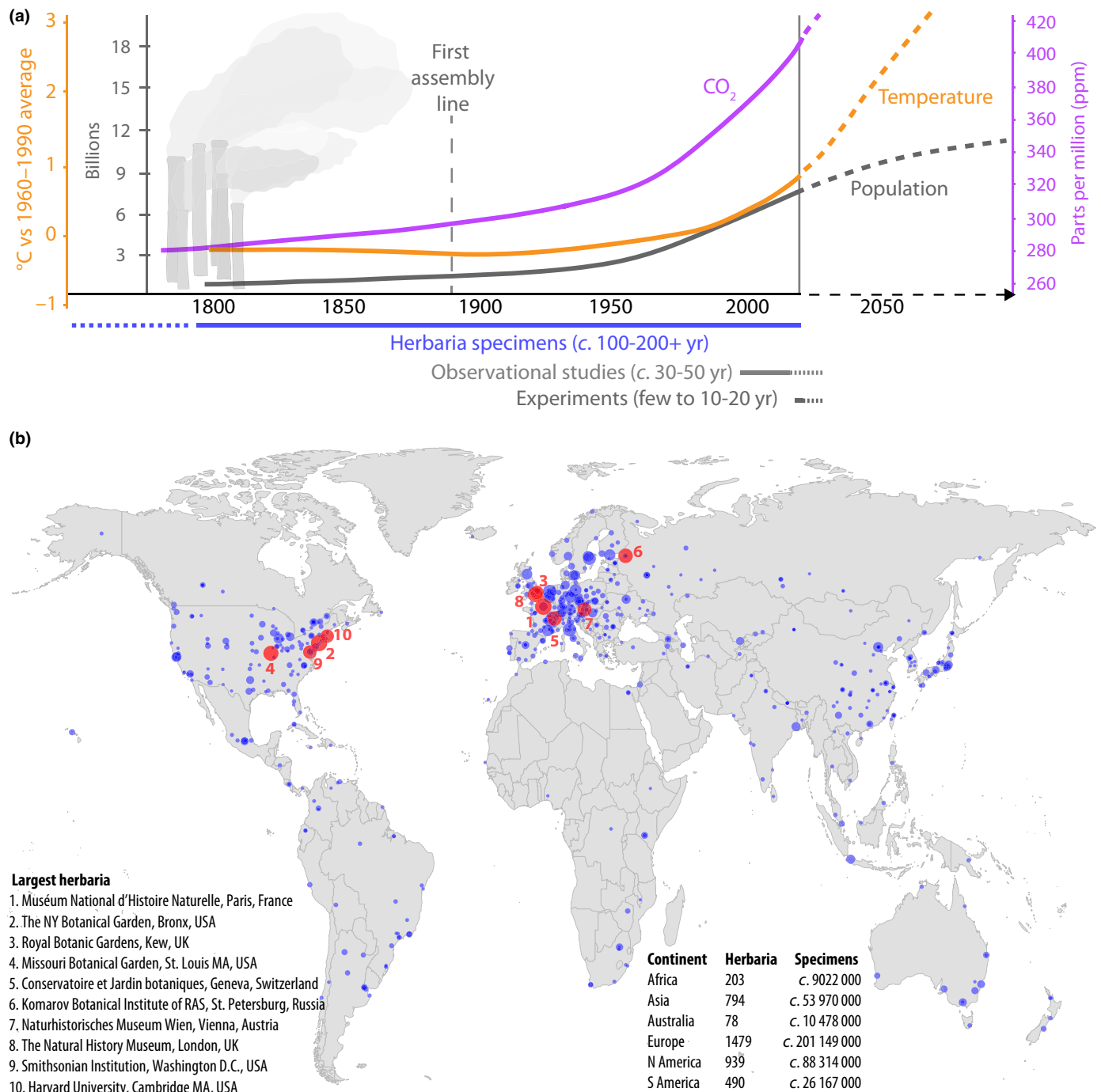
Fig. 1 Herbaria as global change witnesses. (a) Timeline of global change, with lines tracking changes in world population, air temperature and atmospheric CO₂ during the last *c*. 200 years. Dashed line ends indicate future projections. Bars below plot indicate the typical temporal extent of herbarium samples vs observational studies and experiments. (Population growth: United Nations, Department of Economic and Social Affairs, Population Division (2017); World Population Prospects: The 2017 Revision. http://esa.un.org/unpd/wpp/; temperature: representative concentration pathway 8.5, Intergovernmental Panel on Climate Change, www.ipcc.ch; (Marcott *et al.*, 2013); CO₂: (Neftel *et al.*, 1994)). (b) Map with global distribution of herbaria (for visual clarity displaying only herbaria of > 100 000 specimens), names of the largest 10 herbaria, and number of herbaria and herbarium specimens curated per continent (reflecting places of storage of specimens, not their origins; Herbarium data from Index Herbariorum, http://sweetgum.nybg.org/science/api/v1/institutions/. Accessed in April 2018).

Even though herbaria were used as early as in the 1960s to study global change (e.g. Ruhling & Tyler, 1968, 1969), and are in the process of being made available online via digitization (> 46 700 000 specimens in the Integrated Digitized Biocollections portal alone; as of 18 July 2018 https://www.idigbio.org/portal/ (search terms: type of record – PreservedSpecimen, kingdom – Plantae)), the community has not fully adopted herbaria as valuable 'time machines' to the past (Lavoie, 2013; Meineke *et al.*, 2018). Especially with the advent
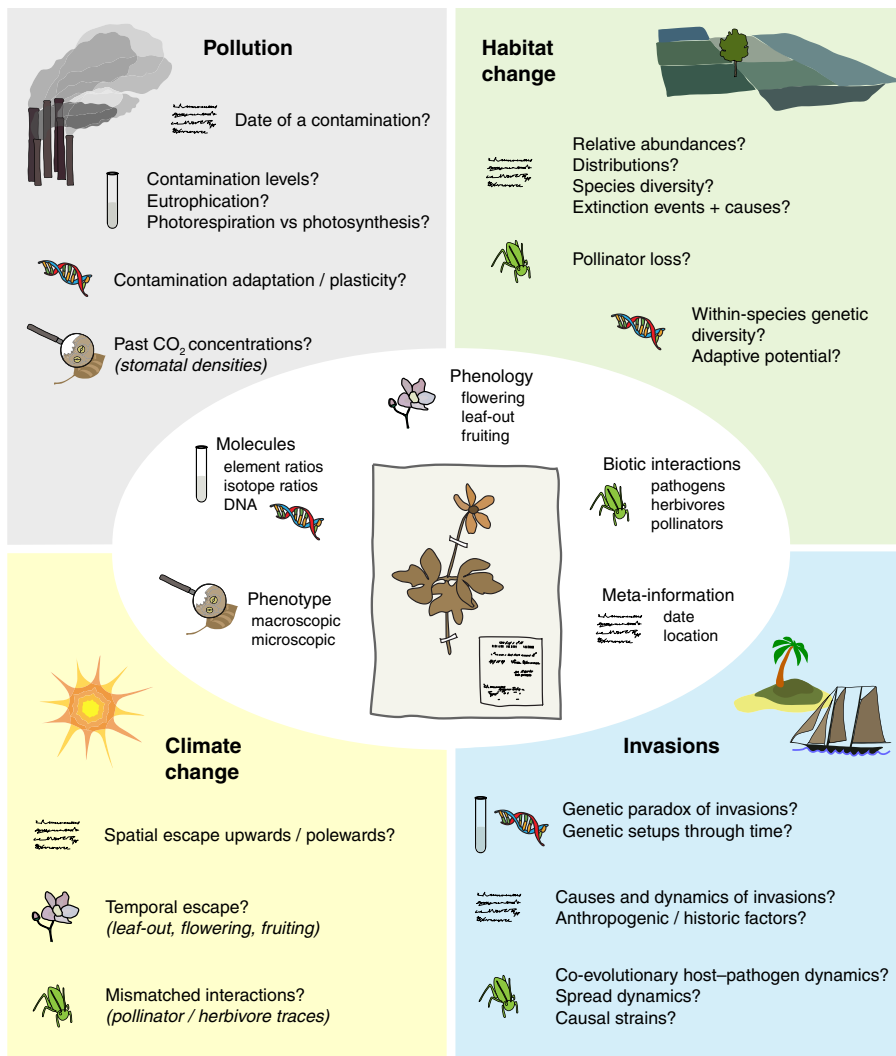
Fig. 2 Diversity of herbarium data and their applications. Herbarium sheet in the centre surrounded by types of data that can be obtained from a specimen, with the questions that these data can help to answer around, ordered by respective global change driver. Symbols indicate the type of data used to address each question.

of high-throughput methods and recent technical developments in image analysis, the value of these collections is now more apparent than ever (Munson & Long, 2017).

Simultaneously, next generation sequencing (NGS) techniques now allow for in-depth genetic analysis of century-old specimens up to whole genome sequencing of plants and even of their equally preserved pathogens (e.g. Martin *et al.*, 2013; Yoshida *et al.*, 2013; Durvasula *et al.*, 2017; Exposito-Alonso *et al.*, 2018a). This extends the spectrum of available long-term data far beyond morphology or phenology. For instance, dense sampling of such full genetic information across time – and geography – enables population genetics studies, to follow speciation processes through time, or to quantify changes in genetic diversity in historical contexts. Working with these small samples of degraded DNA – so-called ancient DNA (aDNA) – retrieved from historic collections is technically challenging and has recently boomed in the animal field (e.g. Shapiro & Hofreiter, 2014; Orlando *et al.*, 2015; Marciniak & Perry, 2017), yet in the plant field it is still rarely used (Gutaker & Burbano, 2017).

Here, we present an overview of the different types of herbaria analyses possible in global change research (Fig. 2). Following a timeline from industrialization onwards, we divide

herbarium-related approaches into four main areas related to four main drivers of global change: industrialization causing increased pollution, which coincides with increasing loss of habitat and changes in land use as well as climate change, and finally global trade and transport resulting in an increasing number of invasive species world-wide. In addition, in excursions dedicated to molecular methods (Box 1), collection biases (Box 2) and the digitization challenge (Box 3), we provide insight into three key methodological issues that herbaria research is currently dealing with, and hopefully inspire with ideas for extended utilization of botanical collections. Our aim is to advocate broader use of herbaria as 'witnesses' of global change. We believe that they have the potential to fast-forward our understanding of the impacts of this unplanned biological experiment, to substantiate our predictions of its long-term outcomes, and to inform conservation measures.

Pollution

Technological developments and the mechanization of work in the second half of the 18th century, known as industrialization, changed the landscape world-wide. Key contributors were improved efficiency

Box 1 Molecular analyses and degradation

The age of herbarium specimens is both their strength and their weakness, as aging is a corrosive process. For most chemicals, the extent, rate and end-results of this process are not defined in herbarium samples. Still, it is clear that age, but also preservation practices or storage conditions can alter tissue chemical contents. This is evident, for example, when N concentrations measured in stored tissues diverge from the results of previous methods and studies – in this case likely due to post-collection contamination (Nielsen *et al.*, 2017). Hence, in-depth analyses of correlations between the age and chemical compound quantities in old samples are necessary in order to make claims about historical absolute abundance values (Nielsen *et al.*, 2017). For DNA from historical samples – aDNA – age-related degradation dynamics are fairly well-characterized (Allentoft *et al.*, 2012; Weiß *et al.*, 2016). Due to chemical modifications, DNA in dead tissue gets increasingly fragmented over time (Fig. B1a), and particularly in fragment ends, aDNA-characteristic deamination drives nucleotide-substitutions of cytosine with thymine ((Weiß *et al.*, 2016); Fig. B1b). This *per se* does not lessen the potential of aDNA-studies (Gutaker & Burbano, 2017): specialized protocols even allow extraction of ultra-short fragments of < 50 bp (Gutaker *et al.*, 2017), and the correlation of nucleotide misincorporations with time enables its use as authenticity criterion of ancient DNA (Sawyer *et al.*, 2012; Weiß *et al.*, 2016). Still, these particular characteristics call for categorical rules for herbarium genetics to minimize contamination risks, verify authenticity and maximize the information gained from precious old plants: samples have to be processed in clean room facilities to avoid contaminations with fresh DNA, and sequenced to a certain depth to yield useful information. Pure PCR analyses on the contrary are inappropriate for aDNA studies, as they do not allow the necessary authenticity verification and, due to the fragmentation of aDNA, are unlikely to yield consistent results.

Such quality requirements are particularly important due to the limitation of available material. Unlike traditional approaches that rely on metadata or morphology of historical samples, molecular analyses require tissue probes and hence destructive sampling of specimens. Therefore, it is the duty of any molecular herbarium scientist to optimize their methods, minimize the amount of sample needed, and employ state-of-the-art analyses to retrieve maximum information from their samples. In the same vein, molecular herbarium scientists and curators should aim to maximize the detail of meta-information that can be gathered from samples. Knowledge, for example, about temporary field collection in alcohol, or post-collection specimen treatments with heavy metals (as insecticides or fungicides) is indispensable to assess the suitability of specimens for molecular approaches. Furthermore, both curators and researchers need to assess specimen-label and specimen-sample pairs for their correctness, and remain cautious particularly regarding the interpretation of trends in (molecular) data observed only in few or single samples.
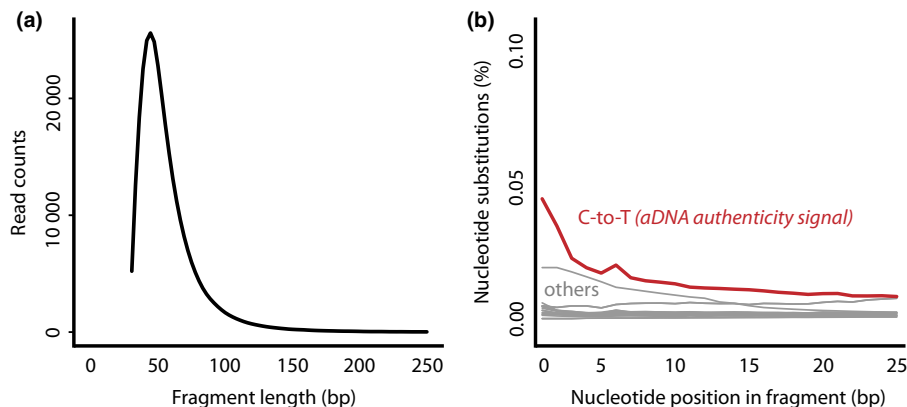

Fig. B1 Typical molecular characteristics of herbarium DNA. (a) Fragment size distribution and (b) damage pattern found in ancient DNA (sample data from Weiß *et al.* (2016), publicly available at ENA ID ERR964451).

of steam engines, the replacement of biofuels with coal and the emergence of a chemical industry. A larger average income, increasing population sizes and accelerated urbanization led to the production of previously unseen quantities of waste and exhausts (Fig. 1a). Herbarium specimens can be used to track historical pollution levels, to serve as a baseline for pre-pollution conditions, and to connect waste production with species' reactions – even at the genetic level in the context of local adaptation, or to study long-term effects of singular events such as the Chernobyl nuclear disaster (Heinrich *et al.* 1994).

Heavy metals

Metals from the atmosphere, soils and groundwater are deposited on or taken up by plants, and remain present in herbarium specimens, so the latter can be used as indicators of pollution, and due to their meta-information facilitate the dating of contamination (Lee & Tallis, 1973; Shotbolt *et al.*, 2007; Rudin *et al.*, 2017). Depending on species, their morphology, physiology and proximity to a pollution source, plants are exposed to and take up more or less pollutants (Lawrey & Hale, 1981; Rudin *et al.*, 2017). Studying lead pollution levels, for example, the isotopic lead composition in moss or lichen samples collected at roadsides reflects fluctuations in local motor vehicle traffic, efforts to reduce lead emissions and changes in petrol origin or composition over time (Farmer *et al.*, 2002). In addition to lead, herbarium samples also track concentrations of other metals such as cadmium, copper and zinc to follow their temporal and spatial trends in relation to anthropogenic activities (Zschau *et al.*, 2003; Shotbolt *et al.*, 2007;

Box 2 Collection biases

Imbalanced sampling is a well-acknowledged issue for the use of herbaria, for example, to map species distributions or assess diversity (e.g. Meyer *et al.*, 2016; Daru *et al.*, 2018). Temporal biases are caused by intense collection periods, and seasonal preferences (Holmes *et al.*, 2016). Also, collections often concentrate on easily accessible or much-frequented sites (geographic bias; e.g. Sofaer & Jarnevich, 2017), and on common or particularly interesting species which – depending on the collectors – can change over time (taxonomic bias; e.g. Feeley, 2012). When working with herbarium data, it is necessary to explicitly test for these biases, for example to avoid a few dominant species generating trends in a dataset (Jácome *et al.*, 2007). Depending on the type of question or analysis, biases may need to be corrected for by different means: normalizing collection efforts with different types of reference sets (e.g. Hedenäs *et al.*, 2002; Law & Salick, 2005; Case *et al.*, 2007), measuring invader distributions in relation to native species (Delisle *et al.*, 2003), or verifying trends with additional, nonherbarium datasets (e.g. Lienert *et al.*, 2002; Kouwenberg *et al.*, 2003; or even those from citizen science, Spellman & Mulder, 2016). In particular when models are based on historical records, comparisons with modern data can support extrapolations or generalizations, but only if biases have been dealt with: models, for example, in the context of invader dynamics and spread, have to take species persistence into account, because historic occurrence does not equal contemporary presence and may cause overestimation of plants' distribution and abundance (Pergl *et al.*, 2012). This is particularly the case for species targeted by eradication measures, such as the human health hazard *Heracleum mantegazzianum*, where herbarium specimens can indicate suitable habitats, but not current occurrence or general invasion dynamics (Pergl *et al.*, 2012). Furthermore, there are often no data on early invasion stages, because herbarium records indicate only the presence of a species, whereas its absence is not reliably documented by a lack of records. Conclusions based on modeling and statistical analysis, particularly of early invasion stages, should hence be used as indications rather than be over-relied upon (Hyndman *et al.*, 2015). Finally, the currently rising bias of low collection effort is a well-known problem for tropical areas (Feeley & Silman, 2011), yet is threatening to become global, via overall declining collections (Prather *et al.*, 2004). Although this particularly jeopardizes studies of new or recent invasions (Lavoie *et al.*, 2012), it strongly affects all herbarium-based research.

Rudin *et al.*, 2017). Combining pollution records and genetic information from historical and contemporary samples from contaminated sites can even enable studies of plants' adaptation to pollution at the genetic, heritable level, for example by studying the association between pollution levels and specific alleles, and thus give indications about long-term adaptation to changing conditions. Such approaches are already well-established for contemporary data alone (Kawecki & Ebert, 2004; Turner *et al.*, 2010; Arnold *et al.*, 2016).

Anthropogenic nitrogen

Similarly, herbaria document human influences on global nitrogen (N) cycling, that started with the rise of the chemical industry and the production of fertilizers, and has peaked since *c.* 1960 (Millennium Ecosystem Assessment, 2005). Moss leaf N-contents (as well as concentrations of phosphate and sulfur) determined from stable isotope ratios enable inferences about realized N sources and further cycling processes (Peñuelas & Filella, 2001). Such analyses show a retention of additional, anthropogenic N within terrestrial ecosystems (Peñuelas & Filella, 2001). Improved knowledge of these nutrient dynamics within different ecosystems helps us to understand eutrophication. Additional detail on the biotic effects of N fluctuations could be retrieved via shotgun-sequencing of historical plant roots, given that *bona fide* microbiomes could be recovered, as it has been shown that the bacterial species composition of roots (and soils) is heavily influenced by overabundance of N (Dynarski & Houlton, 2018).

Increased carbon dioxide

Pollutants such as N or carbon dioxide (CO_2) can influence overall organismal morphology, making their effects partially measurable without destructive sampling. Increased fossil fuel combustion and the concurrent increase in CO_2 concentrations since the industrial revolution, for example, correlate with a reduction of stomatal densities on the leaves of herbarium specimens. This trend was already observed in 1987 in a 200-yr spanning study of woody angiosperm herbaria samples. Further analyses under controlled experimental conditions (Woodward, 1987; Peñuelas & Matamala, 1990) confirm historic samples as proxies to reconstruct past CO_2 concentrations.

In addition to morphological studies, herbarium specimens enable complementary measurement of global change effects on plant carbon metabolism. Using mass spectrometry to estimate the relative abundances of different carbon isotopes, studies indicate increased water-use efficiency – the ratio of photosynthesis to water loss – with rising CO_2 concentrations (Peñuelas & Azcón-Bieto, 1992; Pedicino *et al.*, 2002). With time-series of genetic variation from herbaria, it is now further possible to determine what part long-term adaptive changes or phenotypic plasticity play in such physiological or chemical responses.

There is, however, one *caveat* for measurements of any type of chemical compounds in long-term stored historical samples: Do chemicals suffer degradation processes similar to hydrolytic damages occurring in DNA over time (see Box 1)? If so, to which extent and at what rate do compounds degrade, and what influence do factors like species, specimen mounting or general storage conditions have on such a decay? Systematic studies of chemical degradation through time will permit the assessment of whether absolute or relative values should be used in historical specimens-based long-term comparisons.

Habitat loss and land-use changes

Apart from pollution, increasing human population densities, urbanization and, in particular, modern agriculture have caused extensive losses, fragmentation or changes of natural habitats. This

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Box 3 Digitization challenge

Large-scale digitization is crucial to make biodiversity data more accessible, balance the unequal distribution of collections world-wide (Drew *et al.*, 2017; see also locations of all herbaria with > 100 000 specimens world-wide, Fig. 1b), increase the use of herbaria in general, the number of specimens included per study specifically (Lavoie, 2013), and fuel novel research (see Soltis, 2017; Soltis *et al.*, 2018). Various online databases already offer access to vast amounts of data (e.g. https://www.idigbio.org/, www.gbif.org, http://vh.gbif.de/vh/or http://avh.chah.org.au/), but the digitization task is enormous – with over 350 million specimens to process – and expensive. To optimize and speed up the process, various larger and smaller institutions have developed affordable digitization workflows (Haston *et al.*, 2012; Nelson *et al.*, 2015; Thiers *et al.*, 2016; Harris & Marsico, 2017). Depending on data needs, digitization could be done in a prioritized way. In conservation biology, for instance, a fraction of available specimens appears to be enough to reliably detect threatened species and trigger conservation efforts (Rivers *et al.*, 2011). How and towards which end such prioritization is carried out, and how large-scale digitization projects would be funded, is a question that needs to be addressed.
Apart from cost and speed, the transcription of meta-information, and particularly georeferencing information, is another digitization bottleneck.
  Optical character recognition may help sorting entries by collector or country (Drinkwater *et al.*, 2014), as might the development of semi-automated imaging pipelines (Tegelberg *et al.*, 2014). Other projects use citizen science approaches to transcribe specimen labels ((Hill *et al.*, 2012); https://www.notesfromnature.org/active-expeditions/Herbarium), and computer vision or machine learning (re-)classify specimens that are unidentified, or whose identification was based on an old taxonomy (Unger *et al.*, 2016; Carranza-Rojas *et al.*, 2017; Gehan & Kellogg, 2017). Still, imprecise or wrong georeferencing is common in herbarium data (Yesson *et al.*, 2007), an issue that is particularly problematic in conservation, for species distribution assessments, or prediction approaches (Feeley & Silman, 2010). Although care with location data from herbaria is, hence, necessary, digital field notebook apps such as ColectoR may at least help guarantee complete and correct meta-information for novel collections (Maya-Lastra, 2016). Finally, in light of concerns about misidentification of up to 50% of tropical specimens world-wide (Goodwin *et al.*, 2015) and the continuously evolving taxonomy, such notebooks, together with the aforementioned computerized identification approaches and even molecular methods, as well as rigorous and continuous manual verification of specimen identities, are crucial to ensure the value of herbaria and herbaria databases.

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affects plants' geographic distribution and densities, for example causing range reductions to more pristine environments (Hallingbäck, 1992). Information about such habitat alterations in response to global change are documented in herbaria. Herbarium sheets normally contain information about the presented species and sometimes other, associated species (referred to in accompanying meta-information, or co-sampled with the focal species, e.g. pathogens). Importantly, herbarium sheets also state the time and place of collection. Hence, comparison between past and present localities serves to infer a species' distribution through time (Hallingbäck, 1992).

Distribution changes

Many factors have contributed to converting the landscape into a patchwork of agricultural fields, interspersed with cities and roads: industrialization-associated population growth, urbanization, increasing agricultural acreages due to mechanization of work, or expansion of railroads and other transport systems. Overall, species abundances tend to decrease with habitat and land-use changes, as is the case, for example, for American ginseng (*Panax quinquefolius*), both as a result of deforestation and of heavy harvesting of wild populations (Case *et al.*, 2007). In light of an area's geography, such data also can inform species' conservation and future trends (Case *et al.*, 2007). However, retrospective studies of species' abundance in a certain location based on historical collections are sensitive both to the quality of available georeferencing data, and to fluctuating collection efforts and other biases (see Box 2). A reference set of specimens picked from the herbarium randomly and independent of species identity can be used to establish a general 'expected collecting frequency', which can balance these biases (e.g. Hedenäs *et al.*, 2002).

When herbarium records are used to relocate historical populations, current data complement herbarium-inferred distributions and abundances (Lienert *et al.*, 2002; Stehlik *et al.*, 2007). Herbaria may in some cases be the only documentation of (likely) extinct species (Chomicki & Renner, 2015). Revisiting surveys can detect such local extinction events, and, in correlation with current land-use practices or site protection status, be used to study their causes (Lienert *et al.*, 2002). They can further document changes in overall plant diversity, which, too, is affected by habitat fragmentation (Stehlik *et al.*, 2007). Such approaches are particularly useful to evaluate changes in the local flora and motivate biodiversity monitoring campaigns, and can inform large-scale diversity surveys, as well as modeling-based inferences or predictions.

Indirect effects of habitat fragmentation

Similar to farming-related landscape changes, urbanization is a prominent driver of biotic interaction changes. One of the most crucial, commercially important types of plant–animal interaction jeopardized, among others, by urbanization and diversity loss, is pollination. Depending on a plant's anatomy, herbaria also house documentation of such interactions, and can illustrate pollinator species decrease or loss. Presence or absence of pollinaria in herbarium specimens of the orchid *Pterygodium catholicum*, for example, reflects the historical pollination rate that depends strictly on a specific bee (*Rediviva peringueyi*) (Pauw & Hawkins, 2011). The bee's decrease following urbanization is consistent with a shift in local orchid communities towards selfing species (Pauw & Hawkins, 2011). Impairment of interactions between plants and their pollinators, caused for instance by such abundance decreases or temporal mismatches, likely also leaves genetic signatures. Given that affected biotic interactions could be identified using historical plant and insect collections, these signatures could be traced

through time and inform the potential of other species-pairs to overcome future mismatches.

Besides the apparent decrease of species diversity, losses of within-species genetic diversity are a less conspicuous consequence of habitat loss, and are a result of shrinking and increasingly isolated populations (Ellstrand & Elam, 1993; Young *et al.*, 1996). Improved high-throughput sequencing techniques and novel molecular approaches have recently made within-species genetic diversity – as preserved in herbaria – accessible (see Box 1). This ancient genetic information extends the information on habitat loss and decreasing relative abundances to the genetic level (Cozzolino *et al.*, 2007; Martin *et al.*, 2014b), with already few specimens giving insights into a population's genetic background. This is crucial knowledge for conservation measures, as genetic diversity, especially in times of increasingly fluctuating environmental conditions, is an indispensable resource for heritable phenotypic variation and rapid adaptation (Huenneke, 1991; Exposito-Alonso *et al.*, 2018b). Reduction of genetic diversity via abrupt decimation of a population, referred to as a bottleneck, can hamper the population's persistence, as selection is less efficient in small populations, where there is more stochasticity and less standing variation to act upon (Ellstrand & Elam, 1993; Young *et al.*, 1996; Hartl & Clark, 2007). Comparison of contemporary vs historical genetic diversity can serve to prioritize the conservation of specific populations over others, and to identify genetically diverse source populations for potential reintroductions to balance bottlenecks (Cozzolino *et al.*, 2007).

Climate change

Some factors on the rise since the start of industrialization, and potentially even before that, have less direct, but long-term effects on ecosystems: the so-called greenhouse gases such as methane (CH_4) and CO_2 (Fig. 1). Their atmospheric increase – for CO_2 a result of enhanced fossil fuel burning in factories, power plants and for transportation – causes global warming and as a result climate change (Millennium Ecosystem Assessment, 2005). Thus, in addition to the earlier mentioned direct effects of the pollutant CO_2 on plant morphology and physiology (see the 'Pollution' section), progressive CO_2-related global warming influences plant life cycles, as is observed for instance already in shifts of plant life cycles, as is observed for instance already in shifts of plant phenology (timing of life cycle events such as flowering and fruiting) to earlier dates. However, herbaria not only directly track these climate-related plant responses, but also give insights into their ripple-effects on pollinators, herbivores and even nutrient cycling.

Range shifts as spatial escape

One possible response of plants to global warming can be distributional shifts when plants escape from unfavorable conditions, which is traceable using herbarium time-series. Comparison of field with herbarium data verifies predictions that with progressive global warming, species will move both upslope and poleward, following their original climatic niches. For instance, historic time-series have monitored movements and consecutive

diversity shifts in California, Costa Rica and South America as a whole (Feeley, 2012; Feeley *et al.*, 2013; Wolf *et al.*, 2016), and hence can differentiate successfully moving species from those that may not persist under continuously changing conditions (Feeley *et al.*, 2013).

Phenology timing

Instead of spatial movements, plants also can escape global warming 'in time' by shifting phenological events like flowering or fruiting towards more favorable conditions. To track such changes in the past, flowering timing, for example, can be approximated from collection dates of flowering herbarium specimens. Using a combination of contemporary flowering time observations with a herbarium specimen series across > 100 yr and 37 genera, Primack and colleagues (Primack *et al.*, 2004) were the first to connect meteorological data with earlier flowering, which was to a great part explained by increasing spring temperatures. This trend has been confirmed by multiple analogous studies (e.g. Davis *et al.*, 2015) and also broader approaches that integrated herbarium data with phenology records obtained from field notes and photographs to cover recent years of herbarium record scarcity (Panchen *et al.*, 2012).

Spatial scale and statistical power are important factors for these types of studies. Because phenology also depends on latitude, altitude and other environmental factors, broad sampling is necessary to separate climate change effects from other influences. Moreover, as phenology is partly species- or plant functional type-specific, it is useful to study contrasting flowering seasons, native status, pollination syndromes or growth forms (Calinger *et al.*, 2013). All of this is facilitated by large-scale digitization and hence improved accessibility of specimens world-wide (Lavoie, 2013; Box 3). Such studies, for example, showed that annual plants are generally more responsive to climate change than perennials (Calinger *et al.*, 2013; Munson & Long, 2017). Compilation of large cross-species datasets furthermore allows the search for phylogenetic signals and thus to identify evolutionary processes involved in shaping the observed responses (Rafferty & Nabity, 2017). Apart from interspecies or -family variation, plant responses also vary across geographic regions. Combination of world-wide herbaria allows to capture such responses, enabling to include remote localities across the globe into analyses (Hart *et al.*, 2014; Panchen & Gorelick, 2017).

Flowering is not the only phenological event heavily influenced by climate change that can be tracked from herbarium specimens. Depending on a plant's reproductive structures, seed dispersal timing also can be evaluated. At least for the Arctic, dispersal timing, too, seems to advance with increasing temperatures, in correspondence with associated flowering data (Panchen & Gorelick, 2017). Contrariwise, it was also estimated from collection meta-information (Kauserud *et al.*, 2008) that autumnal mushroom fruiting, especially of early fruiting species, is delayed in Norway, possibly reflecting a prolonged growth period due to warm autumn and winter temperatures.

Another parameter that affects entire communities and ecosystem processes is the leaf-out timing of deciduous trees, as it impacts trophic interactions as well as nutrient and water cycling (Polgar &

Primack, 2011). Such data collected from herbarium records track long-term leaf-out trends (Zohner & Renner, 2014) and, for example, confirm large-scale patterns of earlier leaf-out inferred with satellite data (Everill *et al.*, 2014).

Mismatching biotic interactions

Naturally, these climate change-related phenomena also affect biotic relationships beyond plants, and hence cannot be seen only as isolated processes. Changes of their timing are likely to affect evolutionarily synchronized relationships, and even their breaking-up over time is, together with flowering change, partially recorded in herbaria. Combined with entomological museum specimens, herbaria for example document disruption of the plant–pollinator relationship between the bee *Andrena nigroaenea* and the orchid *Ophrys sphegodes* (Robbirt *et al.*, 2014). In herbivory relationships, herbarium specimens can actually directly reflect insect reactions to warming. For example, increased traces left by the scale insect *Melanaspis tenebricosa* on maple tree leaves collected in warmer years evidence a higher insect density, perfectly in accordance with observations in the field (Youngsteadt *et al.*, 2015). Herbaria can thus help overcome the lack of historical insect abundance records and facilitate evaluation of climate change effects beyond plants alone.

The greatest challenge of most aforementioned approaches investigating species' responses to pollution, and habitat and climate change, is their inability to distinguish between plastic responses and evolutionary adaptation (Leger, 2013; Munson & Long, 2017), and thus whether observed differences among herbaria specimens reflect genetic changes or just environmentally induced phenotypic changes caused, for instance, by physiological processes (Bradshaw, 1965; Nicotra *et al.*, 2010). Quantitative genetics methods using herbarium time-series could help in disentangling these two alternative hypotheses (Gienapp *et al.*, 2008; Tiffin & Ross-Ibarra, 2014). Once the genetic basis of phenotypic differences is identified, local adaptation can be further tested using traditional approaches such as common garden experiments and reciprocal transplant studies (Savolainen *et al.*, 2013).

Biological invasions

Natural long-distance dispersal of plants is rare (Nathan & Muller-Landau, 2000), but as a side effect of global change, plants increasingly move long distances (van Kleunen *et al.*, 2015a). This movement massively increased with human migration waves towards the New World in the 16th century, and further accelerated with growing trade and faster transportation – coinciding with the core time range of herbarium collections. Today, jet-setting plant stowaways establish as 'neophytes', 'aliens' or 'invaders' wherever conditions are favorable enough. With this growing alien species richness, the global species distribution is getting more homogenous (Winter *et al.*, 2009). Local plants lose habitats and thus genetic diversity to the invaders, which are therefore considered a threat to biodiversity (Millennium Ecosystem Assessment, 2005).

Understanding invasion dynamics

Understanding the causes and spatiotemporal dynamics of invasions is indispensable to prevent further damage, preserve natural ecosystems and prioritize management actions (Vilà *et al.*, 2011; van Kleunen *et al.*, 2015b). Although contemporary surveys depict the current status of invasive species, herbaria track invasions from the first recorded colonizer onwards – which can serve as a proxy, even if it is not the actual first colonizer. In conjunction with contemporary collections and literature surveys, herbaria are crucial to establish inventories of introduced species that monitor their status of naturalization – or invasion – and inform management strategies (Magona *et al.*, 2018). With native plants as baseline for collection efforts and abundance, herbaria illustrate geographical and temporal spreads (Crawford & Hoagland, 2009) that may – in search for invasion causes – be connected with historic events. For instance, a map of Chilean alien expansions uncovers two spread peaks, one connected to the spread of agriculture, the other to its increased mechanization (Fuentes *et al.*, 2008). Understanding such causalities can feed early preventive measures: retrospectively mapped invasions identify geographic invasion hotspots, and the environmental and anthropogenic factors crucial for their creation. In this way, herbaria can contribute to understanding the general invasibility of particular habitats (Aikio *et al.*, 2012; Dawson *et al.*, 2017). Furthermore, combined with contemporary data, they can help to identify characteristics of successful invaders, and to quantitatively connect and established naturalization risk with external factors, and rank potential new invaders (Dodd *et al.*, 2016).

Herbaria also provide a means of assessing the continued success of invasive species after establishment in a new environment. Previous studies have used them both to predict and to verify predictions of the climatic niche that plants can potentially occupy. For example, the size of the native range of an invasive species has been found to be highly correlated with its abundance in the new range, as documented for many highly invasive Eurasian species around Québec (Lavoie *et al.*, 2013). Herbaria also can enable estimation of a weediness index – or how much a plant associates with human-caused disturbance – which often also overlaps with plant invasiveness (Robin Hart, 1976). Such estimates hold well in comparison with field surveys (Hanan-A *et al.*, 2015). More precise forecasts of a species' spread can further include its native climate range, again extrapolated from herbarium records, thereby roughly visualizing occupation of a possible climatic niche (Bradley *et al.*, 2015). Much as surveying and modeling the dynamics and spread of invaders is crucial to inform containment measures, it is very sensitive to biases and errors in historical collections – one crucial and common error being misidentification and misnaming (Jacobs *et al.*, 2017) – and increasingly at risk from decreasing collection efforts (see Box 2).

Genetic changes of invaders

Irrespective of whether invasive species stay within their native climatic range or move beyond, they face challenges when establishing in new environments. Successful invasive species often

adjust to the novel conditions, and it is therefore important to understand such changes in the invasive range.

Adjustment of morphological traits to novel environments is often well-captured in herbaria, as demonstrated with Australian invasives where 70% of surveyed species showed at least one phenotypic trait changing over time (Buswell *et al.*, 2011). With NGS, it is now possible to define whether this trait variation is associated with genomic changes – caused either by drift or potentially adaptive – or more likely the result of phenotypic plasticity. In addition, these methods can potentially solve the 'genetic paradox of invasion': the surprising success and spread of colonizers in spite of their reduced genetic diversity (Estoup *et al.*, 2016): Do these species adapt based on their (reduced) standing genetic variation, do they borrow pre-adapted standing variation from native species (adaptive introgression; Keller & Taylor, 2010; Arnold *et al.*, 2016), or do they rely on *de novo* mutations and hence novel variation (Exposito-Alonso *et al.*, 2018a)?

Comparison of historic native and invasive populations with contemporary genetic diversity can also point to diversification or hybridization events before species expansion. A recent herbarium genetics study, for example, has shown strong divergences of flowering time genes particularly during the establishment phase of the invader *Sisymbrium austriacum* ssp. *chrysanthum*, possibly enabling a subsequent spread (Vandepitte *et al.*, 2014). Such patterns change over the course of invasion. In the Eurasian *Alliaria petiolata* invading North America, invasive success declines along with population age and reduced phytotoxin production in late stages of invasion (Lankau *et al.*, 2009). Contrary to that, chemical analyses of herbarium specimens of the phototoxic *Pastinaca sativa*, a European weed also invading North America, displays increased concentrations of phytochemicals over time since invasion, which coincide with the emergence of the herbivore *Depressaria pastinacella* (Zangerl & Berenbaum, 2005). Studies using ancient DNA also have pointed to anthropogenic landscape disturbances causing genetic admixture in *Ambrosia artemisiifolia*'s native populations before its introduction to new habitats, potentially a prerequisite for later invasive success (Martin *et al.*, 2014b). In this sense, herbarium material allows us to compare genetic composition through time, and to identify so-called 'cryptic' (i.e. hidden) invasions, where native genotypes are dispelled by phenotypically indistinguishable but more successful and aggressively spreading non-native relatives (Saltonstall, 2002).

Hitchhiking invaders: pathogens and herbivores

Moving beyond plant invasions, herbaria even harbor information about hitchhikers traveling with the original plant stowaways, pathogens, purposely or unknowingly sampled together with their hosts (Yoshida *et al.*, 2014). Thereby, they track the invasion (success) stories of plant pathogens such as *Phytophthora infestans*, the microbe at the root of potato late blight and the Irish potato famine (Martin *et al.*, 2013, 2014a; Yoshida *et al.*, 2013). Other preserved pathogens of particular interest for agriculture include rust fungi and downy-mildew-causing oomycetes. Herbaria allow identification of causal strains, their genetic characteristics and their tracking to contemporary pathogen diversity. Coupled with host

plant analyses, they provide a (genetic) timeline of host–pathogen dynamics to study and illustrate co-evolutionary principles such as the arms race between hosts and their pathogens. Genetic analysis of such systems can hence provide crucial insight into spread dynamics of pathogens that could have devastating consequences on crop monocultures world-wide.

Even for invasive herbivores, historic samples may contain a genetic record. The horse chestnut leaf-mining moth *Cameraria ohridella*, for example, is preserved pressed and dried in leaves of its host plant (Lees *et al.*, 2011). Genetics can backtrack the moth's spread from its native Balkan region, and in conjunction with host plant analyses may identify resistant cultivars and biocontrol agents for the invasive pest (Lees *et al.*, 2011).

Conclusions and outlook

Plants preserved in herbaria offer unique perspectives on global change and its consequences, as they are directly affected victims (Fig. 2). Thus, they represent an invaluable temporal, geographical and taxonomic extension of currently available data employed to understand global environmental change, predict its course and inform conservation measures. To fully take advantage of this potential, and to increase and sustain the value of herbaria for the future, three core areas demand particular attention: the maintenance and curation of herbaria including continued collection efforts, the digitization of collections, and herbarium genomics (see also Boxes 1–3).

Even though many herbaria are already investing in digitization, only a fraction of the *c.* 350 million specimens world-wide have been digitized so far. Large-scale digitization would both encourage the use of herbaria for research, and strengthen projects (e.g. Munson & Long, 2017), as studies including digitized material are able to use large sample sizes (Lavoie, 2013). Fast processing of specimens at consistently high data quality is crucial for making digital herbaria truly useful (Yesson *et al.*, 2007), as is substantial funding to enable this task and secure databases' continuity. Yet, even with increased digitization, the actual power of herbaria – for climate change study amongst other types of research – lies in their continuity through time. Despite growing recognition of the value of herbaria, this characteristic is currently threatened by declining collection efforts (i.e. Prather *et al.*, 2004; Meyer *et al.*, 2016) and a frequent lack of support for herbaria world-wide. Consequences of reduced data for modeling and other analyses can already be seen in the tropics, where collections are generally sparse (Feeley & Silman, 2011). To maintain herbaria as the treasure they are today, continued and consistent collection world-wide is essential, especially because they have recently revealed themselves as a browsable repository of genetic variation and diversity. This drastically increases the value of herbaria for climate change research, and for understanding principles of adaptation and evolution in this context. To date, herbaria are still underused in this aspect (Lavoie, 2013), and in particular, high-quality sequencing data are scarce. With firm guidelines for protocols and quality standards, pointing also to the necessity of DNA preservation-informed sequencing efforts, this neglect is likely to change in the coming years.

Hence, being aware of the answers herbaria can give if we use the right methods to ask, it is up to us to keep herbaria alive and well, define what we need to know, and start the questioning.

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Chapter III

Forest wildflowers bloom earlier as Europe warms – but not everywhere equally

Franziska M. Willems, J. F. Scheepens, and Oliver Bossdorf

Abstract

Some of the most striking biological responses to climate change are the observed shifts in the timing of life-history events of many organisms. Plants, in particular, often flower earlier in response to climate warming, and herbarium specimens are excellent witnesses of such long-term changes. However, in large-scale analyses the magnitude of phenological shifts may vary geographically, and the data are often clustered, and it is thus necessary to account for spatial correlation to avoid geographical biases and pseudoreplication. Here, we analysed herbarium specimens of 20 spring-flowering forest understory herbs to estimate how their flowering phenology shifted across Europe during the last century. Our analyses show that these forest wildflowers now bloom over six days earlier than at the beginning of the last century, and that these changes were associated with warmer spring temperatures. Plants flowered an average of 3.6 days earlier per 1°C warming. However, in some parts of Europe plants flowered earlier or later than expected. This means, there was significant residual spatial variation in flowering time across Europe, even after accounting for the effects of temperature, precipitation, elevation and year. Including this spatial autocorrelation into our statistical models significantly improved the model fit and reduced bias in coefficient estimates. Our study indicates that forest wildflowers in Europe strongly advanced their phenology in response to climate change during the last century, with potential severe consequences for their associated ecological communities. It also demonstrates the power of combining herbarium data with spatial modelling when testing for long-term phenology trends across large spatial scales.

Introduction

Since the industrial revolution anthropogenic global change threatens species and ecosystems. Climate warming in particular can cause shifts in the timing of annual life-history events of plants and animals (Root et al. 2003, Menzel et al. 2006, Cleland et al. 2007). Such phenological changes, including earlier leaf-out or flowering of plants, are some of the most striking large-scale biological responses to ongoing climate change (Cleland et al. 2007). To understand why and how phenology shifts, it is critical to infer which attributes of the environment are the triggers (cues) or proximate causes (drivers) of life cycle events. As their phenology links plants to their environments, changes in the phenology can affect the local persistence and biotic interactions of plants (Inouye 2008, Willis et al. 2008, Wheeler et al. 2015, Cerdeira Morellato et al. 2016). For instance, Willis et al. (2008) found that plant species whose flowering time poorly tracked temperature variation had declined in abundance during the last century. Unequal shifts of interacting organisms in trophic interactions can result in phenological "mismatches", e.g. when the timing of the activity of consumers aligns less well with the availability of their resources, or the phenology of plants and pollinators shift differently (Renner and Zohner 2018, Visser and Gienapp 2019). Such mismatches can have severe demographic and evolutionary consequences (reviewed e.g. in (Renner and Zohner 2018, Visser and Gienapp 2019).

When studying phenology changes over time, we should keep in mind that phenology, and magnitudes of phenological responses to climate change, not only vary among species but they also vary in space. At smaller scales, phenology can vary because of microclimatic differences (Hwang et al. 2011; Ward et al. 2018; Willems et al. 2021), and at larger scales both (baseline) phenology as well as phenological responses are expected to vary because of macroclimatic variation, because the magnitudes of climatic changes differ geographically (Klein Tank et al. 2002, IPPC 2019), and because phenological sensitivities to cues such as temperature may differ between regions (Riihimäki and Savolainen 2004, Zohner and Renner 2014, Prevéy et al. 2017, Zohner et al. 2020). Robust studies on phenology and climate change therefore require a larger-scale perspective, with spatial variation and autocorrelation explicitly taken into account. However, many previous studies on plant phenological responses to climate change have a limited geographical scope (Pau et al. 2011).

In this context, herbaria offer unique opportunities because they allow tracking phenology at large temporal as well as spatial scales. Herbarium specimens are usually collected when plants

flower, and most herbarium sheets provide collection dates and locations (Fig. 1). With many herbaria dating back to some 200 years, and hundred millions of specimens worldwide, herbaria are a tremendous treasure for studying phenology changes both long-term and large-scale. Previous studies have indeed found strong patterns of long-term phenology changes in herbarium data (Primack et al. 2004, Miller-Rushing et al. 2006, Davis et al. 2015, Willis et al. 2017, Park et al. 2019, reviewed by Jones and Daehler 2018), and they have also demonstrated that phenology trends estimated from herbarium data are comparable to those from field observations (Davis et al. 2015, Jones and Daehler 2018). However, almost all previous studies were done in the US, and there has been little work so far on herbaria and plant phenology in Europe (but see Robbirt et al. 2011, Molnar et al. 2012 and Diskin et al. 2012). Most previous studies also ignored geographic variation in phenology and spatial correlation of herbarium samples (but see Matthews and Mazer 2016 and Park et al. 2019).

In Europe, climatic conditions vary substantially across the ranges of many plant species, especially from north to south, and phenological responses may differ across this latitudinal gradient. For a similar climatic gradient in the eastern US, Park et al. (2019) found that long-term phenological responses estimated from herbarium specimens substantially differed among climatic zones, with greater mean climate sensitivities, as well as greater among-species variability in sensitivities, in the warm and mixed-temperate climatic regions than in the cool-temperate northeast and the Appalachians. Another problem with large-scale herbarium data is that they are often, for historical reasons, strongly clustered, i.e. specimens are more frequently collected where collectors live, and around academic institutions. However, when modelling across a spatial range, ordinary linear regression ignores spatial dependency between sampling locations and treats all data points as independent. This assumption is highly unlikely, since the proximity of spatial points is usually related to their environmental similarity (Tobler 1970), and as explained above, this is certainly also true for climatic conditions. Ignoring spatial dependency thus results in pseudoreplication, and it can strongly bias model results. The solution to this, spatial modeling with explicit incorporation of spatial structure and thus spatial autocorrelation, is computationally challenging, and it has therefore hardly ever been used in analyses of herbarium data. However, recent advances in statistical methods now allow to model spatial data efficiently, e.g. using stochastic partial differential equations (SPDE) and integrated nested Laplac approximations (INLA) as implemented in the R package *R-INLA* (Rue et al. 2017, Bakka et al.

2018), and it is therefore possible to take the next step in herbarium studies and analyse large-scale phenology in relation to climate change in a spatially explicit framework.

Here, we analysed long-term and large-scale trends in the flowering time of 20 common forest understory wildflowers, and their relationships with climate change, across Europe, using over a century of herbarium data. We focused on early-flowering understory plants, because they have a very distinct phenology, with a critical blooming window before the leaf-out of deciduous trees. Because of this, they may be particularly sensitive to climate change and phenology shifts. Furthermore, forest understory plants may also be exposed differently to climate change because macroclimate warming is buffered under forest canopies (De Frenne et al. 2019). However, so far little is known about their phenological responses to climate change. In our analyses, we employed R-INLA (Rue et al. 2009, 2017, Bakka et al. 2018) to account for spatial clustering and autocorrelation of climate and phenology data. We asked two main questions: (A) Did forest understory plants advance their flowering phenology during the last ~100 years? (B) If yes, are these phenological shifts associated with climate change in Europe? We answered both questions with or without accounting for spatial correlation in the statistical models, and thus also addressed the question of how important doing this was for the results and conclusions of our study.

Methods

Phenological data

We mined three large German herbaria and the Global Biodiversity Information Facility (GBIF) for all European specimens of 20 common spring-flowering forest understory herbs (see Table S1). The three herbaria were at the University of Tübingen (international herbarium code TUB), University of Jena (JE) and at the State Museum of Natural History in Stuttgart (STU). Our criteria for including herbarium specimens were that: (i) they had flowers and that open flowers represented at least 50% of the reproductive structures, (ii) they had an exact collection date and (iii) information on the sampling location that we could use to estimate GPS coordinates, and (iv) they were collected in Europe. In addition, we obtained all digital specimens of the same 20 species from GBIF (GBIF 2020) that were from Europe and also had (i) an exact collection date and (ii) GPS coordinates of the sampling location, using the *rgbif* package (Chamberlain and Boettiger 2017) in R (R Core Team 2008). This resulted in an initial 3930 specimens from the

three herbaria and 3511 specimens from GBIF, with the collection years ranging from 1807 – 2017 for these 7441 specimens. However, since reliable, gridded climate data were not available before 1901 we decided to restrict our analyses to data from 1901 onwards. Moreover, because there were only very few specimens from outside of these limits, we truncated our data to 40 to 65 degrees northern latitude and -5 to 30 degrees longitude, covering a broad geographic area in mainly Central and Northern Europe, but also Western and South-Eastern Europe (Fig. 1A). We further discarded all specimens with dates outside of the normal flowering range of our 20 study species (before day of the year (DOY) 50 and after DOY 200), because we suspected these to be recording mistakes. Also, the GBIF data contained unusually many specimens from May 1 and June 1 (DOYs 121 and 152, respectively), which strongly indicated that they were from specimens without exact collection dates that were arbitrarily assigned to the first day of a month, and we excluded these data from our analyses. Lastly, we discarded six datapoints for which the assigned elevation value was below -10 m. Our final set of phenology data contained 6131 herbarium specimens, with 46 to 600 records per species (Table S1).

Climate and elevation data

For associating plant phenology variation with long-term temporal and spatial variation in climate, we used gridded estimates of historic monthly air temperature (°C) and precipitation (mm) that were available for 1901–2017 and with a 0.5° × 0.5° grid resolution from the Climate Research Unit (CRU, https://crudata.uea.ac.uk; (Harris et al. 2020)). We used these data to calculate mean winter (December – February) and spring (March – May) temperatures, as well as annual precipitation values for each year and grid cell. Each herbarium specimen was then assigned to a specific set of values of these three climate variables, based on its collection year and the geographic grid cell it was located in, using custom-made scripts in *python* (Van Rossum and Jr. Drake 2009). We also estimated the elevation a.s.l. of each herbarium specimen using the *raster* package in R (Hijmans 2020).

Statistical analyses

Our statistical analyses generally had a two-step logic, relating to the two main questions of our study. First, we tested for overall phenological shifts, i.e. temporal trends in flowering time, across our 20 study species, using a simpler statistical model (model A). Second, we tested for

phenology-climate associations with a more complex model B (for details see below). Both models were run with and without accounting for spatial correlation.

To test for temporal trends in flowering time (model A) we modelled flowering phenology during the last 120 years as a function of the year of collection, while accounting for the effects of elevation and species. Model A was specified as:

$$Y_{ij} \sim Intercept + \beta_{ij}\chi_{ij} + S_i + T_i \cdot Year_{ij} + U_i + \varepsilon_{ij},$$

where Y_{ij} is the day of flowering of herbarium specimen i and species j, χ_{ij} is a vector containing all the covariates (in the case of model A: collection year and elevation) as linear fixed effects, β_{ij} is the vector of estimated parameters (regression slopes), $S_{(i)} \sim N(0, \sigma^2_{species})$ is the species random intercept, $T_{(i)} \sim N(0, \sigma^2_{species})$ the species random slopes, both with a Gaussian distribution, and $\varepsilon_{ij} \sim N(0, \sigma^2)$ the residuals. The species random intercept accounts for the fact that mean flowering times can differ between species and the species random slope is the species-specific shift over the years, since species might respond differently. $U_{(ij)} \sim N(0, \Omega)$ represents the spatial structure (see below) that is additionally included as a random effect in the models accounting for spatial correlation. In model A, the slope of the linear relationship between the collection dates (= DOY of flowering) of specimens and their collection year is the formal test for long-term phenological shifts.

To test for phenology-climate associations (model B) we additionally included spring temperature, winter temperature and precipitation, plus the interactions between spring temperature (which is presumably the most important driver of spring phenology) and all other variables, into the model described above (see Table 1 for further explanations of the variables, and their expected effects on plant phenology). We thus modified the model equation to:

$$Y_{ij} \sim Intercept + \beta_{ij}\chi_{ij} + S_i + T_i \cdot SpringTemp_{ij} + U_i + \varepsilon_{ij}$$

In model B the slopes of the linear relationships between the collection dates (= DOY of flowering) of specimens and the temperature or precipitation at the corresponding location and year estimates the sensitivities of phenology to climate changes. Here, the species random slopes are the species-specific shifts with temperature, accounting for the fact that some species might be more temperature sensitive than others. As for model A, we also fitted model B with and without including the spatial structure U_{ij}.

To estimate spatial dependency, we used integrated nested Laplace approximation (INLA), an approximate Bayesian technique and faster alternative to MCMC methods for fitting

Bayesian models (Bakka et al. 2018). A key challenge with spatial models is that the Gaussian random field, the most common tool for capturing spatial dependency, is hard to use with large data. *R-INLA* solves this problem through stochastic partial differential equations (SPDEs) that allow to model Gaussian random fields fast and efficiently, and to handle complex spatial data (Lindgren et al. 2011). The SPDE is the mathematical solution to the Matérn covariance function describing the statistical covariance between values at two different points. The covariance matrix of the Gaussian field is approximated as a Gaussian Markov Random Field (GMRF) using a Matérn covariance structure (Bakka et al. 2018). The GMRF models spatial dependence by defining a neighborhood structure on a mesh that divides the study area (in our case Europe) into non-overlapping triangles (see Fig. S1). The data points (in our case sampling locations of herbarium specimens) are then assigned to the adjacent nodes of the mesh according to their proximities (or to only one if they fall directly onto one). This creates an observation matrix for estimating the Gaussian Markov Random Field (Bivand et al. 2015, Cosandey-Godin et al. 2015). The mesh can have different shapes and sizes, and we used the default constrained Delaunay triangulation (a particular way to divide an area into triangles) together with vague priors that have little effect on the posterior distributions of the fixed effects. To select the mesh size, we compared models with different meshes and chose the finest mesh (with a maximum triangle edge length of 20 km and a minimum edge length of 5 km) as it resulted in the lowest DIC/WAIC values. The derived Gaussian Markov Random field is then represented by the term U_{ij} in the model above, a smooth spatial effect that links observations to spatial locations, with the covariance structure Ω estimated via the Matérn correlation. The term U_{ij} is thus spatially variable and captures spatial patterns not already modelled by the fixed covariates, thereby ensuring that the residuals ε_{ij} are independent. We compared the results of models with and without including U_{ij} in the model.

To avoid biased parameter estimates because of unequal scales, we used the covariates in the following forms: year expressed in decades, spring precipitation in mm·10^{-1}, elevation in hundred meters [100 m] and spring and winter temperature in degree Celsius [°C]. We also mean-centered all covariates because this meant that the regression slopes for each covariate were estimated for the case that all other covariates were at their mean value (rather than zero; (Dalal and Zickar 2012), which greatly helped to interpret the results of the regression analysis.

For both models we checked whether the residuals were normally distributed, plotted the distribution of residuals against fitted values and explanatory variables to check for heterogeneity

or other patterns in the variances, and we plotted the observed vs fitted data to evaluate model fit and performance (Zuur et al., 2017). All statistical analyses were done in R version 3.6.2 (R Core Team 2018) using the *R-INLA* package (www.r-inla.org, see also: Rue et al. 2009, Lindgren et al. 2011, Bakka et al. 2018).

Table 1. All explanatory variables (fixed and random effects) that were included in our analyses, together with the reasonings for including them, and their expected effects on plant phenology. Model A included elevation, year and the two random factors, model B also the climate variables, and the interactions of spring temperature with the other covariates.

Variable	Why did we include it?	What do we expect?
Elevation	Climate conditions, including snow-melt patterns, vary with altitude, which should influence flowering patterns (Inouye 2008; Bucher and Römermann 2020).	Plants flower later at higher altitudes.
Year	Long-term trends of rising temperatures should result in corresponding long-term trends in plant phenology.	Plants advanced their flowering during the last century.
Spring temperature	Temperature drives plant phenology (Piao et al. 2019; Tang et al. 2016). For early-flowering understory plants, spring temperature should be particularly relevant.	Plants flower earlier with warmer temperatures.
Winter temperature	To start leaf-out or flowering in spring, some plant species depend on a preceding chilling period (vernalization) indicating that winter has passed (Piao et al. 2019; Tang et al. 2016).	Unclear, if winter chilling requirements are still meet, plants will flower earlier with warmer temperatures if not later.
Precipitation	Since plant growth depends on water availability, precipitation could also influence plant phenology (Matthews and Mazer 2016, Peñuelas et al. 2014).	Precipitation effect alone unclear; maybe temperature-dependent.
Spring temperature × Winter temperature	If plants experience insufficient chilling in warm winters they can be less sensitive to warm spring temperatures (Tang et al. 2016).	We expect a negative interaction, with plants flowering earliest when winters are sufficiently cold but springs are warm.
Spring temperature × Precipitation	Since plant growth depends on both temperature and precipitation, phenology may be driven by the interaction of the two. Mattews and Mazer (2016) showed that (in western North America) phenological responses to warming were strongest where precipitation was high.	We expect a positive interaction, with plants flowering earliest where both temperature and precipitation are increasing.
Spring temperature × Elevation	Previous studies suggested, that that plants at high elevation are more sensitive to temperature changes (Cufar et al. 2012, Chapman 2013, Liu et al. 2014, but see Vitasse et al. 2010, Dai et al. 2014).	We expect a positive interaction, with greater temperature-sensitivity at higher elevations.
Spring temperature × Year	If plants have evolved in response to climate change, then sensitivity to spring temperature might have changed over the years.	We expect an interaction between temperature and collection year.
Spatially dependent random intercept (U_{ij})	Environmental conditions are variable and correlated across space. Plants that are closer to each other experience more similar conditions, and may also show more similar phenological responses (Park et al. 2019).	We expect substantial geographic variation, and that this will influence model estimates for other covariates.
Species (random intercept and slope) (S_i, T_i)	Flowering time, as well as its sensitivity to climate, differs between plant species.	There is variation in mean phenology (intercept) and phenological responses (slopes) of the study species.

Results

Model validation and spatial correlation

The herbarium data analysed in our study covered a broad geographical range in Europe, but their spatial distribution was heterogenous (Fig. 1), and in addition the flowering time data were spatially correlated up to a distance of around 200 km and 100 km in models A and B, respectively (Fig. S2).
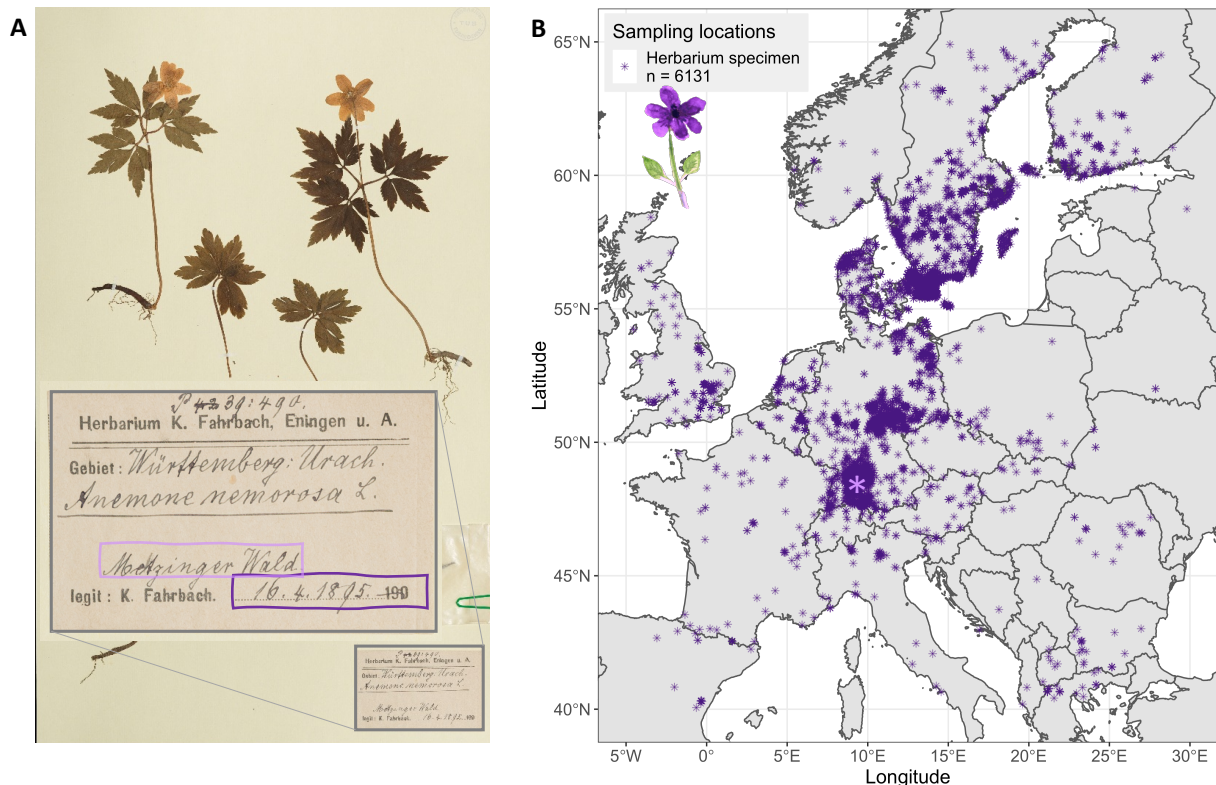
Figure 1. (A) Example of an herbarium specimen, with the collection date and location on the herbarium label. This *Anemone nemorosa* was flowering on April 16 (DOY = 107) in 1895, and it was collected in the "Metzinger Wald" forest close to Tübingen (lighter purple point in the map). (B) Sampling locations of the 6131 herbarium specimens included in our analyses.

If this spatial correlation was not included in the analyses, then the residuals were clearly non-random in space, especially in model A (Fig. S3), and there were other violations of model assumptions, in particular non-random distribution of residuals in relation to several covariates (Fig. S4 and S5). In the models with spatial correlation, these problems were all fixed. Moreover, models that included spatial correlation also generally had a better fit (see Fig. S6 for a comparison of DIC values and regression parameter estimates of model B without spatial

correlation and with spatial correlation, using different mesh sizes), and the fitted values were much closer to the observed values ($r = 0.78$ vs. 0.57 for Model A and $r = 0.82$ vs 0.70 for Model B), respectively for models with and without spatial correlation (Fig. S7). Overall, residuals were smaller when spatial correlation was accounted for (Fig. S8). Thus, models that explicitly incorporate spatial correlation between data points are not only more statistically appropriate, but they are also stronger and more informative. In the next sections, we show that taking spatial correlation into account also strongly affects the model estimates answering the main questions of our study.

Temporal shifts in plant phenology

Overall, the herbarium data indicated that the studied 20 forest understory plants significantly advanced their flowering time during the last century (Table 2, Fig. 2). The estimated advancement of flowering time was -0.56 days per decade (credible interval: -0.74 to -0.39; see Table 2) according to model A with accounting for spatial correlation, and these responses were different from zero (posterior probability > 0.95) for all 20 species (ranging from -0.562 to -0.559). For species-specific residuals see Fig. S9 and for a summary of all hyperparameter see Table S2. The observed phenology shifts corresponded with increasingly warmer spring temperatures during the last century (Fig. 2C). If model A ignored spatial correlation, it severely overestimated the overall magnitude of phenology shifts, with an estimated -1.34 days per decade (CI: -1.69 to -0.98; Table S3, Fig. 2B), i.e. it estimated an average shift of around two weeks during the last century instead of less than half of this in model A with spatial correlation. One reason for this discrepancy is that datapoints from northern vs southern Europe are unevenly distributed in time, with more earlier data from the north, and an overrepresentation of southern data during the last decades (Fig. 2C). When spatial information is ignored in model A, this latitudinal bias thus distorts the estimated shift over time. The opposite is true for the relationship with elevation: in model A with spatial correlation plants flower later at higher altitudes (2.44 days/100m, 95% CI 1.98 to 2.89; Table 2, Fig. 2B), but when spatial correlation is ignored there is no relationship between elevation and flowering time (Table S3, Fig. 2B). Further, the model including spatial correlation shows that there is strong spatial variation in flowering time (after the effect of the covariates – year and elevation – has been accounted for). Plants from Northern and Eastern Europe flower up to ~60 days later than plants from Central and Southern Europe (Fig. 5).
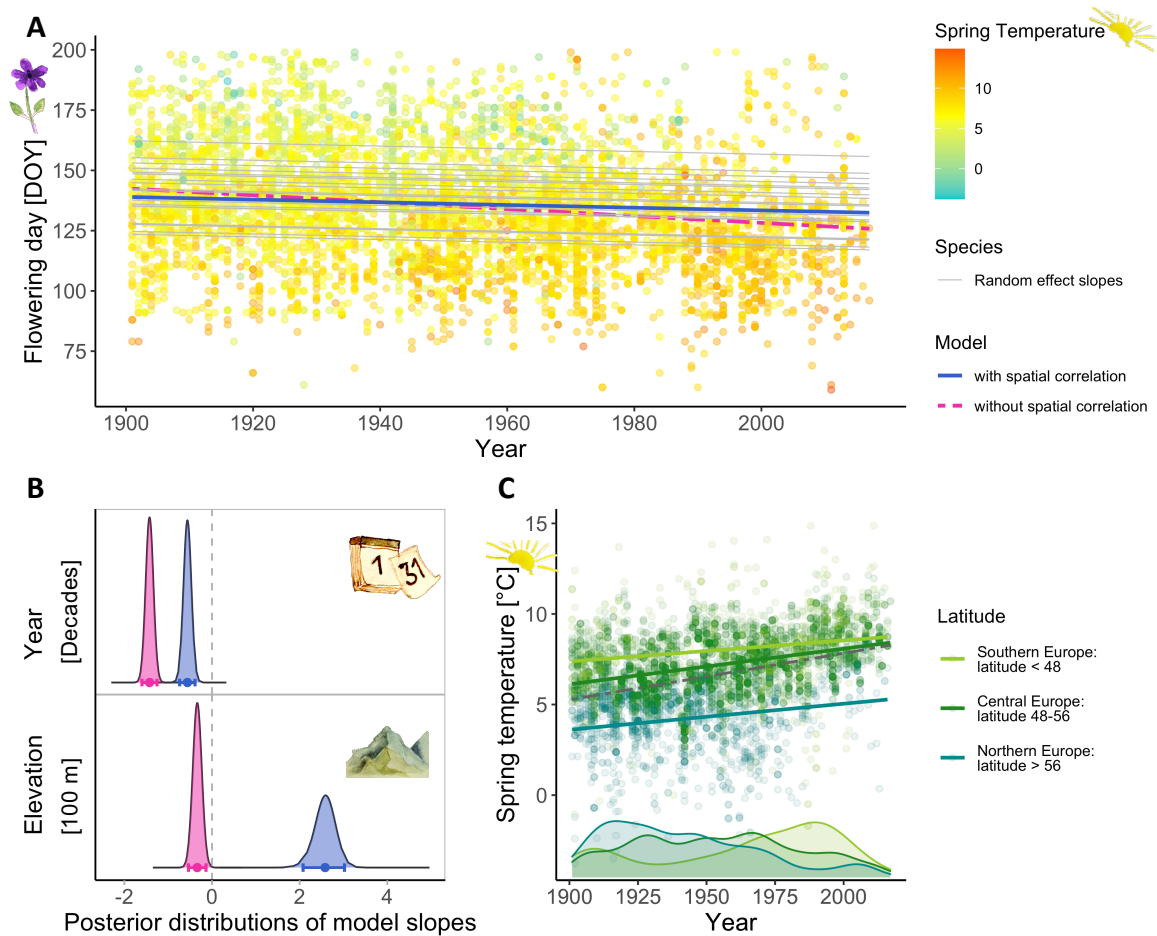
Figure 2. Temporal trends of flowering time and spring temperature over the last century, and the results of model A. (A) Shifts of flowering time since 1901 estimated by model A with spatial correlation (solid blue line) and without spatial correlation (dashed magenta line). With spatial correlation, plants advanced their flowering on average by around six days, and the responses were different from zero (posterior probability > 0.95) for all 20 species (thin grey lines). In the model without spatial correlation the estimated phenology shift is more than twice as large. (B) Differences in parameter estimates (posterior probability distributions) for model for model A without (magenta) and with (blue) spatial correlation. (C) Long-term trends in spring temperature in the locations of the studied herbarium specimens, separately for southern, central and northern European data, with the histograms at the bottom showing the temporal distributions of these data.

Table 2. Model estimates (slopes), with standard deviations and 95% credible intervals, for all variables included in models A and B with spatial autocorrelation.

	Estimate	SD	95% CI
Model A			
Intercept	136.09	4.07	128.02, 144.03
Years [Decades]	-0.56	0.09	-0.74, -0.39
Elevation [100 m]	2.57	0.24	2.08, 3.02
Model B			
Intercept	138.62	2.52	133.57, 143.48
Spring temperature [°C]	-3.61	0.22	-4.04, -3.18
Winter temperature [°C]	-1.05	0.13	-1.31, -0.79
Precipitation [mm/10]	0.07	0.15	-0.23, 0.37
Elevation [100 m]	1.42	0.21	1.00, 1.84
Year [Decade]	-0.22	0.09	-0.40, -0.04
Spring temperature × Year	0.05	0.04	-0.03, 0.13
Spring temperature × Elevation	0.06	0.05	-0.04, 0.16
Spring temperature × Precipitation	-0.04	0.06	-0.16, 0.07
Spring temperature × Winter temperature	-0.06	0.03	-0.12, 0.01

Relationships with climate change

Across the European sampling locations included in our study, spring temperatures increased during the last century (Fig. 2C), and the phenology of the plants was related to these climatic changes. Overall, plants flowered around 3.6 days earlier per +1°C (Table 2, Fig. 3 and 4). If spatial correlation was not included in model B, the strength of this relationship was overestimated with 5.4 days per +1°C (Table S4, Fig. 3 and 4). The observed temperature-phenology relationship was consistent across the 20 studied species, with negative slopes credibly different from zero (posterior probability > 0.95) for all (ranging from -3.395 to -3.416, see also Fig. 3).
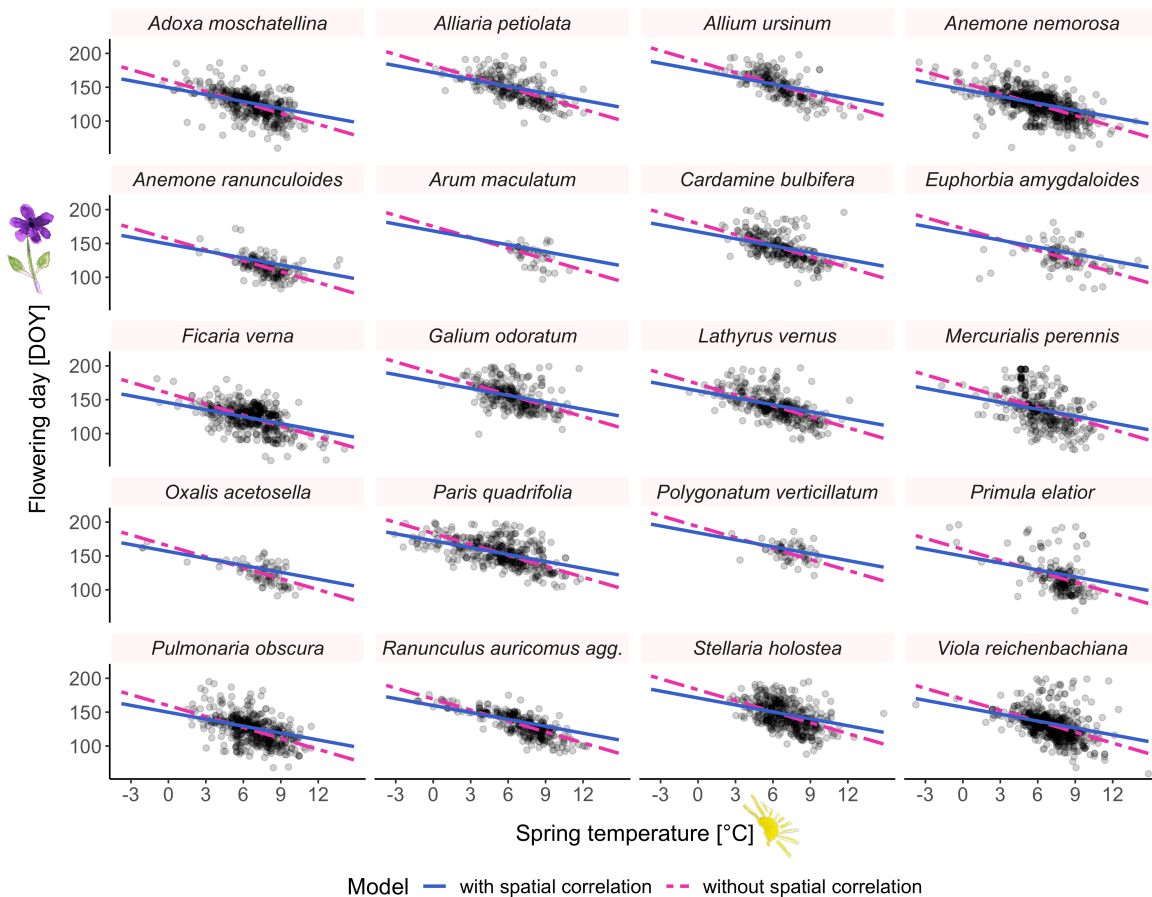
Figure 3. Relationships between the spring (March-May) temperature in the year of collection and the date of collection (= flowering day) of European herbarium specimens of 20 early-flowering forest understory plants. The blue and magenta lines indicate slope estimates from statistical models with and without taking spatial autocorrelation into account.

Besides the relationship with spring temperature, there was also a significant, albeit weaker, relationship with winter temperature, but no relationship with precipitation, in the model B with spatial correlation (Table 2, Fig. 4). There were further relationships of phenology with elevation and the year of sampling (Table 2, Fig. 4). The direction of these results – later flowering at higher altitudes and earlier flowering in more recent specimens – was as in model A, only with smaller effect sizes. This is because both the year of sampling and elevation are systematically related to temperature, so the larger effects in model A are partly temperature effects. None of the interaction terms between spring temperature and the other covariates was significant (Table 2). Ignoring the spatial locations of specimens substantially affected also these parameter estimates: in model B without spatial correlation the relationship with elevation was underestimated, whereas the relationship with winter temperature was lost, and there was now a

relationship with precipitation, and several significant interactions between covariates (Table S3, Fig. 4).

As in model A, there was significant spatial variation in flowering time after the covariates and their interactions had been accounted (Fig. 5, right panel). Although the residual spatial correlation was clearly much less and more small-scale than in model A, there were still several regions with clustering of positive or negative residuals, showing the usefulness and importance of incorporating spatial correlation also in model B.
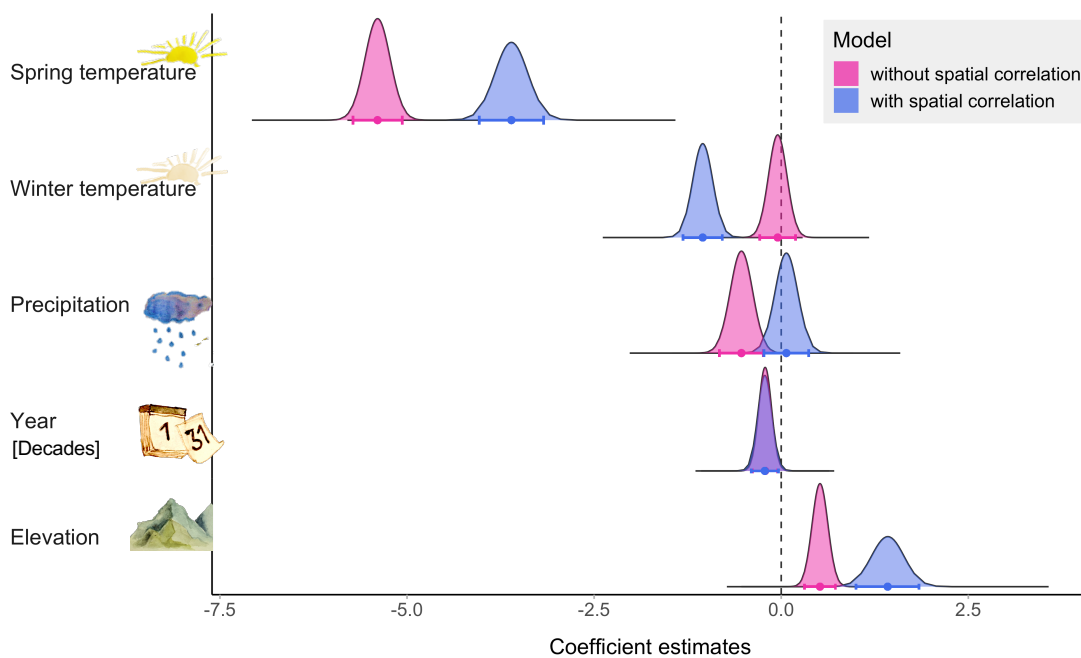
Figure 4. Model coefficient estimates for relationships between different covariates (climate in the year of collection, year of collection, elevation of collection site) and the date of collection (= flowering time) of herbarium specimens of 20 forest wildflowers in Europe. The blue vs. mangenta curves show the differences between the parameter estimates (posterior probability distributions) from model B with and without taking spatial autocorrelation into account.
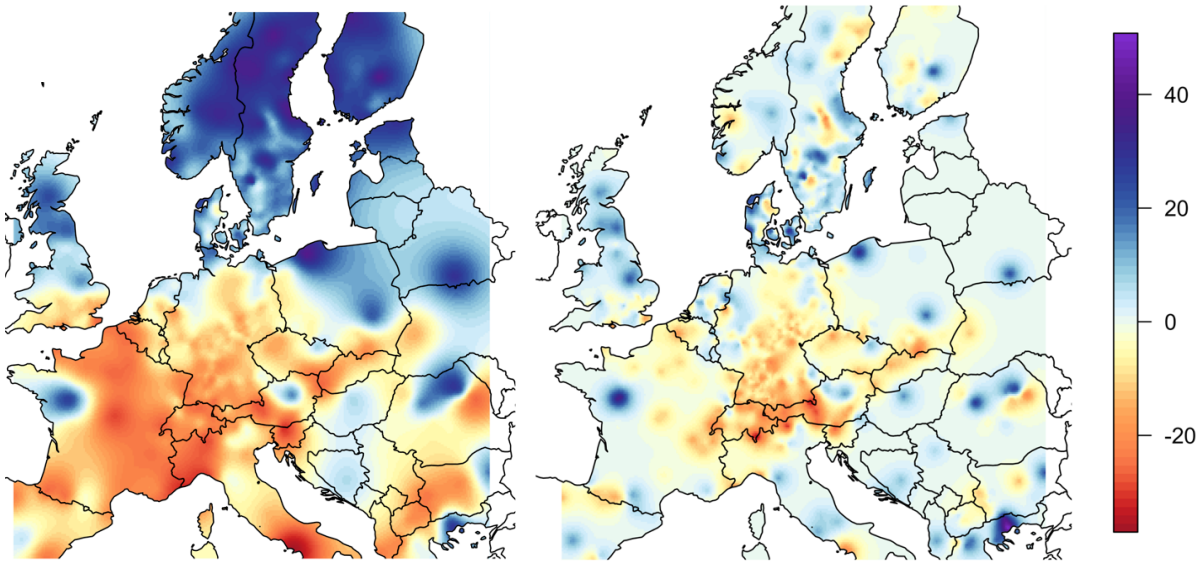
Figure 5. Spatial variation in flowering time [days] in model A (left) and model B (right) after the effects of the covariates (model A: year and elevation; model B: year, elevation, spring and winter temperature, and spring precipitation) have been accounted for.

Discussion

Herbaria are unique archives for studying long-term responses of plant phenology to anthropogenic climate change. We studied herbarium specimens of 20 early-flowering forest herbs across Europe and show that these plants advanced their flowering during the last century, most likely in response to increasing spring temperatures. The herbarium data we used were substantially autocorrelated – even after accounting for elevation, climate and year, and including this spatial structure in our statistical models significantly improved the model fit and parameter estimates. Below, we therefore discuss only the results from models that accounted for spatial correlation.

Temporal shifts in plant phenology

We found that forest understory herbs from Central Europe advanced their flowering by an average of six days during the last century (-0.6 days per decade). This is at the moderate end of what other studies found. Previous herbarium studies conducted in the temperate zone, which included 28-186 herbaceous or woody species and covered 100-170 years of data, estimated flowering time shifts between -0.4 and -1.5 days per decade (Primack et al. 2004, Miller-Rushing et al. 2006, Panchen et al. 2012, Molnar et al. 2012, Bertin 2015, Bertin et al. 2017). All of these studies were geographically very restricted and, except for one study from Hungary (Molnar et al.

2012), all came from the Northeastern US. There have been other longer-term studies on phenology trends in Europe, but these were based on field observations, and they did not go back further than the 1970s. The trends reported in these obervational studies tend to be much stronger (-2.5 to -4.5 days per decade; (Fitter and Fitter 2002, Menzel et al. 2006), possibly indicating that phenological changes have been accelerating during the last decades in response to more rapid climate changes (European Environmental Agency 2020). Interestingly, while herbarium studies from temperate regions were all relatively consistent, studies from other climatic regions found very different results, e.g. weaker or no phenology shifts across >1700 species in the subtropical southeastern US (Park and Schwartz 2015), or much stronger phenology shifts in some Himalayan species (up to -9 days per decade; (Gaira et al. 2011, 2014). The stronger shifts in the Himalayas might at least be partly due to stronger climate changes at higher elevations, or due to greater temperature sensitivity of higher-elevation plants (see also discussion below).

Relationships with climate warming

The long-term changes in plant phenology we detected are likely responses to climate change, in particular rising spring temperatures, which were strongly associated with the average collection dates of our herbarium specimens. For each 1°C of temperature increase, plants were on average collected -3.6 days earlier. In Europe, land temperatures have increased around1.5°C since 1900 (Luterbacher et al. 2004, Harris et al. 2014, European Environmental Agency 2020), so the magnitude of phenological changes we observed is similar than what would be expected based on climate change and the observed temperature sensitivities (1.5°C x 3.6 days/°C = 5.4 days – vs our overserved shift of around 6 days). However, our results for temperature-phenology associations fit well to what others observed. Other herbarium studies from the temperate zone estimated flowering-time advancements of -2.4 to -6.3 days per 1°C temperature increase (Primack et al. 2004, Miller-Rushing et al. 2006, Panchen et al. 2012, Calinger et al. 2013, Hart et al. 2014, Bertin 2015, Davis et al. 2015, Bertin et al. 2017). Again, most of these studies were from the Northeastern US, and they were often geographically very restricted. Two previous herbarium studies from Europe found stronger shifts of -6 to -13 days per 1°C (Robbirt et al. 2011, Diskin et al. 2012), but both were based on a single species in a rather restricted geographic area. More robust European data comes from field observations: a long-term (1954-2000) observational study in England found advances of -1.7 to 6.0 days per 1°C across 385 plant species (Fitter and Fitter 2002), and a meta-analysis of long-term observation data found an

average advancement of plant phenology of 2.5 days per 1°C temperature increase (Fitter and Fitter 2002, Menzel et al. 2006). In a recent monitoring study of a subset of 16 of this study's species, we related plant phenology to the microclimates of forests and found a similar advancement of -4.5 days per 1°C temperature increase (Willems et al. 2021). So, the overall pattern of around 3-4 days earlier phenology per degree warming appears rather robust across a range of species and temperate regions, and our study strongly indicates that this large-scale biological response to anthropogenic climate change has also been taking place in Europe during the last century. As for the temporal shifts, our conclusions are restricted to temperate regions, as some studies from other climatic regions have found very different results, e.g. delayed rather than advanced flowering in response to increased spring temperatures in the subtropical southeastern US (Park and Schwartz 2015), or much stronger climate-related shifts in both directions in studies from Australia (Gallagher et al. 2009, Rawal et al. 2015). That plants also flower earlier with warmer winter temperatures suggests that their potential chilling-requirements are yet still fulfilled.

Other drivers of phenology variation

While temperature may be a key driver of phenology, it is not the only one, and often does not explain all observed phenology variation (Marchin et al. 2015, Piao et al. 2019). In our study, we found that, across the study area, plants flowered later at higher elevation, and this pattern remained significant even if temperature was included as explanatory variable. Thus, the later flowering at higher elevation must be more than a temperature effect, and it indicates that phenology advances are generally slower at higher altitudes. One explanation for this could be that plants at higher elevation are less sensitive to temperature changes (Vitasse et al. 2010, Dai et al. 2014). On the other hand, the residual spatial variation we observed in model B indicates that in some mountainous regions (especially the Alps) plants flowered earlier than expected (after accounting for all covariates) and therefore, on the contrary, might be more sensitive to temperature changes (Chapman 2013, Liu et al. 2014). A solution for this apparent contradiction could be that the relationship between elevation and phenology is non-linear or is confounded with other environmental variables. Several other studies that related phenology to altitude provide mixed results, from slower to faster phenology changes at high elevations (Defila and Clot 2005, Ziello et al. 2009, Čufar et al. 2012). Clearly, the relationship between elevation and phenology changes is not well understood yet, and large-scale herbarium plus climate data that

correct for spatial autocorrelation have the potential to shed more light on this and to help to understand how, when, where and for which species elevation influences phenology.

Besides temperature, another climate factor that could potentially influence plant phenology is precipitation. We had expected a significant interaction with temperature, with strongest phenology advances where both temperature and precipitation were increasing, but there was no evidence for preciptation-phenology relationships in our data at all. Previous research found that changes in rainfall and water availability can influence phenology but with substantial geographical differences, e.g. in Mediterranean forests and shrublands (Peñuelas et al. 2004). Another complication with precipitation effects on phenology is that if precipitation occurs as snow this may influence phenology in very different ways than rain fall. Increased snow fall often delays plant growth and flowering (Park and Mazer 2018), which is another potential explanation for why overall plants flowered later at higher elevations in our study. As global warming is expected to change snow melt more severely at higher elevations, it might have quite different effects on species at higher altitudes than on those at lower elevation (Cornelius et al. 2013), which in turn can cause problems for migrating or hibernating animal species across altitudinal gradients (Inouye et al. 2000).

Spatial variation in phenology

Spatial autocorrelation has so far been largely ignored in herbarium-based studies of long-term phenology changes. However, it is important to take spatial variation into account not only because herbarium data are generally strongly spatially clustered, but also because neither phenology nor phenological responses to climate change are expected to be spatially homogenous across larger geographic scales. For previous studies that were geographically very restricted (Bertin 1982, Primack et al. 2004, Miller-Rushing et al. 2006, Miller-Rushing and Primack 2008, Bertin et al. 2017), the problem may be minor, but larger-scale analyses will require to take spatial variation into account. Recently, Park and Mazer (2018) studied phenological shifts across several climatic zones and Park et al. (2019) explicitly tested for geographic differences in phenological sensitivities. To our knowledge, our study is the first herbarium-based study that modelled and mapped such spatial variation as a continuous variable in an analysis of large-scale phenology variation.

The best studied aspect of geographic variation of phenological responses is how they change with latitude (Chmura et al. 2019). We found that plants from Central Europe (especially around

the Alps) flowered earlier (after accounting for the effects of covariates, see Fig. 5). Such deviations could indicate that we are either missing an important driver, or that the responses to covariates differ geographically. Several previous studies found that phenology shifts (typically advances) more at high latitudes (Root et al. 2005, Parmesan 2007, Ge et al. 2015), likely because temperature has increased more in northern regions (IPCC 2014). However, larger relative shifts (e.g. a stronger advancement given the same temperature increases) are more often observed at lower latitudes, likely due to stronger temperature sensitivities (Dai et al. 2014, Shen et al. 2015, Ge et al. 2015, Wang et al. 2015a, 2015b, Park et al. 2019, but see Pudas et al. 2008, Wolkovich et al. 2012, Dai et al. 2014). Such differences can result from adaptation to local conditions that cause genetic differentiation between populations along a latitudinal gradient with respect to flowering time (Riihimäki and Savolainen 2004). However, there are two opposing hypotheses about the way temperature sensitivity changes with latitude. The first argues that temperature sensitivities are higher at lower latitudes, because plants from colder, high-latitude regions with generally variable (less reliable) climates developed more conservative, (late frost) risk-avoiding responses, by relying more on photoperiod and higher phenological thresholds to temperature (Renner and Zohner 2018). Due to the other hypotheses, plants from northern ecosystems are more sensitive to temperature and require less warming to trigger leaf-out or flowering (Riihimäki and Savolainen 2004, Liang and Schwartz 2014, Prevéy et al. 2017), ensuring that plants start growing as soon as growth conditions become good in early spring, which is crucial in cold regions with short growing seasons. The missing consensus among studies about the association between latitude and phenological shift may be partially due to differences in spatial scale and because their relationship is complex, confounded with other environmental factors such as elevation or non-linear. Phenological sensitivity to temperature could for example decrease from southern to mid-northern latitudes but increase again in far-northern regions. This could explain, why we found no consistent trend with latitude. Furthermore, because temperature and latitude are usually highly correlated it can be difficult to disentangle their effects on phenology. These are challenges, that can be tackled by investigating geographic patterns via a continuous spatial field (as we did here, using R-INLA), that can depict differentiated geographic variability of phenology.

Conclusions

The flowering time of forest herbs in Europe has substantially advanced during the last century, and these advances are strongly associated with climate warming. Our study demonstrates how herbarium specimens can be used to expand not only the temporal but also geographic and taxonomic scope of phenology research, and to contribute to understanding global environmental change (Wolkovich et al. 2014). Herbarium data from large geographic ranges are particularly powerful but they also come with challenges, and we showed that accounting for spatial autocorrelation significantly improved model fits and parameter estimates. Future studies should more frequently employ such spatial modeling techniques when analysing large-scale phenology variation and its different drivers, ideally across multiple climatic regions (Park et al. 2019). The long-term phenology changes we observed in our study reflect physiological responses to climate warming, i.e. plants have adjusted to ongoing climate change (Munguía-Rosas et al. 2011). While this may to some extent be considered good news, the phenological shifts can have further consequences for the species and their associated ecological communities. For individual plant species, phenology shifts could be detrimental e.g. if they do not track warming temperatures well enough (Willis et al. 2008, Munguía-Rosas et al. 2011) or if earlier leaf-out or flowering increases the risk of late-frost damage (Wipf et al. 2006, Inouye 2008, Zohner et al. 2020). In addition, if climate change affects plants and their interacting organisms, such as pollinators or herbivores, unequally, then the phenology shifts of plants could result in temporal "mismatches" between the interacting organisms (Renner and Zohner 2018). Finally, changes in plant phenology also influence ecosystem functions such as productivity or carbon cycling (Menzel et al. 2006, Cleland et al. 2007, Piao et al. 2019). Unterstanding not only phenology changes but also their further consequences for communities and ecosystems is an important goal for future research.

Acknowledgements

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Supplementary material

Table S1. The 20 studied forest understories herbs, their respective families and the number of herbarium specimens of each species that were included in the analyses.

Species	Family	N
Adoxa moschatellina	Adoxaceae	415
Alliaria petiolata	Brassicaceae	252
Allium ursinum	Amaryllidaceae	206
Anemone nemorosa	Ranunculaceae	661
Anemone ranunculoides	Ranunculaceae	165
Arum maculatum	Araceae	46
Cardamine bulbifera	Brassicaceae	272
Euphorbia amygdaloides	Euphorbiaceae	93
Ficaria verna	Ranunculaceae	445
Galium odoratum	Rubiaceae	295
Lathyrus vernus	Fabaceae	343
Mercurialis perennis	Euphorbiaceae	349
Oxalis acetosella	Oxalidaceae	85
Paris quadrifolia	Melanthiaceae	409
Polygonatum verticillatum	Asparagaceae	64
Primula elatior	Primulaceae	193
Pulmonaria obscura	Boraginaceae	460
Ranunculus auricomus agg.	Ranunculaceae	330
Stellaria holostea	Caryophyllaceae	448
Viola reichenbachiana	Violaceae	600
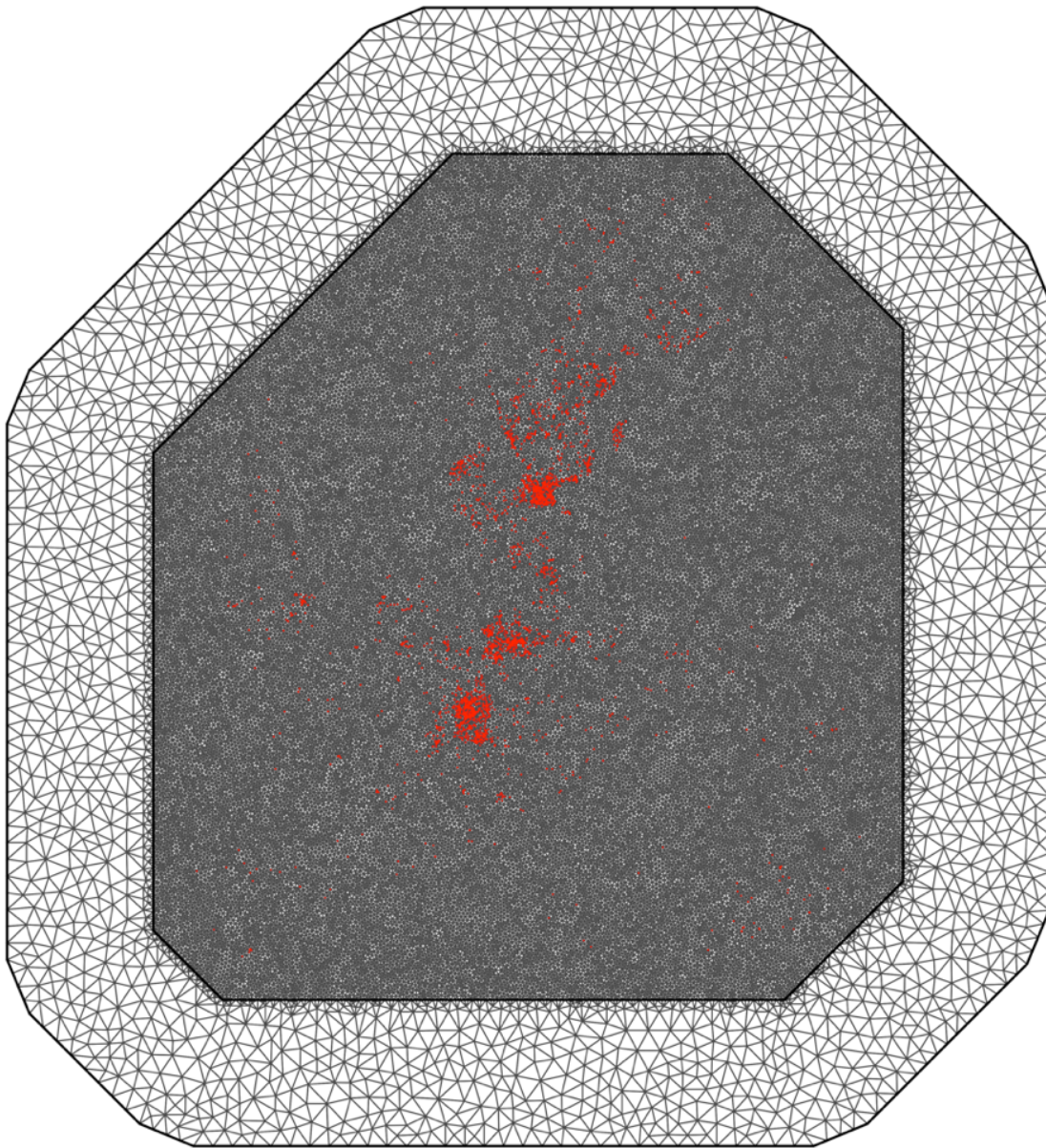
Figure S1. The mesh – based on refined Delaunay triangulation – used to estimate spatial autocorrelation of herbarium specimen data across Europe. The covariance matrix is estimated only for the inner area (see Methods section), and the outer area is a buffer zone against potential boundary effects. The sampling locations (red dots) are projected from latitude/longitude values onto the UTM coordinate system.
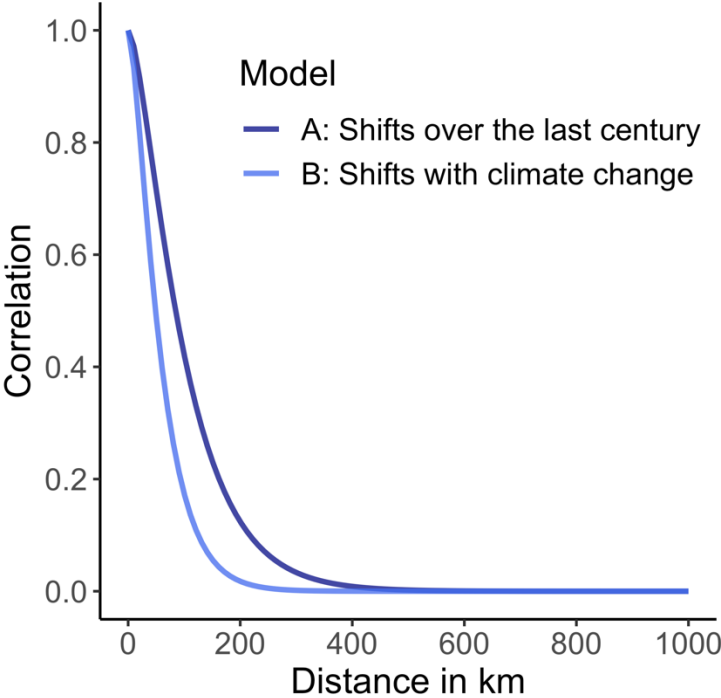
Figure S2. The spatial correlation of flowering time data in models A and model B (after the effect of the covariates have been accounted for).
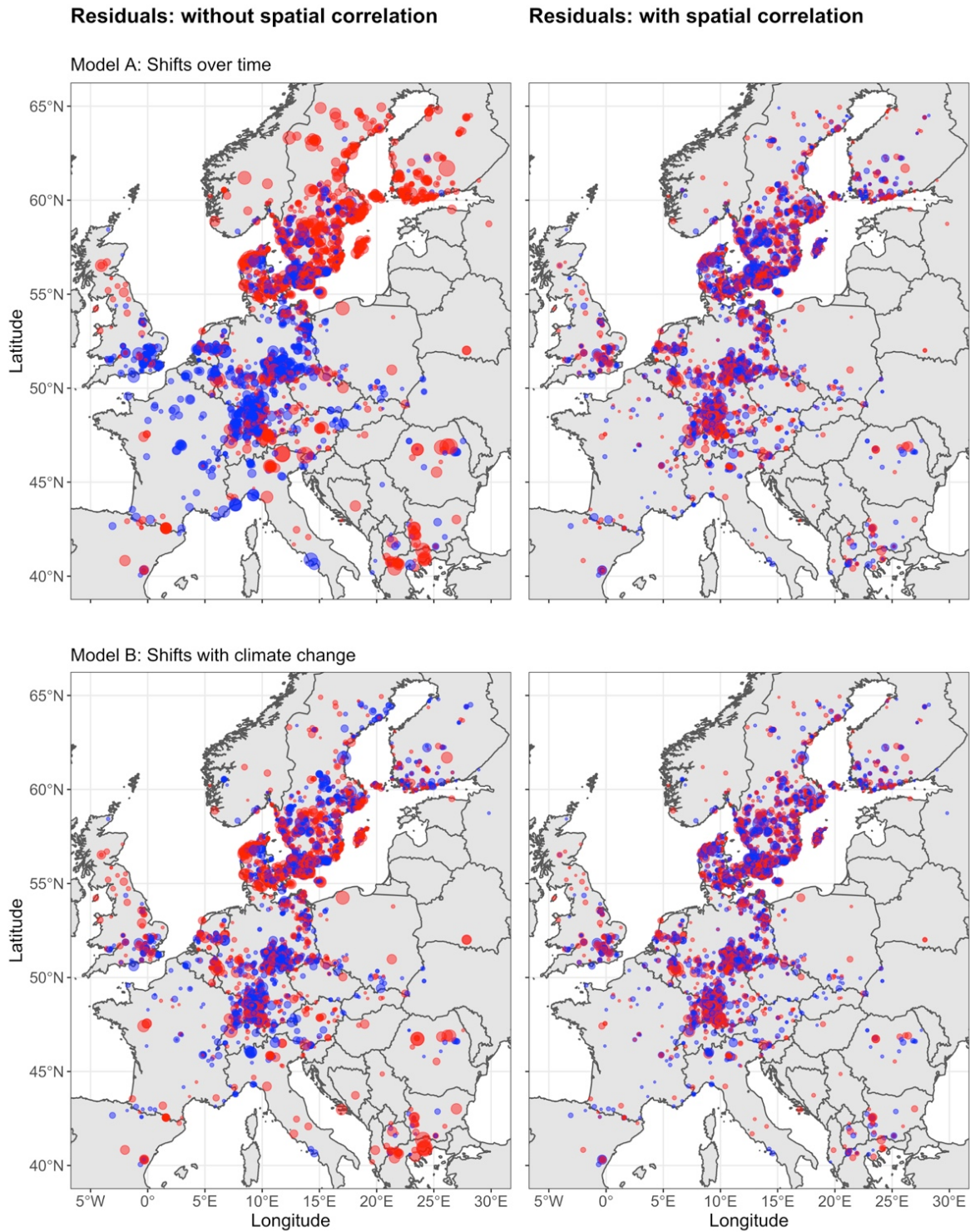
Figure S3. Spatial patterns in the residuals of the model A (top panels) and model B (bottom panels), without (left) and with (right) accounting for spatial correlation in the models. Larger points indicate larger residuals and point color indicates whether residuals were negative (red) or positive (blue).

Model Validation Check for Model A: Phenological shifts over the last century
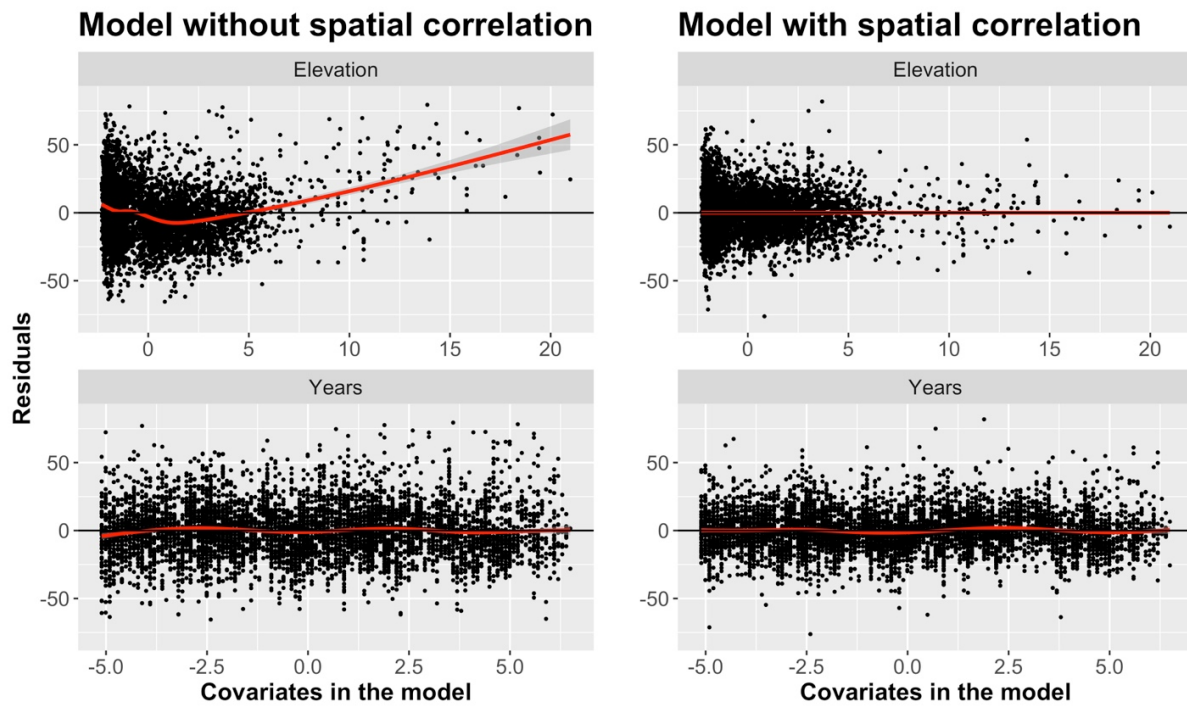
Figure S4. Residuals plotted against each covariate in model A without (left) and with spatial correlation (right). The red lines are thin plate regression spline smoothers, fitted with the *mgcv* package (Wood 2006), with 95% confidence intervals in gray, to aid visual interpretation.
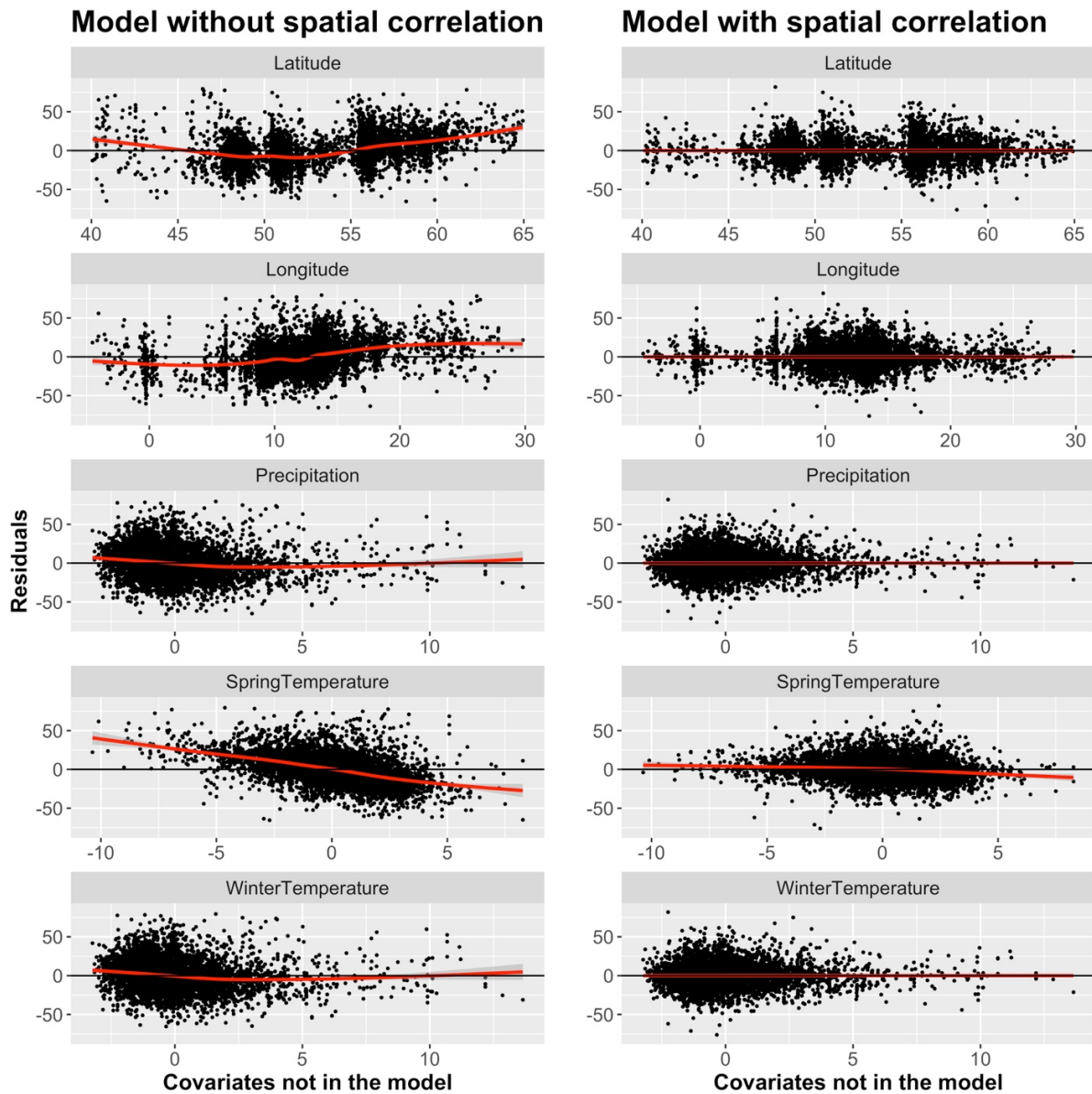
Figure S5. Residuals of Model A plotted against covariate that were not included in model A, without (left) and with spatial correlation (right). The red lines are thin plate regression spline smoothers, fitted with the *mgcv* package (Wood 2006), with 95% confidence intervals in gray, to aid visual interpretation.
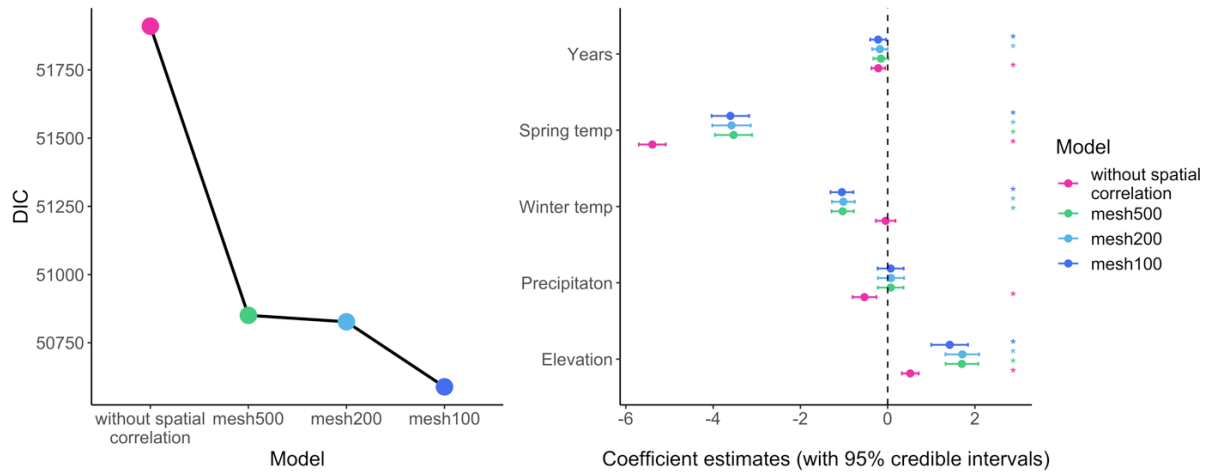
Figure S6. DIC values and model estimates (regression coefficients) of model B without spatial correlation and with spatial correlation, using different mesh sizes. For our analyses with spatial correlation we chose the finest mesh100 (number indicates the assumed range of spatial correlation).
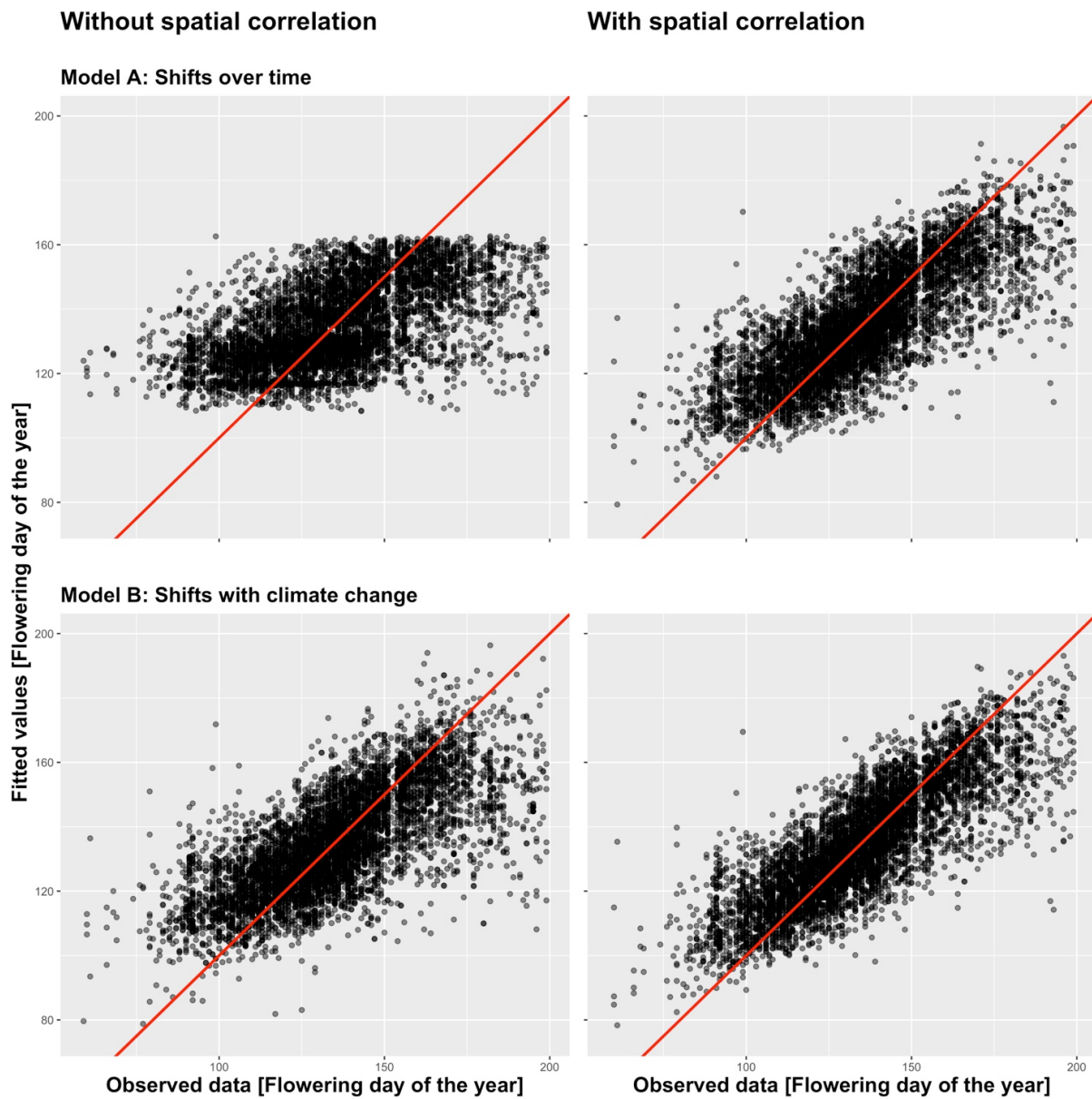
Figure S7. Observed vs. fitted values for model A (top) and model B (bottom), without (left) and with (right) spatial correlation. The red diagonal is the identity line.
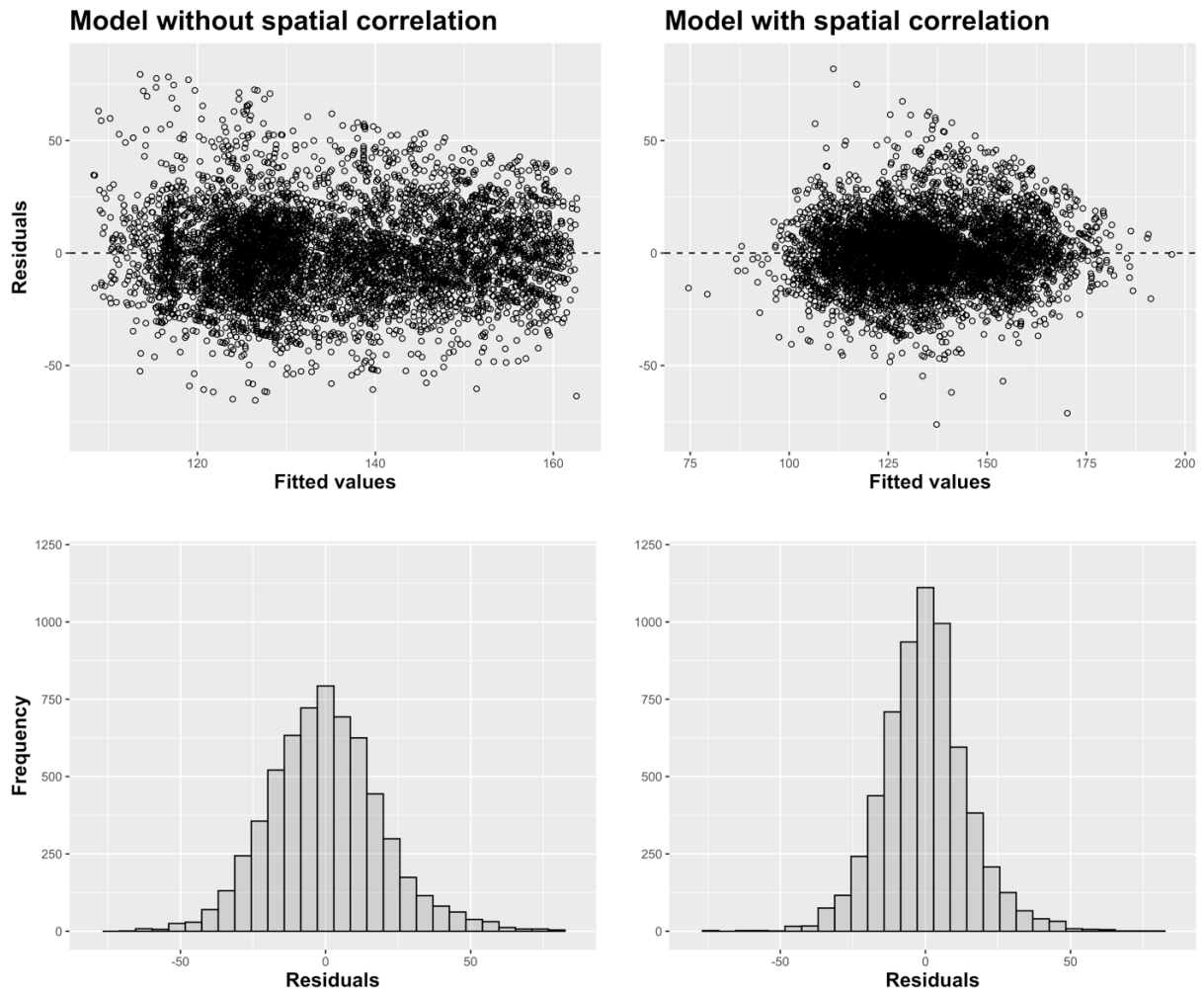
Figure S8: Residuals vs. fitted values (top) and histogram of the residuals (bottom) of model A without (left) and with spatial correlation (right). Residuals and fitted values of model B showed similar patterns.
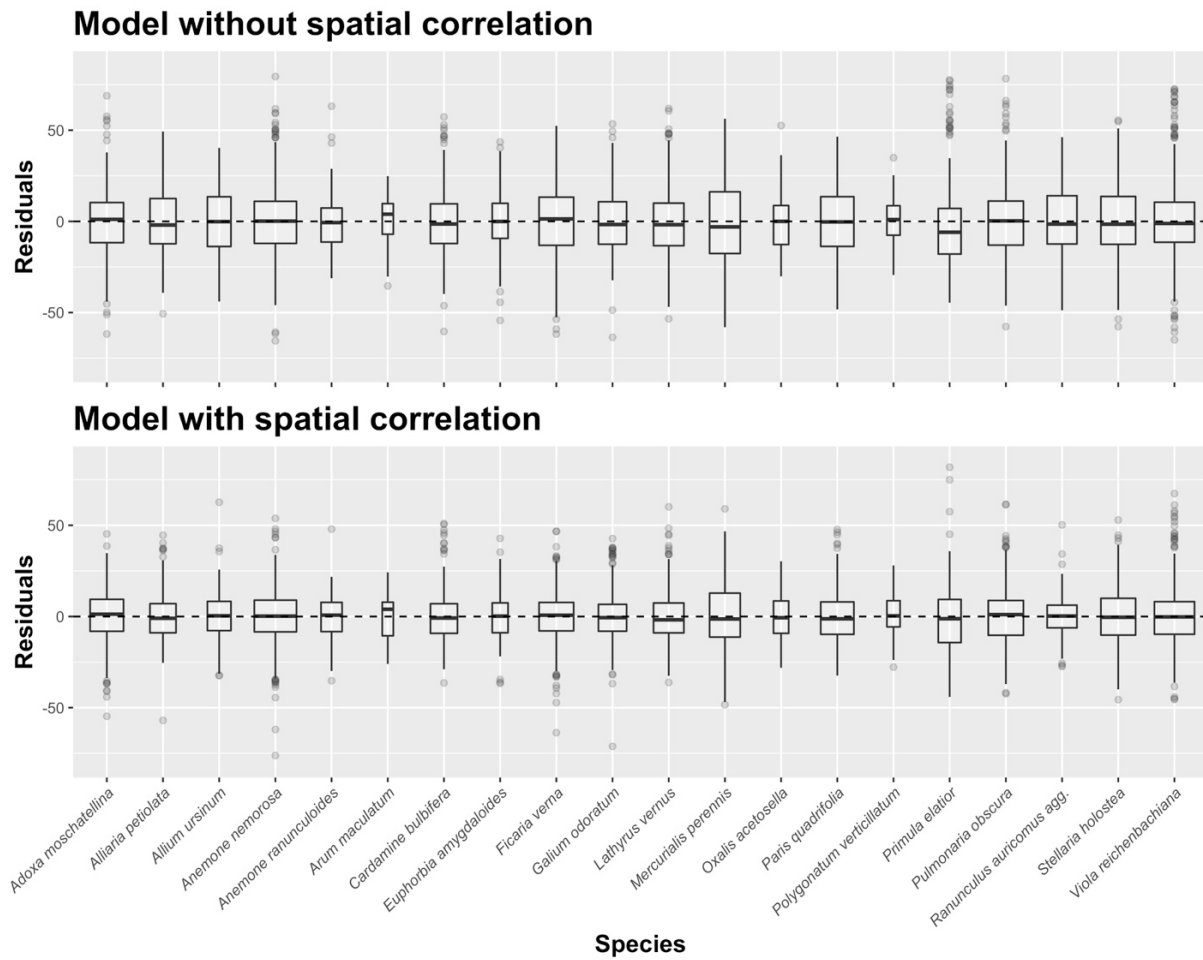
Figure S9. Residuals (of model A) for each species (that were included as a random factor) without (top) and with spatial correlation (bottom). Box widths are proportional to sample size.

Table S2. Estimates, standard deviations and 95% credible intervals for the hyperparameter values in model A with spatial correlation. RE = random effect.

	Estimate	SD	95% CI
Precision for the Gaussian observations	4.27E-03	1.00E-04	4.09E-03, 4.48E-03
Precision for RE species intercepts	8.47E-03	3.99E-03	3.72E-03, 1.88E-02
Precision for RE species slopes	1.83E+04	1.93E+04	2.07E+03, 6.91E+04
Range for spatial RE [km]	214.07	64.94	92.06, 331.46
SD for spatial RE [m]	1.88	1.89	14.8, 22.11

Table S3. Model estimates, standard deviations and 95% credible intervals for the parameters in model A without spatial correlation. RE = random effect.

	Estimate	SD	95% CI
Intercept	135.10	2.55	130.09, 140.10
Years [Decades]	-1.43	0.09	-1.60, -1.25
Elevation [100 m]	-0.34	0.10	-0.53, -0.14
Precision for the Gaussian observations	2.56E-03	4.07E-05	2.49E-03, 2.65E-03
Precision for RE species intercepts	7.86E-03	2.98E-04	7.29E-03, 8.48E-03
Precision for RE species slopes	1.58E+04	5.41E+02	1.48E+04, 1.70E+04

Table S4. Estimates, standard deviation and 95% credible intervals for the parameters and hyperparameters in the model B version without spatial correlation. RE = random effect.

	Estimate	SD	95% CI
Intercept	135.90	2.50	130.94, 140.77
Spring Temperature [°C]	-0.21	0.08	-0.37, -0.06
Winter Temperature [°C]	-5.39	0.16	-5.70, -5.09
Precipitation [mm/10]	-0.53	0.14	-0.81, -0.26
Elevation [100 m]	0.52	0.10	0.33, 0.71
Years [Decade]	-0.05	0.11	-0.27, 0.18
SpTemp:Year	0.06	0.04	-0.01, 0.13
SpTemp:Elevation	0.13	0.04	0.06, 0.20
SpTemp:Precipitation	-0.04	0.05	-0.15, 0.06
SpTemp:WinterTemperature	-0.07	0.02	-0.12, -0.02
Precision for the Gaussian observations	4.0e-03	<0.001	4.00e-03, 4.00e-03
Precision for RE species intercepts	7.0e-03	2.00e-03	4.00e-03, 1.10e-03
Precision for RE species slopes	4.8e+04	1.52e+04	2.56e+04, 8.45e+04

Table S5. Estimates, standard deviations and 95% credible intervals for the hyperparameter values in model B with spatial correlation. RE = random effect.

	Estimate	SD	95% CI
Precision for the Gaussian observations	0.0047	0.0001	0.0046, 0.0048
Precision for RE species intercepts	0.0089	0.0033	0.0043, 0.0170
Precision for RE species slopes	481.9	361.4	76.5, 1414.8
Range for spatial RE [km]	113.61	20.64	75.13, 162.53
SD for spatial RE [m]	16.23	1.23	13.70, 18.94

Chapter IV

Spring understory herbs flower later in intensively managed forests

Franziska M. Willems, J.F. Scheepens, Christian Ammer, Svenja Block, Anna Bucharova, Peter Schall, Melissa Sehrt and Oliver Bossdorf

Abstract

Many organisms respond to anthropogenic environmental change through shifts in their phenology. In plants, flowering is largely driven by temperature, and therefore affected by climate change. However, on smaller scales climatic conditions are also influenced by other factors, including habitat structure. A group of plants with a particularly distinct phenology are the understorey herbs in temperate European forests. In these forests, management alters tree species composition (often replacing deciduous with coniferous species) and homogenizes stand structure, and as a consequence changes light conditions and microclimate. Forest management should thus also affect the phenology of understorey herbs. To test this, we recorded the flowering phenology of 16 early-flowering herbs on 100 forest plots varying in management intensity, from near-natural to intensely managed forests, in Central and Southern Germany. We found that in forest stands with a high management intensity, such as Norway spruce plantations, the plants flowered on average about two weeks later than in unmanaged forests. This was largely because management also affected microclimate (e.g. spring temperatures of 5.9 °C in managed coniferous, 6.7 in managed deciduous and 7.0 °C in unmanaged deciduous plots), which in turn affected phenology, with plants flowering later on colder and moister forest stands (+4.5 days per -1°C and 2.7 days per 10 % humidity increase). Among forest characteristics, the percentage of conifers had the greatest influence on microclimate, but also the age, overall crown projection area, structural complexity and spatial distribution of the forest stands. Our study demonstrates that forest management alters plant phenology, with potential far-reaching consequences for the ecology and evolution of understorey communities. More generally, our study suggests that besides climate change other drivers of environmental change, too, can influence the phenology of organisms.

Key Words: Climate change, forest structure, global change, land-use change, microclimate, phenological shifts, structural equation modelling, temperature

Introduction

Phenology is the study of the timing of recurrent biological events, the biotic and abiotic drivers of this timing, and its variation within and among species (Lieth, 1974). It includes the seasonal timing of key life events, such as animal migration or reproduction, or the leaf-out, flowering and fruiting of plants, which are important for individual fitness. In plants, many phenological events are triggered by abiotic environmental factors, especially temperature, and are therefore sensitive to climate change (Schwartz, Ahas, & Aasa, 2006; Tang et al., 2016). Long-term observational studies have found earlier leaf-out and changes in the start of flowering associated with climate change across the world (Fitter and Fitter, 2002; Schwartz et al., 2006). Spring-flowering plants seem to be particularly responsive to climate change and often show the largest phenological shifts (Chmielewski, Müller, & Bruns, 2004; Fitter and Fitter, 2002; Renner & Zohner, 2018).

Plants play a key role in many ecosystems, and they interact with many other species. Therefore shifts in plant phenology can have significant consequences for pollinators, food webs, agricultural yields, as well as many ecosystem functions and services such as productivity and carbon cycling (Chmielewski et al., 2004; Cleland et al., 2007; Reilly et al., 1996; Tang et al., 2016). Understanding the drivers of phenology variation is thus important to predict future states of species abundance and distribution, biogeochemistry and ecosystem productivity, as well as ecosystem services such as pollination (Chuine, 2010; Durant et al., 2005; Høye, Post, Eric, Schmidt, Trøjelsgaard, & Forchhammer, 2013; Kharouba et al., 2018; McKinney et al., 2012; Memmott, Craze, Waser, & Price, 2007; Richardson et al., 2010), and it should also help to inform environmental conservation (Cerdeira Morellato et al., 2016) and to develop adaptive management strategies in a changing world (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012; Enquist, Kellermann, Gerst, & Miller-Rushing, 2014; Pacifici et al., 2015; Walther, 2010).

However, our mechanistic understanding of the impact of environmental change on plant phenology is still limited (Richardson et al., 2012). In particular, besides climate warming, the influences of other global change drivers – such as land use change – on plant phenology have received little attention. Climatic cues that have been shown to influence phenology can be influenced by topography or forest cover at small spatial scales (Geiger, Aron and Todhunter, 2003). As a consequence, microclimates can differ from regional climate patterns and affect the timing of phenological events on small spatial scales (Hwang et al., 2011; Ward, Schulze, and Roy, 2018). Forest understorey microclimates are often buffered against extreme heat or cold and macroclimatic warming (De Frenne et al., 2013,

2019; Zellweger et al., 2019). Valdés et al. (2015) showed that in European forests plant diversity depends more on forest habitat features than on landscape structure or microclimate. Within forests, differences in stand structure affect the microclimate and light availability (Chen et al., 1999; Baker et al., 2014; Ehbrecht et al.,2019) and is thus likely to impact flowering phenology of understory herbs. Forest stand structure can be defined as the distribution of trees in space and their variability in size, arrangement, consistency and time (Schall et al., 2018). Stand structure can, for example, be characterized by the main tree species, the ages of trees, the mean and variation in diameter at breast height, the basal area covered, or their crown projection area (Schall et al., 2018). Furthermore, stand structural complexity indices (SCI, see e.g. Zenner and Hibbs (2000)) can combine several structural attributes (Gossner et al., 2014; del Río et al., 2016) or take the spatial distribution of trees into account (Ehbrecht, Schall, Ammer and Seidel 2017; Penttinen, Stoyan, and Henttonen, 1992). Zellweger at al. (2019) showed that changes in canopy cover and composition change understory temperature in temperate forest across Europe.

Changes in forest management alter stand structure in temperate forests and, as a consequence, microclimate conditions. While thinnings and selection cuttings lead to only small increases of radiation at the forest floor (Aussenac 2000; Hale, 2003), clear-cuttings radically reduce the canopy cover and thus result in drastic and persistent changes of the microclimate. In deciduous forests, there is a time window during spring when the leaf-out of trees is not yet completed that allows early spring-flowering species to take full advantage of the available sunlight, moisture and nutrients of the forest floor (Lapointe, 2001). Planting of evergreen coniferous trees – such as Norway spruce (*Picea abies* (L.) H. Karst)*,* one of the most economically important tree species in Europe (Spiecker, 2003) – reduces the light availability during early spring and changes microclimatic conditions. Furthermore, Ehbrecht et al. (2017) showed that structural complexity was higher in even-aged, mature European beech stands and uneven-aged, single-tree selection systems than in coniferous stands. Actually, structural complexity of coniferous stands increased linearly with increasing proportion of broad-leaved tree species such as European beech (Juchheim et al. 2020). More generally, all management changes that alter tree species composition and stand structure are likely to also affect the phenology of forest understory herbs through changes in radiation, microclimate or other factors. Because of their narrow and distinct flowering period, spring-flowering forest herbs should thus be particularly susceptible to management changes, and therefore they are a particularly relevant study system for exploring forest management effects on plant phenology.

Here, we hypothesized that forest management would change forest structure and thereby microclimatic conditions and, as a consequence, flowering phenology. To investigate this, we studied the phenology of 16 early-flowering forest herb species on 100 forest plots of different management type and intensity. The studied forest plots ranged from protected unmanaged forests and extensively managed selection system forests to managed deciduous age-class forests (from young thickets to mature timber plots) and intensively managed Norway spruce plantations. For each study plot, we obtained detailed phenology, forest structure and microclimate data. We first looked at the overall effect that management intensity had on phenology, and we then analysed the effects of different individual microclimatic and forest structural traits on phenology. Finally, we used structural equation modelling to get a better understanding of the possible underlying causal relations, and to disentangle direct and indirect effects of forest characteristics and microclimatic variation on plant phenology. We expect unmanaged, old and structurally complex deciduous forests to have sheltered, warmer microclimatic conditions during spring than age-class forests (especially young thickets which have a drastically reduced crown projection area of remaining mature trees) or the highly managed and homogenous spruce plantations. Thus, we generally expected understory plant species to flower later on more disturbed and more intensively managed forests. Specifically, we asked the following questions: (i) Does forest management intensity affect plant phenology? (ii) Which forest characteristics are the strongest drivers of phenological variation? And (iii) to what extent does forest management affect phenology through changing microclimate?

Methods

Study system

Most forests in Central Europe have a rather low tree diversity and are dominated by only few deciduous tree species (Schulze et al., 2016). Therefore, variation in stand composition is to a substantial degree related to the effects of forest management (Schall et al., 2018). We studied the forest plots of the Biodiversity Exploratories project (www.biodiversity-exploratories.de) in Germany, a large-scale platform for ecological research that includes a broad range of forests plots of different management types and intensities (Fischer et al. 2010). We focused on 100 forest plots (100 × 100 m) located in equal parts in two of the three regions of the Biodiversity Exploratories, the Schwäbische Alb in Southwest Germany (long: 9.39°, lat: 48.44°) and the Hainich-Dün in Central Germany (long: 10.47°, lat: 51.16°). The elevation a.s.l. ranges from 285–550 m in the Hainich-Dün area to 460–860 m on the Schwäbische Alb.

Further details on the characteristics of the regions are provided in Fischer et al. (2010). The forests in the study areas are dominated by native deciduous trees, mainly European beech (*Fagus sylvatica* L.). However, decades ago some forests had been converted to plantations of Norway spruce (*Picea abies*), a coniferous species originally restricted to montane and subalpine regions, but cultivated for timber in the lowlands since 250 years (see Fig. 1 & Schall et al., 2018).

Phenological monitoring

From March to June 2017, we monitored the phenology of 20 early-flowering herbs in the understories of our study plots (Appendix S1: Table S1). The monitored species included all common spring-flowering herbs in the plots. For all further analyses, we only considered the 16 species that were flowering on at least 10 plots (see Fig. 2). Most of the studied species (12 out of 16) grew on both deciduous and coniferous forest plots. We visited all 100 forest plots once per week and monitored the phenology of all plants within a 3 m wide strip outside the 20×20 m core area of each plot, corresponding to an area of 224 m^2 within each 1 ha plot. For each species in each plot, we recorded flowering start as the day of the year with the first fully open flower, and flowering end as the time when no fully open flowers could be found anymore. To be able to determine flowering peaks, we counted the number of open inflorescences or, if plants were abundant on a plot, we estimated the percentages of flowering individuals. We then defined the day of the year with the highest number or percentage of open inflorescences as the day of flowering peak. If there were two days with equal maximum flowering, we used their median as the time of peak flowering. If it was apparent during a weekly visit of a plot that a start, peak or end of flowering had been well between the present and past visit, we dated this record back between the two visits, resulting in an effective half-weekly resolution of our data. For an overview of the overall and species specific number of plots and data points per region and forest type see Appendix S1:Table S2.

Figure 1: Impressions from the study plots. Top: beech plot at the Hainich-Dün. Bottom: spruce plot at the Schwäbische Alb. Both photos were taken in April 2017

Figure 2: The 16 early-flowering forest understorey species included in our phenology monitoring. For each species, the number of plots with flowering individuals is indicated in the bottom right corner.

Forest characteristics

The structure of the studied forests is strongly influenced by management, and it can be characterized by differing forest attributes. The required data have been collected in two forest inventories that were conducted on the forest plots of the Biodiversity Exploratories at single-tree level for all living trees with a diameter at breast height ≥ 7 cm. We used the data from the most recent inventory (2014-2016). Specifically, we used the following individual variables: main tree species (deciduous vs. coniferous), the mean age of the main tree species, the richness and diversity (inverse Simpson's index) of tree species, crown projection area of mature trees, the share of conifers based on crown projection, stand density, the mean

diameter at breast height and its standard deviation, and the basal area covered with trees. Furthermore, we used Morisita's index of dispersion as well as Clapham's variance mean ratio as measures of horizontal heterogeneity (for both <1: regular, >1: clumping, 1: random; 20 m × 20 m raster cells), and Zenner's Structural Complexity Index based on tree height as a proxy for vertical structural complexity (Zenner, 1998). We selected these variables because they characterize stand structure, and we expected them to have an influence on microclimatic conditions as well as on light availability and other abiotic and biotic factors.

In addition to these individual forest variables, we also used a synthetic index for silvicultural management intensity (SMI) developed by Schall and Ammer (2013). This index combines data on tree species, stand age and stand biomass into a quantitative measure of forest management intensity. The main idea of the SMI index is that it has a component related to tree density (SMI_d) based on the discrepancy between potential and actual basal area, i.e. how far away a forest stand is from equilibrium biomass, and a component related to "risk" (SMI_r) based on stand age and tree species, which determine the susceptibility of a stand to natural disturbances and thus the need to manage such stands more intensively. A recent study by Gossner et al. (2015) demonstrated that SMI is indeed strongly negatively correlated with other estimates of forest "naturalness". For more details on the SMI index please see Schall and Ammer (2013), and for an overview of all forest characteristics used in our study see Table 1.

Microclimate and other environmental data

Besides the data on forest structure, there is detailed information on local microclimate available for all plots in the Biodiversity Exploratories (Fischer et al., 2010). On every plot, air temperature is measured at 10 cm above ground as well as several depths below ground using a multi-layer temperature sensor (Meier-NT GmbH, Zwönitz, Germany), air temperature and air humidity at 2 m above ground using a Mela KPC1/5-ME sensor (MELA Sensortechnik GmbH, Mohlsdorf-Teichwolframsdorf, Germany), and soil moisture at 10 cm below ground using a DeltaT ML2X soil humidity probe (Delta-T Devices Ltd, Cambridge, UK). All data were taken at hourly intervals and stored on an ADL-MX datalogger system (Meier-NT GmbH, Zwönitz, Germany). To be able to test for relationships between microclimate, forest management and phenology, we compiled data for two different potentially relevant time periods, the spring months during which our phenology monitoring took place, and the preceding winter months. For the spring months (February–May 2017), we calculated the average air temperature at 10 cm and 2 m above ground, the growing days

(=days with mean temperatures between 10°C to 30°C), the growth sum (= sum of mean day temperatures > 5°C (minus 5)), the warm sum (= sum of mean day temperatures with > 10°C (minus 10)), mean relative air humidity (measured at 2 m), as well as mean soil moisture and soil temperature (both measured at 10 cm depth). For the winter months (October 2016 - January 2017), we also calculated the mean air temperature (measured at 2 m height and 10 cm height), the number of cold days (= days with a temperature minimum < 0°C), the cold sum (= sum of mean day temperatures < 0°C), the number of cool days (= days with a temperature maximum < 10°C), the number of ice days (= days with a temperature maximum < 0°C), mean relative air humidity (measured at 2 m), as well as mean soil moisture and soil temperature (both measured at 10 cm depth).

In addition to the microclimate data, we also included several geographical variables that we expected to influence abiotic conditions at the stand level, such as region and a slope variable – calculated by multiplying inclination (in degrees; average over the plot area) by 1 for south-, -1 for north-, and 0.5 for east- and west-facing slopes, to be able to distinguish slopes in the four cardinal directions which are known to differ in their microclimatic conditions (Dahlgren, Zeipel, and Ehrlén, 2007). Elevation above sea level is confounded with region and therefore not included as an explanatory variable. For an overview of all explanatory variables, see Table 1.

Data analysis

Our data analyses following a three-step logic. First, to get an overview of the overall effect forest management intensity had on flowering time we employed a linear mixed effect model, pooling all data, with silvicultural management intensity (SMI) as the explanatory variable and species as random factor. Second, we used univariate linear regression to test the effects of forest management intensity, as well as individual forest characteristics and microclimatic variables on flowering time for each species separately. Third, we selected a subset of these variables for structural equation modelling, to understand the relationships between forest characteristics and microclimate, and disentangle direct and indirect effects on plant phenology. Prior to the data analyses, we checked all variables for outliers, and if outliers clearly resulted from measurement errors, we removed them from our data set. Such outliers were generally very rare; we removed only a few data points from three different plots. For the statistical analyses we excluded four (out of the original 20 species, see Appendix S1: Table S1) species that were flowering on less than ten plots (*Adoxa moschatellina* L. and *Euphorbia amygdaloides* L., *Polygonatum verticillatum* L. and *Pulmonaria obscura* Dumort).

Table 1: Overview of all explanatory variables used in our study. Variables in bold were included in the structural equation model (SEM).

Geographic variables	
Slope	Inclination multiplied by 1 for south-, -1 for north-, and 0.5 for east- and west-facing slopes
Region	Schwäbische Alb vs. Hainich-Dün
Forest variables	
Age [years]	Mean age of the main tree species
Basal area [m^2 ha^{-1}]	Total basal area covered by trees
Clapham's variance mean ratio	A measure of horizontal dispersion, < 1: regular, ≈ 1: random, > 1: clumping
Crown projection area [m^2 ha^{-1}]	Cumulative crown projection area of trees
Coniferous basal area [%]	Percentage of conifers based on basal area
Coniferous crown projection [%]	Share of conifers based on crown projection area
Diameter at breast height [cm]	Mean diameter at breast height
Standard deviation of DBH [cm]	Standard deviation of the diameters at breast height of trees
Main tree species	Main tree species: deciduous vs. coniferous
Morisita's index of dispersion	Horizontal dispersion, < 1: regular, ≈ 1: random, > 1: clumping
Silvicultural Management Intensity (SMI)	Synthetic index of forest management intensity developed by Schall and Ammer (2013), 0 = lowest to 1 = highest
Species diversity	Species richness = number of tree species
Species diversity 2D	Tree species diversity based on abundance = inverse Simpson's index
Stand density [trees ha^{-1}]	Total number of trees
Structural Complexity [m^2 m^{-2}]	Zenner's structural complexity index based on tree height. A proxy for vertical structural complexity
Microclimatic variables Spring (Feb-May)	
Air temperature 2 m [°C]	Air temperature measured at 2 m above ground
Air temperature 10 cm [°C]	Air temperature measured at 10 cm above ground
Growing days	Number of days with temperatures between 10°C and 30°C
Growth sum	Sum of mean day temperatures > 5°C (minus 5)
Warm sum	Sum of mean day temperatures > 10°C (minus 10)
Soil temperature [°C]	Soil temperature at 10 cm below surface
Relative air humidity [%]	Mean relative air humidity measured 2 m above ground
Soil moisture [%]	Soil moisture at 10 cm below surface
Microclimatic variables Winter (Oct - Jan)	
Air temperature 2 m [°C]	Air temperature measured 2 m above ground
Air temperature 10 cm [°C]	Air temperature measured 10 cm above ground
Ice days	Number of days with a temperature maximum < 0°C
Cold days	Number of days with a temperature minimum < 0°C
Cold sum	The sum of days with a mean day temperature < 0 °C
Cool days	Number of days with a temperature maximum < 10°C
Soil temperature [°C]	Soil temperature at 10 cm below surface
Soil moisture [%]	Soil moisture at 10 cm below surface
Relative air humidity [%]	Mean relative air humidity measured 2 m above ground

Using linear regression analyses, we calculated R^2-values, standardized regression coefficients and P-values (corrected for multiple testing using false discovery rate (FDR)) for the relationships between each forest trait and microclimatic variable and the phenology of each studied species. We used these results to make an informed preselection of variables for the subsequent structural equation modelling (see next section), since especially the microclimatic variables included several temperature proxies with high levels of collinearity. All data analyses were conducted using R (R Core Team, 2018). Standardized regression coefficients were derived using the *QuantPsyc* package (Fletcher, 2012).

Next, we conducted confirmatory path analysis across all species based on piecewise fitting of component hierarchical linear mixed-effects models (Lefcheck, 2016; Shipley, 2009). Path analysis or structural equation modelling is a powerful, multivariate technique used increasingly in ecology to evaluate complex multivariate causal relationships, particularly with observational data that often includes substantial collinearity. Structural equation models (SEMs) differ from many other modelling approaches as they test the direct and indirect effects in pre-assumed causal relationships (Fan et al. 2016). In our analysis we used the *piecewiseSEM* package (Lefcheck, 2016). In *piecewise SEM*, each set of relationships is estimated independently (or 'locally'). For each response variable, the process decomposes the network into the corresponding simple or multiple linear regressions, which are evaluated separately, and then re-combined afterwards to draw conclusions about the full model (Lefcheck, 2016). The relationships between variables can then be visualized through path diagrams where arrows denote which variables are influencing (and are influenced by) other variables.

Prior to our path analyses we checked for additivity and linearity of individual variables. We used correlation matrices (Appendix S1: Fig. S1) and variance inflation factors (with a cut-off value of 4) to check for collinearity among the explanatory variables, to avoid inclusion of highly correlated variables, and we used simple regression plots to confirm linearity. Furthermore, to check the statistical assumptions of linear models – normality and homogeneity of residuals – we visually inspected histograms of the standardized residuals, Q-Q-Plots and residual scatter plots, as well as calculations of skewness and kurtosis. The skewness and kurtosis values were all within the guidelines set by Kline (2015) and also below the more conservative threshold set by Ryu (2011).

The subset of forest characteristics that we included in the SEM, after checking for collinearity, were: crown projection area, variance mean ratio, structural complexity index, the diameter at breast height and its standard deviation, and the percentage of coniferous trees.

We selected diameter at breast height as an explanatory variable over tree age and density because it was the best proxy for the developmental stage of a forest. After the exclusion of highly correlated variables and based on the simple linear regressions results (considering average r^2-values and standardized regression coefficients), mean spring air temperature and spring relative air humidity (both measured at 2 m above ground) were the only microclimatic variables we included in the SEM. Because other geographical or environmental factors might also influence plant phenology, we additionally included slope as well as region as explanatory variables in the SEM. In the sub-model with flowering peak as a response variable and forest characteristics and microclimatic variables as explanatory variables, we included species identity as a random variable. To test whether the forest characteristics influence the local microclimate, we set both spring air temperature and spring relative humidity also as response variables, while using the forest characteristics as well as other geographical factors as explanatory variables. The complete dataset included 687 data points, but since 45 rows had missing values for at least one of the variables, we analysed the full SEM with 642 data points.

We evaluated the overall path model using Shipley's test of directed separation (Shipley 2009), which yields a Fisher's C statistic comparable to a χ^2-value. A P-value above 0.05 indicates that a model can adequately reproduce the hypothesized causal network. Fisher's C is then used to calculate the Akaike Information Criterion (AIC), or a corrected AIC for small sample sizes (AICc), to compare model fits. We calculated both marginal and conditional R^2-values, where the former describes the proportion of variance explained by only fixed factors, whereas the latter describes the variance explained by fixed and random factors. Starting with a full model based on *a priori* knowledge of interactions that included all the above-mentioned variables, we used a backwards stepwise elimination process based on AICc to remove non-significant pathways. Additionally, we used d-separation tests to evaluate whether any non-hypothesized independent paths were significant, and whether the models could be improved by including any of the missing paths.

Results

The onset of flowering in our study species ranged from mid-March (*Mercurialis perennis* L., *Primula elatior* (L.) Hill, *Anemone nemorosa* L.) to the beginning of May (*Galium odoratum* (L.) Scop.), *Arum maculatum* L., *Polygonatum verticillatum* (L.) All.). Similarly, the peak flowering time of the different species ranged from the end of March until the end of May. For some species, the flowering period ended already in mid-May while others continued to

flower until mid-June. Besides these species differences in mean onset, peak and end of flowering, we also found large differences among species in their levels of among-plot variation. Some species had very narrow ranges, e.g. the flowering peak of *Galium odoratum* varied only by 10 days across the 79 studied plots, whereas for *Anemone nemorosa* (n = 87) and *Mercurialis perennis* (n = 71) the flowering peaks differed by up to 42 and 46 days, respectively. Of the studied species 75 % (12 out of 16) flowered on both deciduous and coniferous forest plots (Appendix S1: Table S2). The average peak flowering time of those four species that grew on deciduous plots only (day of the year =118.3) did not differ significantly from those that grew on both deciduous and coniferous plots (day of the year = 119.9). For an overview of mean flowering start, peak and end, as well as the respective N, of all species see Appendix S1: Table S1 and S2.

Impact of forest management on phenology

Across all studied species, forest understory herb species growing on plots with a high silvicultural management intensity had a significantly delayed start, peak and end of their flowering periods (Fig. 3: Flowering start: regression coefficient ± SE β = 11.45 ± 2.00, conditional R^2 = 0.86, P-value < 0.001. Flowering peak: regression coefficient β = 18.14 ± 1.78, conditional R^2 = 0.87, P-value < 0.001. Flowering end: regression coefficient β = 19.60 ± 1.90, conditional R^2 = 0.78, P-value < 0.001.); for detailed results of the corresponding linear mixed effect models see Appendix S1: Table S3). On plots with the highest management intensity, the average peak of flowering was over two weeks later than on plots with the lowest management intensity (average days of year of 128, 119 and 113 in managed spruce forests, managed beech forests, and unmanaged beech forests, respectively). Generally, plants flowered later on plots dominated by coniferous trees than on deciduous forest plots (Fig. 3 and 4). These general patterns were also reflected at the level of individual species: in all but one of the studied species, there was a positive (albeit not always significant) relationship between silvicultural management intensity and peak flowering (Table 2), with some of the strongest effects observed in *Primula elatior*, *Anemone nemorosa* and *Galium odoratum*. For detailed regression results, see Table 2 and Appendix S1: Tables S4 and S5.
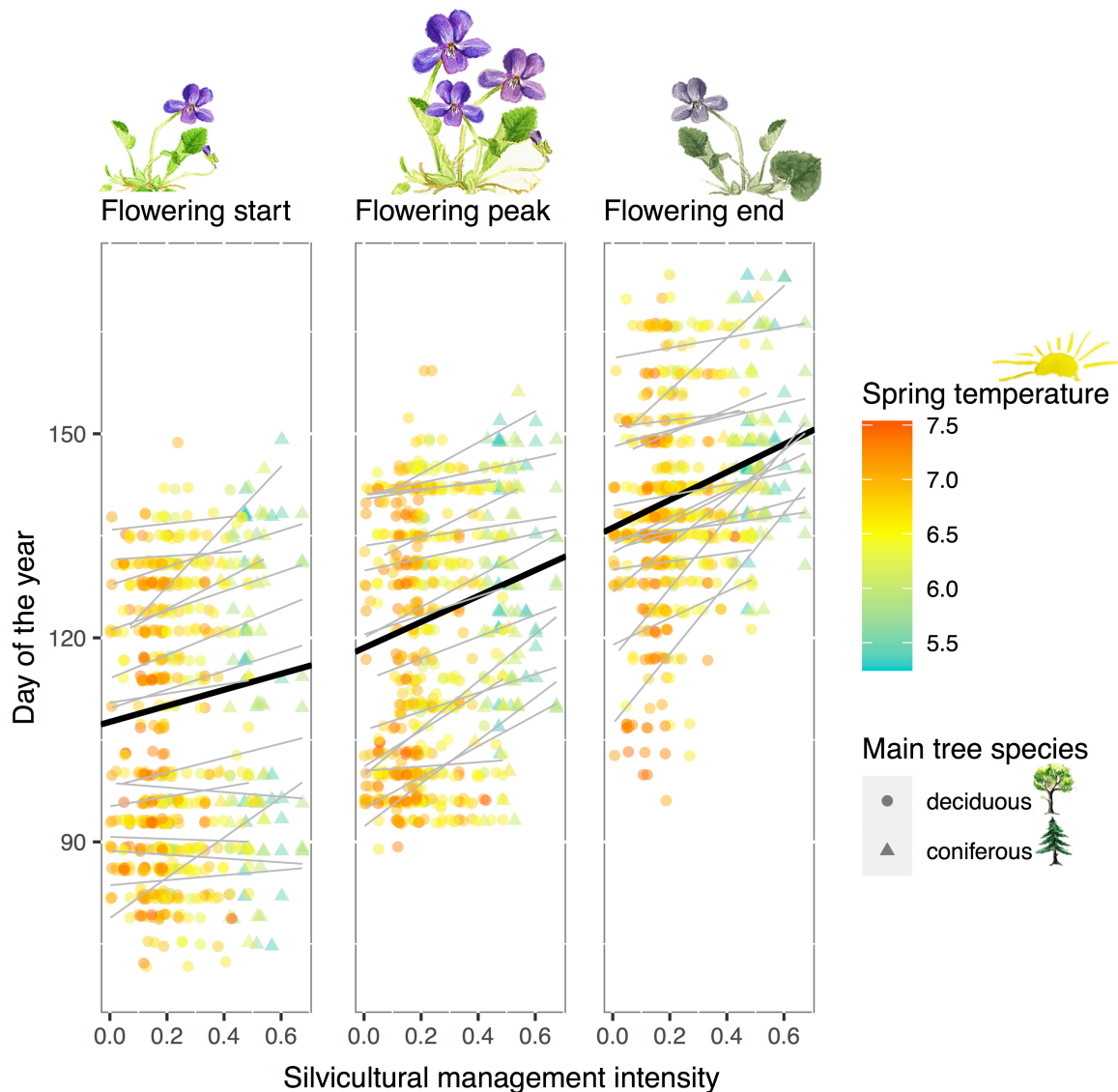
Figure 3: The relationships between silvicultural management intensity and flowering start, peak and end, respectively, across all 16 species and 100 plots. Each point represents a plot by species combination. Silvicultural management intensity is a synthetic index mainly based on tree species, tree density and stand age, with values from 0 (lowest management intensity) to 1 (highest management intensity). The shape and colour of the symbols code for main tree species and mean spring temperature (see legend). The fitted regression lines are derived from a linear mixed model with species as random factor. Species specific slopes are shown in grey.

While the strongest impact of management intensity on phenology can be attributed to the percentage of conifers, i.e. spruce plantations, there are other aspects of forest management that also have effects on phenology and microclimate. Even if analyses are restricted to deciduous-dominated plots only, higher management intensity is still connected with significantly colder spring temperatures (F-value = 7.221, df = 80, P = 0.009, regression coefficient = -0.85) and 75% of the studied species still show a tendency to flower later on plots with higher management intensity.

Since forest management affects many aspects of forest structure simultaneously (see Appendix S1: Table S6), we used linear regressions to understand which specific forest characteristics were most related to variation in plant phenology. We found the strongest statistical associations with flowering peak for the percentage of the crown projection area of mature trees and the basal area that is taken up by coniferous trees (with an average standardized regression coefficient of 0.41 and 0.40, mean R^2 = 0.20 and 0.21, and maximum R^2 = 0.67 and 0.67, respectively; Table 2). The higher the percentage of coniferous trees was, the later the understory herbs tended to flower (see also Fig. 3). Furthermore, plants flowered later in younger forest stands (average standardized regression coefficient -0.26, with a mean R^2 = 0.11, maximum R^2 = 0.30) and those with a low structural complexity (average standardized regression coefficient = -0.19, with a mean R^2 = 0.15 and a maximum R^2 = 0.89). Table 2 gives an overview of the standardized regression coefficients of all forest characteristics, and the corresponding R^2 values and unstandardized regression coefficients are provided in Appendix S1: Table S4 and S5.

Impact of microclimate on phenology

We found that microclimatic conditions varied substantially between different forest plots, and that this was partly related to forest management (Fig. 3 and Tables 2 and S7). For instance, on managed forest plots the mean spring temperatures were significantly lower than on unmanaged forest plots (5.9°C on managed coniferous, 6.7 on managed deciduous and 7.0°C on unmanaged deciduous plots, $F_{2,96}$ = 32.82, P < 0.001), for all pairwise comparisons between the three categories, and the patterns were also very similar for the two regions. Microclimate, in turn, was significantly correlated with plant phenology. Higher spring and winter temperatures were generally associated with earlier flowering, whereas higher humidity was correlated with later flowering (Tables 2 and S7-S9, Fig. 4 and S2). Of all tested microclimatic variables, mean spring temperature at 2 m height explained most of the variability in peak flowering across all species (mean R^2 = 0.25, maximum R^2 = 0.52, for R^2 values of all linear regression see: Appendix S1: Table S8). Per 1°C temperature increase, the plants reached the flowering peak on average 4.5 days earlier. At the level of individual species there was a significant negative relationship between spring temperature and peak flowering in 11 out of the 16 analysed species, and for most of the other species there was a non-significant negative trend (Table 1, Fig. 4). The magnitudes of the responses varied substantially among species, ranging from a change of over 12 days per 1°C for *Mercurialis perennis* to only minor changes in flowering time of around 3 days per 1°C for *Cardamine*

bulbifera L. For a comparison of all standardized and unstandardized regression coefficients of all microclimatic variables see Appendix S1: Tables S7 and S9. Of all moisture-related variables, relative humidity during spring was the best predictor of peak flowering (mean R^2 = 0.15, maximum R^2 = 0.47, see Appendix S1: Tables S7-S9 and Fig. S2) and was therefore also included in the SEM. On average, plants flowered 2.7 days later per 10 % increase of relative humidity.
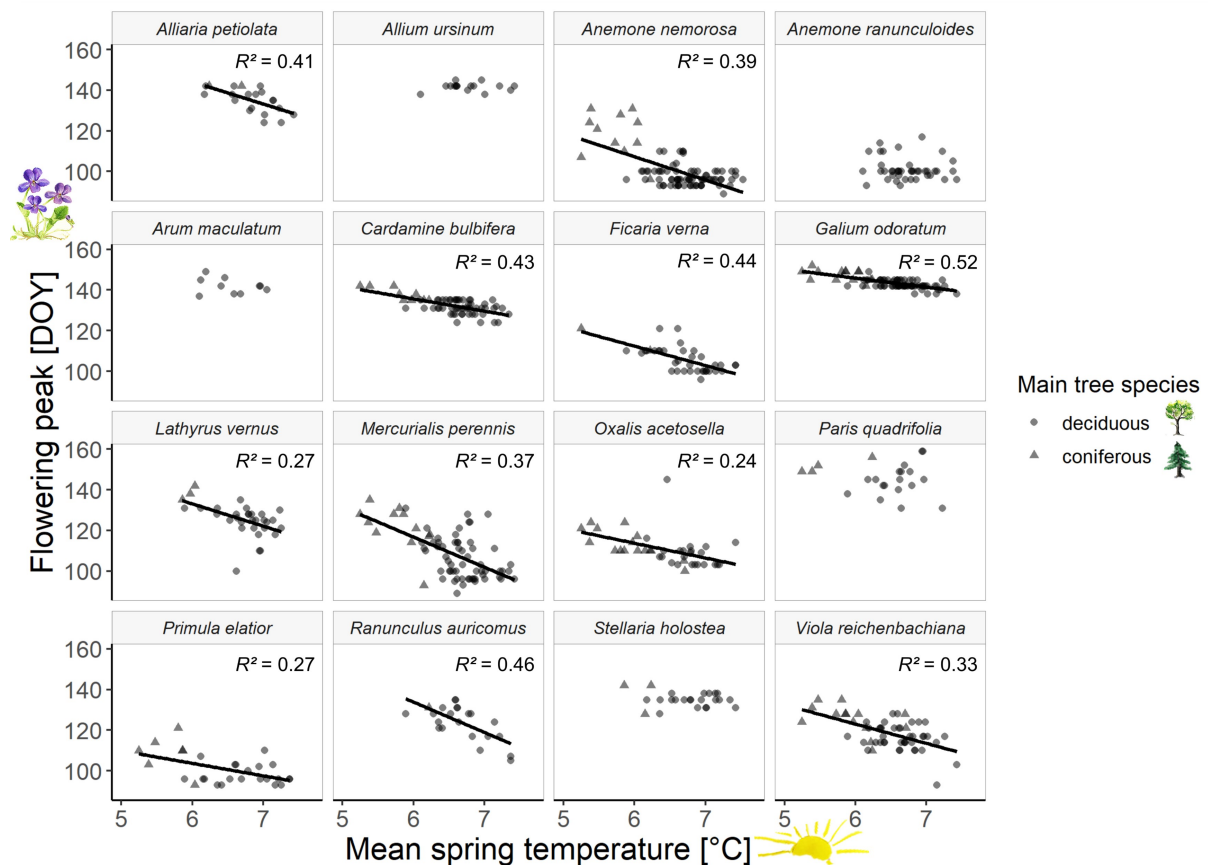

Figure 4: Regression of flowering peak (DOY, day of year) against mean spring temperature. Each point represents a forest plot, and the shape of each point indicates whether the main tree species is deciduous (circle) or coniferous (triangle). For significant regressions, the fitted regression lines are plotted. All regression coefficients are listed in Appendix S1: Table S2.

Table 2: Relationships between forest characteristics and microclimate (last two columns), and the peak flowering of different plant species. The values are standardized regression coefficients derived from linear regressions of flowering peak against the different forest trait and microclimate variables, with significant values in bold (corrected using FDR). Age = mean age of main tree species, dbh SD = standard deviation of dbh, density = stand density, Div = species richness of trees, Div 2D = inverse Simpson's index for trees, Moristia = Morisita's index of dispersion, SCI = Zenner's structural complexity index, Slope = a combination of inclination and exposition, BA = basal area covered by trees, CPA = crown projection area, Con = Coniferous, dbh = diameter at breast height, SMI = silvicultural management intensity, VMR= Clapham's variance mean ratio. Ta = air temperature and rH = relative humidity, with both climate variables measured at 200 cm height during February – May 2017. See Table 1 for a more detailed explanation of the different explanatory variables, and Appendix S1: Tables S3-S6 for the corresponding R^2 values and unstandardized regression coefficients for all regressions. * = Species that covered the whole range of the different forest types. N = number of peak-flowering data points per species.

	N	Age	Slope	BA	Con BA	Con CPA	CPA	Dbh	Dbh SD	Density	Div	Div 2D	Morisita	SCI	SMI	VMR	Ta	rH
Alliaria petiolata	20	-0.41	-0.08	0.10	0.42	0.41	**0.06**	-0.04	-0.27	0.23	0.32	0.26	-0.19	-0.27	0.41	-0.16	**-0.64**	0.26
Allium ursinum	16	-0.52	0.13	-0.43			**-0.39**	-0.10	-0.29	0.19	0.24	0.55	-0.14	-0.33	0.26	0.15	0.06	-0.17
Anemone nemorosa *	**87**	**-0.37**	0.01	**0.46**	**0.82**	**0.82**	0.08	0.16	-0.22	-0.04	-0.14	-0.17	-0.13	**-0.34**	**0.54**	-0.13	**-0.63**	**0.37**
Anemone ranunculoides	42	-0.11	**-0.41**	0.00	0.32	0.34	**0.08**	-0.19	-0.20	0.28	0.20	0.03	0.08	-0.07	0.07	0.22	-0.11	0.01
Arum maculatum	10	-0.55	-0.63	-0.10	0.19	0.18	**0.06**	-0.36	-0.35	0.57	0.11	0.06	0.06	0.22	0.22	-0.12	-0.29	0.07
Cardamine bulbifera *	62	-0.22	**-0.33**	**0.48**	**0.58**	**0.59**	0.11	0.31	-0.01	-0.22	-0.31	**-0.35**	**-0.35**	-0.21	0.32	**-0.37**	**-0.65**	**0.56**
Ficaria verna *	37	-0.31	-0.02	0.01	0.37	0.38	**-0.16**	0.01	-0.18	0.08	-0.03	-0.06	-0.18	-0.36	**0.45**	-0.16	**-0.67**	0.34
Galium odoratum *	**79**	**-0.39**	-0.24	**0.36**	**0.60**	**0.61**	0.08	0.14	-0.19	0.03	-0.21	-0.21	-0.17	**-0.42**	**0.49**	-0.19	**-0.72**	**0.36**
Lathyrus vernus *	32	-0.14	**-0.28**	0.13	**0.50**	**0.51**	-0.28	0.09	-0.17	-0.22	-0.28	-0.40	-0.10	-0.37	0.42	0.12	**-0.52**	**0.53**
Mercurialis perennis *	**71**	**-0.39**	-0.23	0.19	**0.53**	**0.53**	-0.08	0.07	-0.27	0.02	-0.06	-0.08	-0.22	-0.32	**0.48**	-0.15	**-0.61**	**0.45**
Oxalis acetosella *	39	-0.16	**0.12**	0.05	0.28	0.29	**-0.24**	0.02	-0.08	0.03	-0.21	-0.13	-0.13	-0.18	0.30	-0.11	**-0.49**	0.38
Paris quadrifolia *	22	-0.14	0.49	0.19	0.45	0.43	-0.14	0.24	-0.06	-0.12	0.24	0.04	0.02	-0.40	0.41	0.02	-0.12	-0.07
Primula elatior *	28	-0.26	-0.09	0.46	**0.63**	**0.63**	-0.04	0.24	-0.10	-0.09	-0.25	-0.19	-0.13	-0.21	**0.57**	-0.11	**-0.52**	**0.63**
Ranunculus auricomus	22	-0.29	0.24	-0.25	0.23	0.23	-0.36	0.11	-0.20	-0.10	-0.22	-0.24	-0.25	-0.32	0.26	-0.15	**-0.68**	0.06
Stellaria holostea *	24	-0.38	-0.42	0.07	0.32	0.35	**0.04**	-0.23	-0.42	0.21	0.27	0.05	0.08	-0.19	0.28	0.16	-0.14	0.07
Viola reichenbachiana *	55	-0.24	-0.28	**0.45**	**0.51**	**0.52**	0.08	0.31	0.01	-0.24	**-0.36**	**-0.41**	-0.24	-0.20	0.31	-0.33	**-0.57**	**0.50**

Interactions among forest management, microclimate and phenology

The piecewise SEM confirmed that, on average, plants flowered earlier on warmer, south-facing and less humid plots, and that most of the forest characteristics – percentage of coniferous trees, crown projection area of mature trees, variance mean ratio and structural complexity index – had a significant influence on the forest microclimate (Fisher's $C = 5.078$, df $= 10$, $P = 0.886$, see Fig. 5 and Appendix S1: Table S10). As expected, spring temperatures were lower on coniferous forest plots than on deciduous forest plots. Moreover, forest plots with a lower crown projection area of mature trees (also reflecting forest age) and structural complexity were also colder than plots with older and more heterogeneous and structurally complex forest stands. Further, plots with north-facing slopes were colder than south facing ones. The relative humidity was higher in forest stands with a higher percentage of conifers, but lower crown projection area of mature trees and variance mean ratio (reflecting horizontal heterogeneity). Further, it was lower on warmer, south-facing plots. Plots located in the Hainich region were generally warmer and more humid and plants tended to flower earlier there than on the Schwäbische Alb (Fig. 5 and Appendix S1: Table S10). Furthermore, a high percentage of coniferous trees had an equally strong direct effect on the timing of flowering peak, with plants growing on forest stands dominated by Norway spruce flowering later than those in deciduous forests. All unstandardized and standardized estimates of the path coefficients, their degrees of freedom, standard errors, critical values and P-values are listed in Appendix S1: Table S10. We re-ran the same SEM with data from deciduous plots only, but this analysis had very similar results, with even stronger effects of structural complexity and crown projection on temperature, except that the effect of horizontal heterogeneity and crown projection area of mature trees on relative humidity became non-significant (Appendix S1: Fig. S3 and Table S11)
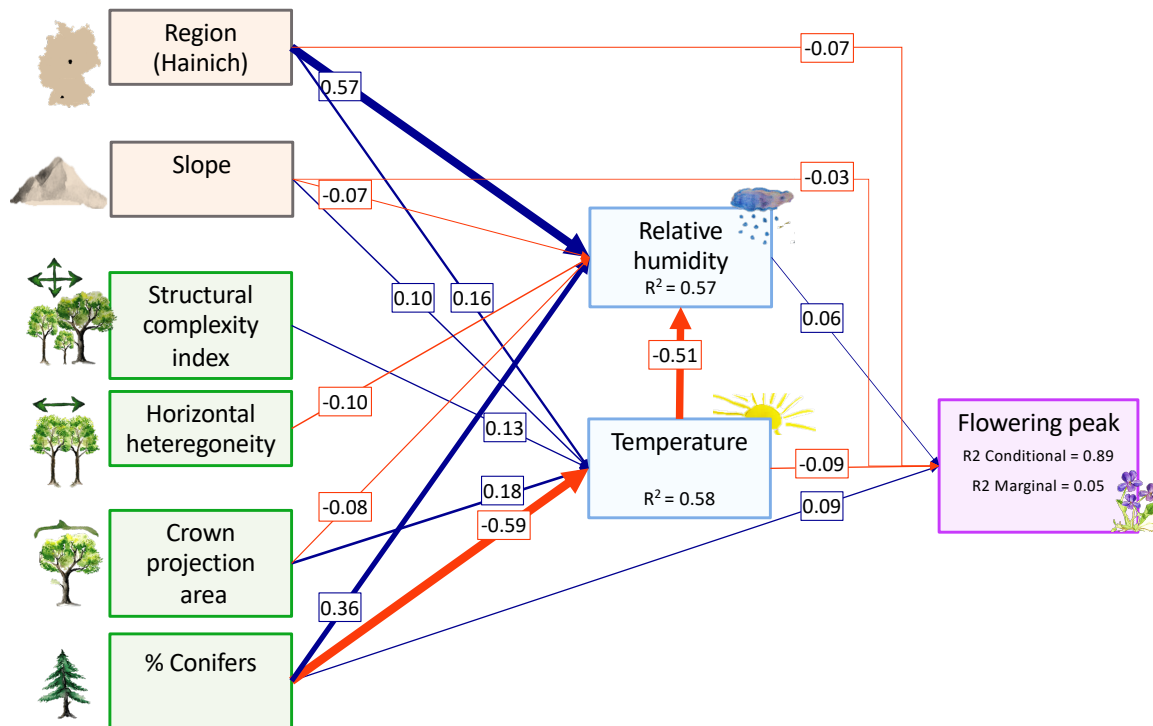
Figure 5: Results of the piecewise structural equation model (SEM) testing for direct and indirect relationships among forest characteristics, geographic parameters, microclimatic variables and the timing of peak flowering of forest understory herbs. Arrows represent unidirectional relationships among variables; only significant paths ($P < 0.05$) are shown. Blue arrows are positive relationships, red arrows negatives ones. The thickness of the arrows is proportional to the magnitudes of the standardized regression coefficient, which are also plotted on the arrows. The R^2 values for component models are also given for each response variable. In the model with flowering peak as a response variable, we included the species as random factor. The overall model is a good fit to the data: Fisher's C = 8.364, *df* = 12, *P*-value = 0.756.

Discussion

Many organisms respond to anthropogenic environmental change through shifts in their timing of phenological events, and these changes can have important consequences for the ecology and evolution of ecological communities (Rudolf 2019). It is therefore key to understand the different potential drivers of phenological changes. Here, we disentangled direct and indirect effects that microclimate and forest management have on the phenology of forest understory herb species. We found that plants flowered later in intensely managed forests than in unmanaged forests. Much of this was because forest management affected microclimate, which in turn affected phenology, with plants flowering later on colder and moister forest stands. Our study thus demonstrates that besides climate change other drivers of environmental change, such as forest management, can influence the phenology of organisms.

Impact of forest management and forest characteristics on phenology

While climate-related shifts of phenology are widely studied and accepted (e.g Fitter and Fitter, 2002; Parmesan and Yohe, 2003; Wolkovich et al., 2012; Cook, Wolkovich, and Parmesan, 2012), the impacts of other global change drivers, such as land use, have received much less attention. However, land use can also influence life-history traits, such as phenology, and can even cause genetic differentiation in phenological traits (Völler et al., 2013; Völler et al., 2017). Our study demonstrated that understory herbs occurring on forest plots with a high silvicultural management intensity had a significantly delayed start, peak and end of their flowering periods. On forest stands with the highest forest management intensity, the plants flowered on average about two weeks later than those growing in unmanaged forests. Among the different forest characteristics, the percentage of coniferous trees, the age of the trees and the structural complexity of a forest stand were the strongest drivers of phenological variation. Plants generally flowered latest on plots dominated by coniferous trees that were relatively young and structurally less complex.

During the last years, there has been cumulating evidence for land-use effects on the phenology of plants and animals. Zhang, Liu and Herebry (2019) showed that land cover and land use change can lead to a delayed start of the growing season in intensively managed agricultural landscapes. Similarly, Altermatt (2012) showed that temperature-related phenological shifts of butterflies depend on their habitat, with delayed phenology in settlement habitats, even though such habitats are generally associated with higher temperatures. Moreover, Leong, Ponisio, Kremen, Thorp and Roderick (2016) found that bee phenology differed between urban and agricultural habitats, with seasonal patterns of abundance and species richness varying less in human-altered landscapes compared to more natural habitats. For plants it has been suggested that climate change and land-use change, alone and in combination, cause growing seasons to start earlier, with human-managed ecosystems greening up particularly faster than their natural counterparts (Wang et al., 2018).

One might argue that the prolongation of the flowering period through diverse forest management at the landscape scale may ultimately improve resource availability and heterogeneity for consumers such as bees. However, this is unlikely to be the case since the abundances of many species (and thus total resource availability) appeared lower on the intensely managed plots we monitored (pers. obs.).

A challenge with the design of our study was that high management intensity was inevitably to some degree confounded with changes in the main tree species. Within our study regions, planting Norway Spruce is a measure of forest management, and there are no

unmanaged coniferous forests. However, to tease apart management types from tree species identity, it would be scientifically ideal to compare managed with unmanaged spruce plots, if the latter existed. Therefore, comparing plant phenology also between unmanaged and managed coniferous forest (in other regions) would be a worthwhile focus for future research.

Impact of microclimate on phenology

Our studied forest plots differed not only in the management regime, but, as a consequence, in their microclimate. Both simple linear regressions and the SEMs confirmed that the flowering phenology of spring-flowering understory herbs was affected by the microclimatic conditions, with higher spring (and winter) temperatures resulting in earlier flowering, and higher relative humidity associated with later flowering. The plants flowered on average 4.5 days earlier per +1°C temperature difference. This magnitude of change corresponds very well with the response of plants to interannual temperature variation observed in previous studies. For example, Heikinheimo and Lappalainen (1997) suggested that a springtime temperature increase of 1°C can result in flower buds bursting approximately 4 days earlier, based on phenological long-term data for eleven plant taxa (trees, shrubs and forest understory herbs) in Finland. In Britain, the average first flowering of 385 plant species (trees, shrubs and herbs) was advanced by 4.5 days in the 1990s compared to the previous four decades, and in relation to climate the effect size was also 4.3 to 6 days per 1°C increase in mean monthly temperature for spring flowering species (Fitter and Fitter, 2002). Moreover, an analysis of a large phenological network data set showed that across Europe phenological shifts match the warming pattern in Europe (Menzel et al., 2006). Our data show that such climatic differences, and the associated very similar changes in phenology, can also occur on much smaller scales. However, microclimatic patterns can differ substantially from regional climate patterns (Hwang et al., 2011; Ward et al., 2018), and we therefore need to take them into account when projecting effects of climate change on phenology (De Frenne et al., 2013; Franklin et al. 2013). Especially in forests, these microclimate dynamics have a stronger impact than macroclimate warming on plant responses to climate change (Zellweger et al. 2020).

We also found that the magnitudes of the temperature-associated phenology changes varied substantially among species. This is consistent with several previous studies. Fitter and Fitter (2002), for example, found that annual plants are more likely to flower earlier than congeneric perennials, and insect-pollinated species more likely than wind-pollinated ones. Such differences in the phenological response might ultimately alter the diversity and

composition of plant communities. Roberts et al. (2015) predicted that interspecific differences would change the order of spring phenology in temperate forests, which in turn would change hierarchies of light competition and thus potentially the composition of temperate forests. Furthermore, even if the majority of species flower earlier, some may still show non-significant trends or even delayed flowering. In a long-term study of 490 species, Cook et al. (2012) demonstrated that the interaction of fall/winter chilling (i.e. vernalization) and spring warming sensitivities explains much of the apparently paradoxical behaviour of non-responding species, or of species that show delayed spring events despite local warming. As both warmer spring and winter temperatures are correlated with earlier flowering in our study, the potential vernalization requirements are probably met for (most of) our plants.

High humidity delayed flowering on average by 2.7 days per 10% increase of relative humidity, and the phenological responses of plants to humidity changes were fairly consistent. The findings of previous studies were ambiguous. While some suggested that humidity is crucial for plant phenology (Laube et al., 2014; Matthews and Mazer, 2016), others found no evidence for a significant role of air humidity for plant phenology (Abu-Asab et al., 2001; Zipf and Primack, 2017). Phenological responses to humidity generally seem to be more complex and species-dependent, and they may depend on interactions with other factors.

Interactions among forest management, microclimate and phenology
The SEM confirmed that variation in microclimatic conditions – spring temperature and relative humidity – was strongly influenced by several aspects of forest structure determined by forest management, with forest structure generally having stronger effect on temperature than on relative humidity. Our results confirm those of Nihlgard (1969) and Augusto, Dupouey, and Ranger (2003) who showed that forests dominated by Norway spruce tended to be colder and moister than those dominated by European beech. As hypothesized, our results show that less spatially heterogeneous and structurally complex forest plots with a low crown projection area of mature trees are colder. This is in accordance with Zellweger at al. (2019) who also found that canopy cover increases daily absolute minimum temperatures during the spring. This may seem counterintuitive at first, because during the day plots with a lower crown projection area of mature trees should allow more light to penetrate the canopy and therefore to be warmer. However, this trend reverses during the night where plots with a low crown projection area are colder (see Appendix S1: Fig. S4), presumably because of a sheltering effect of large tree crowns, which reduce convection, mixing of air and infrared

reflection (Geiger et al. 2003; von Arx et al., 2013). Since the night effect is stronger than that during the day the net effect is a cooling under lower crown projection areas.

Planting of Norway spruce instead of European beech profoundly alters ecological properties of the forests in our study system. Besides their narrower crown-width-to-diameter-ratio in comparison to beech, spruce plantations differ from beech forests in many other characteristics such as stand density, size distribution, age, horizontal/spatial- and vertical patterns (Schall et al., 2018). One reason why forest stands dominated by conifers are colder is that particularly in early spring, when deciduous trees have not yet completed their leaf-out, they allow much less light to reach the forest floor and thus do not warm up to the same temperatures as deciduous forest stands during the day – while both cool down during the night (see Appendix S1: Fig. S3). De Frenne et al. (2013) argue that in Europe current conservation actions are often directed toward restoring traditional management (e.g., coppicing in ancient forests), which results in canopy opening and thus potentially increased temperatures at the forest floor and thereby could accelerate the increasing dominance of warm-adapted species.

In our study, the dominant tree species affected plant phenology not only indirectly, through altering microclimate, but also directly. This direct effect is almost as strong as the effect of temperature, and it must result from other abiotic or biotic factors, that are affected by the dominant tree species in a forest. The two most likely candidate explanations are light and soil conditions. If not heavily thinned, evergreen, coniferous trees create much darker conditions on the forest floor during spring, which may be crucial for the development of the understory vegetation (Tinya et al., 2009). Moreover, coniferous forests are also known to differ in other biotic and abiotic traits – many soil properties, including soil moisture, pH, nutrients and mycorrhizae (Messenger, 1980; Ranger and Claude, 1992; Augusto, Dupouey, and Ranger, 2003) – all of which could affect the phenology of understory plants. Wolf, Zavaleta and Selmants (2017) showed that biotic interactions can affect the timing of flowering, with plants flowering earlier after (experimentally manipulated) biodiversity loss.

Potential consequences of phenological shifts

A phenology that is fine-tuned to environmental conditions is crucial for plants. Plants that fail to track seasonal temperatures or climatic long-term changes are prone to decline in abundance (Willis et al., 2008). As a consequence, microclimate warming in temperate forests can cause a shift in biological communities favouring warm-affinity species (i.e.,thermophilization) (Zellweger et al., 2020). On the other hand, Scheepens and Stöcklin

(2013) showed that earlier flowering as a response to climatic changes can also be maladaptive and lead to a fitness decline due to a more rapid development and therefore lower flower numbers. Phenological shifts can alter reproduction and survival, leading to demographic changes (Miller-Rushing et al., 2010), and potentially favouring exotic species (Abu-Asab et al., 2001). For instance, Dreiss and Volin (2013) found that later leaf growth of deciduous trees can facilitate the establishment of invasive understory species. Furthermore, a review by Elzinga et al. (2007) argues that that biotic interaction with mutualists and antagonists, e.g. pollinators or pollinator-transmitted fungi, can change plant phenological patterns. It is likely that the biotic and abiotic drivers that determine phenology vary between interacting groups of organisms (or species) such as plants, insects or vertebrates (Parmesan and Yohe, 2003; Voigt et al., 2003). Phenological shifts can alter species interactions and thereby influence the potential for persistence and coexistence of competing species and change biodiversity patterns in natural systems (Rudolf 2019). If overstory tree leaf out advances more with increased spring temperature than understory wildflower phenology, those wildflowers must deal with a shorter period of high light before they are shaded by tree canopies. This can reduce their carbon budgets (Heberling et al. 2019), Further, asynchronous changes could potentially lead to mismatches between the phenology of interacting organisms (Kharouba et al., 2018; McKinney et al., 2012; Stenseth & Mysterud, 2002; Visser & Both, 2005; Visser, Both, & Lambrechts, 2004), which could exacerbate the effects of climate change on organisms. Several studies found that spring warming can cause plants to flower earlier (Cleland et al., 2007; Parmesan & Yohe, 2003) and create a phenological mismatch between plants and pollinators (Kudo and Ida, 2013; Settele, Bishop, and Potts, 2016), with detrimental effects on plant reproduction (Forrest, 2015) and pollinator fitness (Schenk, Krauss, and Holzschuh, 2018). However, there is no consensus on how likely such mismatches are. Renner and Zohner (2018) argue that mismatches due to climate change are most likely in antagonistic interactions, whereas there is only limited evidence of phenological mismatches in mutualistic interactions. A literature review by Kharouba et al. (2018) suggests that a majority (57%) of interacting species changed their phenologies fairly synchronously whereas 43% showed a trend toward asynchrony. Furthermore, because pollinator activities are low during early spring, due to cool temperatures, pollinator limitation is already common in spring-flowering forest species. Thus, climate change may have a particularly strong impact on bee-pollinated spring bloomers, because their reproductive success is highly susceptible to seasonal fluctuation (Kudo, Ida & Tani 2008). Besides affecting the distribution and fitness of interacting species, changes in plant phenology can

also affect ecosystem functions such as productivity and carbon cycling, and they can therefore also effect yields in agriculture, horticulture, viticulture, and forestry (Cleland et al., 2007; Menzel et al., 2006).

Conclusions

Our study shows that plant phenology is affected by forest management. It thus contributes to the growing evidence that, besides climate change, other drivers of current environmental change, such as land use, influence phenology. Forest management interventions – e.g. planting certain tree species, thinning, selective removal of target trees or even clearfellings – change many forest characteristics such as crown projection area, spatial dispersion of trees and the structural complexity of a forest. Thus, forest management alters forest structure, and thereby changes the microclimatic conditions of a forest stand, its light conditions as well as most likely other environmental factors that impact flowering phenology of understory herbs. These phenology changes in turn can have wide-ranging implications for forest ecosystems and their long-term composition, stability and evolution.

Acknowledgements

Data Availability

This work is based on data from several projects of the Biodiversity Exploratories programme (DFG Priority Program 1374). All data used for analyses are publicly available from the Biodiversity Exploratories Information System (https://doi.org/10.25829/bexis.27746-1) at https://www.bexis.uni-jena.de/PublicData/PublicDataSet.aspx?DatasetId=27746. Raw data are publicly available from the same repository (with identifiers 20826 ("Basic Information of all Experimental Plots (EPs)"), 17706 ("New forest type classification of all forest EPs, 2008-2014"), 17486 ("Stand age of all forest EPs, 2012"), 17746 "SMI - Silvicultural management intensity index on all forest EPs, 2008-2014" and 22766 "Stand structural attributes based on 2nd forest inventory, all forest EPs, 2014-2018").

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Supplementary material

Table S1: The 20 studied forest understorey species and their mean dates of flowering start, peak and end, with the respective sample sizes (plot numbers) in brackets. All statistical analyses were restricted to the 16 species flowering on at least 10 plots.

Species	Flowering start		Flowering peak		Flowering end	
Adoxa moschatellina	April 8	(7)	April 22	(6)	May 2	(6)
Alliaria petiolata	May 4	(22)	May 15	(20)	May 30	(18)
Allium ursinum	May 12	(16)	May 22	(16)	June 1	(16)
Anemone nemorosa	March 27	(88)	April 10	(87)	May 16	(87)
Anemone ranunculoides	March 31	(43)	April 11	(42)	May 11	(41)
Arum maculatum	May 17	(11)	May 22	(10)	May 31	(10)
Cardamine bulbifera	May 5	(60)	May 12	(62)	May 22	(62)
Euphorbia amygdaloides	April 20	(10)	May 18	(6)	June 12	(3)
Ficaria verna	April 7	(43)	April 16	(37)	May 3	(36)
Galium odoratum	May 12	(79)	May 24	(79)	June 12	(79)
Lathyrus vernus	April 23	(35)	May 5	(32)	May 18	(31)
Mercurialis perennis	March 26	(66)	April 19	(71)	May 16	(71)
Oxalis acetosella	April 8	(50)	April 21	(39)	May 13	(39)
Paris quadrifolia	May 11	(24)	May 26	(22)	June 8	(9)
Polygonatum verticillatum	May 26	(9)	May 31	(8)	June 5	(8)
Primula elatior	March 29	(25)	April 11	(28)	May 1	(29)
Pulmonaria obscura	April 4	(9)	April 15	(8)	May 13	(8)
Ranunculus auricomus	April 22	(22)	May 4	(22)	May 17	(22)
Stellaria holostea	April 28	(24)	May 15	(24)	May 31	(22)
Viola reichenbachiana	April 11	(58)	April 29	(55)	May 16	(54)

Table S2: The number of plots each species was flowering on per region and forest type, as well as the overall number of data points, plots and species per region and forest type. Management intensity is increasing from left to right.

Species	Total	Region		Forest type						
		Alb	Hainich	Un-managed	Selection forest	Age-class forest				Spruce plantation
						mature	immature	pole wood	thicket	
Alliaria petiolata	22	10	12	0	6	6	4	3	1	2
Allium ursinum	16	1	15	6	5	1	3	0	1	0
Anemone nemorosa	90	45	45	16	13	11	15	9	15	11
Anemone ranunculoides	44	8	36	10	11	6	7	5	5	0
Arum maculatum	11	6	5	2	3	0	2	1	3	0
Cardamine bulbifera	62	33	29	6	11	9	13	6	9	8
Ficaria verna	43	16	27	9	11	8	9	4	1	1
Galium odoratum	79	47	32	6	13	11	14	9	11	15
Lathyrus vernus	35	19	16	5	6	4	9	2	6	3
Mercurialis perennis	73	41	32	14	9	8	13	7	11	11
Oxalis acetosella	53	32	21	4	9	7	11	4	3	15
Paris quadrifolia	25	23	2	2	0	4	8	3	4	4
Primula elatior	29	17	12	6	3	4	6	1	2	7
Ranunculus auricomus	23	9	14	7	2	2	5	2	5	0
Stellaria holostea	24	3	21	3	11	4	1	1	1	3
Viola reichenbachiana	58	38	20	2	8	8	13	6	8	13
# Data points	687	348	339	98	121	93	133	63	86	93
# Plots	100	50	50	18	13	11	15	10	17	16
# Species	16	16	16	15	15	15	16	15	16	12
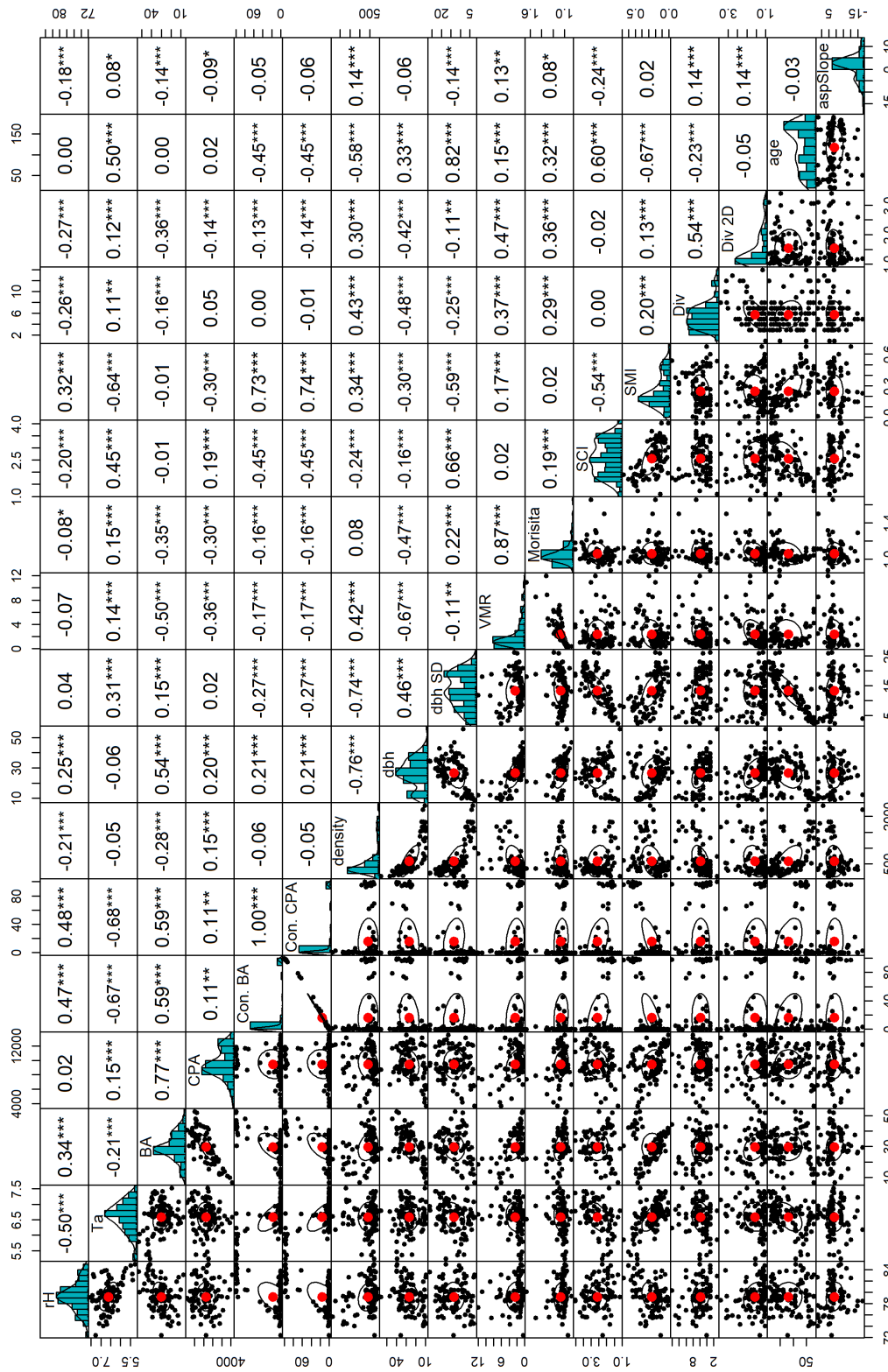
Figure S1: Pearson's correlations among all forest variables, spring temperature (Ta) and relative humidity (rH) and the respective scatterplots and histograms. See Table S8 for details on the variables. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table S3: The effect of silvicultural management intensity on the time of flowering start, peak and end. Linear mixed model results with species as random factor. Slope estimates with their respective standard errors (SE), *P*-values and the number of observations (N) and standard deviation (SD) of the random effects (RE).

Response variable	Estimate	SE	R² conditional	R² marginal	N	*P*-value	SD of RE
Flowering start	11.45	2.00	0.86	0.01	701	<0.001	18.87
Flowering peak	18.14	1.78	0.87	0.02	674	<0.001	17.19
Flowering end	19.60	1.90	0.78	0.04	651	<0.001	13.17

Table S4: Relationships between forest traits and the peak flowering of different plant species. The values are R²-values derived from linear regressions of flowering peak against the different forest trait variables. Age = mean age of the main tree species, BA = basal area covered with trees in m² ha⁻¹, CPA = crown projection area in m² ha⁻1, Con = Coniferous, dbh = diameter at breast height in cm, dbh SD = the standard deviation of dbh, density = stand density (trees ha⁻¹), Div = species richness, Div 2D = inverse Simpson's index, Morisita = Morisita's index of dispersion, SCI = Zenner's Structural Complexity Index (SCI) based on tree height, Slope = the inclination multiplied by 1 for south-, 1 for north-, and 0.5 for east- and west-facing slopes, SMI = silvicultural management intensity, VMR= Clapham's variance mean ratio (VMR).

	Age	Slope	BA	Con BA	Con CPA	CPA	Dbh	Dbh SD	Density	Div	Div 2D	Morisita	SCI	SMI	VMR
Alliaria petiolata	0.166	0.006	0.01	0.178	0.171	0.003	0.002	0.074	0.053	0.101	0.07	0.037	0.07	0.164	0.027
Allium ursinum	0.272	0.018	0.181			0.149	0.009	0.086	0.036	0.057	0.297	0.019	0.109	0.066	0.023
Anemone nemorosa	0.136	0	0.208	0.668	0.669	0.007	0.025	0.046	0.002	0.018	0.029	0.017	0.113	0.288	0.017
Anemone ranunculoides	0.012	0.169	0	0.104	0.118	0.006	0.038	0.039	0.08	0.04	0.001	0.006	0.005	0.005	0.048
Arum maculatum	0.303	0.401	0.009	0.036	0.032	0.003	0.131	0.121	0.325	0.012	0.003	0.003	0.049	0.048	0.015
Cardamine bulbifera	0.047	0.111	0.226	0.339	0.351	0.013	0.094	0	0.049	0.098	0.122	0.124	0.043	0.102	0.138
Ficaria verna	0.098	0	0	0.139	0.146	0.027	0	0.03	0.007	0.001	0.003	0.034	0.13	0.203	0.025
Galium odoratum	0.15	0.056	0.132	0.365	0.373	0.007	0.019	0.035	0.001	0.045	0.042	0.029	0.173	0.237	0.034
Lathyrus vernus	0.019	0.079	0.016	0.254	0.263	0.081	0.007	0.028	0.047	0.08	0.16	0.011	0.14	0.173	0.014
Mercurialis perennis	0.151	0.051	0.035	0.276	0.283	0.007	0.004	0.073	0	0.003	0.006	0.048	0.099	0.226	0.023
Oxalis acetosella	0.024	0.013	0.002	0.078	0.082	0.058	0.001	0.006	0.001	0.042	0.018	0.016	0.033	0.09	0.013
Paris quadrifolia	0.018	0.24	0.036	0.199	0.183	0.02	0.058	0.004	0.015	0.058	0.002	0	0.16	0.17	0
Primula elatior	0.066	0.009	0.208	0.392	0.402	0.002	0.056	0.01	0.008	0.062	0.036	0.018	0.043	0.32	0.012
Ranunculus auricomus	0.086	0.058	0.061	0.053	0.052	0.13	0.012	0.038	0.01	0.05	0.056	0.061	0.101	0.067	0.021
Stellaria holostea	0.142	0.178	0.004	0.102	0.122	0.002	0.054	0.177	0.045	0.074	0.002	0.007	0.037	0.077	0.027
Viola reichenbachiana	0.059	0.079	0.199	0.263	0.27	0.006	0.098	0	0.059	0.128	0.166	0.057	0.04	0.099	0.11

Table S5: Relationships between forest traits and the peak flowering of different plant species. The values are regression coefficients derived from linear regressions of flowering peak against the different forest trait variables. Significant values are written in bold. Age = mean age of the main tree species, BA = basal area covered with trees in m² ha⁻¹, CPA = crown projection area in m² ha⁻¹, Con = Coniferous, dbh = diameter at breast height in cm, dbh SD = the standard deviation of dbh, density = stand density (trees ha⁻¹), Div = species richness, Div 2D = inverse Simpson's index, Morisita = Morisita's index of dispersion, SCI = Zenner's Structural Complexity Index (SCI) based on tree height, Slope = the inclination multiplied by 1 for south-, 1 for north-, and 0.5 for east- and west-facing slopes, SMI = silvicultural management intensity, VMR= Clapham's variance mean ratio (VMR).

	Age	Slope	BA	Con BA	Con CPA	CPA	Dbh	Dbh SD	Density	Div	Div 2D	Morisita	SCI	SMI	VMR
Alliaria petiolata	-0.05	-0.06	0.09	0.10	0.10	$4.04 \cdot 10^{-4}$	-0.02	-0.27	$24.15 \cdot 10^{-4}$	0.63	2.03	-13.82	-2.13	20.71	-0.75
Allium ursinum	-0.02	0.09	-0.10			$-3.11 \cdot 10^{-4}$	-0.02	-0.11	$18.69 \cdot 10^{-4}$	0.32	**2.20**	-5.15	-1.08	4.35	0.22
Anemone nemorosa	-0.06	0.01	**0.45**	**0.23**	**0.24**	$2.71 \cdot 10^{-4}$	0.14	**-0.33**	$-9.53 \cdot 10^{-4}$	-0.55	-2.63	-10.00	**-4.57**	**31.56**	-0.49
Anemone ranunculoides	-0.01	-0.44	0.00	**0.78**	**1.17**	$2.13 \cdot 10^{-4}$	-0.11	-0.19	$28.80 \cdot 10^{-4}$	0.64	0.30	3.37	-0.51	3.18	0.51
Arum maculatum	-0.04	-0.28	-0.04	0.17	0.17	$1.49 \cdot 10^{-4}$	-0.15	-0.26	$41.04 \cdot 10^{-4}$	0.16	0.26	0.95	1.34	6.52	-0.09
Cardamine bulbifera	-0.02	-0.24	**0.22**	**0.07**	**0.08**	$2.90 \cdot 10^{-4}$	**0.12**	-0.01	$-17.44 \cdot 10^{-4}$	**-0.51**	**-2.32**	**-10.94**	-1.21	**9.01**	**-0.64**
Ficaria verna	-0.05	-0.03	0.01	**0.12**	**0.13**	$-6.69 \cdot 10^{-4}$	0.01	-0.22	$9.87 \cdot 10^{-4}$	-0.08	-0.65	-19.75	**-3.91**	26.42	-0.79
Galium odoratum	-0.02	-0.10	**0.10**	**0.05**	**0.05**	$1.53 \cdot 10^{-4}$	0.04	-0.09	$2.87 \cdot 10^{-4}$	-0.23	-0.96	-3.95	**-1.81**	**9.05**	-0.23
Lathyrus vernus	-0.02	-0.36	0.15	**0.15**	**0.15**	$-6.73 \cdot 10^{-4}$	0.07	-0.25	$-24.19 \cdot 10^{-4}$	-0.91	**-4.79**	-5.64	-3.73	**22.42**	0.30
Mercurialis perennis	-0.09	-0.42	0.24	**0.18**	**0.18**	$-3.57 \cdot 10^{-4}$	0.08	**-0.55**	$9.45 \cdot 10^{-4}$	-0.26	-1.49	-21.58	**-5.53**	**34.55**	-0.85
Oxalis acetosella	-0.03	0.21	0.05	0.05	0.05	$-13.13 \cdot 10^{-4}$	0.03	-0.13	$11.84 \cdot 10^{-4}$	-0.70	-2.55	-9.00	-2.14	13.74	-0.50
Paris quadrifolia	-0.02	0.56	0.17	**0.09**	**0.09**	$-1.53 \cdot 10^{-4}$	0.19	-0.09	$-17.02 \cdot 10^{-4}$	0.63	0.50	1.20	-4.88	22.96	0.06
Primula elatior	-0.04	-0.15	**0.41**	**0.11**	**0.11**	$1.85 \cdot 10^{-4}$	0.24	-0.17	$7.48 \cdot 10^{-4}$	-0.83	-2.51	-7.27	-2.20	**22.75**	-0.37
Ranunculus auricomus	-0.05	0.46	-0.20	0.11	0.13	$-9.71 \cdot 10^{-4}$	0.09	-0.29	$-25.39 \cdot 10^{-4}$	-0.73	-3.32	-23.54	-3.61	13.96	-0.50
Stellaria holostea	-0.03	-0.40	0.03	0.04	0.04	$0.73 \cdot 10^{-4}$	-0.09	**-0.29**	$21.26 \cdot 10^{-4}$	0.37	0.33	2.15	-0.97	6.37	0.25
Viola reichenbachiana	-0.04	-0.36	**0.36**	**0.10**	**0.10**	$5.41 \cdot 10^{-4}$	**0.23**	0.02	$-36.90 \cdot 10^{-4}$	**-0.96**	**-4.65**	-17.68	-2.30	**16.09**	**-1.24**

Table S6: Mean values of estimated management intensity, structural traits and microclimatic conditions for the different forest management types. Higher colour saturation indicates higher values. AC = age class forest consisting mainly of trees of the same age, Age = mean age of the main tree species, BA = basal area covered with trees in m² ha⁻¹, CPA = crown projection area in m² ha⁻1, Con = Coniferous, dbh = diameter at breast height in cm, dbh SD = the standard deviation of dbh, density = stand density (trees ha⁻¹), Div = species richness, Div 2D = inverse Simpson's index, Morisita = Morisita's index of dispersion, SCI = Zenner's Structural Complexity Index (SCI) based on tree height, SMI = silvicultural management intensity, VMR= Clapham's variance mean ratio (VMR). Ta = air temperature in °C and rH = relative humidity, both measured during the spring period February – May 2017 at 200 cm height.

Forest management type	N	SMI	Age	BA	Con BA	Con CPA	CPA	Density	Dbh	Dbh SD	Div	Div 2D	Morisita	SCI	VMR	Ta	rH
Beech unmanaged mature timber	18	0.10	173.93	35.68	2.12	1.67	11607.10	447.15	27.03	18.39	6.38	1.62	1.12	3.06	3.14	6.99	78.93
Beech AC mature timber	11	0.16	138.39	28.84	0.33	0.23	9118.52	236.87	37.23	15.33	4.46	1.47	1.01	2.17	1.30	6.75	78.70
Beech AC immature timber	8	0.17	101.39	30.58	3.12	2.51	9732.36	349.27	31.61	12.41	4.65	1.20	0.99	2.59	0.82	6.57	78.54
Beech selection system	13	0.17	171.32	27.46	0.00	0.00	8710.53	300.76	29.18	19.47	4.59	1.17	1.11	3.17	2.59	6.80	79.90
Beech AC mixed immature timber	7	0.23	102.78	29.39	22.07	17.65	8988.78	431.62	27.33	13.03	5.41	1.90	1.01	2.85	1.12	6.52	77.92
Beech AC pole wood	10	0.29	69.42	26.82	0.93	0.77	11124.93	1679.36	13.22	5.60	8.21	1.86	1.05	2.35	4.56	6.66	77.33
Beech AC thicket with shelterwood	3	0.34	158.25	18.11	0.54	0.33	6470.21	534.42	16.20	15.35	4.83	2.12	1.15	2.74	3.93	6.50	79.24
Beech AC thicket	13	0.39	71.76	14.91	2.82	2.60	6803.31	1197.95	11.07	7.04	7.89	2.15	1.16	1.96	6.94	6.59	78.66
Spruce AC mature timber	5	0.48	84.26	46.47	94.43	91.39	10470.38	326.12	40.49	13.69	5.71	1.39	0.99	2.06	0.78	5.69	83.19
Spruce AC immature timber	10	0.54	56.01	40.76	95.90	93.95	10082.35	649.43	27.41	8.23	5.94	1.27	1.04	1.81	2.09	5.94	82.01

Table S7: Relationships between microclimate and the peak flowering of different plant species. The values are standardized regression coefficients derived from linear regressions of flowering peak against the different microclimate variables, with significant values in bold. Blue colours indicate positive relationships, orange colours indicate negative relationships, and colour intensity is proportional to effect size. The winter period encompasses October 2016 – January 2017 and the spring period February – May 2017. Ice days = the number of days with a maximum temperature < 0°C, cold days = the number of days with a temperature minimum < 0°C, cold sum = the sum of days with a mean day temperature < 0 °C, cool days = the number of days with a temperature maximum < 10°C, Ta = air temperature in °C, rH = relative humidity, Ts = soil temperature, SM = soil moisture, GD = days with temperatures between 10°C to 30°C, growth sum = the sum of day temperatures between 10°C and 30°C, warm sum = the sum of mean day temperature > 5°C, warm sum = sum of day temperatures with a mean > 10 °C. See Table S8 for a more detailed explanation of the different explanatory variables, and Tables S3 + S4 for the corresponding R^2 values and unstandardized regression coefficients for all regressions.

Winter

	Ice days	Cold days	Cold sum	Cool days	Ta 10 cm	Ta 200 cm	Ts	rH	SM
Alliaria petiolata	0.31	**0.45**	**0.50**	0.04	-0.28	**-0.57**	-0.15	0.16	0.33
Allium ursinum	-0.42	0.20	0.25	-0.41	-0.19	-0.01	-0.12	-0.23	0.22
Anemone nemorosa	**0.32**	**0.53**	**0.60**	0.05	**-0.38**	**-0.60**	0.12	0.10	-0.10
Anemone ranunculoides	0.11	0.17	0.29	-0.06	-0.09	-0.22	0.02	-0.01	0.12
Arum maculatum	-0.30	0.51	0.30	0.06	0.08	-0.37	0.14	-0.08	-0.19
Cardamine bulbifera	**0.31**	**0.37**	**0.32**	**0.41**	**-0.45**	**-0.42**	**-0.29**	**0.36**	0.21
Ficaria verna	0.32	**0.53**	**0.57**	0.01	**-0.37**	**-0.61**	-0.17	0.22	0.11
Galium odoratum	**0.28**	**0.54**	**0.60**	0.09	**-0.45**	**-0.64**	0.03	0.04	-0.06
Lathyrus vernus	0.34	**0.37**	0.34	0.18	-0.33	**-0.40**	-0.08	0.32	0.08
Mercurialis perennis	0.17	**0.55**	**0.61**	-0.06	**-0.49**	**-0.62**	-0.19	0.23	0.06
Oxalis acetosella	-0.04	**0.35**	**0.40**	-0.04	**-0.43**	**-0.44**	-0.06	0.10	0.09
Paris quadrifolia	-0.24	0.00	0.10	-0.19	-0.01	-0.07	0.06	-0.23	-0.20
Primula elatior	**0.54**	**0.44**	**0.39**	0.35	-0.12	**-0.43**	0.08	**0.50**	0.05
Ranunculus auricomus	**0.44**	**0.53**	**0.52**	0.00	**-0.38**	**-0.60**	-0.17	0.02	-0.01
Stellaria holostea	0.09	0.17	0.34	-0.26	-0.26	-0.27	-0.28	-0.10	-0.16
Viola reichenbachiana	**0.49**	**0.40**	**0.40**	0.35	**-0.33**	**-0.45**	0.13	**0.33**	0.08

Spring

	GD	growth sum	warm sum	Ta 10 cm	Ta 200 cm	Ts	rH	SM
Alliaria petiolata	-0.25	**-0.62**	**-0.54**	-0.37	**-0.64**	-0.09	0.26	0.35
Allium ursinum	-0.28	-0.01	-0.09	-0.01	0.06	0.15	-0.17	0.17
Anemone nemorosa	**-0.61**	**-0.62**	**-0.67**	**-0.53**	**-0.63**	-0.04	**0.37**	-0.21
Anemone ranunculoides	-0.14	-0.11	-0.18	-0.26	-0.11	-0.12	0.01	0.19
Arum maculatum	-0.37	-0.37	-0.51	-0.20	-0.29	0.01	0.07	-0.01
Cardamine bulbifera	**-0.52**	**-0.67**	**-0.57**	**-0.53**	**-0.65**	**-0.41**	**0.56**	0.11
Ficaria verna	**-0.47**	**-0.65**	**-0.66**	**-0.44**	**-0.67**	-0.11	**0.34**	0.07
Galium odoratum	**-0.59**	**-0.73**	**-0.72**	**-0.65**	**-0.72**	-0.16	**0.36**	-0.16
Lathyrus vernus	**-0.53**	**-0.52**	**-0.50**	**-0.45**	**-0.52**	-0.25	**0.53**	0.08
Mercurialis perennis	**-0.56**	**-0.62**	**-0.66**	**-0.61**	**-0.61**	**-0.31**	**0.45**	0.03
Oxalis acetosella	**-0.33**	**-0.49**	**-0.48**	**-0.52**	**-0.49**	-0.19	**0.38**	0.10
Paris quadrifolia	0.03	-0.13	-0.16	0.11	-0.12	0.22	-0.07	-0.30
Primula elatior	-0.33	**-0.51**	**-0.48**	**-0.44**	**-0.52**	-0.16	**0.63**	-0.04
Ranunculus auricomus	**-0.51**	**-0.66**	**-0.54**	**-0.47**	**-0.68**	-0.06	0.06	0.03
Stellaria holostea	-0.37	-0.15	-0.19	-0.15	-0.14	-0.25	0.07	-0.07
Viola reichenbachiana	**-0.49**	**-0.55**	**-0.47**	**-0.47**	**-0.57**	-0.17	**0.50**	-0.09

Table S8: Relationships between microclimate and the peak flowering of different plant species. The values are R²-values derived from linear regressions of flowering peak against the different microclimatic variables. The winter period encompasses October 2016 – January 2017 and the spring period February – May 2017. Ice days = the number of days with a maximum temperature < 0°C, cold days = the number of days with a temperature minimum < 0°C, cold sum = the sum of days with a mean day temperature < 0 °C, cool days = the number of days with a temperature maximum < 10°C, Ta = air temperature in °C, rH = relative humidity, Ts = soil temperature, SM = soil moisture, GD = days with temperatures between 10°C to 30°C, growth sum = the sum of mean day temperature > 5°C, warm sum = sum of day temperatures with a mean > 10 °C.

Winter

	Ice days	Cold days	Cold sum	Cool days	Ta 10 cm	Ta 200 cm	Ts	rH	SM
Alliaria petiolata	0.10	0.20	0.25	0.00	0.08	0.32	0.02	0.03	0.11
Allium ursinum	0.17	0.04	0.06	0.17	0.04	0.00	0.02	0.05	0.05
Anemone nemorosa	0.10	0.28	0.35	0.00	0.14	0.36	0.02	0.01	0.01
Anemone ranunculoides	0.01	0.03	0.09	0.00	0.01	0.05	0.00	0.00	0.02
Arum maculatum	0.09	0.26	0.09	0.00	0.01	0.14	0.02	0.01	0.04
Cardamine bulbifera	0.10	0.14	0.10	0.17	0.20	0.18	0.09	0.13	0.04
Ficaria verna	0.10	0.28	0.33	0.00	0.14	0.38	0.03	0.05	0.01
Galium odoratum	0.08	0.29	0.37	0.01	0.20	0.41	0.00	0.00	0.00
Lathyrus vernus	0.11	0.14	0.12	0.03	0.11	0.16	0.01	0.10	0.01
Mercurialis perennis	0.03	0.31	0.37	0.00	0.24	0.38	0.04	0.05	0.00
Oxalis acetosella	0.00	0.13	0.16	0.00	0.19	0.20	0.00	0.01	0.01
Paris quadrifolia	0.06	0.00	0.01	0.04	0.00	0.00	0.00	0.05	0.04
Primula elatior	0.29	0.20	0.15	0.12	0.01	0.18	0.01	0.25	0.00
Ranunculus auricomus	0.19	0.28	0.27	0.00	0.15	0.36	0.03	0.00	0.00
Stellaria holostea	0.01	0.03	0.11	0.07	0.07	0.07	0.08	0.01	0.03
Viola reichenbachiana	0.24	0.16	0.16	0.12	0.11	0.20	0.02	0.11	0.01

Spring

	GD	growth sum	warm sum	Ta 10 cm	Ta 200 cm	Ts	rH	SM
Alliaria petiolata	0.07	0.38	0.29	0.14	0.41	0.01	0.07	0.13
Allium ursinum	0.08	0.00	0.01	0.00	0.00	0.02	0.03	0.03
Anemone nemorosa	0.37	0.39	0.45	0.28	0.39	0.00	0.14	0.04
Anemone ranunculoides	0.02	0.01	0.03	0.07	0.01	0.01	0.00	0.03
Arum maculatum	0.14	0.14	0.26	0.04	0.09	0.00	0.00	0.00
Cardamine bulbifera	0.27	0.44	0.32	0.28	0.43	0.17	0.31	0.01
Ficaria verna	0.22	0.42	0.43	0.19	0.44	0.01	0.12	0.00
Galium odoratum	0.35	0.53	0.52	0.43	0.52	0.02	0.13	0.02
Lathyrus vernus	0.28	0.27	0.25	0.20	0.27	0.06	0.28	0.01
Mercurialis perennis	0.31	0.38	0.43	0.37	0.37	0.10	0.21	0.00
Oxalis acetosella	0.11	0.24	0.23	0.27	0.24	0.03	0.14	0.01
Paris quadrifolia	0.00	0.02	0.03	0.01	0.01	0.05	0.01	0.09
Primula elatior	0.11	0.26	0.23	0.19	0.27	0.03	0.40	0.00
Ranunculus auricomus	0.26	0.44	0.29	0.23	0.46	0.00	0.00	0.00
Stellaria holostea	0.14	0.02	0.04	0.02	0.02	0.06	0.01	0.01
Viola reichenbachiana	0.24	0.31	0.22	0.22	0.33	0.03	0.25	0.01

Table S9: Relationships between microclimate and the peak flowering of different plant species. The values are regression coefficients derived from linear regressions of flowering peak against the different microclimatic variables, with significant values in bold. The winter period encompasses October 2016 – January 2017 and the spring period February – May 2017. Ice days = the number of days with a maximum temperature < 0°C, cold days = the number of days with a temperature minimum < 0°C, cold sum = the sum of days with a mean day temperature < 0 °C, cool days = the number of days with a temperature maximum < 10°C, Ta = air temperature in °C, rH = relative humidity, Ts = soil temperature, SM = soil moisture, GD = days with temperatures between 10°C to 30°C, growth sum = the sum of mean day temperature > 5°C, warm sum = sum of day temperatures with a mean > 10 °C.

Winter

	Ice days	Cold days	Cold sum	Cool days	Ta 10 cm	Ta 200 cm	Ts	rH	SM
Alliaria petiolata	0.49	0.47	**0.11**	0.05	-4.70	**-10.89**	-0.65	0.58	0.50
Allium ursinum	-0.19	0.14	0.04	-0.23	-0.05	0.34	0.65	-0.14	0.08
Anemone nemorosa	**0.70**	0.70	**0.15**	0.09	**-8.70**	**-11.45**	-0.44	1.31	-0.30
Anemone ranunculoides	0.15	0.19	0.07	-0.12	-3.48	-1.77	-0.84	0.03	0.20
Arum maculatum	-0.30	0.40	0.05	0.04	-1.99	-3.17	0.07	0.11	-0.01
Cardamine bulbifera	**0.33**	0.22	**0.04**	**0.33**	**-3.96**	**-6.05**	**-2.39**	**0.86**	0.07
Ficaria verna	0.52	0.51	**0.10**	0.02	**-5.14**	**-9.52**	-0.75	1.03	0.08
Galium odoratum	**0.22**	0.22	**0.05**	0.05	**-3.30**	**-4.41**	-0.52	0.38	-0.07
Lathyrus vernus	0.72	0.48	0.09	0.29	-6.83	**-10.95**	-2.06	1.91	0.09
Mercurialis perennis	0.54	0.90	**0.20**	-0.13	**-13.68**	**-14.81**	-4.85	2.06	0.06
Oxalis acetosella	-0.11	0.41	**0.08**	-0.14	**-6.83**	**-7.27**	-1.76	1.30	0.11
Paris quadrifolia	-0.49	0.00	0.02	-0.28	1.45	-1.71	3.07	-0.19	-0.41
Primula elatior	**0.95**	0.39	**0.07**	0.76	-4.74	**-6.15**	-1.60	**1.61**	-0.05
Ranunculus auricomus	**1.05**	0.64	**0.13**	0.00	-8.06	**-14.92**	-0.62	0.24	0.06
Stellaria holostea	0.10	0.11	0.05	-0.37	-1.01	-1.21	-0.96	0.20	-0.03
Viola reichenbachiana	**1.10**	0.46	**0.09**	**0.56**	**-6.35**	**-9.49**	-1.61	**1.42**	-0.10

Spring

|	GD	growth sum	warm sum	Ta 10 cm	Ta 200 cm	Ts	rH	SM
Alliaria petiolata	-0.70	**-0.13**	**-0.22**	-3.02	**-7.29**	-0.86	0.32	0.49
Allium ursinum	-0.42	0.00	-0.02	-1.01	-0.08	-0.41	-0.19	0.11
Anemone nemorosa	**-1.20**	**-0.14**	**-0.35**	**-5.81**	**-9.12**	1.09	**0.33**	-0.15
Anemone ranunculoides	-0.22	-0.02	-0.09	-1.04	-3.08	0.13	-0.02	0.13
Arum maculatum	-0.56	-0.05	-0.17	0.73	-3.70	0.52	-0.11	-0.10
Cardamine bulbifera	**-0.44**	**-0.08**	**-0.14**	**-2.84**	**-3.08**	**-1.40**	**0.53**	0.14
Ficaria verna	**-0.76**	**-0.12**	**-0.26**	**-4.05**	**-6.89**	-1.04	**0.61**	0.14
Galium odoratum	**-0.35**	**-0.06**	**-0.12**	**-2.07**	**-3.24**	0.08	**0.04**	-0.03
Lathyrus vernus	**-1.12**	**-0.14**	**-0.28**	**-3.96**	**-6.35**	-0.52	**0.94**	0.10
Mercurialis perennis	**-1.36**	**-0.19**	**-0.43**	**-9.37**	**-12.32**	**-2.41**	**0.97**	0.11
Oxalis acetosella	**-0.55**	**-0.09**	**-0.19**	**-6.24**	**-5.74**	-0.55	**0.40**	0.11
Paris quadrifolia	0.04	-0.03	-0.07	-0.06	-0.87	0.67	-0.70	-0.28
Primula elatior	-0.44	**-0.08**	**-0.16**	**-1.29**	**-4.20**	0.62	**1.33**	0.05
Ranunculus auricomus	**-1.12**	**-0.18**	**-0.33**	**-5.68**	**-9.41**	-1.46	0.08	-0.02
Stellaria holostea	-0.32	-0.02	-0.05	-1.61	-2.27	-0.97	-0.27	-0.09
Viola reichenbachiana	**-0.84**	**-0.11**	**-0.20**	-3.46	**-6.15**	0.96	**0.90**	0.09
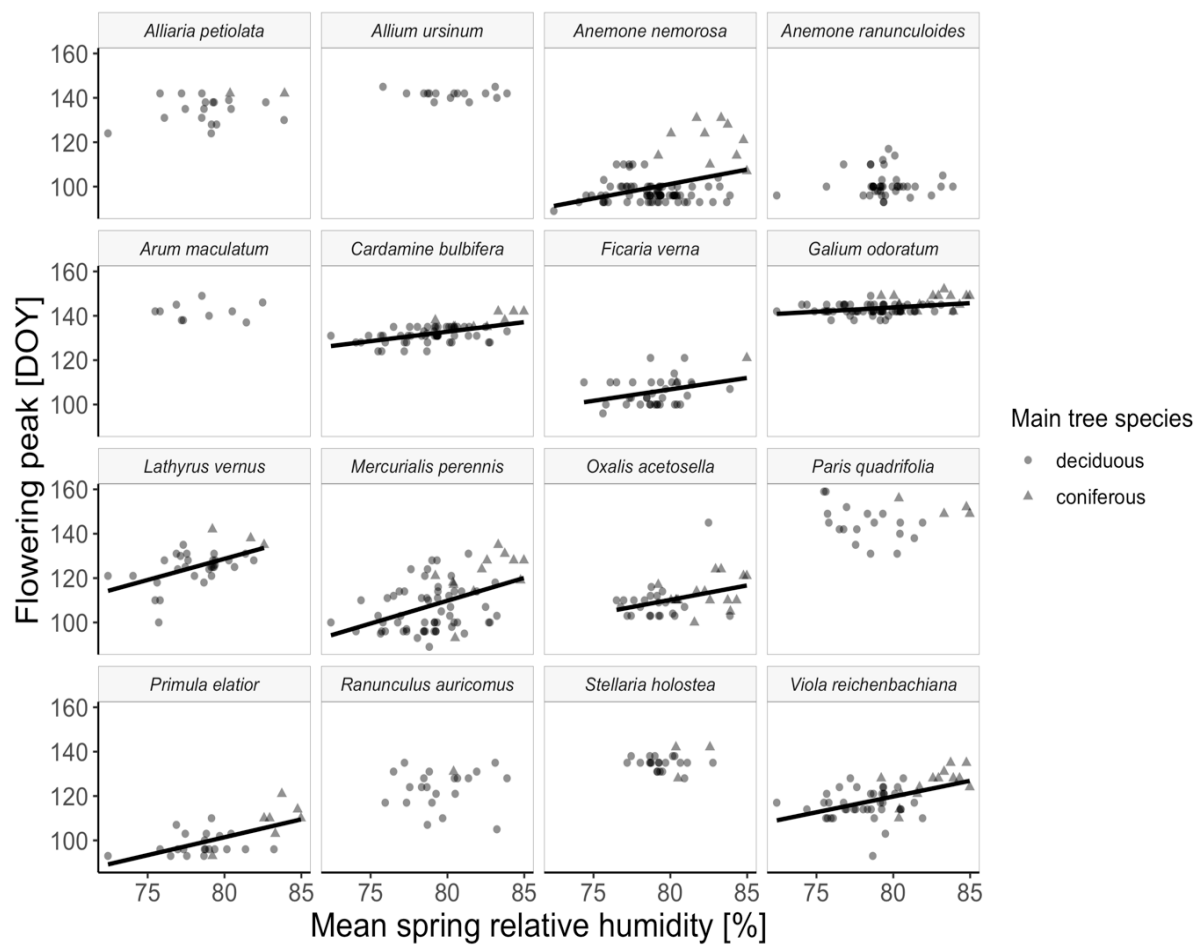
Figure S2: Regression of flowering peak against mean spring relative humidity. Each point represents a forest plot, and the shape of each point indicates whether the main tree species is deciduous (circle) or coniferous (triangle). For significant regressions, the regression lines are plotted. All regression coefficients are listed in Table S2.

Table S10: Unstandardized and standardized estimates of the path coefficients derived from the piecewise SEM, with their respective standard errors (SE), degrees of freedom (DF), critical- and *P*-values.). Only significant paths are listed. CPA = crown projection area, SCI = Zenner's Structural Complexity Index, Slope = the inclination multiplied by 1 for south-, 1 for north-, and 0.5 for east- and west-facing slopes, VMR = Clapham's variance-mean ratio, % conifers = percentage of coniferous trees and Exploratory = region.

| Response	Predictor	Estimate	SE	DF	Critical Value	P-value	Std. Estimate
peak	Temperature	-3.54	0.86	621.00	-4.10	<0.001	-0.09
peak	Relative humidity	0.44	0.15	621.00	3.01	0.003	0.06
peak	coniferous CPA	0.05	0.01	621.00	4.16	<0.001	0.09
peak	Exploratory (HAI)	-2.52	0.71	621.00	-3.54	<0.001	-0.07
peak	Slope	-0.09	0.04	621.00	-2.05	<0.001	-0.03
Temperature	CPA	0.0001	<0.001	636.00	6.39	<0.001	0.18
Temperature	coniferous CPA	-0.01	<0.001	636.00	-19.03	<0.001	-0.59
Temperature	SCI	0.09	0.02	636.00	4.19	<0.001	0.13
Temperature	Exploratory (HAI)	0.15	0.03	636.00	5.27	<0.001	0.16
Temperature	Slope	0.01	0.00	636.00	3.82	<0.001	0.10
Relative humidity	CPA	-0.0001	<0.001	635.00	-2.68	0.008	-0.08
Relative humidity	coniferous CPA	0.03	0.00	635.00	9.20	<0.001	0.36
Relative humidity	Temperature	-2.71	0.21	635.00	-12.90	<0.001	-0.51
Relative humidity	Exploratory (HAI)	2.85	0.15	635.00	18.51	<0.001	0.57
Relative humidity	Slope	-0.03	0.01	635.00	-2.70	0.007	-0.07
Relative humidity	VMR	-0.09	0.02	635.00	-3.73	<0.001	-0.10
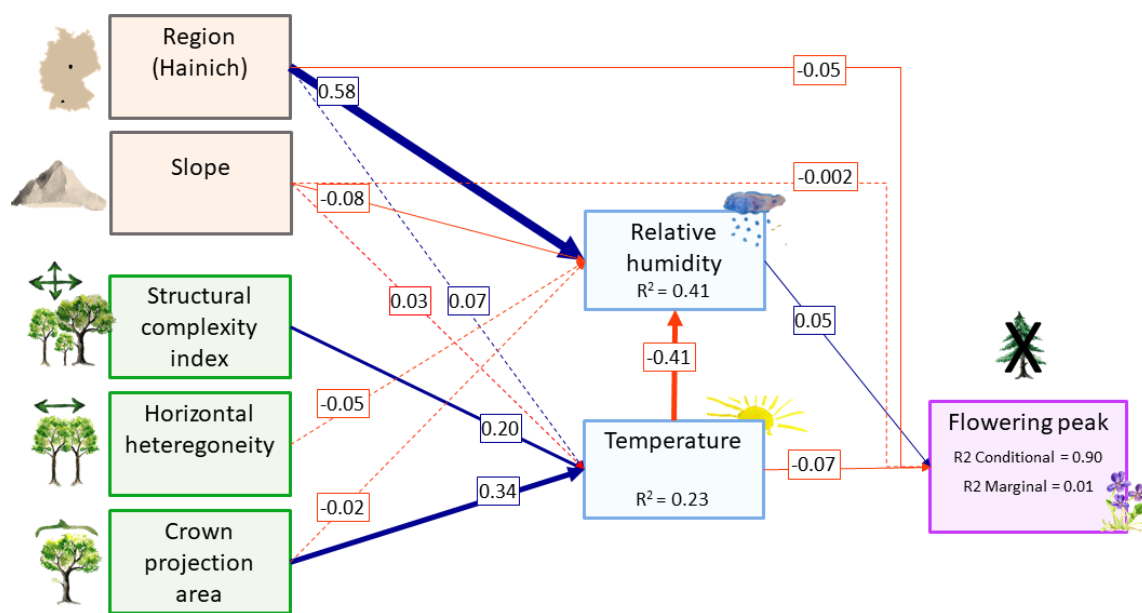
Figure S3. Results of the piecewise structural equation model (SEM), using data from deciduous plot only, testing for direct and indirect relationships among forest characteristics, geographic parameters, microclimatic variables and the timing of peak flowering of forest understory herbs. Arrows represent unidirectional relationships among variables; non significant paths ($P > 0.05$) are dashed. Blue arrows are positive relationships, red arrows negatives ones. The thickness of the arrows is proportional to the magnitudes of the standardized regression coefficient, which are also plotted on the arrows. The R^2 values for component models are also given for each response variable. In the model with flowering peak as a response variable, we included the species as random factor. The overall model is a good fit to the data: Fisher's C = , 4.135 df = 10, P-value = 0.941.

S11: Unstandardized and standardized estimates of the path coefficients derived from the piecewise SEM analysing deciduous plots only, with their respective standard errors (SE), degrees of freedom (DF), critical- and *P*-values.). Only significant paths are listed. CPA = crown projection area, SCI = Zenner's Structural Complexity Index, Slope = the inclination multiplied by 1 for south-, 1 for north-, and 0.5 for east- and west-facing slopes, VMR = Clapham's variance-mean ratio, % confers = percentage of coniferous trees and Exploratory = region.

Response	Predictor	Estimate	SE	DF	Critical Value	P-value	Std. Estimate
peak	Temperature	-3.687	1.02	328.00	-3.62	<0.001	-0.07
peak	Relative humidity	0.436	0.18	328.00	2.36	0.019	0.05
peak	Region (HAI)	-2.181	0.96	328.00	-2.28	0.023	-0.05
peak	Slope	-0.006	0.07	328.00	-0.09	0.928	0.00
Temperature	CPA	0.0001	<0.001	343.00	6.54	<0.001	0.34
Temperature	SCI	0.094	0.02	343.00	3.89	<0.001	0.20
Temperature	Region (HAI)	0.063	0.04	343.00	1.49	0.137	0.07
Temperature	Slope	-0.002	<0.001	343.00	-0.56	0.574	-0.03
Relative humidity	CPA	0.000	<0.001	342.00	-0.31	0.760	-0.02
Relative humidity	Temperature	-2.556	0.28	342.00	-8.99	<0.001	-0.41
Relative humidity	Region (HAI)	3.055	0.23	342.00	13.35	<0.001	0.58
Relative humidity	Slope	-0.041	0.02	342.00	-1.97	0.050	-0.08
Relative humidity	VMR	-0.043	0.04	342.00	-1.09	0.276	-0.05
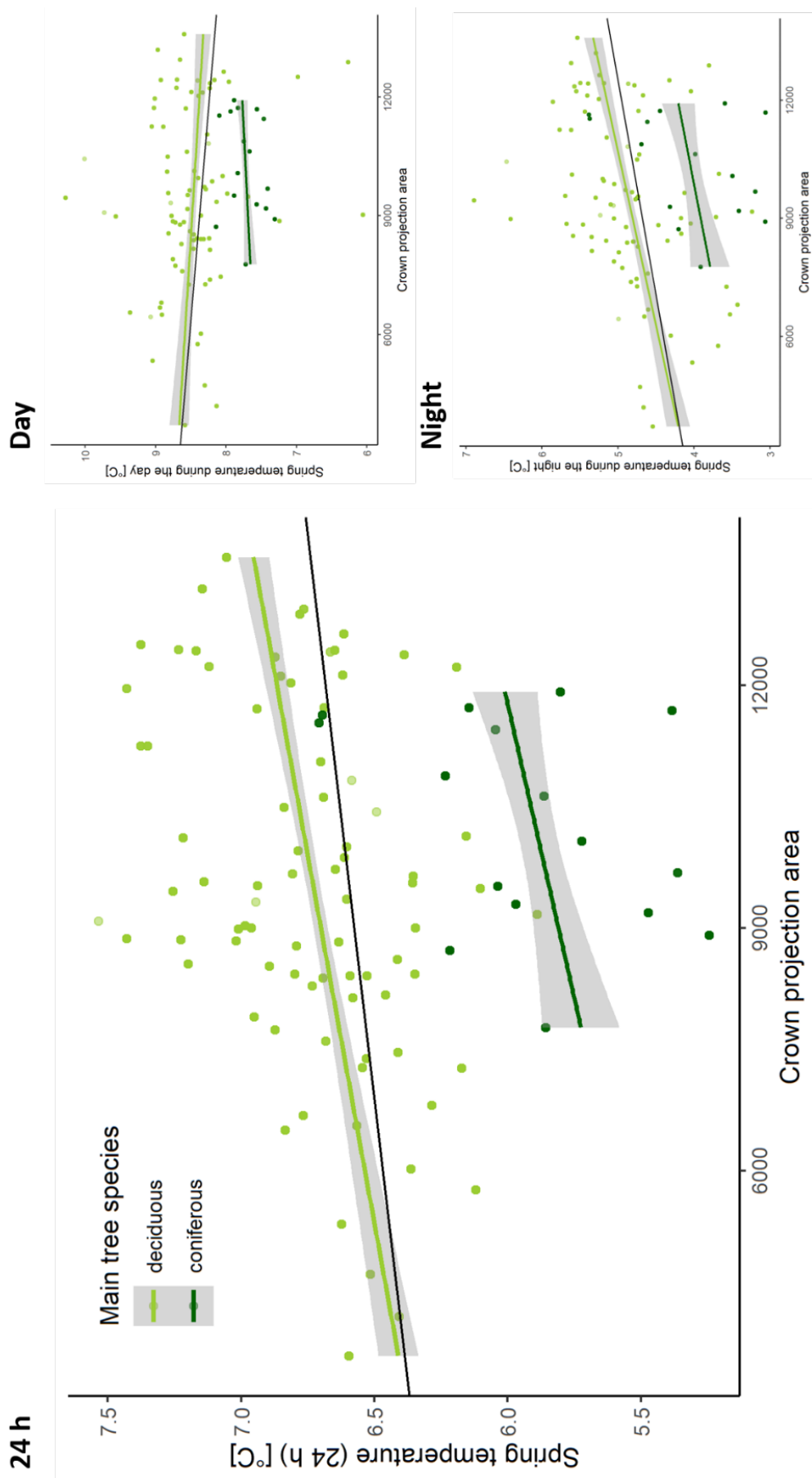
Figure S3: Relationship between crown projection area and spring temperature over 24 hours, during the day (8 a.m. – 8 p.m.) and during the night (8 p.m. – 7 a.m.). Regression of mean spring temperature against crown projection area for deciduous (light green) as well as coniferous plots (dark green), with 95% confidence interval and over all plots together (black line).

Chapter V

Climate warming changes synchrony of plants and pollinators in Germany

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Abstract

Climate warming changes the phenology of many species. When interacting organisms respond differently, climate change may disrupt their interactions and affect the stability of ecosystems. Here, we used GBIF occurrence records to examine phenology trends in plants and their associated insect pollinators in Germany since the 1960s. We found strong phenological advances in plants, but differences in the extent of shifts among pollinator groups. The temporal trends in plant and insect phenologies were generally associated with interannual temperature variation, and thus likely driven by climate change. The phenological advancement of plants did not depend on their level of pollinator dependence. When examining the temporal co-occurrence of plant-pollinator pairs from 1980 onwards, the temporal trends in their synchrony again depended on the pollinator group: while the synchrony of plant-butterfly interactions remained unchanged, interactions with bees and hoverflies tended to become more synchronized, mainly because the phenology of plants responded more strongly to climate change and plants caught up with these pollinators. If the observed trends continue, these interactions are expected to become more asynchronous again in the future. Our study demonstrates that climate change affects the phenologies of interacting groups of organisms, and that it also influences their synchrony.

Keywords: asynchrony, GBIF, mismatch, phenology, pollination mode

Introduction

Phenological events are periodically occurring events in the life cycle of organisms. The timing of these events often depends on environmental factors such as temperature or photoperiod, and it is well known that climate change affects some of these and thus changes the phenologies of many organisms (Cleland et al. 2007). With such phenology shifts, there is increasing risk of phenological mismatches between interacting organisms, potentially exceeding the natural resilience of ecosystems (Memmott et al. 2007). Climate change-induced phenological shifts have been documented extensively for individual species (Parmesan 2007), but we still know much less about how these shifts affect ecological interactions. Kharouba et al. (2018) recently reviewed 54 published interaction studies across ecosystems and interaction types and found no clear general trend, with about half of the studied interactions becoming more asynchronous but the other half becoming even more synchronized through climate change.

Plant-pollinator systems are among the biotic interactions expected to suffer most from a mismatch of phenological events (Scheffers et al. 2016). Several previous studies have observed mismatches (Miller-Rushing et al. 2010, Robbirt et al. 2014), but in others pollinators and plants seemed to be able to keep up with each other (Bartomeus et al. 2011). An interesting question in this context is also which of the two partners is advancing faster if there is an increasing mismatch. So far, the evidence here is also mixed. For instance Gordo and Sanz (2006) found pollinators to advance faster than trees, and Parmesan (2007) that butterflies advanced faster than herbaceous plants, but in a study by Kudo & Ida (2013) it was the plants – spring ephemerals – that advanced faster than their bee pollinators.

Mismatches of plant-pollinator interactions can have negative consequences for both partners. For the pollinators, this can include lower survival rates, a decreased overall fitness and higher parasite loads (Schenk et al. 2018). Moreover, mismatches might also impact pollinator demography, the body sizes (Miller-Rushing et al. 2010) and frequencies of sexes, and thus population viability (Schenk et al. 2018). On the plant side, desynchronized pollinator interactions are mainly expected to impact plant fitness and thus long-term population growth and survival. For instance, Kudo & Ida (2013) found that seed counts were reduced in early-flowering spring ephemerals after desynchronization with their bee pollinators. However, in another study fly-pollinated plants did not show similar responses (Kudo et al. 2004).

Plants differ in their level of dependence on plant pollinators, and an intriguing question therefore is to what extent phenology responses to climate change are linked to the pollinator

dependence of plants. Bond (1995) theorized that wind-pollinated plants might experience little negative consequences of climate change as they do not depend on interactions with animals. Conversely, insect-pollinated plants may be subject to strong selection toward phenologies that are in synchrony with their pollinators. This hypothesis was later corroborated in an empirical study by Fitter and Fitter (2002). A more recent study on orchids (Molnár et al. 2012) found that pollination mode influenced the degree of plant advances in flowering phenology, indicating that self-pollinating and thus pollinator-independent plants were not constrained by pollinator phenology. The main idea of these previous studies is that all else being equal, pollinator-independent plants should exhibit stronger phenological shifts in response to the same climate changes.

Testing hypotheses about plant-pollinator responses to climate change is not trivial. Since changes in phenology take place on the scale of decades (Parmesan 2006), we need long-term data. A possible source of long-term data on plant phenology are herbarium specimens (Jones and Daehler 2018, Lang et al. 2019), which can indicate the day of year that a specific species was flowering in a given location and year. Herbarium data provide unique historical depth, but they need to be treated with caution because of the sampling biases associated with them (Daru et al. 2018, Maldonado et al. 2015). In recent years the digitization of herbaria as well as other collections and observation data, including on other taxa such as pollinating insects, e.g. from long-term monitoring networks, is creating an increasing number of public data bases that contain vast amounts of natural history data that cover large spatial and temporal scales (Newbold 2010). These data bases are increasingly being used for analyses of broad ecological trends and global changes (Maldonado et al. 2015, Chapman 2005). One of the largest and most important hubs of large-scale and long-term ecological data sets is the Global Biodiversity Facility (GBIF), an intergovernmental initiative and public data base that provides access to biodiversity data compiled from various individual sources like academic institutions, government agencies or independent collections (GBIF 2019).

Another matter is finding a measure for changes in phenology. Primack et al. (2004) demonstrated that the average collection date of the herbarium specimens of a plant species in a year can be used as a proxy for peak flowering time in that year. The same approach of using occurrence records in natural history collections or other data bases can in principle be used to estimate the activity times of other groups of organisms such as insects (Kharouba et al. 2018 and references therein). For instance, analyses of natural history collections in the UK have demonstrated phenology changes in bees (Robbirt et al. 2014) and butterflies

(Brooks et al. 2014). Thus, the peak occurrences of plants and insects in GBIF may be used to estimate activity shifts of different groups, as well as their synchrony. When we use the term 'activity' in this paper, we refer to the period in an organism's life when it can interact with its ecological partner. For plants this is the period of flowering, for insect pollinators the period of flight.

We used data from GBIF to study phenological mismatches between plants and pollinators in Germany, at the level of taxonomic groups as well as individual interactions. We asked the following questions: (i) Are there long-term trends in the phenology of plants and pollinators? (ii) If yes, are phenology trends related to climate change? (iii) How are phenological changes of plants related to their pollinator dependencies? (iv) How does climate change affect the synchrony of plant-pollinator interactions?

Methods

Phenology data

We worked with occurrence records of plants and insects available from the GBIF database (GBIF: The Global Biodiversity Information Facility 2019, GBIF.org 2020*i*, GBIF.org 2020*k*, GBIF.org 2020*c*, GBIF.org 2020*h*, GBIF.org 2020*d*, GBIF.org 2020*a*, GBIF.org 2020*b*, GBIF.org 2020*g*, GBIF.org 2020*j*, GBIF.org 2020*e*, GBIF.org 2020*f*). For the plants, we restricted ourselves to species covered by the BioFlor database of plant traits (Klotz et al. 2002), because we needed to be able to classify plants by their level of pollinator dependence (see below). For the insects we restricted ourselves to beetles (Coleoptera), flies (Diptera), bees (Hymenoptera) as well as butterflies and moths (Lepidoptera), as these groups contain most insect pollinators (Kevan and Baker 1983). We used the R package *rgbif* (Chamberlain and Boettiger 2017) to download all available records of the above taxa from GBIF. Our basic criteria for including records were that they originated from Germany, and that they referred to either a living specimen (e.g., a captured insect), a human observation, just an observation (i.e., when the exact type of observation was not clear), or a preserved specimen (e.g., an herbarium record). If names of plant species were not accepted names, we used the R package *taxsize* (Chamberlain and Szöcs 2013) to check the names against the GBIF backbone taxonomy and determine the actual accepted.

Prior to the data analyses, we subjected the data to several steps of quality control (Fig. S1). First, we removed all records from before 1960 as these turned out to be too inconsistent, with few records per year and large gaps between years with records. We also removed the records from 2020 as the year had not been complete at the time of our analysis. Second, we removed

all records from the first and last days of years because the high number of records on those days indicated that records without a recorded collecting date had been given these as default dates. Next, we removed all records from "GEO Tag der Artenvielfalt", a German bioblitz event where large numbers of records are taken on a specific day of the year. Including these data would have strongly biased the intra-annual distributions of our records. Finally, we removed the records from several collections which appeared to have misclassified these as being of German origin, probably through a combination of coordinate rounding and determining countries of origin automatically from these coordinates. We identified these sets of records by visually inspecting the geographic distributions of the records of each institution; most of these erroneous data sets were from Luxembourg (Table S1). There were a few records just outside the boundaries of Germany that we did not remove from our data set because the country information appeared trustworthy and we suspected errors with the recording of the coordinates. Obviously, the latter steps of our quality control were possible only for georeferenced records, which made up 99.97% of the total amount of records. After these data curation steps, we maintained around 11 million plant records and over one million insect records for our data analysis. There were large differences between plants and insects not only in the numbers of records but also in their temporal distribution across the studied period (Fig. S2). While plants, but also beetles, had relatively even record numbers across decades, the other insect groups, in particular flies and bees, were strongly underrepresented in the earlier decades, and record numbers increased rapidly only in the last 20 years, probably due to the advent of platforms like iNaturalist.org and naturgucker.de, which allow logging of species occurrences by citizen naturalists, and which make up most of our insect data. Beetles were represented, save for one species from the Orsodacnidae, by the Chrysomelidae family.

Climate data, pollinator dependence, and individual interactions
Besides the main phenology data from GBIF, we obtained several other data sets required for our analyses. To test for associations with climate, we used climate data from Deutscher Wetterdienst (DWD, https://www.dwd.de/), specifically the historical (until 2018) and recent (2019) monthly station observations data set (DWD Climate Data Center 2020*a,* DWD Climate Data Center 2020*b*) to calculate the Germany-wide average annual temperatures for 1960-2019. The exact climate data sets used are available at the repository under data availability.

To classify plants by their level of pollinator dependence we used plant trait data from BiolFlor (Klotz et al. 2002). A species was assigned as pollinator-dependent when it was either known to be self-incompatible and pollinated by an insect, dioecious and pollinated by an insect, or protogynous/protandrous while also being pollinated by an insect. In contrast, species that were pollinated abiotically or through selfing, that exclusively reproduced vegetatively, or were apomicts, were classified as pollinator-independent. If none of the above applied, we assigned an intermediate pollinator dependence. If part of the information above was missing, no pollinator dependence was determined, and the species was excluded from the analyses involving pollinator dependence.

Finally, we obtained data on individual plant-pollinator interactions from a UK database on plant-pollinator interactions hosted by the Centre for Ecology and Hydrology (CEH). This database included all known interactions between plants and flower-visiting bees, butterflies, and hoverflies (but unfortunately neither beetles nor moths) in the UK, a country similar to Germany in terms of climate and species composition. While these interaction data are unlikely to represent all possible species interactions in Germany, we could not find similar data for our study area.

Calculation of plant and insect phenology

For our analyses of plant flowering phenology and pollinator activity times, we averaged all records of a plant or insect species in a year to calculate each year's mean day of the year (DOY) of the occurrence of a species. As discussed above, this occurrence measure was used as an estimate of each year's peak flowering or peak activity time of plants and insects, respectively. Each annual mean DOY was calculated from at least five records of a species per year. To avoid extreme shifts based on too little data, we included only species with records in at least 40% of the years. The median number of records per year for a species in our analyses was 47.

Since our analyses of individual plant-insect interactions (see below) were done at the level of decades, we additionally calculated the decadal means, based on nominal decades (0-to-9), of species DOYs for each of the included species, and only when at least five records existed per decade. These decadal interaction analyses were done only from 1980 onwards, i.e. for four decades, as too few data were available prior to 1980. To be included in our analyses, an interaction's records needed to span the entire period examined.
After clean-up and averaging, a total of 58,895 annual and 1,336 decadal peak DOYs, with the latter based on a median number of 1,686 records, remained in our data set (Fig. S1). The

annual activity data included 1,274 plant and 88 insect species. For 948 of the plant species we had information about pollinator dependence: 144 were pollinator-dependent, 204 pollinator-independent, and 600 were classified as intermediate. The 88 insect species consisted of 40 species of beetles, 44 butterflies and moths, three bees and just one fly species. The decadal data included 245 plant and 26 insect pollinator species. All data wrangling and analysis was done in R (R Core Team 2008).

Data Analysis

To understand phenology changes in plants versus insects, we first estimated the average phenological shifts in each group. We defined phenological shifts as the slope of the linear regression linking the peak activity (= mean annual) DOY of an individual species to the year of observation. We visually confirmed approximate normal distribution of the individual-species slopes, and that no improbable outliers were present. There were some plants with rather extreme values (Fig. S3), however these were mostly early-flowering plants which likely experience stronger pressures and therefore stronger phenology shifts (Forrest 2015), and we therefore did not exclude them from our analyses. We compared the mean phenological shifts between plants and insects using an independent-sample Welch's *t*-test, and we further examined the temporal trends between different insect orders and plants in an ANOVA, using a Tukey post-hoc test to determine pairwise differences. We excluded bees and flies from the last step as their numbers were too small to be representative for their respective groups.

Different climatic factors likely affect the timing of early and late activity periods, which might complicate the interpretation of the peak shifts. We therefore also assessed the extent of shifts of first and last day of activity for each species (and consequently the duration of their activity) to understand how asymmetries in the shifts might affect the peak shifts in phenology. For this we estimated the shifts of the decadal average first and last activity day of the year over time in a linear model. We also estimated the shifts of duration of the activity period by first calculating the yearly duration of the activity period as the difference between the last recorded day of activity and the first for each species, taking the decadal average of said duration and then estimating the shift over time in a linear model. We used decadal averages to ensure the differences were due to long-term trends, as the absolute first and last day of activity is just the first and last record of a species in that year and therefore subject to fluctuation.

In addition to the temporal trends in phenology, we also tested for the climate sensitivity of plant and insect phenology. These analyses were analogous to the ones above, except that the explanatory variable was annual mean temperature instead of year of observation, i.e., the data were regression slope parameters of mean annual DOY of a species over the average temperature in that year.

Next, we tested whether phenology trends differed between plant groups with different levels of pollinator dependence. For this, we used the same data as above (slope parameters of individual-species regressions), but we analyzed it with a linear model that included pollinator dependence (dependent, independent, or intermediate) as a fixed factor, and then determined pairwise differences between groups with a Tukey post-hoc test. In addition, we also tested whether mean activity DOY differed significantly between the three pollinator-dependence levels.

Finally, we analyzed asynchrony between plants and pollinators using the data on individual plant-pollinator interactions. For each plant and presumed insect pollinator, we calculated the absolute difference in peak activity times for each decade. A value of zero thus indicated perfect asynchrony, and higher values indicated increasing asynchrony. To test whether asynchrony changed over time we estimated the slopes of the relationship between differences in peak activities and time (decades) for each plant-insect interaction with a linear model. Here, negative slope values indicated a shift towards greater synchrony, and a positive slope a shift towards greater asynchrony. Altogether, there were 1,797 interactions involving 245 plants and 26 insect pollinators, one insect usually associated with multiple plants but seldomly plants with multiple insects. To test for differences in average asynchrony and change of asynchrony between insect groups, we used an ANOVA and assessed pairwise differences with a Tukey post-hoc test.

Results

Temporal trends in plant and insect phenology

The analysis of the peak activity data showed a strong difference in the average temporal shifts of plant and insect phenology (Welch's $t_{100.929} = 6.644$, $P < 0.001$). The phenology of plants generally advanced much more strongly, with an average shift of -4.5 ± 0.2 days per decade (mean ± SE), while across all insects the shift was only -0.4 ± 0.6 days per decade. 84.8% of all plant species but only 56.8% of all insect species advanced their phenology (Fig. 1). However, these numbers across all insects obscured different trends among the insect orders: when considered separately, butterflies/moths exhibited a strong phenology shift of -

3.2 ± 0.8 days per decade (mean ± SE), with 79.5% of the species advancing, whereas the beetles in contrast delayed their peak activity on average by 2.0 ± 0.7 days per decade, with 65.0% of the species following this trend. When plants, butterflies/moths and beetles were analyzed as separate groups, ANOVA indicated significant differences among them ($F_{2, 1355} = 25.16$, $P < 0.001$), with significant pairwise differences (Tukey post-hoc, $\alpha = 0.05$) between the phenology shifts of beetles and plants, and beetles and butterflies/moths, respectively (Fig. S4A and Fig. S5.).
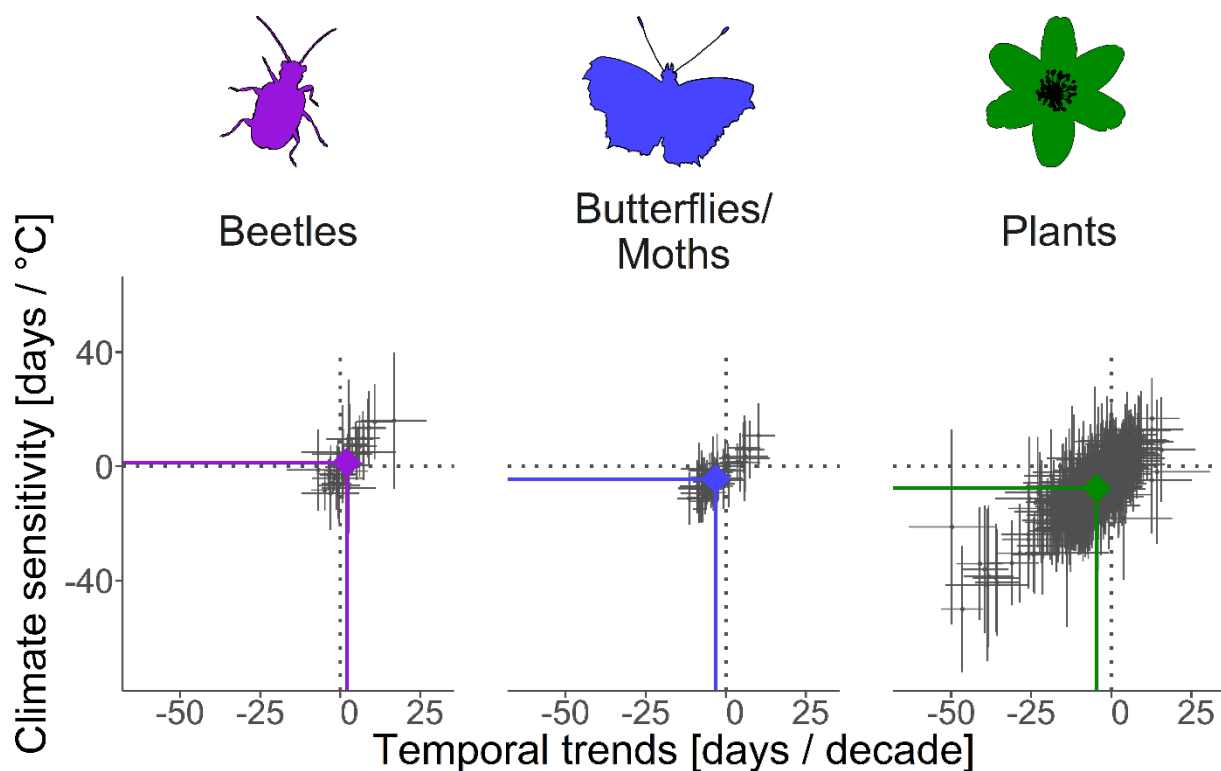
Figure 1. Temporal trends (days per decade) versus climate sensitivities (days per °C temperature change) of the phenology (peak flowering/activity) of plants, beetles, and butterflies/moths, with the colored lines indicating the averages for each group. Grey dots indicate individual species means with the vertical and horizontal bars representing the 95% confidence intervals. For all three groups the relationship between temporal trend and climate sensitivity is highly significant at with $r > 0.8$ and a $P < 0.001$.

We found asymmetries between the slopes of first and last day of activity over time (Fig. S6). In plants, the symmetry was generally skewed towards a stronger shift of the first day of activity (First: -1.2 ± 0.0 mean days/decade ± SE, Last: 0.5 ± 0.0 mean days/decade ± SE) with butterflies/moths behaving similarly (First: -1.4 ± 0.1 mean days/decade ± SE, Last: 0.5 ± 0.2 mean days/decade ± SE), whereas in beetles the last day of activity shifted more strongly (First: 0.1 ± 0.1 mean days/decade ± SE, Last: 0.5 ± 0.1 mean days/decade ± SE). It

is also notable that the plants' and butterflies/moths' day of first activity generally advanced while the beetles' day of first activity was rather delayed (Fig. S7).

Climate sensitivity of plant and insect phenology

The climate sensitivities of the phenologies of plants, butterflies/moths and beetles generally resembled their temporal trends (Figure 1), and group differences in climate sensitivities matched those in temporal trends described above. Again, there was a significant difference between plants and all insects (Welch's $t_{96.026} = 8.027$, $P < 0.001$), with plants showing a strong negative association between peak activity and temperature, but a much weaker association for all insects together. On average, plant peak flowering shifted by -7.6 ± 0.2 days per °C (mean $\pm$ SE), and 92.5% of the individual species showed earlier flowering with increasing temperature, whereas for insects it was only -1.3 ± 0.8 days per °C, and 63.6% showing a trend towards earlier peak activity (Figure 1). When the butterflies/moths were considered separately, however, they showed a fairly strong association with temperature, with an average peak activity shift of -4.4 ± 0.8 days per °C (mean $\pm$ SE) and 80% of the individual species advancing, whereas the beetles showed an opposing trend of delayed peak activity, with an average of $+1.4 \pm 1.1$ days per °C temperature change. There were significant differences among the three groups (ANOVA, $F_{2, 1355} = 45.701$, $P < 0.001$), with significant differences between all pairwise combinations (Tukey post-hoc, $\alpha = 0.05$). (For an overview over all groups, see Fig. S4B and Fig. S8)

Pollinator dependence

The phenology of plants, and its temporal trends, differed very little among plant groups of different levels of pollinator dependence (Fig. S9). The peak flowering of pollinator-independent plants (average DOY 199.5) advanced on average by -3.9 days per decade, while pollinator-dependent plants (average DOY 196.2) advanced by -5.1 days per decade, and intermediate plants (average DOY 199.5) advanced by -4.5 days per decade. In all three groups, the percentage of plants advancing was 85-86%. None of the differences between groups was statistically significant.

Synchrony of plant-pollinator interactions

When examining the synchrony of individual plant-pollinator interactions, we found that the three pollinator groups differed in their average levels of asynchrony with the plants, but that

interactions did not become more asynchronous but rather more synchronized during the last decades (Figure 2A).
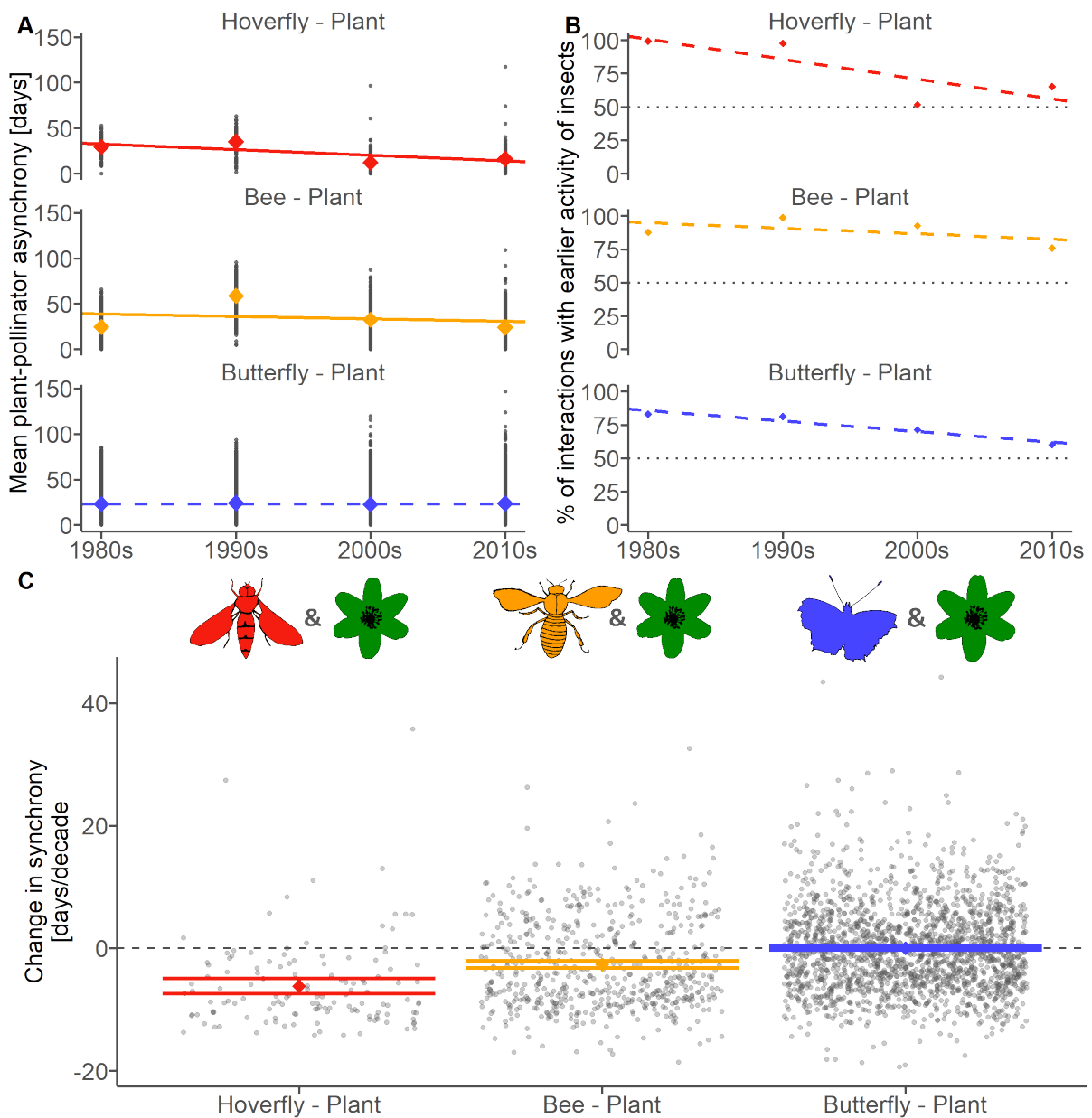

Figure 2. Asynchrony of individual plant-pollinator interactions, and their temporal trends, separated by pollinator groups. (A) Decadal changes of asynchrony (grey dots/lines: individual interactions; colored diamonds/lines: linear regression for each group. (B) Fraction of interactions with earlier insect activity. (C) Average decadal asynchrony changes of individual interactions (grey dots), and the means for each group (colored dots and 95% CI whiskers). Solid lines in (A) and (B) indicate significant linear regressions, dashed lines non-significant ones.

The temporal trends differed strongly among the pollinator groups (ANOVA, $F_{2, 2522} = 67.750$, $P < 0.001$; Tukey's post-hoc test significant at $\alpha = 0.05$ for all pairwise comparisons:

Figure 2C): while the synchrony of plant-butterfly interactions remained on average unchanged, plant-pollinator interactions involving bees shifted on average by -2.7 days per decade, with 68% of individual interactions decreasing asynchrony over time. The strongest shifts were in plant-hoverfly interactions which shifted by -6.2 days per decade, with 89% of all interactions showing decreasing asynchrony (Figure 2A, C). In all three plant-pollinator groups, asynchrony was mostly due to earlier peak activity of the insects (Figure 2B). Interestingly, however, there was a tendency for these patterns to disappear in all three groups over time, presumably because of the stronger phenology shifts of plants (Figure 1). Plant-hoverfly interactions ($n_{Insect} = 1$, $n_{Plant} = 132$, $n_{total} = 132$) became on average synchronous in the last decade. For the plant-butterfly interactions ($n_{Insect} = 36$, $n_{Plant} = 231$, $n_{total} = 1,819$) the linear model predicts the point of synchrony to be reached in 2029, and for the plant-bee interactions ($n_{Insect} = 4$, $n_{Plant} = 214$, $n_{total} = 574$) in 2050.

Discussion

In this study, we took advantage of large collections of occurrence records to examine phenological trends of flowering plants and insect pollinators in Germany. We asked whether phenology changes affected the synchrony of plants and insects, and whether observed changes in phenology, and variation therein, were related to the different groups' responses to climate warming. We also examined whether the phenology responses of plants depended on their levels of pollinator dependence. Our results showed that the phenological shifts of plants and insects indeed differed, with plants shifting by several days per decade while insects on average shifting hardly at all. As peak flowering historically occurred after peak insect activity, these trends imply an increase in plant-pollinator synchrony during the last decades, but a potential for future desynchronization if climate change continues.

Plants and insects also differed in their overall temperature sensitivity. While plants shifted on average by over a week per degree of warming, insects shifted by only one day. There were large differences between insect orders in their phenology trends and temperature sensitivities. As groups with greater temperature sensitivity also showed larger phenology shifts over time, it seems likely that the two are causally related, i.e., that anthropogenic climate warming is responsible for the observed phenology shifts. Lastly, there were no differences between pollinator-dependent and -independent plants, suggesting that plants either responded passively to temperature, with advanced flowering in warmer years irrespective of pollinator dependence, or that most plants have sufficient generalist pollinators

that can fill in for other, desynchronized pollinator species and thereby reduce selection pressure on plant phenology.

Caveats

When interpreting the results of our study, it is important to consider some caveats of the collections data and occurrence records we used. For instance, the temporal distribution of collections data is usually quite heterogenous, and so was our data (Fig. S2). Our analysis of the shifts of the first and last days of activity may thus be influenced by varying observation efforts over the years. Particularly, the increasing popularity of nature observation platforms such as www.naturgucker.de, whose records are contained in GBIF, may have resulted in higher probability of detecting early and late occurrences. Besides temporal heterogeneity, occurrence records are usually also not homogenously represented in space. Our study's measure of phenology, peak occurrence time, does not account for temporal variation of spatial representation of records within Germany, although some areas might be over- or underrepresented in some parts of the studied period. Moreover, our study also does not account for spatiotemporal variation in macro- and microclimate which can influence intraspecific variation in phenology shifts (Song et al. 2020) and could therefore potentially induce local mismatches.

When estimating insect peak activity, we did not account for the earlier life stages of insects appearing in the data, despite being not important for pollination. This bias could be most relevant for butterflies and moths, as their larval stages are more conspicuous than fly and beetle larvae. Butterflies/moths are, however, the group with the latest peak activity times for large parts of the studied period, so this bias is either not strong or we are underestimating how late in the year butterflies and moths occur. Similarly, some plants occurrences may have been recorded when plants were not flowering. Flowers are important for plant species identification, and herbarium records are usually made from flowering specimen, but we cannot rule out that some plant occurrence records were based on vegetative plants alone. Finally, in our analyses we focused on peak activity and therefore did not consider the degree of overlap between the flight times of pollinators and the flowering of plants. However, if the durations of activity periods change, then the relative overlap of two interacting groups could change in spite of identical activity peaks, or vice versa. Testing such possibilities with occurrence data, however, requires even higher-resolution data for individual species than in our study.

Phenological shifts over time

The general differences between plants and insects in their advancement of phenology seem to indicate a shift in the synchrony between plants and their pollinators, with plants generally advancing faster than insects. However, the insect groups differed strongly in the extent of their shifts of activity over time, and the overall pattern of a slower phenological shifts was largely driven by the beetles, whereas butterflies/moths kept pace with the phenology changes of plants.

The extent to which plants advanced their phenology in our data is comparable to that found by Fitter and Fitter (2002) in their long-term observation study of changes in first flowering dates of hundreds of plant species in England. They compared flowering during 1991-2000 to that between 1954 and 1990 and found an average advancement of 4.5 days. This is surprisingly congruent with our observation of 4.5 days advancement per decade over the whole period from 1960 to 2019. A more recent long-term analysis of phenology changes in subalpine meadow plants in the Rocky Mountains was undertaken by CaraDonna et al. (2014) who found an even stronger average advancement of first flowering of 6.4 days per decade. Since CaraDonna et al. (2014) also analyzed peak floral abundance, their data should be particularly comparable to our estimation of peak flowering through the DOY of peak occurrence. They found a rate of advancement of 5.3 days per decade in spring peak abundance but only 3.3 days for the summer peak floral abundance. Our results of peak occurrence across the whole year thus fall in between these two estimates.

For insects, previous studies seem to be less consistent, with widespread but not universal advances in springtime phenology (mostly associated with warming) over the last decades (Forrest 2016). For butterflies, long-term records showed that their times of first flights (correlated with peak appearance) advanced on average by -3.7 days per decade in the 2000s compared to the previous decades in England (Roy and Sparks 2000), and by -7.7 days per decade in California (Forister and Shapiro 2003). The magnitude of the shifts observed in England is similar to what we estimated for butterflies/moths in Germany (on average -3.2 days per decade).

Temperature sensitivities of plant and insects

We found that associations between temperature and phenology differed among groups but that the magnitude of these associations generally reflected the different groups' phenology shifts observed over time. This strongly suggests a link between the phenology shifts and climate change, corroborating previous studies such as the ones by CaraDonna et al. (2014)

and Song et al. (2020). We found that plants were generally more sensitive to temperature, i.e., their phenology advanced more strongly, than insect pollinators. Previous studies on insect phenology in the temperate zone (reviewed in Forrest 2016) have shown that increased spring temperatures are often associated with earlier insect emergence, but that this pattern cannot be generalized as easily as for the plants, as temperature–phenology relationships of insects are more complex. While many insects plastically respond to warmer temperatures by speeding up their rates of development (and thus potentially emerge earlier), others have been found to respond in counterintuitive ways and delay their phenology. This might be due to dependence on other cues such as rainfall (Bonal et al. 2015), due to cold period requirements of insects during their diapause (climate warming can cause a loss or reduction of this chilling period, and this tends to increase the amount of warming required for subsequent emergence (Forrest 2016)), or because species overwinter in a diapause state in which they are not temperature sensitive (Fründ et al. 2013). Fründ et al. (2013) also showed that bees overwintering in larval stages responded to higher winter temperatures with delayed emergence, while bees overwintering as adults showed advanced emergence (but had greater weight losses during overwintering). We did see delayed phenology in some of our data, particularly for beetles and bees. This also connects well to some of the findings reviewed by Forrest (2015), for instance that during winter above-ground nesting bees experience different temperatures than the plants they feed on during the summer. Such microclimate differences between insects and plants during overwintering may sometimes explain contrasting climate responses. In other cases, delays in the first appearance of adults may result from longer growing seasons. For example, longer growing seasons have reduced selection for rapid development in some high-elevation grasshoppers, in such a way that they reach maturity later — but at a larger size — than in the past (Buckley et al. 2015). Furthermore, warming can change the number of generations per year (voltinism; Forrest 2016). All the above-mentioned mechanisms can cause variation in the phenology shifts of insects with climate warming and may therefore explain why climate change is not always accompanied by phenological advances but might also cause delays – as we observed for the beetles.

Another interesting idea is that the phenological advancement of the plants itself could cause delayed phenology of some pollinators. Wallisdevries & van Swaay (2006) found that advanced plant growth led to delayed development of butterflies since the cooling created by shading leaves worsened foraging conditions for the larvae. However, in our study we did not see this effect for butterflies/moths as their phenology shifts closely tracked the shifts of

plants, perhaps because of the high levels of specialization of many butterfly larvae (Gilbert and Singer 1975).

Pollinator-dependence of plants

We did not find any differences in the phenological changes of pollinator-dependent versus pollinator-independent plants. This result is consistent with Rafferty & Ives (2011) who found that the phenology shifts of plants were not constrained by their pollinators, because these kept pace with the plants. In contrast, Kudo et al. (2008) found a negative effect of flowering advancement in bee-pollinated but not fly-pollinated plants. Fitter and Fitter (2002) found significant differences between insect-pollinated plants (-4.8 days shift in day of first flowering) versus wind-pollinated plants (-3.5 days shift) and suggested this was because shifting pollinator activity forced plants to flower earlier. In our study we did not find any such differences, indicating that plant responses to temperature are either entirely passive, or that most plants have generalist pollinators with a long period of activity, so that there is little selection pressure on plant phenology. The data set used in our analysis is larger than those used in the studies cited above, so our results may be regarded as more conclusive and more general, bearing the limitations of the collections data in mind.

Changes in plant-pollinator synchrony

When we analyzed the synchrony of plant-pollinator interactions, we found clear trends in shifting synchrony, but they strongly varied among insect pollinator groups. Since the phenology of plants generally advanced faster than that of the insects during the last decades, but plants had generally been the later partner in most plant-pollinator interactions, these shifts lead to greater synchrony overall. However, if the observed trends continue, then many of the studied interactions will soon reach points of perfect synchrony, and after that the interactions may become more asynchronous again, albeit in the other direction. For plant-hoverfly interactions this point has already been reached. With linear trends and if we assume that observed trends will continue, the points of reversals are expected in approximately 10 years for plant-butterfly interactions and in around 30 years for plant-bee interactions. If interactions will become more asynchronous again in the future, then resilience of pollinator networks, in particular through pollinator generalism, could buffer some of the impact of phenological mismatches (Miller-Rushing et al. 2010), and our finding of no differences between pollinator-dependent and pollinator-independent plants support this idea. However, while generalist pollinators make up the larger part of the interactions in most pollination

networks, some plant-pollinator interactions are highly specialized, and these might be the ones suffering most from future mismatches (Bascompte and Jordano 2007).

Acknowledgements

Data availability

The R code used to conduct the analysis is available at https://github.com/jonasfreimuth/Phenological-shifts-germany.

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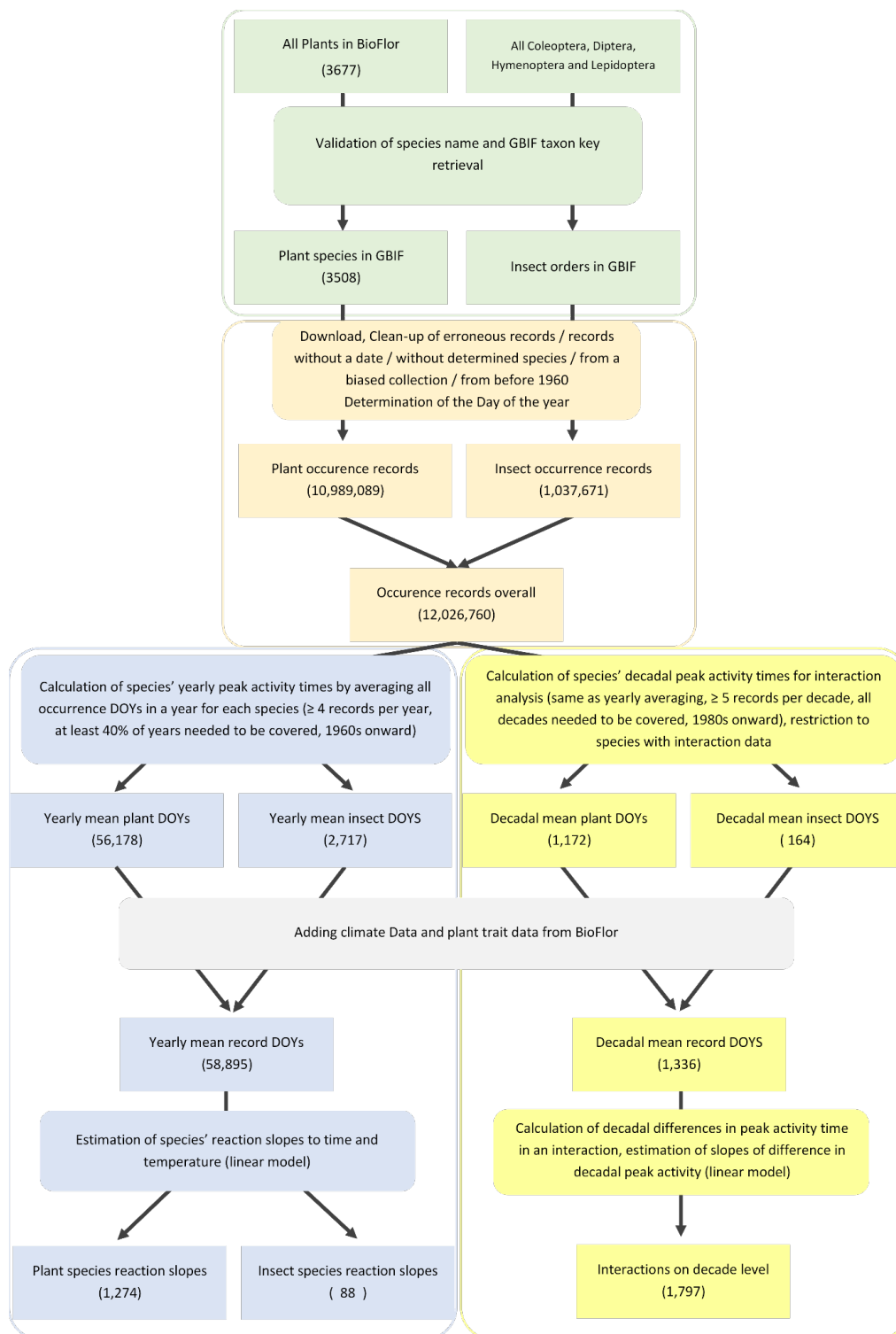
Supplementary material

Figure S1. Schematic of data collection and the different steps of quality control, data selection and data aggregation. First, we selected and aggregated the plant and insect data (green), then these data were cleaned (orange), and after that we created a data set on activity shifts of individual species using the yearly data (blue) and another data set on species interactions using decadal data (yellow).
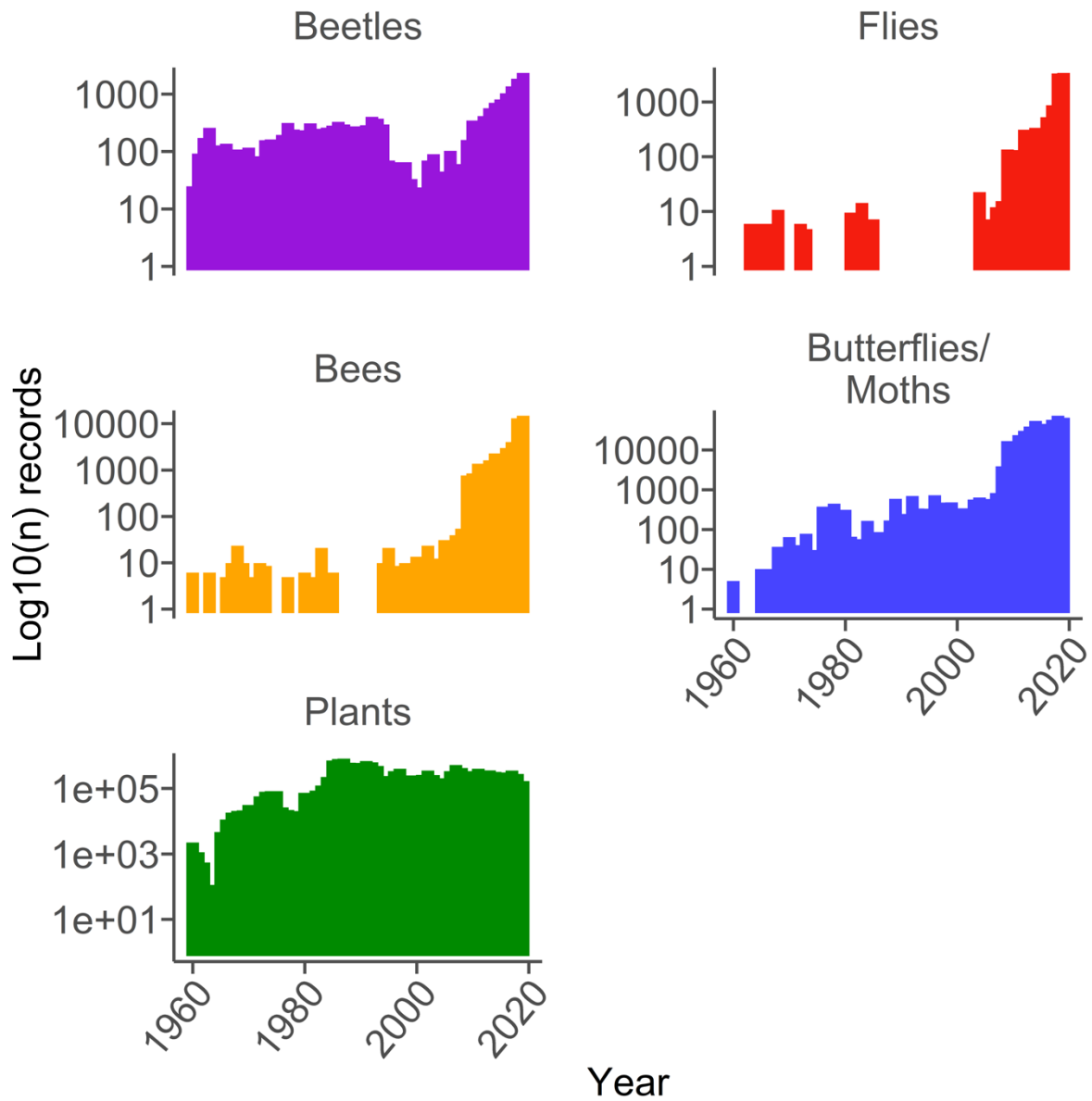
Figure S2. The numbers of occurrence records per year for each of the studied taxonomic groups. Note the different scales of the y-axes, and their log-transformation.
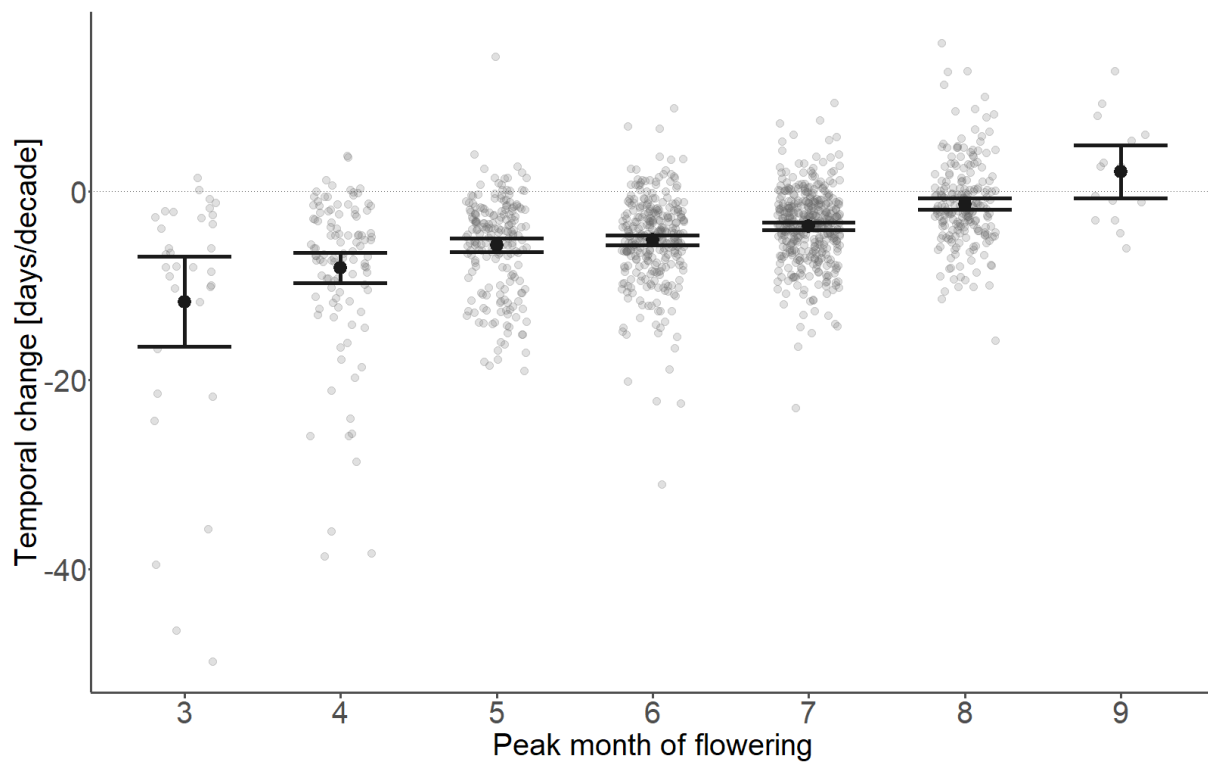
Figure S3. Temporal changes of plant phenology, with species grouped by their peak month of flowering. Grey dots are regression slopes (= days/decade) of individual species; black dots with whiskers are group means with 95% confidence intervals.
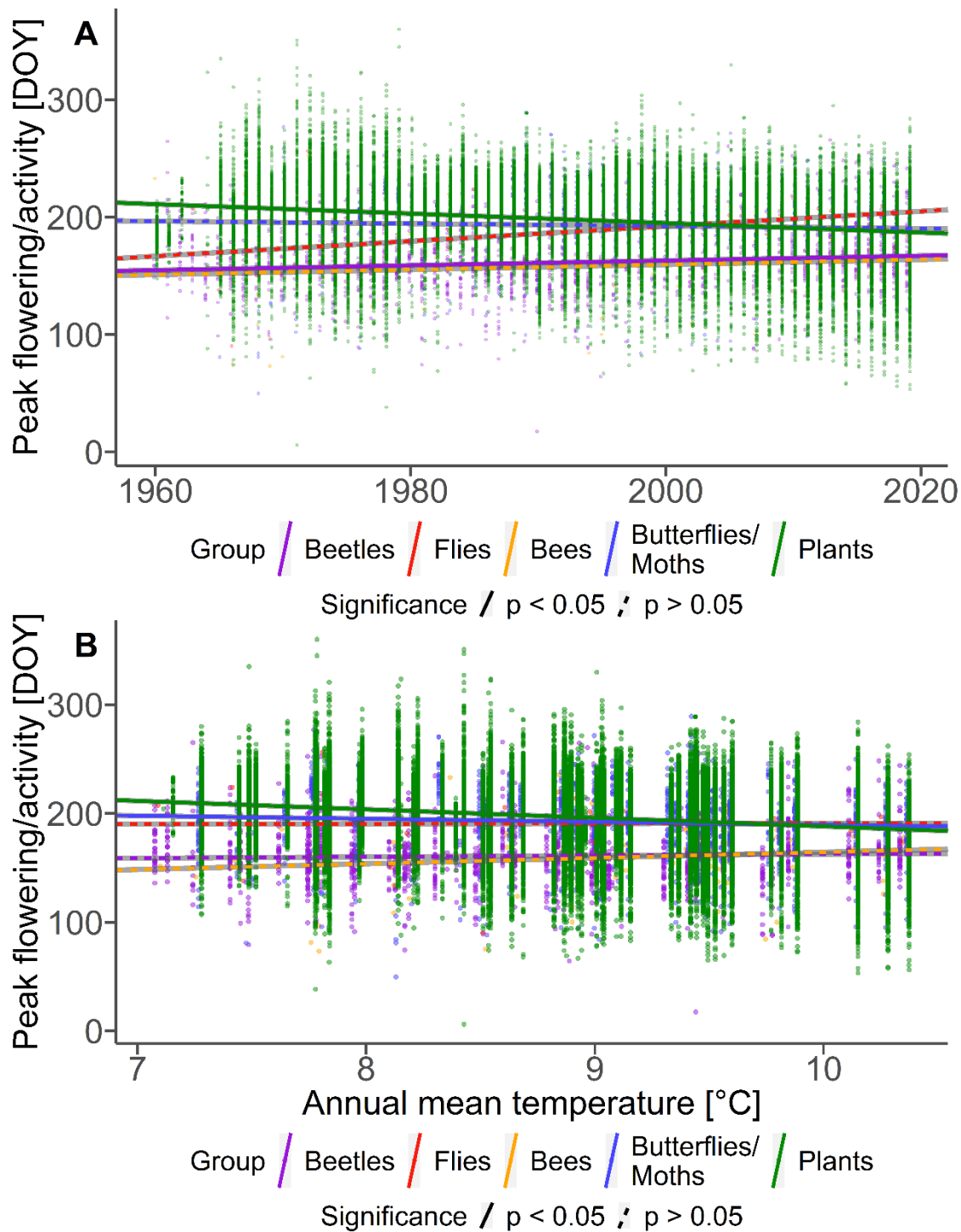
Figure S4. Temporal trends (A) and relationships with climate (B) of the peak flowering/activities of plants (green) and insects (purple: beetles; red: flies; orange: bees; blue: butterflies/moths), with each dot representing the average peak activity of an individual species in a specific year. Solid lines represent significant linear regressions, dashed lines non-significant trends for taxonomic groups.
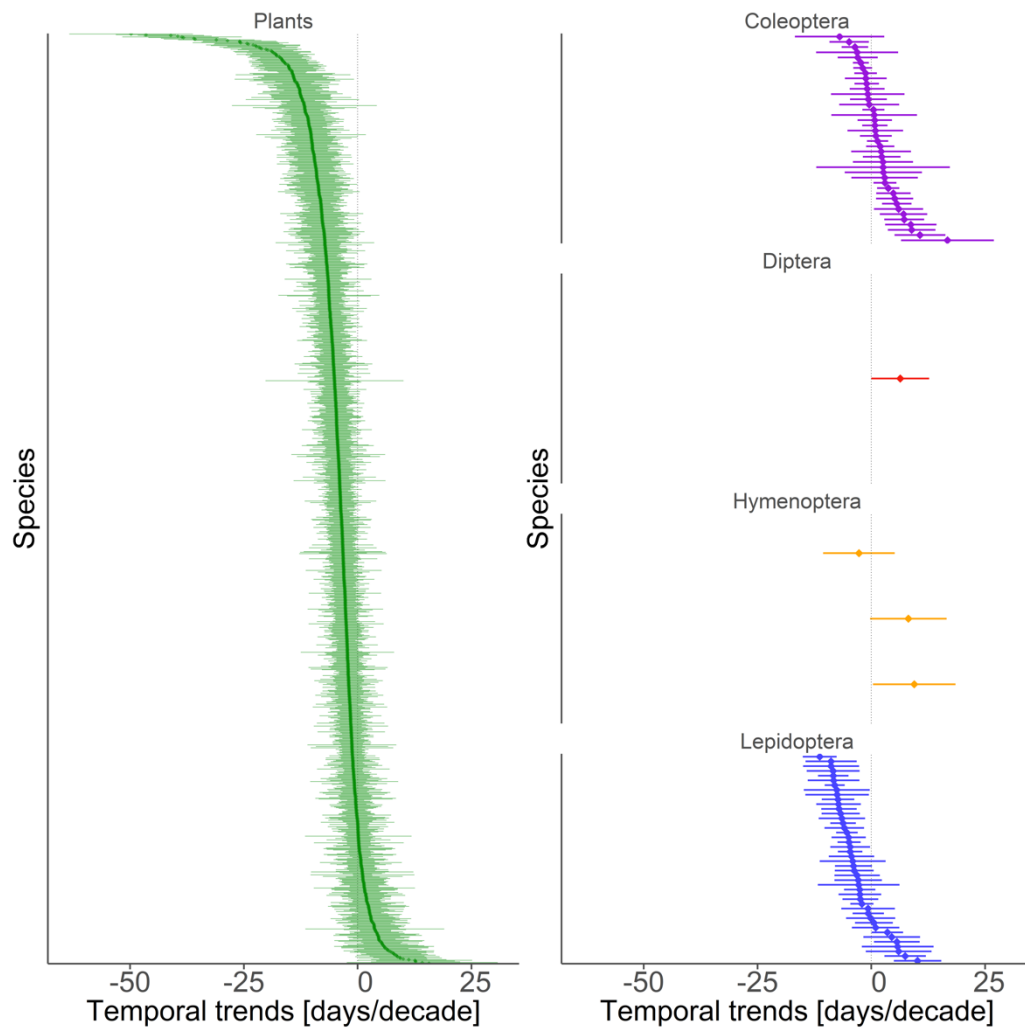
Figure S5. Forest plot of plant and insect species' temporal trends ordered by the strength of the trend. Lines represent 95% confidence intervals.
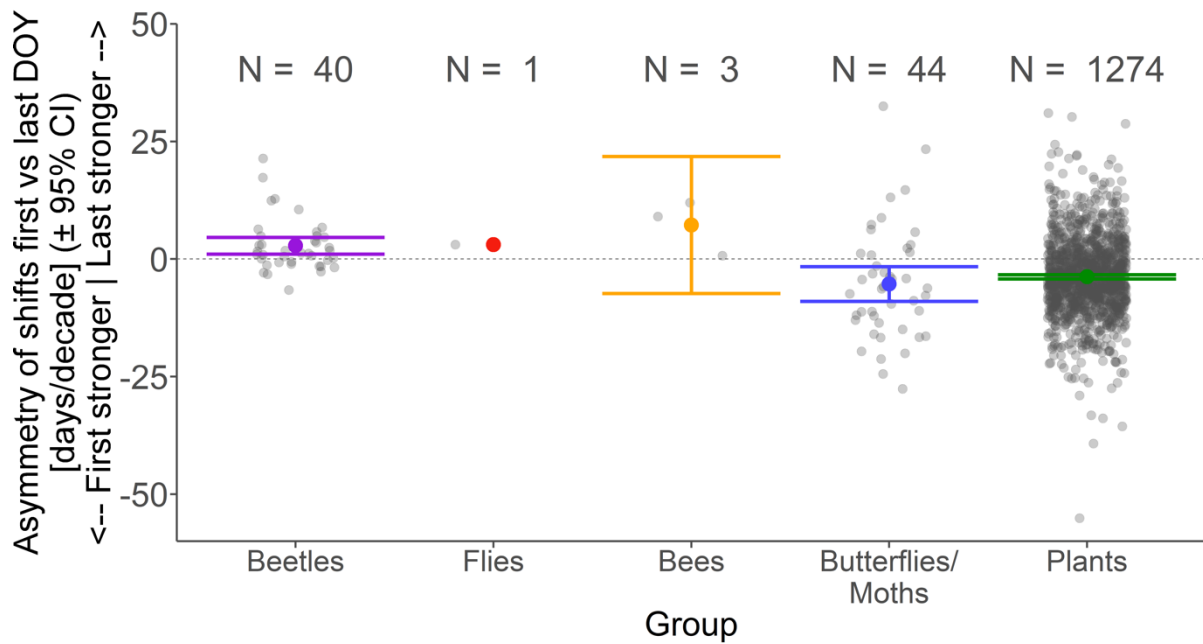
Figure S6. Asymmetry in slope of the first and last day of occurrence over time by species group (colored dots with 95% confidence intervals) and by individual species (grey dots). Asymmetry is the difference between the slope of the decadal average first and last occurrence of a species in a year over time, with values close to zero representing low asymmetry.
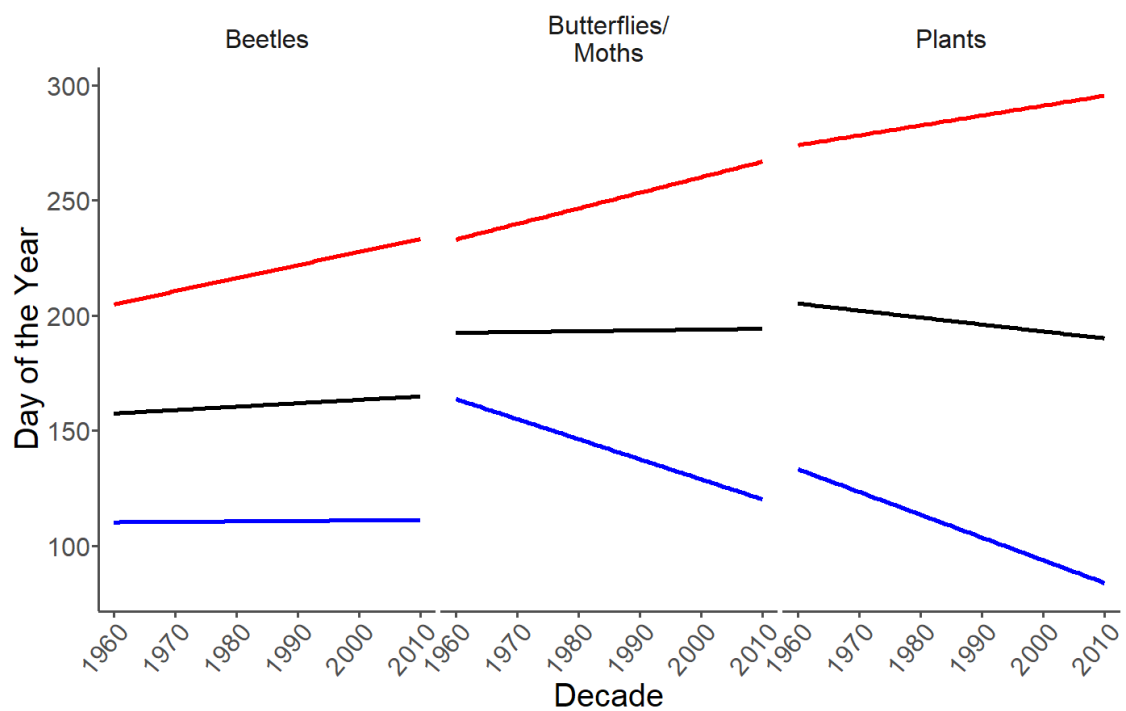
Figure S7. Mean shifts in first (blue) and last (red) occurrences of beetles, butterflies/moths, and plants over the studied decades. Black lines represent trends of the peak flowering/activity times.
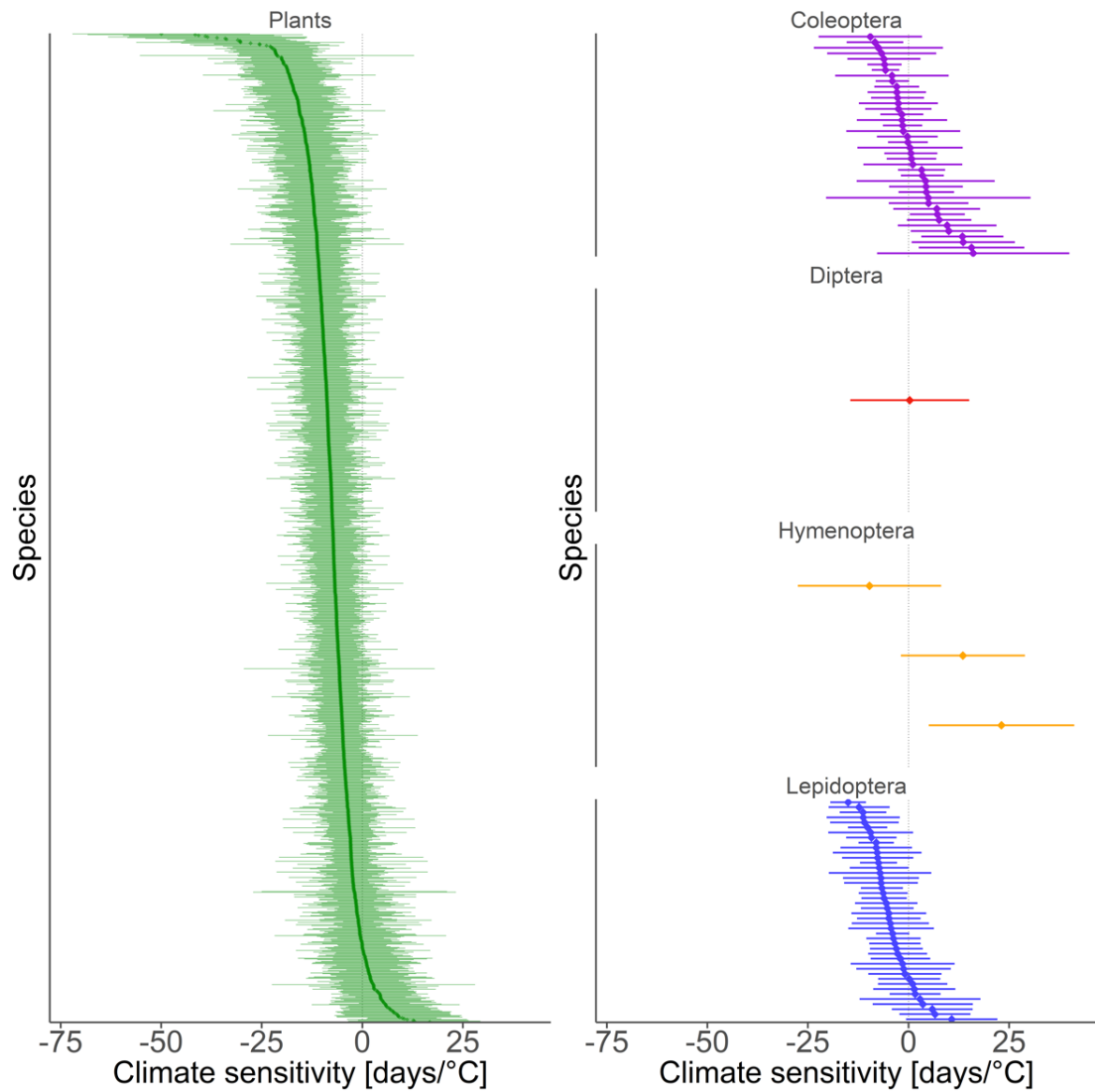
Figure S8. Forest plot of plant and insect species' sensitivity to climate ordered by the strength of the sensitivity. Lines represent 95% confidence intervals.
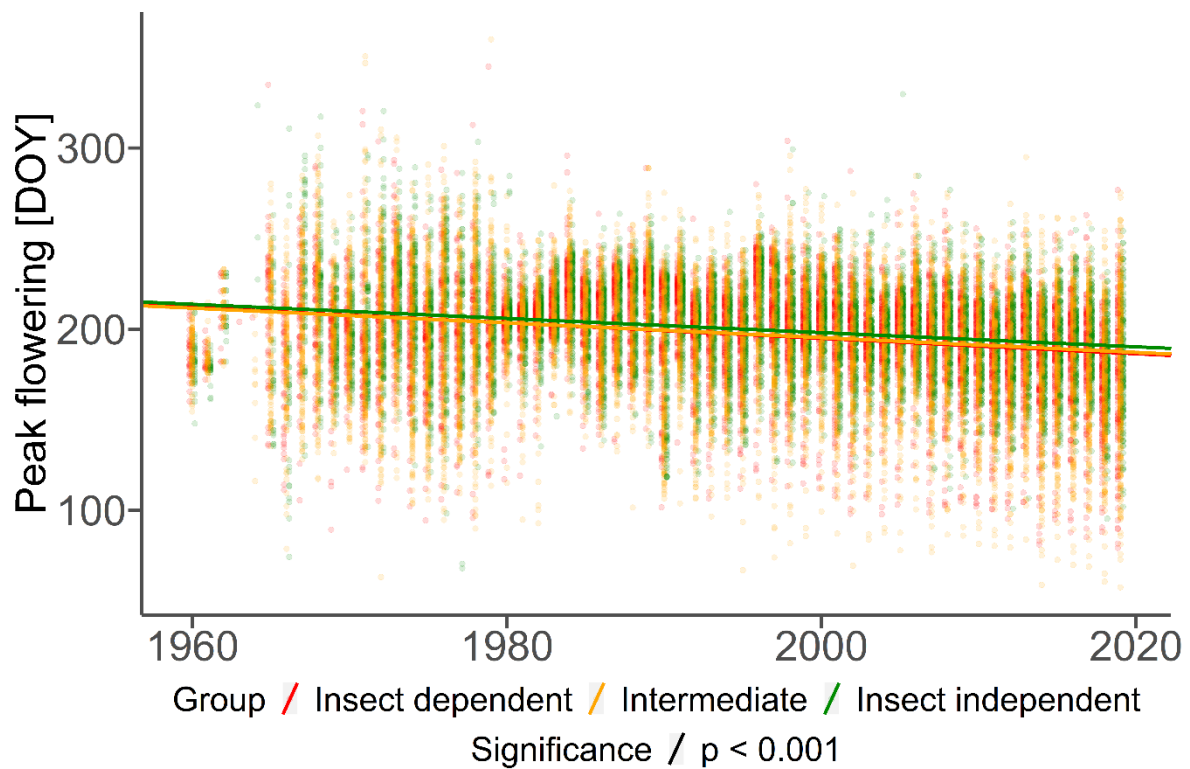
Figure S9. Temporal trends in plant phenology, separated by levels of pollinator dependence of the plants. Red dots: pollinator-dependent plants; green dots: pollinator-independent plants; yellow dots: intermediate levels of pollinator dependence. The lines are linear regressions; all are significant at *P* < 0.001.

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Table S1. Institutions whose records we excluded from our analyses. For "GEO Tage der Artenvielfalt" the reason was that there was an extreme number of records on a single day; for all other institutions the reason was that many records listed as being of German origin were probably misclassified and actually not from Germany.

Institution	Description
Administration de la Gestion de l'Eau (AGE)	Water administration of Luxembourg
Administration de la nature et des forêts (ANF)	Nature and forest administration of Luxembourg
GEO Tag der Artenvielfalt	Annual Biodiversity Day of the GEO magazine in Germany
Ministère de l'Environnement, du Climat et du Développement durable (MECDD)	Ministry of Environment, Climate and Sustainable Development of Luxembourg
Musée national d'histoire naturelle du Luxembourg (MnhnL)	National Museum of Natural History, Luxembourg
Naturpark Öewersauer	Upper Sûre Natural Park, Luxembourg
SICONA - Naturschutzsyndikat	Communal organization for nature conservation in Luxembourg
SPW-DEMNA	Department of Natural and Agricultural Environment, Wallonia, Belgium
STOWA	Foundation for Applied Water Research, Netherlands

Chapter VI

Synthesis

There is mounting evidence that human-induced global change – which started with the industrial revolution and accelerated during the last decades – affects and deteriorates ecosystems world-wide. One of the prime examples for such biological consequences of global change are shifts in the timing of life-history events. Phenological shifts (or the lack thereof) can have sweeping consequences for species' survival and fitness, and whole ecosystems (Post and Forchhammer 2008, Willis et al. 2008, Heberling et al. 2019, Visser and Gienapp 2019). While data on shifting phenologies with climate warming have accumulated rapidly, there is still insufficient research to explain the diversity and complexity of phenological responses observed across latitudes and species (Wolkovich et al. 2014). Long-term data to trace these changes through time and space, reaching back to the beginning of the industrial revolution, is hard to obtain. Additionally, the impact of other global change drivers besides climate change, such as land-use change, on phenology has so far been largely neglected.

In this thesis I tracked the effects that global change has on the life rhythms of plants, and I showed that the footprints of these effects can be found both in herbaria and in today's forest understories. Specifically, I investigated how land use and climate change shift the flowering time of plants, as well as the phenology of the insects that pollinate them. **Chapter II** summarized how herbaria can be used to track how plants are affected by these human-induced transformations of global ecosystems over the last centuries. Herbaria harbour plants collected throughout the last centuries, providing otherwise hard-to-obtain long-term data for deciphering ecological and evolutionary changes during this period of intense global change. They help to deduce how plants are affected by at least four of the main drivers of global change: pollution, habitat/land-use change, climate change and invasive species. In **Chapters III** and **VI** I focused on the impact of two of these global change drivers on phenology: **Chapter III** showed how climate change advances flowering phenology in Europe (analyzing herbarium data) and **Chapter IV** unraveled how flowering time is affected by environmental variation that resulted from spatially variable forest management in German forests. These two chapters highlight that using long-term and large-scale herbarium data in combination with fine-scaled field data makes it possible to draw a differentiated picture of how different drivers of global change influences plant phenology. Finally, **Chapter V** compared the

phenological shifts of plants and insect groups that pollinate plants, showing that the phenology of plants shifts on average stronger and more consistently compared to the phenology of the studied insect groups. Below, I shortly summarize the main results from my research concerning: (1) phenological shifts over time, (2) the role climate and (3) land use change (i.e. forest management) play in these shifts and (4) their consequences, such as the potential for phenological mismatches.

Phenological shifts over time within the last century

In **Chapter III,** I tracked long-term flowering time shifts of forest understory herbs in Europe by mining historical data from herbaria. I found that flowering time advanced ~6 days within the last century (-0.6 days per decade). I showed that it is crucial to account for geographic variability, i.e. spatial correlation, to estimate such large-scale and long-term shifts correctly. When analyzing herbarium data this is especially essential, since herbarium collections usually do not consist of statistically unbiased samples of plant diversity across space and time, but often have widespread sampling biases (Daru et al. 2017, Panchen et al. 2019, see also **Chapter II**). Collectors of herbarium specimens, for example, are likely to repeatedly collect plants within a limited region that is close to their home, academic institution or favorite holiday location. Thus, herbarium data is likely to be "patchily" distributed over space and time (see **Chapter II** and **III**). Incorporating this spatial correlation is needed to prevent pseudoreplication and map geographic variation that cannot be explained by the model; the latter due to biological causes rather than methodological ones. By comparing the results from models including and ignoring spatial correlation, I demonstrated that phenological shifts over time were substantially overestimated, with flowering shifting more than twice as much during the last century, and model assumptions were violated when spatial correlation was ignored.

It seems that phenological shifts have accelerated within the last decades. The large-scale comparison of temporal shifts of plant and insect phenology over the last ~50 years in **Chapter V** (based on records from GBIF, the Global Biodiversity Information Facility) found that in Germany, plant phenology (of 1,274 plants, ranging from grasses and annual herbs to trees) advanced by -4.5 days per decade (compared to -0.6 days per decade over the last ~100 years for the forest understory wildflowers analyzed in **Chapter IV**). An explanation for this much stronger shift might be that climate warming is rapidly gathering pace since the 1960s (IPPC 2019). However, when limiting the data in **Chapter V** to the same forest herbs that are analyzed in **Chapter III** and **IV**, the observed flowering time shifts are almost identical (-0.7

days per decade) to those observed in **Chapter III**. This suggests that the phenology of forest understory plants has shifted less compared to other plants. A cause for this discrepancy could be that forests can buffer climate warming and have distinct and on average colder and more stable microclimates that curb flowering time shifts (Zellweger et al. 2019, 2020) and see **Chapter IV**).

Effects of climate change on phenology

European land temperatures have increased by at least 1.7°C compared to pre-industrial times (European Environmental Agency 2020). In **Chapter III,** I showed that the advancement of flowering time in Europe over the last ~100 years is likely driven by warmer spring temperatures due to climate change, with forest wildflowers blooming around -3.6 days earlier per 1°C spring temperature increase. The 1,274 plant species analyzed in **Chapter V** advanced their phenology stronger (-7.6 days) per 1°C warming. This trend is even more distinct (-12.9 days per +1°C) when the dataset is subset to the forest herb species analyzed in **Chapter III** and **IV**. This deviation could be due to differences in the underlying data and analysis: In **Chapter III** I analyzed herbarium data spanning Europe (and over 100 years) while correcting for spatial correlation and elevation, while **Chapter V** uses data from GBIF, including both herbarium and observational data from Germany (over the past ~50 years) only, and without incorporating spatial dependency or elevation. In **Chapter III**, I demonstrated that ignoring spatial correlation resulted in overestimations of the temperature related shifts. Alternatively, or additionally, the temperature sensitivities of plant populations could have changed over time or may differ geographically (between Germany and overall Europe).

In **Chapter III**, I showed that indeed flowering time varied substantially across Europe, even after accounting for the effects of spring and winter temperature, spring precipitation, elevation and year. In Central Europe, including Germany, plants flowered on average earlier than in Northern, Southern, Western and Eastern Europe. This discrepancy can have three causes: (1) there are other drivers of phenology that vary geographically that were not accounted for, or (2) the phenological responses to environmental variables (such as temperature) vary geographically between populations because the sensitivity to these cues differs (as speculated above). The latter might be a consequence of local or regional adaptation and genetic differences between populations. Furthermore (3), the actual local microclimatic conditions could deviate from the climate data used (that is interpolated on a 0.5° latitude by 0.5° longitude grid resolution; ~ 60 × 40 km). Macroclimate data is measured

in meteorological stations, which usually record free-air temperature above short grassland – that can differ profoundly from temperature conditions in the surrounding landscape, especially forests (Valdés et al. 2015, De Frenne et al. 2019, Zellweger et al. 2019).

Effects of land use on phenology

In **Chapter IV,** I showed that within the same year plants flowered about two weeks later in highly managed forest stands, such as Norway spruce plantations, compared to unmanaged forests. One of the main causes for this is that highly managed (coniferous) forest were significantly colder than unmanaged forests (within the same region). An explanation for this difference is that forest management can alter tree species composition and canopy cover, shape the age and structure of a forest, and thereby cause distinctive microclimates that vary a lot across space, over time and with forest management practices (Zellweger et al. 2020). Understory plants flowered earlier in forest stands with warmer microclimates (-4.5 days per +1°C) within a single year in German forests. A comparison with **Chapter III** shows that macroclimate warming over the last century resulted in similar shifts (-3.6 days per +1°C). This indicates that both long-term macroclimate change (due to global warming) and local microclimate (shaped by forest management) can influence flowering time of forest understory herbs in similar, and equally strong, ways.

It is especially noteworthy about my results that the influence of forest management on phenology cannot be fully attributed to microclimatic changes. The dominant tree species (Norway spruce vs. European beech) affected plant phenology as strongly as temperature did – not only indirectly, through altering microclimate, but also directly. This profound effect must result from other abiotic or biotic factors, influenced by the dominant tree species in a forest, such as light and soil conditions, associated mycorrhizal fungi or microbial communities. A change in the foundational tree species can alter the structure and dynamics of forest ecosystems (Ellison et al. 2005). Non-local (often coniferous) tree species such as Norway spruce are often planted for timber production, where otherwise deciduous trees, mainly European beech, would grow. Consequently, land-use in forests alters many ecological properties of Central European forests – for example, the phenology of the understory vegetation (see **Chapter IV**, and Schall et al. 2018). I studied only current land use, however, there are likely also persistent imprints of historical land use that still influence forest understories. For example, traces of the historical use of forests as wood pastures in Europe, one of the oldest land-use practices in human history, where open forests served as shelter and forage for grazing animals, are detectable in many old-growth forests until today.

Such past land use and forest management practices shape today's ecosystems and their legacy can affect phenology, biodiversity and ecosystem dynamics today (De Frenne et al. 2013). Legacies of past management are particularly strong in forests because of the long life-span of trees, where planting a certain tree species will impact decades if not centuries of forest ecosystems (in contrast to e.g. agricultural practices that might change from year to year). This long-term legacy of management effects is something that needs to be kept in mind for forest management, especially since near-natural Central European deciduous forests have dwindled due to human activities, making it crucial to preserve them, the species they are home to and their ecological processes (Bengtsson et al. 2000).

Why phenology shifts matter

Climate change can affect the phenologies of interacting groups of organisms differently, and this can influence their synchrony, for example, between the overstory and understory of forests. It has been suggested that trees in deciduous forests advance their leaf-out phenology more strongly than understory wildflowers do. These wildflowers then get less light in spring because they are shaded by the tree canopies. This could reduce the amount of carbon they can assimilate via photosynthesis and thus negatively impacts their fitness, leading to population decline in these ecologically important species (Heberling et al. 2019). Furthermore, if plant phenology changes, this could have consequences for animal species that depend on these plants. When the phenology of consumers and their resources (such as pollinators and plants) changes differently this can reduce their synchrony. Such phenological mismatches can have far-reaching ecological consequences. It has been shown that in forests such shifts in plant pollinator interactions since the beginning of the last century contributed to a degradation of interaction network structure e.g. because quantity and quality of pollination services declined (Burkle et al. 2013).

The large-scale comparison of the phenological shifts of plants and insects in **Chapter V** showed that the extent of the phenological shifts of plants and their insect pollinator groups, indeed varied substantially. Per decade, plants advanced their phenology over 4 days, butterflies/moths advanced ~3 days and beetles delayed their phenology ~2 days. That the phenology of some organisms shifts more (or even diametrical) compared to others, happens because of different responses to (potentially different) phenological cues, such as temperature (and thus climate change) or daylength. I showed that plants indeed responded stronger to temperature changes than the investigated insect groups. Overall, plant phenology advanced 7.6 days per +1°C warming, while the phenology of butterflies and moths advanced

only ~4 days and beetles even delayed theirs ~1 day per +1°C. A recent review of similar phenology datasets found that phenological sensitivity to climate differed between trophic levels, with primary consumers responding more strongly to climatic changes than secondary consumers, thus the phenology of the former is likely to shift more (Thackeray et al. 2016). Since forest understory herbs, and plants in general, interact with many other species and play a key role in many ecosystems, shifts in plant phenology could have substantial consequences for pollinators, food webs, agricultural yields, and ecosystem functions and services such as productivity and carbon cycling (Chmielewski et al., 2004; Cleland et al., 2007; Reilly et al., 1996; Tang et al., 2016).

Outlook

My work and recent reviews (Tang et al. 2016, Chuine and Régnière 2017, Chmura et al. 2019, Piao et al. 2019) stress that phenology is complex and that our understanding of the mechanisms that determine plant and animal phenology is still limited. Phenology is influenced by macroclimate (see **Chapters II** and **V**), microclimate and other abiotic and biotic factors that can themselves be altered by land use, such as forest management (see **Chapter IV**). That multiple drivers of phenology (e.g. temperature and daylength) interact, and that the influence of these drivers can vary between species, populations or geographic regions, makes it challenging to model and predict shifts in plant phenology caused by climate and land use changes. My results showed that in Europe flowering time varies, even after accounting for geographic differences of spring and winter temperature, precipitation and elevation – that are known drivers of phenology. This left-over geographic variation clearly indicates that we are either still missing some significant drivers of phenology that shape these geographic differences, or that plants have adapted to local or regional conditions in such a way that they respond differently to the same cues (**Chapter III**). Further research should build on such spatial-temporal modeling, to determine how the known phenological cues interact and how their influence differs geographically or might have changed over time. Further, spatial-temporal modeling could also help to identify potential additional phenological drivers that we are still missing for explaining variation in phenological responses. In this context, so called varying coefficient models (that can also be employed in R-INLA) could be very promising, since they allow to let the regression coefficients vary systematically and smoothly in more than one dimension, e.g. geographically with latitude and longitude. In this type of model, the estimated effects (i.e. the regression coefficients) of phenological cues, e.g. the influence of temperature on flowering time, could be different

among regions, for instance in Scandinavia compared to the Mediterranean, in the Alps compared to the Northern Lowland in Germany or in regions with old-growth deciduous forests compared to regions with coniferous plantations. Herbaria could provide the long-term data that cover large parts of the world (see **Chapter II**) and thus make these types of analyses feasible. Ongoing digitization will make the data increasingly accessible. With growing amounts of data – from herbaria, natural history collections, field observations, or large infrastructures such as the Pan European Phenology Project (Templ et al. 2018) – available in online databases (e.g. GIBIF.org or PEP725) it will be more and more achievable to employ complex models. This will allow us to put together more and more of the puzzle pieces for understanding what shapes phenology, and to project future changes.

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