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Doctoral Thesis No. 2024:65 • Boreal pollinators in a changing climate • Joan Díaz Calafat

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The effects of forest composition, structure, and microclimate

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Boreal pollinators in a changing climate – the effects of forest composition, structure, and microclimate

Abstract

The boreal biome, the world's largest contiguous forested region, is undergoing significant changes due to rising global temperatures. This warming is impacting the interactions between trees, understory plants, and pollinators, which determine the species composition of understory plants and their pollinators, as well as essential ecosystem services such as berry production and pollination. However, our current knowledge is insufficient to predict how changes in climate and land use, the main drivers of global change, will affect this system. The objective of this thesis was to enhance our understanding of the interplay between forest structure, microclimate, and pollinators. My studies highlighted the limited understanding of climate change effects on boreal pollinators (**I**), emphasized the crucial roles of forest density and overstory species composition in regulating understory microclimates (**II**), which in turn impacted pollinator foraging behaviour (**III**) and their species diversity (**IV**). When assessing the effects of climate change on boreal plant-pollinator interactions, most studies use proxies to measure pollinators, omitting their taxonomic identities and limiting our ability to evaluate the resilience of specific interactions (**I**). Forests buffer extreme temperatures, providing refugia for species affected by warming, with higher forest density offering the best temperature regulation relative to macroclimate. Both broadleaf and conifer trees provided these benefits, though most broadleaf trees allow more light (**II**). Since most boreal pollinators are ectothermic insects, ambient temperature was the most important factor driving their foraging rates, while microclimate affected their foraging duration (**III**). However, the richness of flower-visiting arthropods was positively influenced by low forest density and broadleaf dominance, whereas microclimate may play a smaller role (**IV**). Collectively, these findings suggest that forest management could enhance pollinator habitats and resilience against climate change, but further research is needed to refine these strategies and understand species-specific responses.

Keywords: *forest management, Diptera, Syrphidae, understory, temperature buffering, climatic refugia, phenological mismatch*

Boreala pollinatörer i ett föränderligt klimat – effekter av skogens trädslagssammansättning, struktur och mikroklimat

Sammanfattning

Det boreala biomet, världens största sammanhängande skogsområde, genomgår betydande förändringar på grund av stigande globala temperaturer. Uppvärmningen påverkar samspelet mellan träd, markvegetation och pollinatörer, samt viktiga ekosystemtjänster som bärproduktion och pollinering. Vår nuvarande kunskap är dock otillräcklig för att förutsäga hur förändringar i klimat och markanvändning, kommer att påverka detta system. Syftet med denna avhandling var att öka vår förståelse för samspelet mellan skogsstruktur, mikroklimat och pollinatörer. Mina studier belyste den begränsade förståelsen av klimatförändringens effekter på pollinatörer i boreala områden (**I**) och betonade den avgörande roll som skogens täthet och artsammansättning i trädskiktet spelar för att reglera mikroklimatet under trädkronorna (**II**), vilket i sin tur påverkade pollinatörernas födosöksbeteende (**III**) och deras artrikedom (**IV**). När man bedömer klimatförändringarnas effekter på interaktioner mellan växter och pollinatörer i boreala områden använder de flesta studier proxyer för att mäta pollinatörers frekvens, det vill säga utelämnar deras taxonomiska identiteter och därmed begränsar vår förmåga att utvärdera motståndskraften hos artspecifika interaktioner (**I**). Skogar buffrar extrema temperaturer och erbjuder refugier för arter som påverkas av uppvärmningen, där en högre täthet av skogen ger den bästa temperaturregleringen i förhållande till makroklimatet. Både löv- och barrträd ger dessa fördelar, även om de flesta lövträd släpper in mer ljus (**II**). Eftersom de flesta pollinatörer i boreala områden är ektoterma insekter var omgivningstemperaturen den viktigaste faktorn för deras födosökshastighet, medan mikroklimatet påverkade födosökets varaktighet (**III**). Rikedomen av blombesökande leddjur påverkades positivt av låg skogstäthet och lövträdsdominans, medan mikroklimatet verkar spela en mindre roll (**IV**). Sammantaget tyder dessa resultat på att förändrad skogsförvaltning kan förbättra pollinatörers livsmiljöer och motståndskraft mot klimatförändringar, men ytterligare forskning behövs för att förfina dessa strategier och förstå effekterna därav på specifika arter.

Keywords: *skogsförvaltning, Diptera, Syrphidae, markvegetation, temperaturbuffring, klimatiska refugier, fenologisk missmatchning*

Pol·linitzadors boreals en un clima canviant – els efectes de la composició, l'estructura i el microclima del bosc

Resum

El bioma boreal, la regió boscosa contigua més gran del món, està experimentant canvis significatius a causa de l'augment global de les temperatures. Aquest escalfament està afectant les interaccions entre els arbres, les plantes del sotabosc i els pol·linitzadors. Això, alhora, determina la composició d'espècies de les plantes del sotabosc i la dels seus pol·linitzadors, així com serveis ecosistèmics essencials com la producció de baies i la pol·linització. No obstant, el nostre coneixement actual és insuficient per predir com els canvis en el clima i en l'ús del sòl – els principals motors del canvi global – afectaran aquest sistema. L'objectiu d'aquesta tesi és millorar la nostra comprensió sobre la interacció entre l'estructura del bosc, el microclima i els pol·linitzadors. Els meus estudis manifesten la poca comprensió que tenim sobre els efectes del canvi climàtic en els pol·linitzadors boreals (**I**), així com el paper crucial de la densitat forestal i la composició d'espècies arbòrees en la regulació del microclima del sotabosc (**II**), que al seu torn afecta el comportament de pecoreig dels pol·linitzadors (**III**) i la seva diversitat d'espècies. (**IV**). Quan s'avaluen els efectes del canvi climàtic en les interaccions entre plantes boreals i els seus pol·linitzadors, la majoria dels estudis realitzats fins ara utilitzen mètodes indirectes per mesurar els pol·linitzadors, ometent la seva identitat taxonòmica i limitant la nostra capacitat d'avaluar la resiliència d'interaccions planta-pol·linitzador específiques vers el canvi climàtic (**I**). Els boscos regulen les temperatures ambientals extremes, proporcionant refugi a espècies afectades per l'escalfament global. Una densitat forestal alta ofereix la millor regulació de la temperatura en relació amb el macroclima. Aquests beneficis són proporcionats tant pels arbres de fulla ampla com per les coníferes, tot i que la majoria dels arbres de fulla ampla permeten que més llum es filtri al sotabosc (**II**). Com que la majoria dels pol·linitzadors boreals són insectes ectotèrmics, la temperatura ambiental és el factor més important que impulsa la seva freqüència de pecoreig, mentre que el microclima afecta la durada de les seves visites florals (**III**). No obstant això, la riquesa dels artròpodes que visiten flors està influenciada positivament per baixes densitats forestals i la dominància d'arbres de fulla ampla, mentre que el microclima en té un paper menor (**IV**). Col·lectivament, aquestes troballes suggereixen que la gestió forestal podria millorar els hàbitats dels pol·linitzadors i la seva resiliència davant el canvi climàtic, però fa falta més recerca per refinar aquestes estratègies i entendre les respostes específiques de cada espècie.

Dedication

To my younger self – look what we did!

There are always flowers for those who want to see them.

— Henri Matisse

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Díaz-Calafat, J.***, Felton, A., Öckinger, E., De Frenne, P. Cousins, S. A. O. and Hedwall, P. O. The effects of climate change on boreal plant-pollinator interactions are largely neglected by science (manuscript)

II. **Díaz-Calafat, J.***, Uria-Diez, J., Brunet, J., De Frenne, P., Vangansbeke, P., Felton, A., Öckinger, E., Cousins, S. A. O., Bauhus, J., Ponette, Q. and Hedwall, P. O. (2023). From broadleaves to conifers: The effect of tree composition and density on understory microclimate across latitudes. *Agricultural and Forest Meteorology*, 341, 109684.

III. **Díaz-Calafat, J.***, Öckinger, E., Felton, A., De Frenne, P., Cousins, S.A.O., Uria-Diez, J. and Hedwall, P. O. Climate, forest structure and flower resources drive pollinator foraging behavior in boreal forests (manuscript)

IV. **Díaz-Calafat, J.***, Peterson, D.L., De Frenne, P., Felton, A., Öckinger, E., Cousins, S. A. O., Roger, F., Cleary, M. and Hedwall, P. O. Using flower eDNA metabarcoding to identify the effects of forest structure and microclimate on flower-visiting arthropods (manuscript)

Paper II is published open access.

*corresponding author

The contribution of Joan Díaz Calafat to the papers included in this thesis was as follows:

I. JDC developed the research idea together with POH, AF, EÖ, PDF and SAOC. JDC conducted the literature searches, screened the resulting papers and developed the first draft of the manuscript. All authors contributed to the final version of this manuscript.

II. JDC participated in planning and conducting fieldwork together with POH, JUD, PDF and JB. JDC conducted the statistical analyses together with POH, and wrote the manuscript in collaboration with all other co-authors.

III. JDC developed the research idea together with POH, AF, EÖ, PDF and SAOC. JDC and POH conducted the fieldwork. JDC classified all the images and developed the vision AI model. JDC conducted the statistical analyses together with POH. JDC wrote the first draft of the manuscript and all other co-authors contributed to the final version.

IV. JDC developed the research idea together with POH, AF, EÖ, PDF and SAOC. JDC collected the eDNA samples in the field and performed DNA extractions and PCRs in the lab with the help of DLP. FR helped with the bioinformatics. JDC conducted the statistical analyses together with POH. JDC wrote the manuscript in collaboration with all other co-authors.

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1. Introduction

1.1 Climate change and the boreal biome

The boreal biome is the largest contiguous forested region of the world, positioned in a circumpolar zone situated between the temperate and arctic or alpine biomes (Tuhkanen, 1984). Global mean surface temperatures have increased by on average 1.1 °C for the period 2011-2020 above those of the pre-industrial period of 1850-1900 (IPCC, 2023). Temperature increase is not uniform across the globe. Land areas are experiencing more warming than the global average, having increased by about 1.59°C and increasing even faster in some boreal regions (SMHI, 2023). Recent evaluations, including the Arctic Climate Impact Assessment (ACIA, 2005), warn that climate change may significantly alter the function and structure of boreal forests more than any other biome on Earth.

In response to climate change, species can either adapt locally, for example, by changing their phenology, or shift their distribution into cooler, more suitable environments, thereby increasing their presence in regions where they were once rare or absent. These distribution shifts typically occur polewards (Taheri et al., 2021) or towards higher altitudes (Frei et al., 2010). As a result, some species are colonizing new areas, while others that fail to do so, or to adapt to novel climatic conditions, may face local or total extinction. Additionally, when species are hindered to disperse to new areas or their current suitable area shrinks due to environmental change, this may result in a reduction in species' distribution area. Evidence of these distributional changes is increasingly being reported across various biotic groups (Pecl et al., 2017) and geographical regions (Chen et al., 2011), but are more pronounced and occur more rapidly at higher latitudes (Virkkala & Lehikoinen, 2014), such as in the boreal biome. In fact, boreal forests show an increase in vegetation productivity at their coldest northern limits, and a decrease at their warmest southern limits, consistent with the shift of this biome northwards (Berner & Goetz, 2022). Additionally, in response to climate change, the local abundance of some species may change. This does not necessarily involve colonization or extinction events but rather occurs as species are pushed or pulled away from their environmental optima (Antão et al., 2022). This adjustment involves species moving to areas within their existing range that better match their climatic preferences as conditions change. Therefore, in response to warming, the composition of species communities can shift, favoring species that thrive in warmer conditions over those that prefer colder environments. This process is known as "thermophilization" (De Frenne et al., 2013). In European boreal forests, two of the most abundant understory shrub species (*Vaccinium myrtillus* L. and *V. vitis-idaea* L.), are expected to have their climatic niches shrunk due to climate change, and consequently, their geographic distribution (Nielsen et al., 2007; Puchałka et al., 2023). These shrubs are considered foundation species (Hedwall et al., 2019), since they play a crucial role in maintaining the structure, stability, and function of boreal forest ecosystems. The decline of these shrubs could lead to cascading effects throughout the ecosystem, potentially reducing biodiversity and altering ecosystem processes.

Species may react differently to a warming environment. For instance, when they use different environmental cues or have different adaptive capabilities and phenotypic plasticity. For species that depend on other species for survival or reproduction (*e.g.,* predators and prey, plants and pollinators), this can lead to spatial or temporal mismatches. Spatial mismatch takes place when interacting species shift their distributions in a diverging way that prevents them from interacting (*e.g.,* Schweiger et al., 2008). In contrast, temporal mismatch arises when there is a misalignment in the timing of key events in the life cycles of interacting species (Visser & Gienapp, 2019). This can appear as changes in the timing of migration, reproduction, or flowering, disrupting the synchrony between species. Both these types of mismatch are distinct dimensions of climate change-driven disruptions that can compromise the survival of the affected species, and ultimately ecosystem functioning (Beard et al., 2019), as this depends largely on species interactions rather than on biodiversity *per se* (Stanworth et al., 2024).

Depending on pollinator availability (or lack thereof), some plants have evolved local adaptations that may differ within their distribution range and determine the population responses to climatic fluctuations (Boulanger-Lapointe et al., 2017) or even their reproductive strategies. For instance, *Orthilia secunda* (L.) House (Ericaceae) reproduces successfully in the absence of pollinators in New Brunswick, Canada (Barrett & Helenurm, 1987) but not in Scandinavia (Knudsen & Olesen, 1993).

1.2 Climate change and pollinating insects

Declines in arthropod abundance have been reported and attributed to global warming (Fitzgerald et al., 2021) and other anthropogenic disturbances, yet population trends and responses to warming may differ between different arthropod groups or areas (Høye et al., 2021). Although climate change is only one factor of the multifaceted causes of insect decline, its negative effect on insect pollinator populations across the Northern Hemisphere may have been underestimated thus far (Vasiliev & Greenwood, 2021).

Despite these declines, some pollinators may also take advantage of climate change and expand their distribution (Ghisbain et al., 2021). For such expansions to occur, a general flexibility in dietary and nesting requirements are often needed, although exceptions do occur (Bogusch et al., 2021). General habitat associations may also influence the extent of such expansions. For instance, in Northern Europe, forest butterfly species show greater range expansions than species associated with open habitats (Betzholtz et al., 2013).

As explained above, changes in spatial or temporal patterns can cause a mismatch in the synchrony between interacting species. In this regard, pollination is a particularly sensitive ecosystem service, since temperature is considered the main driver of spring phenology for plants in temperate and boreal ecosystems (Kramer et al., 2000). When the synchrony between flowering and pollinator phenology is disrupted, the fitness of both plants and pollinators can suffer (Kudo & Cooper, 2019). Over the past 130 years, spring emergence of certain bees and bumblebees in North America has advanced by approximately 10 days (Bartomeus et al., 2011), and by 5 days over the past 20 years in Sweden (Blasi et al., 2023). Meanwhile, warmer springs have led to an advancement in flowering time by 0.49 days per decade in Sweden (Auffret, 2021) and by 2.4 days per degree Celsius in north-central North America (Calinger et al., 2013). However, plant phenological responses to warming and changes in precipitation appear to be species-specific (Rice et al., 2021). Despite both pollinators and plants having accelerated their emergence and flowering, it is when the extent of these responses differs that mismatch takes place.

Since specialization in mutualistic interactions tends to increase with latitude (Schleuning et al., 2012), the rapidly warming boreal regions – already characterized by low diversity in both plants and pollinators (Esseen et al., 1997) – are particularly vulnerable to shifts in species composition that can disrupt plant-pollinator interactions (Antão et al., 2022). Furthermore, this vulnerability can be compounded by concurrent changes in habitat and the decline of resources or other species. For example, it seems that the potential nectar production of the herb layer in temperate forests can potentially decline due to climate change (De Schuyter et al., 2024). Moreover, the forest understory vegetation used by queen bumblebee foundresses as an early resource to start their colonies (a critical phase in bumblebee demography) is declining in both North America and Europe (Hedwall et al., 2021; Jonsson et al., 2021; Mola, Richardson, et al., 2021), which may intensify the negative effects of phenological mismatch on plant and pollinator populations.

Although research on multi-species plant-pollinator assemblages suggest that the overall structure of pollination networks is probably resistant to the extent of climatic change experienced in the early decades of this century (Hegland et al., 2009), a degradation in both the structure and function of plant-pollinator networks over time has nevertheless been documented in some temperate forests in North America (Burkle et al., 2013). Similarly, Kudo and Ida (2013) report a reduction in seed production by an understory plant species owing to phenological mismatch in a cold-temperate forest in Japan. This indicates that some pollination networks may already be deteriorating due to current climatic stressors, and the resilience of other networks is likely to diminish under additional climatic pressure. However, the implications of phenological mismatch for plant and pollinator demographics, as well as for ecosystem functionality, remain unclear both inside and outside forests.

1.3 The importance of forests for pollinators

In many cold-temperate and boreal forests, wind pollination dominates over other types of pollination among trees, being the strategy followed by many coniferous and deciduous species in these forests (Kevan et al., 1993). Despite this, wind-pollinated trees can serve as a pollen source for some bees during the early part of the season when the availability of resources is typically low (Saunders, 2018; Yourstone et al., 2021). However, it is still not very clear how nutritionally beneficial this particular pollen is for pollinators, nor what conditions make this pollen preferred over other pollen sources (Wood et al., 2022). On the other hand, the herbaceous and woody understory vegetation of the boreal forest is dominated by species which have conspicuous flowers and rely on insect pollination as a pollination strategy. Many of these understory flowering plants are crucial for the establishment of bumblebee colonies, as they are the only resource available when queens emerge from hibernation (Mola, Hemberger, et al., 2021). Moreover, forests also provide other essential resources besides flowering plants, such as dead wood for nesting, tree resins, and various non-floral sugar sources, like honeydew (M. Ulyshen et al., 2023).

Forests support a diverse range of pollinators, including forest-dependent species, and even play a crucial role in enhancing pollination in nearby habitats, such as croplands (M. Ulyshen et al., 2023). Nevertheless, not all types of forests may benefit pollinators in the same way. Bees and butterflies typically prefer open forest habitats (Hanula et al., 2016), and some groups benefit from a high canopy composition diversity (Traylor et al., 2024). Moreover, given the temperature buffering capacity of forests, these environments can provide favourable abiotic conditions that can ameliorate climate change (Mola, Hemberger, et al., 2021).

1.4 Forest microclimatic temperature buffering

The microclimate a species experiences may clearly differ from the macroclimate. In fact, the variation in microclimate over just a few meters can be as pronounced as the differences in macroclimates across 5,000 kilometers (Ackerly et al., 2010; Stoutjesdijk & Barkman, 2014), meaning that the thermal extremes in which some species may live can be found at rather local scales.

Tree canopies buffer ambient temperatures, decreasing high temperatures and increasing low temperatures. Under forest canopies, maximum and average temperatures are typically cooler than the macroclimate, while minimum temperatures are warmer compared to more open habitats (De Frenne et al., 2019). This creates microclimatic conditions that affect organisms inhabiting the forest understory (De Frenne et al., 2013). Therefore, forests may serve as climatic microrefugia, enabling the existence of species unable to survive in the surrounding areas, thereby potentially reducing the pace or impact of thermophilization on understory communities (De Frenne et al., 2013; Zellweger et al., 2020). The microclimate buffering capacity of boreal forests is expected to increase due to climate change by the end of this century (De Lombaerde et al., 2022). Consequently, the significance of microclimate buffering for the boreal biome, as well as their role as microclimatic refuge, is anticipated to grow.

Forest understory microclimate is influenced by a multitude of factors, including canopy openness, vegetation type, and soil properties. Canopy openness plays a critical role by regulating light penetration, temperature, and moisture levels in the understory. Dense canopies can reduce light availability and lower temperatures, while also maintaining higher humidity by limiting evaporation (Geiger et al., 1995). Moreover, forest structure offers resistance to wind, hindering the mixing of air with the surrounding atmosphere, which creates a temperature insulating effect (Geiger et al., 1995). The type of vegetation in the understory, such as shrubs and herbaceous plants, can further modify microclimate conditions through their own evapotranspiration and by affecting ground shading (Stickley & Fraterrigo, 2021). Moreover, deciduous species in both the overstory and understory produce litter, which contributes to regulate soil microclimate (Fekete et al., 2016). Soil properties, including composition and moisture content, also significantly impact the microclimate by influencing heat retention and water availability (von Arx et al., 2013). Additionally, topographical features like slope and orientation can cause variations in sunlight exposure and water drainage, thereby altering microclimate conditions (Hoylman et al., 2018).

Identifying what factors contribute to understory forest microclimate is crucial to achieve forest management goals that benefit biodiversity under climate change strains. Climate-smart management has been suggested as a decision-making framework for forest landscape management, focusing on the understanding of landscape vulnerability to future climatic change (Sterck et al., 2021; Pradhan et al., 2023). However, there are still significant gaps in our knowledge regarding the factors that contribute to the understory forest microclimate, their functioning scale, and how they interact with the biotic and abiotic requirements of understory biota.

1.5 Microclimate in plant-pollinator interactions

Insect activity is largely regulated by temperature (Williams, 1961). Therefore, microclimate can affect insect abundance and activity (Beattie, 1971). For instance, solar exposure and temperature are key factors influencing insect activity (Bovee et al., 2021), and insects may only be available for pollination during short time spans when the sun is shining directly over the forest floor where the flowers are located (Beattie, 1971). Open forest canopies have correspondingly been related to a higher number of pollinator visits (Eckerter et al., 2019). This can be driven, not only by the increase in pollinator activity linked to a higher light availability and direct sunshine, but also by different factors affecting understory vegetation. Plants under open canopies have a higher flowering probability, and also produce more flowers (Eckerter et al., 2019). Then, a higher flower abundance has been related to an increased pollinator density and diversity (Blaauw & Isaacs, 2014), and flowers on low density forest areas may be easier to spot by pollinators compared to flowers in more closed areas. Therefore, depending on the forest structure and microclimate at a plant individual's location, the plant may be more or less likely to be visited by an insect (Herrera, 1995). However, different pollinators may respond differently to the forest floor irradiance depending on their thermal requirements (Herrera, 1997).

Additionally, microclimate can also alter plant growth and the insects' development. For instance, *Pieris napi* L. caterpillars that are located just a few meters apart can differ by more than a week in the timing of pupation because of microclimatic differences caused by forest canopies (Greiser et al., 2022). These effects of microclimate on insects' physiology can ultimately have an impact on their phenology, potentially leading to phenological mismatch. Similarly, flowering phenology can be driven by microclimate, *e.g.,* when flowering time is determined by degree day accumulation (Jagadish et al., 2016); or by forest structure, *e.g.,* when flowering is triggered by photoperiod (Johansson & Staiger, 2015). See Figure 1 for a conceptual model of the relationship between forest structure, microclimate and plant-pollinator interactions.

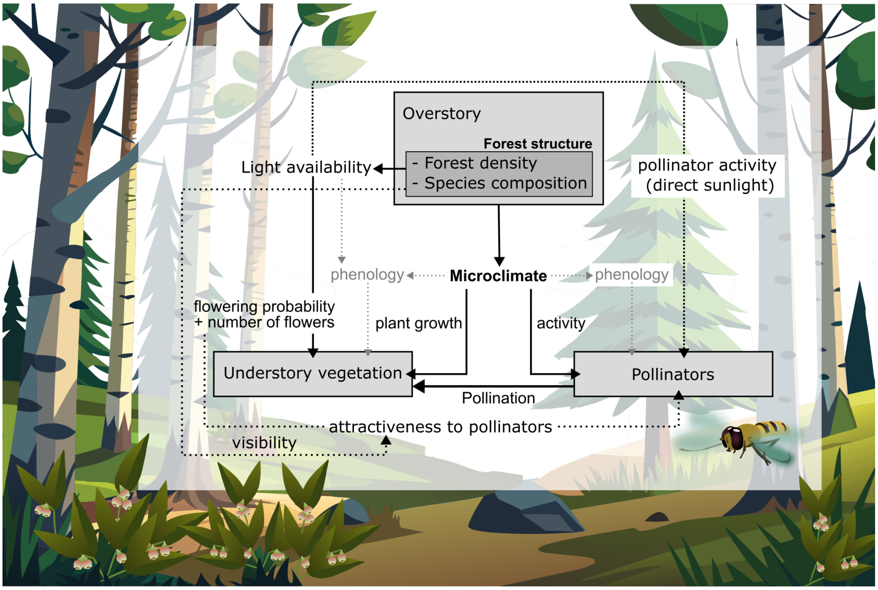


Figure 1. Conceptual model of the relationship between forest structure, microclimate and plant-pollinator interactions. Direct interactions are depicted with solid lines and involve immediate relationships between elements, such as between forest structure and microclimate. Indirect interactions, shown with dotted lines, represent relationships that are mediated by other factors, like how forest structure influences pollinators through light availability.

As climate change continues to reshape ecosystems, the complex dynamics between plants and pollinators in boreal regions are becoming more apparent, mainly by the species-specific responses to warming and the lack of data on the pollinator side. However, we currently lack the knowledge to be able to predict how climate change will affect plant-pollinator interactions in boreal forests, their resilience, and the role of forest canopies in mitigating the negative impacts of warming.

2. Thesis aims

Current research on the relationship between climate, forest composition and boreal pollinators reveals significant gaps in our understanding. These knowledge gaps include the specific responses of species to climate change and the potential for phenological mismatch in their interactions, insufficient understanding of how forest structure buffer temperatures, the role of microclimate in shaping pollinator behaviour in boreal forests, and the impact of forest structure and microclimate on the diversity of flower-visiting arthropods. The overall objectives of this thesis were to address these gaps in order to provide some of the information necessary to develop effective forest management strategies that support biodiversity and ecosystem services in the face of ongoing climate change. The specific aims of each chapter are the following:

I. To identify research gaps on the impacts of climate change on plant-pollinator interactions in the boreal forest, focusing on species responses at individual and community levels.
II. To understand how forest structure and composition, specifically the density and proportion of conifers and broadleaves, influence the buffering of understory microclimatic temperatures against macroclimate changes across a broad latitudinal gradient.
III. To identify the effects of macroclimate, microclimate, and forest vegetation on pollinator visitation rates, foraging duration, and overall activity using a novel approach that combines camera traps with standardized artificial flowers.
IV. To study the impact of forest structure and microclimate on the diversity of flower-visiting arthropods in a mixed forest using eDNA from flower samples.

3. Methods

This section provides an overview of the methodology used in each of the papers that comprise this thesis. In Paper I, a systematic review was conducted on the effects of climate change on boreal plant-pollinator interactions. In Paper II, models were built to assess the effect of forest density and overstory species composition on the microclimate buffering capabilities of forests. In Paper III, time-lapse cameras were used in combination with artificial plastic flowers and high resolution microclimate data to monitor pollinator activity and find its drivers in the forest understory. In Paper IV, flower eDNA was used to reveal arthropod flower visitor richness and relate it to forest structure. For further details, see the corresponding papers.

Note that despite referring to the insects recorded with the artificial plastic flowers or eDNA as pollinators, it is important to keep in mind that we never recorded any evidence of pollination in our experimental sites. Therefore, visiting insects should be considered as potential pollinators or just flower visitors.

3.1 Literature review (Paper I)

In this chapter, I gathered a baseline of information regarding the effects of climate change on plant-pollinator interactions that have been reported in literature. For this, I conducted a literature review based on peer-reviewed articles including all databases available to the Swedish University of Agricultural Sciences through the Web of Science, namely: the Web of Science Core Collection, BIOSIS Citation Index, Current Contents Connect, CABI: CAB Abstracts®, Data Citation Index, Derwent Innovations Index, KCI-Korean Journal Database, MEDLINE®, ProQuest™ Dissertations & Theses Citation Index, SciELO Citation Index and Zoological Record. Results from the Preprint Citation Index were excluded. Only works written in English were considered.

I conducted four complementary searches in November 2023. All search queries (see i-vii in Table 1) included a section on (i) climate change, (ii) forest categories, and (iii) regions for inclusions and (iv) exclusion (*e.g.,* tropical forests). In addition, depending on the focus of the search, an additional section criteria was added that included (v) pollinators, or (vi) understory vegetation, including the common and scientific name of the most common species, or both (v) and (vi). The primary search included both pollinators and understory vegetation (*i.e.,* elements i-vi in Table 1). To ensure no relevant studies involving pollinators were missed, a secondary search focused solely on pollinators (*i.e.,* elements i-v in Table 1) was performed. Additionally, a search for review articles specifically about understory vegetation in boreal forests was conducted. This involved two separate searches using elements i-iv + vi in Table 1. In one search, review articles were filtered from the results using Web of Science's ‘review articles’ feature. In the final search, a new section (vii in Table 1) was added to the query, incorporating keywords such as "review" and "meta-analysis."

3.2 Study area (Papers II-IV)

To increase the generality of the findings, five mixed forests within a latitudinal gradient in North-Western Europe were selected, spanning from 49° N to 64° N (ca. 1,800 km and 7°C difference in mean annual temperature). Of these five forest areas (“sites” from now on), three were located in Sweden, spreading over the north (NS), mid (MS) and south (SS) of the country. The other two sites were located in western Germany (G) and eastern Belgium (B) (Figure 2A). The forests in sites G and B are temperate, whilst SS is hemiboreal and MS and NS are in the boreal zone (Ahti et al., 1968). All sites had a similar overstory species composition and were similar in their successional stage. The dominating conifer species was Norway spruce (*Picea abies* H. Karst), and the dominating broadleaf was birch (*Betula pendula* Roth and *B. pubescens* Ehrh.). However, in B, oak (*Quercus*

Table 1. Search query for the four literature searches conducted. Different combinations of the elements i-vii were used in each search. See text for details. The overlap between the different searches, as well as the PRISMA diagram with exclusion criteria are shown on the right.

(i) TS=((Climat* OR "Climat* gradient*" OR “Climat* change*” OR “Climat* warming” OR Temperature* OR Drought* OR “Global warming” OR mismatch* OR Phenolog* OR snow* OR “rain” OR precipitation)

(ii) AND (Forest* OR Tree* OR Woodland*)

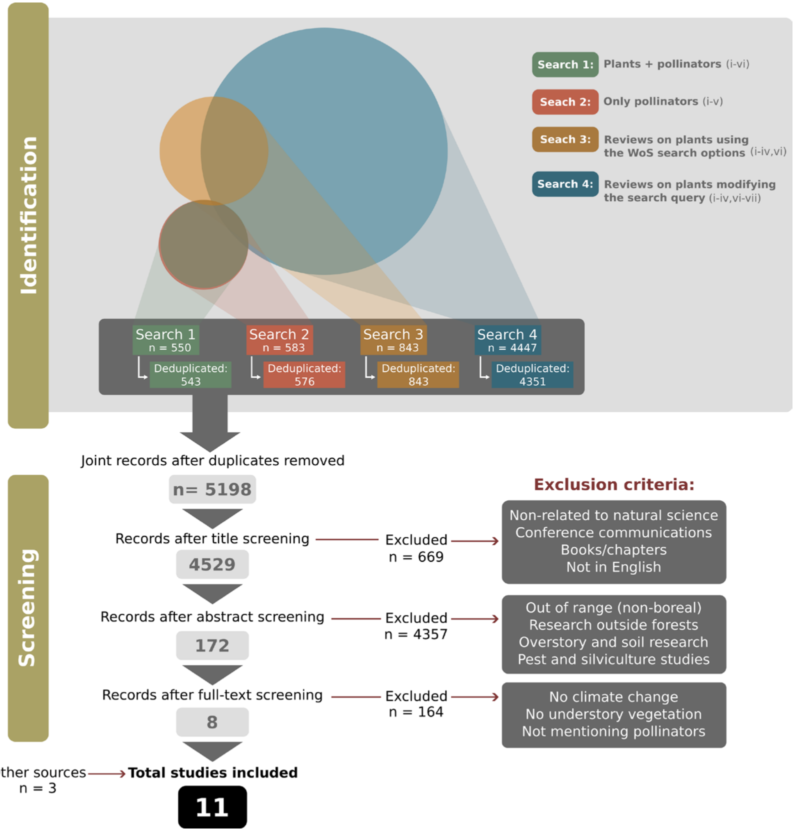
(iii) AND (*boreal* OR taiga OR "north* hemisphere" OR circumpolar OR Fennoscan* OR Scandin* OR "north* europ*" OR "nord* countr*" OR Eurasia* OR "north* asia" OR Canad* OR Finland OR Finnish OR Japan* OR Norw* OR Russia* OR Swed* OR "United States" OR "USA" OR "U.S.A" OR "north America*" OR Alaska OR Ontario OR Quebec OR "Nova Scotia" OR "New Brunswick" OR Manitoba OR "British Columbia" OR "Prince Edward Island" OR Saskatchewan OR Alberta OR "Newfoundland” OR “Labrador")

(v) AND (Pollinat*)

(vi) AND (Understor* OR "Fieldlayer*” OR Field layer* OR “Field-Layer*” OR Herb* OR Flower* OR Groundlayer* OR “Ground-layer*” OR “Ground layer*” OR Vegetation* OR Plant* OR Seed* OR Pollen*)

(vii) AND (revis* OR review* OR synthesi* OR meta-analys* OR metaanalys* OR summar*)

(iv) NOT (*tropic* OR desert* OR rainforest* OR savanna* OR pampas* OR ethnic* OR archeolog* OR medic* OR allerg* OR marine* OR fisher* OR horticultur* OR agronom* OR orchard OR cultiv* OR breed* OR hybr* OR mast* OR polic* OR simulat* OR *water))



robur L. and *Q. petraea* (Matt.) Liebl.) was the most common broadleaved species.

Within each of these sites, 40 plots were established along gradients of forest density (*i.e.,* from open to closed canopies) and overstory species composition (from conifer-dominated plots to broad-leaf dominated plots; Figure 2B). In the center of each plot, we established a microclimate station that recorded microclimatic temperature in the understory (see details in section 3.3). Data for Paper II was collected in all sites, data for Paper III in MS and SS, and data for Paper IV in SS.

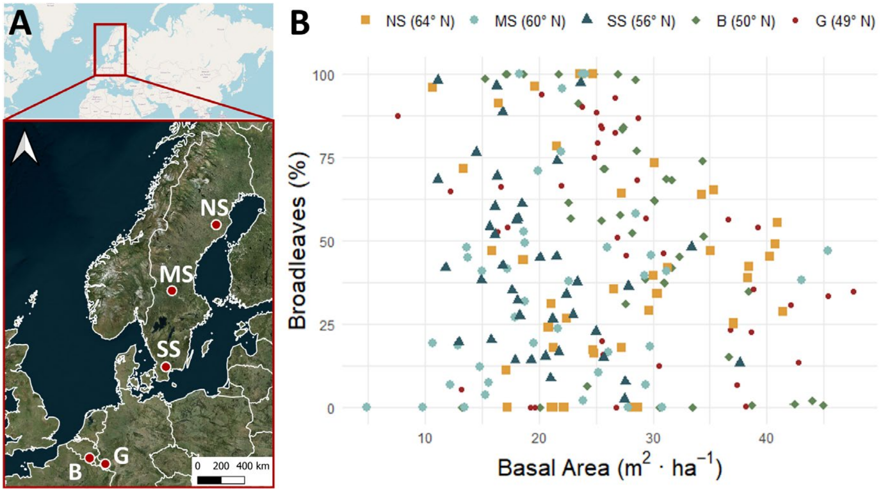


Figure 2. **A.** Geographical location of sites used across Europe to discern how understory microclimate is affected by tree species composition and density. The sites were located in North Sweden (NS), Mid Sweden (MS), South Sweden (SS), Germany (G) and Belgium (B). **B.** Forest density estimated by basal area ($m^2 \cdot ha^{-1}$) and share (% of basal area) of broadleaved trees in 200 plots from the 5 sites (40 in each) included in the study

Table 2. Description of the five forest sites across a 1,800 km latitudinal gradient in which microclimate was measured. Tree species composition values represent the mean basal area ($m^2 \cdot ha^{-1}$) per species across all plots within site. Annual mean temperature and annual mean precipitation were extracted from the WorldClim version 2.1 climate data for 1970-2000 (Fick & Hijmans, 2017)

		Northern Sweden (NS)	Mid Sweden (MS)	Southern Sweden (SS)	Germany (G)	Belgium (B)
	Location	64°18′ N, 19°44′ E	60°50′ N, 14°40′ E	56°17′ N, 13°58′ E	49°36′ N, 7°01′ E	50°02′ N, 5°13′ E
	Mean annual temperature (°C)	1.50°C	4.52°C	6.79°C	7.51°C	8.58°C
	Total annual precipitation (mm)	599 mm	589 mm	738 mm	1029 mm	961 mm
	Distance to used weather station	~ 6 km	~ 15 km	~ 1 km	~ 17 km	~ 13 km
	Elevational difference to used weather station	35 m	-39 m	9 m	-33 m	-161 m
	Average tree diameter ± SD (cm)	16.7 ± 6.3	16.1 ± 5.3	12.9 ± 4.6	13.9 ± 6.4	35.7 ± 14.0
	Stem density (trees ha^{-1})	1061.56	946.17	1356.0	1522.17	248.57
	Average basal area ($m^2 \cdot ha^{-1}$)	26.8	21.6	19.9	27.9	28.7
Conifer species	*Picea abies*	14.8 $m^2 \cdot ha^{-1}$ (55.2%)	13.5 $m^2 \cdot ha^{-1}$ (62.4%)	12.1 $m^2 \cdot ha^{-1}$ (60.7%)	13.7 $m^2 \cdot ha^{-1}$ (48.8%)	14.6 $m^2 \cdot ha^{-1}$ (50.8%)
	Pinus sylvestris	1.0 $m^2 \cdot ha^{-1}$ (3.8%)	0.1 $m^2 \cdot ha^{-1}$ (0.6%)	-	<0.1 $m^2 \cdot ha^{-1}$ (0.3%)	-
	Pseudotsuga menziesii	-	-	-	0.2 $m^2 \cdot ha^{-1}$ (0.7%)	0.5 $m^2 \cdot ha^{-1}$ (1.8%)
	Larix spp.	-	-	-	1.1 $m^2 \cdot ha^{-1}$ (3.8%)	-
	Picea sitchensis	-	-	-	0.4 $m^2 \cdot ha^{-1}$ (1.5 %)	-
Broadleaf species	*Betula pubescens/pendula*	11.0 $m^2 \cdot ha^{-1}$ (41.0%)	8.0 $m^2 \cdot ha^{-1}$ (36.9%)	7.1 $m^2 \cdot ha^{-1}$ (35.6%)	11.4 $m^2 \cdot ha^{-1}$ (40.7%)	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)
	Alnus incana/glutinosa	-	-	0.4 $m^2 \cdot ha^{-1}$ (1.9%)	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)	-
	Fagus sylvatica	-	-	-	0.3 $m^2 \cdot ha^{-1}$ (1.1%)	0.3 $m^2 \cdot ha^{-1}$ (1.0%)
	Carpinus betulus	-	-	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)
	Sambucus spp.	-	-	-	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)	-
	Quercus robur/petraea	-	-	0.3 $m^2 \cdot ha^{-1}$ (1.7%)	0.3 $m^2 \cdot ha^{-1}$ (1.1%)	13.2 $m^2 \cdot ha^{-1}$ (46.0%)
	Sorbus spp.	-	-	-	0.4 $m^2 \cdot ha^{-1}$ (1.5%)	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)
	Salix spp.	-	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)	-	-	-

3.3 Microclimate stations and measurements

Microclimate stations consisted of a wooden pole with a well-ventilated radiation shield attached at approximately 1.2 m from the ground level. This shield did not affect microclimatic measurements compared to temperature measured through weather stations. Two HOBO Pendant® MX Water Temperature Data Loggers (Onset Computer Corp, Bourne, MA, USA) were placed in each microclimate station: one inside the shield (air logger) and one in the humus layer (soil logger). The soil logger was placed in a jar to protect it from humidity and animals, and the jar was attached to the pole through fishing wire, both to make the logger easier to find and to avoid animals taking the jar. No soil loggers were established in B due to frequent wild boar rooting in this site. Microclimatic temperature measurements were taken every 15 minutes between August 2019 and June 2023, except in SS, where air loggers recorded temperature every 30 minutes. For Paper II, only the microclimate data from the warmest and coldest months in 2020 and/or 2021 in each site were used. For Paper III, data collected in SS and MS between July and September 2021 were used. For Paper IV, data from site SS collected in June and July 2022 were used.

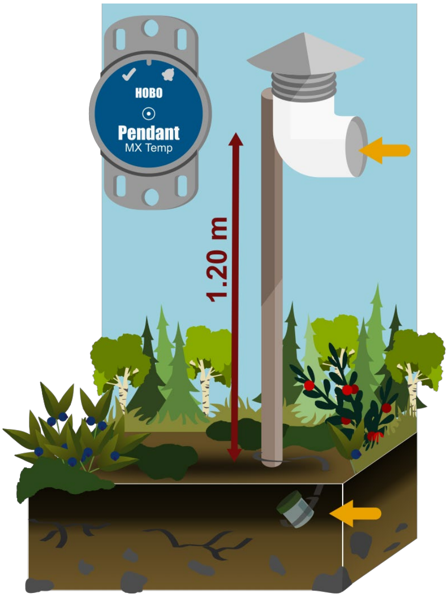


Figure 3. Microclimate station. Loggers (top left) recorded both air (top yellow arrow) and soil (bottom yellow arrow) temperatures. A radiation shield (top) was used to protect the air temperature loggers from direct exposure to sunlight and animals. Soil loggers were placed inside hermetic plastic jars to protect them from direct contact with the soil.

3.4 Macroclimate data (Papers II & III) and temperature offsets (Paper II)

Macroclimate data was used in Papers II and III. In Paper II, we calculated daily averaged macroclimatic temperatures from three different sources: the closest available weather stations to each site, the ERA5-Land climatic model (Hersbach et al., 2020) downloaded for reanalysis from the Copernicus Climate Data Source (cds.climate.copernicus.eu), and ERA5 data downloaded through the 'mcera5' R package (Klinges et al., 2022). The 'mcera5' package applies an inverse distance weighting calculation and diurnal temperature range corrections to the ERA5 data. Daily temperature offsets (*i.e.,* difference between macroclimate and microclimate) were calculated based on the temperatures extracted from these three different macroclimatic sources. Temperature offsets were calculated by subtracting the macroclimate temperature from the microclimate temperature, so that negative offset values showed days in which the forest temperature was lower than the ambient temperature, and positive value days in which the forest temperature was higher. Weather station data followed microclimatic trends better and was therefore used in the analyses of Paper II. Soil temperature offsets were calculated only from ERA5 data, as macroclimate soil temperature was not available through the other sources. Additionally, for Paper II, three meteorological variables were downloaded from the ERA5 climatic model: maximum wind gust per day, daily snow depth and daily precipitation. Previous averaged temperature and previous averaged precipitation were calculated as a five-day rolling mean, *i.e.,* temperature and precipitation of the past five days.

In Paper III, macroclimatic temperature and precipitation data were downloaded from the ERA5 climatic model using the 'mcera5' R package. These variables were downloaded at an hourly resolution and matched with the microclimatic measurements. Moreover, total precipitation was calculated per day.

3.5 Overstory measurements (Papers II-IV)

For all trees within a 10-meter radius (20 meters in site B) from the temperature loggers, we documented the species, diameter at breast height (DBH), and the distance to the logger. These measurements were used to calculate the total basal area (indicating forest density) per plot, as well as the percentage of basal area composed of broadleaved trees per plot (referred to as the "proportion of broadleaves" hereafter). This data was used for Papers II-IV. However, in Paper II, in order to determine the scale at which the overstory affects understory microclimate, we computed basal area and the proportion of broadleaves at various radii between 4 and 10 meters (4 and 20 meters in site B), meter by meter.

In addition, hemispherical photographs were taken in each plot during summer to determine canopy openness, using a Nikon D5300 camera equipped with a fish-eye lens and tripod. All photographs were captured from above the air temperature logger, at an approximate height of 1.75 meters. Pictures were taken either on clear days with no clouds or on overcast days to ensure that the contrast between the trees and the sky was optimal. These images were processed and analyzed in R following the method outlined by ter Steege (2018).

Therefore, we used two different proxies for forest density: basal area and canopy openness. There are two reasons for this. While basal area is a common forestry metric and is used in decision support systems, canopy openness is more easy to interpret ecologically and, in some cases, predicts forest understory microclimate better (Meeussen et al., 2021; Zellweger et al., 2019). Furthermore, canopy openness data may be more readily available due to remote sensing. In our data, these two variables were found to be correlated with an r = -0.44.

3.6 The flower problem (Paper III and IV)

Flowers were not naturally present in all plots in the forest. As both in Paper III and Paper IV we were interested in measuring flower-related variables across our double gradient of forest density and overstory species composition, we had to devise a methodological solution to address this issue.

In Paper III we wanted to determine how forest structure and microclimate influenced pollinator foraging rates and durations, and to do so we needed to record the potential presence of pollinators in the plots of SS and MS. For this, we built artificial plastic flowers. These artificial plastic flowers were inspired by the design used in Thomson et al. (2012) and consisted of a plastic jar filled with a saturated sucrose solution. This solution was in contact with a weighted cotton thread that supplied sucrose to an upper knot, functioning as a nectary. The upper knot was located within a tube representing the base of the flower's corolla, and it would automatically refill the sucrose solution through capillary action whenever it was depleted. To attract pollinators, the lid of the jar was painted with UV-reactive colors, including blue, yellow, and white (Figure 4). Two artificial flowers of each color were then placed in a tray partially filled with water to avoid ant visitation, since ants can negatively affect pollinator flower visitation by insects in both real (Junker et al., 2007) and artificial flowers (Cembrowski et al., 2014).

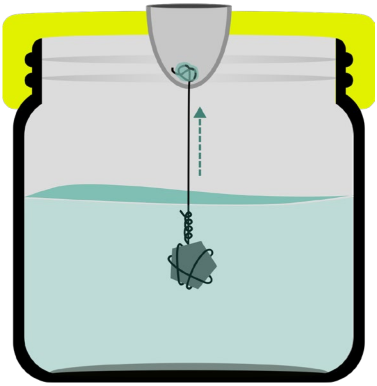


Figure 4. Structure and function of the artificial plastic flowers. A plastic jar is filled with a saturated sucrose solution, which is in contact with a weighted cotton thread that provides sucrose to an upper knot that works as a nectary. This upper knot is found in a tube that represents the bottom of the flower's corolla, and will refill the sucrose solution through capillary action every time it gets depleted. The lids of the jars were painted in UV-reactive colours (blue, yellow and white) to attract pollinators.

In Paper IV, we translocated flowering plants grown in a greenhouse into the plots of SS. For this, two different flowering plant species were chosen: wild strawberry (*Fragaria vesca* var. *semperflorens* Jamin) and red clover (*Trifolium pratense* L.). These particular species were selected based on their diverging flower morphology, which may make them available to different sets of pollinators. Moreover, this particular variety of wild strawberry produces flowers throughout the growing season. Both plant species are also commercially grown, which facilitated procurement. We grew *T. pratense* from seed indoors until seedlings reached approximately five centimeters of height. Then, these seedlings were potted in 20 cm pots and moved outdoors.

Grown *F. vesca* seedlings were purchased directly, placed in the greenhouse and transplanted to the same type of pots as *T. pratense*. Potted plants were placed in a sunny spot to induce flowering and were monitored daily. Once the plants developed flower buds, but before any flowers opened, the entire pot was covered with a mesh bag (mesh size ~0.3 mm) to prevent insect visitation prior to placement in the forest. Before covering, the plants were thoroughly checked for insects, including between the leaves. Once the plants flowered, they were relocated to the forest stand. The pots were placed in holes in the ground to prevent them from drying out or tipping over. Two pots of each flowering plant species were positioned in each plot, as close as possible to the microclimate station, resulting in a total of 80 strawberry and 80 red clover plants. In the field, the mesh bag was removed from one pot of each plant-species pair, allowing insect visitation to the flowers, while the other pot remained covered (Figure 5). This paired design served as a control for false-positive detections of insects by accounting for airborne eDNA and any insect DNA already present on the plant before the experiment, such as from the outdoor greenhouse (see 3.8 for details).

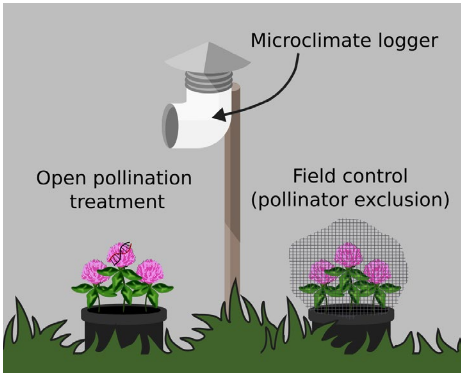


Figure 5. Experimental setup for the eDNA experiment. In the center of each plot, a microcliamte station measuring understory temperature was installed, and two pots of planted *Trifolium pratense* and *Fragaria vesca* were placed, one of which was covered with a 0.3 mm mesh to avoid pollinator visitation (field control), and the other was uncovered and thus available to all pollinators (open pollination treatment).

As wildflowers still may have contributed to pollination attraction in the plots where they could be found, we recorded the species richness of flowering plants and abundance of individual flowers within a five-meter radius of each artificial flower station in Paper III. In Paper IV, we used flower abundance data from another experiment that was running in the same forest stand.

3.7 Camera traps and vision AI model (Paper III)

In Paper III, we used time-lapse cameras (Wingscapes TimelapseCam Pro WCT00126, Pradco Outdoor Brands, AL, USA) with an automatic LED flash to record pollinator activity in all plots from MS and SS. Cameras captured images of the artificial flowers (Figure 6A-B) every 10 seconds from 7 am to 8 pm. The flash was activated only under low light conditions. The images were taken at a resolution of 4224x2376 pixels.

To prevent pollinators, particularly social species such as bumblebees, from memorizing the locations of the artificial flowers and thereby biasing our results through repeated visitation (Keasar, 2000), we utilized 13 cameras and sets of artificial flowers, which we relocated among 39 plots in each forest every 3-4 days according to a pre-set schedule. The color order of the artificial flowers was randomized each time they were moved. Whenever the cameras and flowers were relocated, camera batteries and memory cards were exchanged, and the sucrose solution levels of the artificial flowers were replenished if needed. Each camera was rotated among a subset of three plots.

The YOLOv5 vision AI model (Jocher et al., 2022) was trained to detect pollinators in our images. This machine-learning model uses a convolutional neural network as its backbone and functions as an object detection tool, creating features from input images and passing them through a prediction system that draws boxes around objects and identifies their classes. YOLOv5 has previously been successful in detecting insects in camera trap images (Bjerge et al., 2023; Stark et al., 2023).

Before training our YOLOv5 model, we cropped the background of all images to reduce noise from different background vegetation types and cover (Figure 6C). To achieve this, we created masks for each combination of plot, camera, and recording date, and automated the clipping process using a MACROS script with the ImageJ software (Abramoff et al., 2004). We then selected a subset of 250 pictures in which pollinators were present as the training dataset for the YOLOv5 model. This dataset included images representative of the pollinators found in the study area (Diptera, Hymenoptera, Coleoptera) in various positions and under different lighting conditions. Each image was labelled using the Yolo_Label software (Kwon, 2023), drawing a bounding box around all pollinators. These labelled images were used as a training dataset in the YOLOv5 model (v7.0-240-g84ec8b5), splitting those in 80% for training and 20% for validation.

Once the vision AI model processed all images, those in which pollinators were detected (*e.g.,* Figure 6D) were checked manually, and false positives removed. Meta-data such as forest site, plot, date, and time were extracted from the selected pictures. Due to varying picture quality, precise taxonomic identification was not always possible, so pollinators were sorted into families or the finest taxonomic resolution achievable. Flies (Diptera) were categorized into two groups: hoverflies (Syrphidae) and non-syrphid flies (all other families). The duration of each pollinator visit was estimated by counting the number of consecutive pictures in which the pollinator appeared and multiplying by the picture interval of ten seconds up to a maximum of 20 minutes.

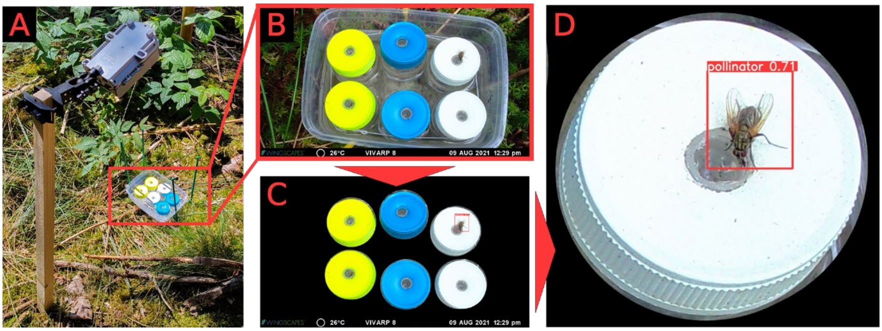


Figure 6. **A.** Camera and artificial flower setup. **B.** Example of a photo of a pollinator obtained through the camera trap. **C.** Processed photo through the YOLOv5 vision AI model. The background has been removed and the model finds potential pollinators in the image. **D.** A zoom in on a fly detected by the vision AI model. Detection accuracy appears on top of the detection square.

3.8 Flower eDNA metabarcoding (Paper IV)

Environmental DNA (eDNA) refers to genetic material obtained indirectly from environmental samples such as soil, water, or air, rather than from an individual organism. This DNA can be sourced from cells, tissues, secretions, and excretions shed by organisms into their surroundings (Ruppert et al., 2019). In Paper IV, we filtered, extracted, and sequenced arthropod eDNA from *Trifolium pratense* and *Fragaria vesca* flowers (see 3.6 for details).

3.8.1 Flower collection

Flowers of *F. vesca* and *T. pratense* were collected from both the plants that were meshed (field controls) and the ones that were not (open-pollinated plants) during their flowering period from June 17th to July 29th, 2022. Flowers were sampled every third day, with nitrile gloves that were changed between collections to avoid cross-contamination. Samples were stored in individual sterile vials and kept in ice in the field and were later transferred to a -20°C freezer until it was time to process them.

3.8.2 Environmental DNA filtering and extraction

Samples belonging to the three most open and three most dense plots were processed individually, whilst the rest were pooled by plot and plant species. Field controls were pooled by collection date across all plots. Flowers were vigorously shaken in milliQ water to suspend the eDNA found on their surface. This water was then filtered through a nylon mesh to remove big pieces of debris, and finally through a polycarbonate membrane (Whatman, Pittsburg, PA) to capture the eDNA. If filters became clogged (*e.g.,* due to an excess of pollen), the water was passed through a second polycarbonate filter. Negative controls with only milliQ water were included for every ten flower samples. Filters were stored at -20°C until DNA extraction using the Qiagen DNeasy Blood and Tissue kit, with careful attention to maintaining sterile conditions throughout the process. The manufacturer's protocol for animal tissue was followed, except for the final elution step, where we split the 200 µl elution volume into two steps of 100 µl to maximize DNA recovery.

3.8.3 PCR amplification

Mini-barcode arthropod-specific primers for the Cytochrome Oxidase I (COI) gene were used. These primers – see Zeale et al. (2011) for details – produce an amplicon of 157 bp and perform well for amplifying degraded DNA while still maintaining the ability to resolve taxa to species level. Primers were ordered with attached Illumina adapters with a separation of 3, 5 or 7 random nucleotides to increase sequence diversity and throughput (Wu et al., 2015). PCR reactions were carried out in volumes of 25 µl consisting of 3 µl of template DNA, 12.3 µl of ddH2O, 1 µl of each primer (10 µM) and 7.7 µl of AmpliTaq Gold™ 360 Master Mix (Applied Biosystems; catalog no. 4398881). Thermocycler parameters were 95°C for 10 min, 55 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1 min, and a final elongation of 72°C for 7 min. Reactions were performed in duplicates to maximize detected diversity. Negative controls with water instead of template DNA were used in each PCR plate. PCR products were then verified on agarose gel and pooled for sequencing.

3.8.4 Library building and sequencing

Library construction and Illumina sequencing were conducted by Macrogen (Amsterdam), producing 171 libraries across various categories. Libraries were built using the Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2 and following the 16S Metagenomic Sequencing Library Preparation Part # 15044223 Rev. B. A total of 171 libraries were built, one for each plot with pooled flowers (34 for *F. vesca* and 32 for *T. pratense*), one for each individual flower in the most open and most dense plots (49 for *F. vesca* and 42 for *T. pratense*), one for each field control (six for *F. vesca* and four for *T. pratense*), three PCR blanks and one filter negative control. These libraries were then sequenced on using Illumina NovaSeq (150 bp) for comprehensive analysis.

3.8.5 Bioinformatics and taxonomic annotation

Sequences were demultiplexed using dual unique indices and processed with the nfcore/ampliseq bioinformatics pipeline V2.8 (Straub et al., 2024), which included quality control with FastQC (Andrews, 2010), primer trimming with Cutadapt (Martin, 2011), and ASV inference with DADA2 (Callahan et al., 2016). A length filtration of 157±6 bp was applied, as we detected a peak of sequences at 149 bp corresponding to the co-amplification of strawberry chloroplasts. ASVs were clustered using Swarm v3 (Mahé et al., 2015) with a conservative threshold to prevent over-clustering. Taxonomic assignment was performed with BOLDigger (Buchner & Leese, 2020) for species-level matches and Sintax (Rognes et al., 2016) for probabilistic assignments, with a consensus classification approach for disagreements. Non-arthropod sequences, herbivores (*e.g.,* Aphididae), and sequences found in the lab control samples were removed. Sequences from field controls were also filtered to account for airborne eDNA contamination by subtracting twice the highest read count found in controls from all samples.

3.9 Data analyses

All analyses for Papers I-IV were run in R version 4.2.2-4.3.3 (R Core Team, 2022, 2023, 2024). For Paper III, Python 3.10.12 (Van Rossum & Drake, 2009) was used to build the YOLOv5 vision model.

3.9.1 Paper I

We utilized the R package ‘synthesisr’ (Westgate & Grames, 2020) to eliminate duplicates from each of the four searches in the literature review. Initially, duplicates were identified by exact title matches. Subsequently, we applied optimal string alignment distance calculations to all titles to identify potential duplicates. To do so, titles were transformed to lowercase and punctuation marks were disregarded. These were manually reviewed and removed as needed. Afterwards, we calculated the overlap between the results found in our four searches and identified additional duplicates across the entire pool of articles collected from all searches by repeating the same process.

3.9.2 Paper II

To explore the effects of forest structure and macroclimate on temperature offsets, we used Generalized Additive Mixed Models (GAMMs). These models extend Generalized Linear Mixed Models (GLMMs) by relaxing the assumption of a linear relationship between predictors and the response variable. We also ran Linear Mixed Models (LMMs) to compare their performance against the GAMMs. Daily mean, minimum, and maximum offsets for air temperature, and daily mean offsets for soil temperature, were the response variables. Predictors included forest density (as canopy openness or basal area) and the proportion of broadleaves, which were used interactively and non-interactively in LMMs and within two-dimensional smoother terms in GAMMs. Models were run for both the warmest (July) and coldest (January) months, accounting for temporal autocorrelation using date as a unidimensional smoother term. Plot nested within site was used as a random intercept effect, and model selection was based on Akaike’s Information Criterion (AIC). Once the best smoother configuration was identified, additional covariates like wind, previous averaged temperature and previous averaged precipitation for air models, as well as previous averaged temperature and snow depth for soil models, were included.

This study also assessed the scale at which overstory affects air and soil temperature offsets by running GAMMs with basal area and proportion of broadleaves calculated at different plot radii (4 to 10 m), both in all combinations of these metrics and with each predictor alone. These analyses were conducted for mean daily air and soil temperatures, using either bidimensional smoothers for both metrics or unidimensional smoothers when used alone. In site B, trees were measured within a 20 m radius, allowing for independent analysis of this site with data combinations from 4 to 20 m. A minimum radius of 4 m was chosen based on data availability to avoid significant reduction in the number of plots with trees.

3.9.3 Paper III

In this study, we assessed the factors influencing pollinator foraging rates and duration using Generalized Linear Mixed Models (GLMMs) with the 'glmmTMB' function in the R package with the same name (Brooks et al., 2017). The analysis focused particularly on flies (Diptera), with separate evaluations for hoverflies (Syrphidae) and non-syrphid flies (all other families). Additionally, the analyses included the most common and easily identifiable single-species *Episyrphus balteatus* (De Geer, 1776). A Hurdle GLMM approach was employed due to the dataset's characteristics, including zero inflation and positively skewed duration data. The model included two components: a binomial model with logit link function that estimated the probability of a zero in our data (*i.e.,* the probability of an insect not being on the artificial plastic flowers), and a continuous component (Gamma distribution with log link) that predicted the duration of foraging events once insects visited artificial flowers. Therefore, in the binomial component, a negative estimate should be interpreted as a positive effect of a specific variable on the occurrence, and vice versa. Fixed effects included flower species richness and flower abundance at the plot level, daily and hourly precipitation, time since rain, and either microclimatic or macroclimatic temperature. Random effects accounted for plot nested within-site variability.

To ensure robust analysis, multiple model configurations were tested. Temperature variables (microclimate and macroclimate, first and second degree polynomials) were included in different components of the model due to their high correlation (r = 0.81). This led to four main model structures, each tested with and without additional predictors such as basal area and proportion of broadleaves, resulting in a total of 12 models. All predictors were standardized for comparability, and model selection was based on Akaike's Information Criterion (AIC). Model performance was assessed using simulated residuals from the 'DHARMa' V0.4.6 package (Hartig, 2022) to evaluate goodness-of-fit. Finally, the predicted binomial response for the models with the lowest AIC values was plotted as an effect of macroclimate.

3.9.4 Paper IV

Rarefaction curves were generated for the three most dense and three most open plots for each flower species, treating individually collected flowers as independent samples. The 'iNEXT' V3 package (Hsieh et al., 2022) was utilized to estimate asymptotic diversity estimates and their standard errors via bootstrapping with 999 replicates.

To investigate the influence of environmental factors on pollinator richness, Generalized Additive Models (GAMs) with a poisson error distribution and log-link function were applied separately for *F. vesca* and *T. pratense*. Models were built using the 'gamm4' function from the gamm4 V0.2-6 package (Wood & Scheipl, 2020). These models incorporated a bidimensional smoother term for forest density (basal area or canopy openness) and percentage of broadleaves. Parametric covariates included the number of flowers sequenced, plot type (used for rarefaction or not), and wildflower abundance within plots. The best model for each plant species was selected based on likelihood ratio tests with the function ‘buildgamm4’ in the buildmer V2.11 package (Voeten, 2023).

Additionally, General Linear Models (GLMs) were fitted, using a poisson distribution and log-link function. Initially, a full model was run including microclimate variables, number of sequenced flowers, plot type, and wild flower abundance. To handle collinearity between the microclimate variables, a full model was run separately for the mean, minimum, and maximum microclimatic temperatures over the study period. Model selection was based on likelihood ratio tests using the 'drop1' function from the stats package. Residuals were also evaluated for overdispersion in these models.

Finally, to assess airborne eDNA contamination in field controls and its potential increase over time, arthropod richness in these controls was compared using sequence data.

4. Main results and discussion

4.1 Boreal pollinators (Paper I)

Knowledge regarding the effect of climate change on boreal pollinators is quite limited. In Paper I, an information baseline on the consequences of climate change on boreal pollinators and their interactions with flowering plants was created by conducting a literature review. However, only 11 out of 5,198 articles fulfilled our search criteria.

Among the limited number of articles that were relevant to our research question, hardly any studies identified pollinators to the taxonomic level of species. For instance, most articles use plant reproductive success as a proxy for pollinator effectiveness, which successfully assesses ecosystem functionality, but overlooks species roles and potential changes in species communities that may be relevant to projecting climate change related impacts. Increasing temperatures may result in species extirpations and extinctions, as well as colonization of new species (Ghisbain et al., 2021). It is possible that a pollinator species that is shifting its distribution because of climate change takes the role of another species that has become locally extinct, especially since warming promotes more generalized pollinator foraging (de Manincor et al., 2023). In this case, we might not see any differences in plant reproduction success (*e.g.,* through seed set). Therefore, if pollinator identity is not taken into account, we might miss whether conservation measures towards any pollinator species should be taken to avoid its decline and subsequent possible extinction. In addition, knowing the interactions between plants and pollinators may be advantageous from a plant conservation point of view, since different pollinators may be affected differently by climate change (Kudo et al., 2004).

In this review, we suggested two main potential reasons for this lack of information regarding boreal pollinators and their interactions with flowering understory plants: the frequent omission of flies as important pollinators at higher latitudes, likely because they are not as charismatic as other insects, and the difficulties in recording pollinators in low temperature areas.

Despite flies being more abundant flower visitors than bees and other pollinators at higher latitudes (Elberling & Olesen, 1999) and elevations (McCabe et al., 2019), they are often neglected in pollinator studies (Orford et al., 2015). Moreover, considering that bees remain understudied in northern Europe (Leclercq et al., 2023; Marshall et al., 2024), it is to be expected that other pollinators that have historically received less attention are likely understudied as well. Flies also appear to be frequent flower visitors under cold conditions where other pollinators are inactive or have reduced activity (Kearns, 2001). The dominance of flies over bees at high elevations may be due, at least partially, to greater environmental restrictions on bees when it comes to their adaptation to colder environments (Elberling & Olesen, 1999; McCabe et al., 2019). In fact, flies seem to be more resilient to environmental constraints than bees in some cases. For instance, Kudo et al. (2004) observed that seed production did not decrease in fly-pollinated plants in an early flowering year, when it drastically decreased for bee-pollinated plants.

Another interesting group of insects that are related to flies are mosquitoes, which are highly abundant in boreal forests when snow melts and water accumulates in vernal ponds (Danks & Foottit, 1989). Mosquitoes typically feed on sugary secretions for energy, suggesting that they could also function as pollinators (Peach & Gries, 2016), and more research is needed on plant-mosquito interactions to better understand their role as potential pollinators (Peach, 2024). Some research suggests that mosquitoes and other small Diptera are nectar thieves that do not contribute to the pollination of the flowers they visit. However, being so abundant, their potentially higher flower visitation rates may result in comparatively higher pollen deposition than that of other pollinators, as happens with flies when compared to non-fly pollinators (Kearns, 2001). Moreover, even if mosquitoes and other Diptera do not contribute to pollination directly, and are instead nectar thieves, this may increase the foraging rate of the actual pollinators through nectar competition, ultimately increasing fruit set (Hunter et al., 2000).

Another possible reason for the current lack of information on boreal pollinators is the difficulty of recording insects due to their limited activity at low temperatures (Beattie, 1971). Recording pollinator diversity and interactions in these conditions may be time-consuming, as pollinators may only be available when specific environmental conditions are met (Herrera, 1995). This may be particularly challenging in heterogeneous forests, where structural complexity creates a wide arrange of microclimates (Kovács et al., 2017). Nevertheless, recent advancements in technology have introduced new approaches to overcome these challenges and record insect activity and their interactions with relatively low efforts. For instance, high resolution cameras can be used to capture images or videos of insects visiting flowers (see Paper III), offering a non-invasive and efficient way to monitor pollinator populations. Additionally, flower eDNA can be used to detect the presence of flower-visitors (see Paper IV), which may be potential pollinators. These two alternative sampling methods provide valuable insights into plant-pollinator interactions and insect diversity without the need for direct observation.

4.1.1 Climate change effects

Although the omission of pollinator identities in most studies restricts our capacity to assess the resilience of specific plant-pollinator interactions to climate change, some impacts of increasing temperatures were reported in the articles we selected for our review. Warmer temperatures and altered snowmelt patterns can disrupt the synchronized timing between flowering plants and their pollinators, leading to phenological mismatches as observed in studies such as Kudo & Cooper (2019), Kudo & Ida (2013) or Nishikawa, (2009). These mismatches can result in reduced plant reproductive success when pollinators are not available during critical flowering periods, or in low resources for pollinators if their emergence from hibernation takes place prior to flowering (Mola, Richardson, et al., 2021). Plants and pollinators may respond differently to climate change depending on their sensitivity to temperature and precipitation patterns, adaptive capacity, and the nature of their mutualistic relationships and other dependencies. When it comes to phenology, species whose environmental cues are related to temperature will be more affected by global warming than those that use other cues (*i.e.,* photoperiod) to regulate the timing of their activities. For instance, a plant that relies on photoperiod for flowering may not react strongly phenologically to an increase in temperature, while insects may alter their emergence time and activity, as temperature drives the phenology and activity of most pollinating insects (Williams, 1961). Moreover, responses to increasing temperature can be species-dependent. For instance, Rivest & Vellend (2018) observed that different plant species that rely on distinct pollinator groups respond uniquely to environmental changes. This highlights the complexity of species-specific responses to climate change, and stresses the importance of recording pollinator identity in order to be able to assess the resilience of specific interactions.

Finally, pollen limitation was documented in six out of the 11 articles in the literature review (Barrett & Helenurm, 1987; Blinova, 2002; Kudo & Cooper, 2019; Kudo & Ida, 2013; Nishikawa, 2009; Rivest & Vellend, 2018), and attributed this to reduced pollinator activity due to low temperatures, in-turn limiting seed set. However, cold temperature can constrain plant reproductive success beyond its limiting effects on pollinator activity. For instance, low temperatures can negatively affect pollen viability (Huang et al., 2022), stigma receptivity or photosynthetic activity (Banerjee & Roychoudhury, 2019), amongst other physiological processes. These factors fluctuate annually with climatic conditions, together with variation in temperature and snowmelt timing. With increasing temperatures, these cold-related issues are expected to decrease, and pollinator activity to increase, thus lessening the limitations related to plant reproductive success. However, this increase in pollinator activity may be mostly driven by thermophilic species that are able to cope with higher temperatures, and at the expense of cold-adapted pollinators that fail to do so. Therefore, our focus should be on how climate change currently affects and reshapes pollinator communities, so that we are better able to predict its impacts on specific plant-pollinator relationships. Research on multi-species plant-pollinator assemblages suggests that the overall structure of pollination networks has likely remained resilient to the extent of climatic changes experienced so far

(Hegland et al., 2009). However, studies conducted in the temperate forests of Illinois (USA) show a decline in both the structure and function of plant-pollinator networks over time (Burkle et al., 2013). This indicates that some pollination networks may already be degrading under current climatic stressors, and the resilience of other networks is likely to diminish with additional climatic pressure. In the case of the boreal biome, it seems that we are lacking the information baseline to be able to assess such impacts at a multi-species network point of view, since data on insect flower visitation at the species level is scarce.

4.2 The effect of forest structure on understory microclimate (Paper II)

In this chapter, an assessment on how forest density and overstory species composition influenced soil and air ambient temperature buffering was done. Here, "buffering" refers to forest microclimates that maintain a narrower temperature range compared to the broader macroclimate, irrespective of the season. A greater buffering capacity for maximum temperatures results in cooler forest understory temperatures relative to the macroclimate, whereas for minimum temperatures, it results in warmer forest temperatures (De Frenne et al., 2021).

According to our models, forest density was the strongest factor affecting microclimate, and interacted with the proportion of broadleaves when driving understory temperatures. The largest buffering of forest temperatures occurs with high basal area and broadleaf proportion in summer, and high basal area with low broadleaf proportion in winter (Figure 7). With increased forest density, mean air temperatures are cooler in summer and warmer in winter, with broadleaves amplifying this effect in the warmest month (Figure 7 A-B). Maximum temperatures in winter are minimally impacted by broadleaves but increase linearly with density in summer, maximizing cooling effects in dense forests (Figure 7C-D). On the other hand, for the minimum air temperatures, the proportion of broadleaves affects offsets differently by season: higher proportions enhance insulation in the warmest month but increase energy loss in the coldest month due to leaf loss. When it comes to soil temperatures, these exhibit greater sensitivity to broadleaf proportion in summer, moderating offsets, while basal area consistently influences offsets across seasons (Figure 8).

Despite our *ca*. 1,800 km macroclimatic gradient, the effects of forest density and tree species composition on understory microclimate were independent of the macroclimatic differences between sites. Therefore, our results may be relevant and applicable beyond our specific study sites. Additionally, since our primary overstory tree species (spruce and birch) were chosen to respectively represent late successional evergreen conifers and early successional broadleaves, our findings offer insights applicable to other forest systems with tree species that have similar ecological and structural characteristics.

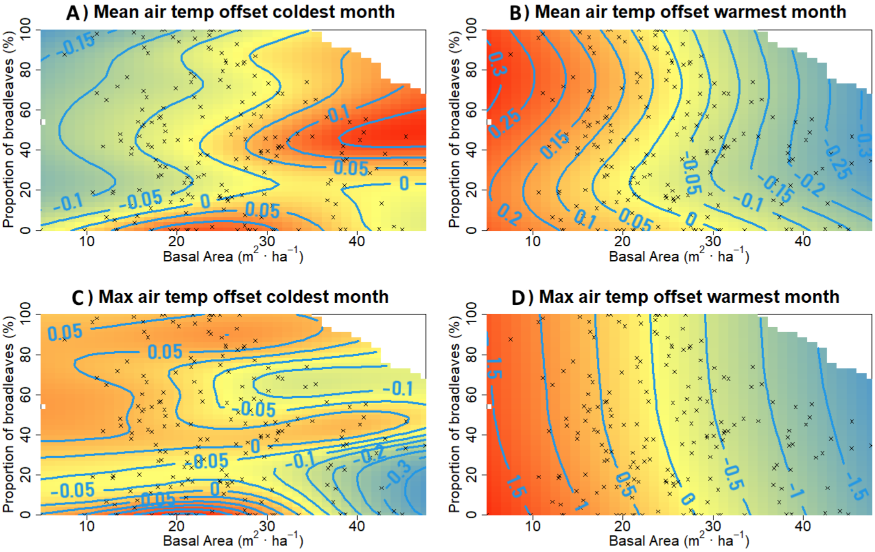


Figure 7. Model predictions for the mean and maximum air temperature offsets (forest temperatures minus weather station data) for the warmest and coldest months in our study. In these predictions, basal area was used as a proxy for forest density. The numbers on the isolines indicate the predicted offsets in degrees Celsius. The predictions are centered at the mean and the zero isoline thus depicts the mean of the model predictions. The offsets of these models were calculated from weather station macroclimatic data. The colours represent a gradient from more positive (red) to more negative (blue) offsets. Gaps (*i.e.,* white spaces) represent the model predictions that were too far from our data points.

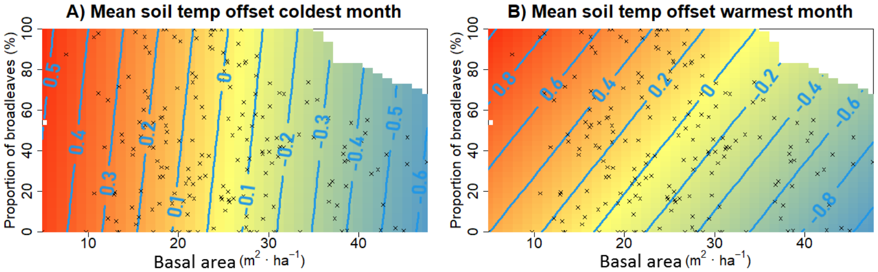


Figure 8. Model outputs for the mean soil temperature offsets for the warmest and coldest months in our study, using basal area as a proxy for forest density. The numbers on the isolines indicate the predicted offsets in degrees Celsius where the zero isoline depicts the mean of the predictions of the model. The offsets of these models were calculated from the ERA5 macroclimatic data. The colours represent a gradient from more positive (red) to more negative (blue) offsets. Gaps (*i.e.,* white spaces) represent the model predictions that were too far from our data points.

4.2.1 Managing microclimate and potential of forests as climatic refugia (Paper II)

To assess the scale at which the overstory affected air and soil temperature offsets, we calculated the minimum radial distance from a temperature logger in which all trees should be measured in order to predict temperature offsets. For air temperature, the distance from the data loggers for which basal area and the proportion of broadleaves best predicted mean air temperature in both the warmest and coldest months was close to 10 m (*i.e.,* the maximum we measured). Neither basal area nor the proportion of broadleaves was more important in determining this area of influence, as the most parsimonious models included both variables. For soil temperature, the best summer models included the proportion of broadleaves at its maximum resolution, and basal area between 7-9 m. However, winter models did not include the proportion of broadleaves, and the distance at which basal area best predicted temperature offsets was 6-8 m.

Our findings suggest that managing microclimate through forest density and broadleaf proportion adjustments could be doable for conservation and climate change adaptation strategies. For instance, our models suggested that in a northern Sweden forest with a basal area of 25 m²/ha and 50% broadleaves, increasing basal area to 40 m²/ha could lower maximum summer temperatures by 1.3°C to 2.5°C. Similar cooling effects could be achieved by replacing all trees with broadleaves under comparable basal area conditions. However, increasing forest density may reduce light availability in the understory, and despite achieving microclimate goals, it may end up negatively impacting understory plant diversity and ecosystem function. Broadleaves, on the other hand, may offer climate buffering and biodiversity benefits without compromising light availability as much as spruce (Felton et al., 2010, 2016)

However, temperature buffering may be detrimental to some thermophilic species. For instance, microclimate cooling has been identified as a threat to thermophilic butterflies (Oliver et al., 2012; Filz et al., 2013), despite macroclimate warming. It is for this reason that management decisions should be taken from a holistic perspective, considering both the benefits of temperature buffering and the potential negative effects on light-dependent and thermophilic species. Careful planning and monitoring are essential to ensure that climate-smart management practices support specific targeted species, biodiversity in general, and ecosystem health. Future research should explore these dynamics further, particularly how different forest management practices influence microclimate at varying spatial scales, and what are the implication of temperature buffering for the organisms living in the understory. Ultimately, integrating such insights into forest management plans can optimize the role of forests as climate refugia amidst changing environmental conditions.

4.3 The effect of microclimate and forest structure on pollinator activity (Paper III)

Paper III evaluated the effect of macroclimate, microclimate and forest structure on pollinator flower visitation rates and foraging duration. In total, the camera traps took 2,185,092 pictures (1,111,818 in MS and 1,073,274 in SS), representing a total of 6,070 h of sampling. Potential pollinators were found in roughly 6.5% of these pictures. Of these, 97% were flies, mostly from the Muscidae, Phoridae and Syrphidae families. As already explained in 4.1, flies are expected to dominate plant-pollinator networks at high latitudes (Elberling & Olesen, 1999), and therefore their predominance in the samples was not unexpected.

The models with the lowest AIC values for hoverflies (Syrphidae), non-syrphid flies, and *Episyrphus balteatus* included microclimate in the continuous component of the model and macroclimate in the binomial one, indicating that macroclimate best predicted fly presence (or, rather: [artificial] flower visitation rate), while microclimate was the best predictor of their foraging duration (Figure 9). Flies are mostly ectothermic, and thus rely heavily on external temperatures to regulate their activity (Heinrich, 1973). Higher macroclimatic temperatures increased the likelihood of flower visitation, having a greater influence than any other variable and suggesting that rising temperatures due to climate change will affect flower visitation rates by insects more than changes in other macroclimatic variable such as the rain regime. According to the predicted responses of our models, the probability of a flower visit increased steeply between *ca*. 10°C - 15°C, with no or little change at higher temperatures (Figure 10). These responses were similar for all groups of flies, but especially between Syrphidae and *E. balteatus*. On the other hand, higher microclimatic temperatures decreased foraging duration in Syrphidae and *E. balteatus*, but lengthened it in non-syrphid flies. Although temperature plays a crucial role in insect flight, foraging behaviour may likely involve a balance of costs and benefits rather than strict adherence to absolute temperature thresholds. For instance, pollinators may need to gather more energy when temperatures are low, prompting them to forage for a longer duration. Microclimate temperature varies largely in space and time, and changes on small scales depending on factors such as direct sunlight or shade (Kovács et al., 2017). It is possible that the effect of this small-scale variation on an insect's thermics may be smaller while it flies through a forest than after landing in a certain place, which may explain the discrepancy between the effects of macro- versus microclimate.

Considering the strong response of pollinators to macroclimatic temperatures in our study, we expect changes in flower visitation rates under climate change to be significant, potentially increasing pollination rates. A rise in macroclimatic temperatures will also likely cause a rise in microclimatic temperature inside forests, which may reduce the foraging time of some groups of pollinating flies, while lengthening it in others. These changes in foraging duration may boost pollination rates, as more flowers are being visited per unit of time. However, shorter flower visits caused by increases in microclimatic temperatures may lead to smaller pollen loads, since foraging time on flowers is associated with the pollen loads carried by pollinating insects (Baur et al., 2019). This may potentially impact pollination negatively. Moreover, prolonged exposure to higher temperatures might exceed optimal thermal limits of insects, likely reducing visitation rates over time, provided that such temperature increases do not lead to a phenological mismatch.

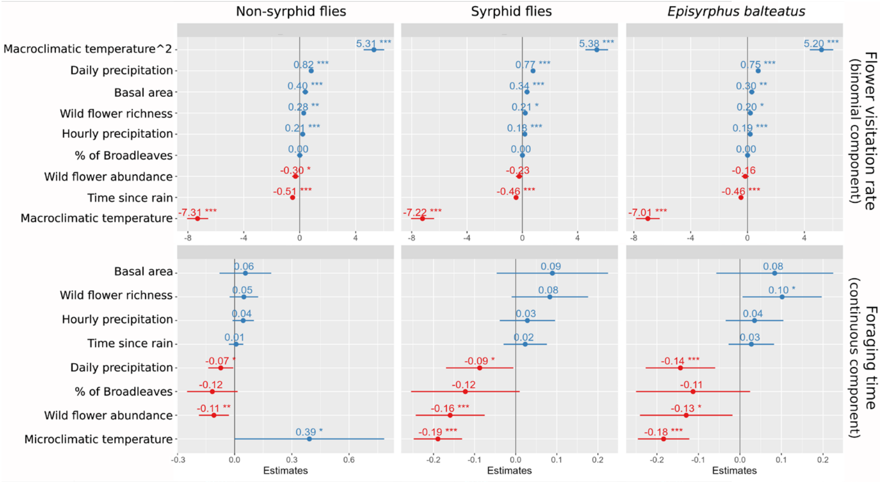


Figure 9. Standardized estimates of the fixed effects of the selected GLMMs for non-syrphid flies (left), syrphid flies (center) and *Episyrphus balteatus* (right). Blue estimates are positive and red estimates are negative. Note that the estimated model coefficients of the binomial part of the models indicate the probability of a zero-observation and thus a negative estimate should be interpreted as a positive effect of a specific variable on the occurrence, and vice versa. Lines departing from each point correspond to one standard error. Asterisks represent statistical significance (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$.

Forest structure also influenced pollinator foraging activity (Figure 9). Forest density negatively impacted fly visitation rates regardless of the proxy used for forest density (*e.g.,* basal area or canopy openness). Forest patches with a high canopy openness have been related to higher pollinator activity and flower abundance than denser forest patches (Eckerter et al., 2019), presumably due to increased light availability. This suggests that some management practices that increase canopy openness could enhance pollinator habitats, which may be especially relevant for maintaining or increasing biodiversity in managed forests. Mixed-species forests and broadleaf dominance also positively influence pollinator communities (Knuff et al., 2020; Traylor et al., 2024). In this study, tree species composition did not have an effect on pollinator activity, although a trend could be observed where the proportion of broadleaves negatively influenced fly foraging duration across the different groups.

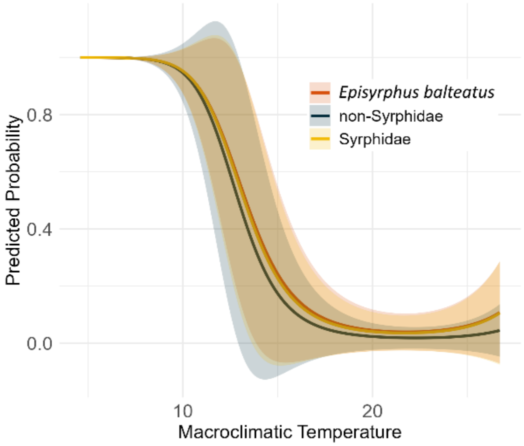


Figure 10. Predicted flower visitation rates from the selected models for non-Syrphidae, Syrphidae and *Episyrphus balteatus* against macroclimatic temperatures. Note that the model predictions indicate the probability of a zero-observation and thus a probability of 1 (=100%) should be interpreted as the total absence of pollinators visiting the artificial flowers, and vice-versa.

Increased wildflower species richness decreased pollinator visitation rates to artificial flowers, and higher wildflower abundance reduced the foraging time of all groups of flies (Figure 9). This suggests that abundant natural floral resources can divert pollinators away from artificial flowers, likely because wildflowers are more attractive or rewarding (Fowler et al., 2016). Moreover, pollinators prefer visiting large patches of wildflowers (Santos et al., 2021) but spend less time there, as finding unvisited flowers is easier in smaller patches (Goulson, 2000).

Precipitation disrupted pollinator foraging activity (Figure 9), possibly by lowering insect activity and affecting sensory mechanisms related to flower attraction. Rain dilutes nectar and degrades pollen, reducing flower attractiveness (Lawson & Rands, 2019). In this study, rain decreased both artificial flower visitation rates and foraging duration of all flies, but post-rain periods increased flower visitation rates.

Finally, it was found that the artificial plastic flower design from Thomson et al. (2012) is effective for use outside greenhouses and suitable for studying a broader range of pollinators, particularly flies. However, most insects visiting the artificial flowers did not appear interested in the sucrose solution, often staying on the lid without approaching the fake nectaries. This could be due to the rainy experimental period causing the sucrose solution to overflow and leave an attractive coating on the lid, or because insects were using the lid's reflective surface for thermoregulation. The reasoning for the use of sucrose was to keep insects busy within the camera's frame during the time needed for the time-lapse camera to take a picture. Some automated monitoring systems use UV-colored platforms to attract insects without a reward solution (Sittinger et al., 2024), indicating that color alone can attract insects. However, these systems typically monitor their targets continuously and need constant power, which was not feasible for our setup. Further exploration is needed to understand the advantages and disadvantages of different approaches to using artificial flowers outdoors.

In conclusion, the findings of this study reveal that macroclimatic temperature is the primary factor influencing flower visitation rates by pollinators, with a much stronger impact than precipitation. As climate change leads to rising temperatures, significant shifts in pollinator behaviour are expected. Although increased temperatures may extend or reduce the foraging times of different pollinators, potentially boosting pollination rates, shorter flower visits could decrease pollen loads, negatively affecting pollination. Forests may act as climatic refugia, buffering some of the temperature increases and mitigating climate change impacts on pollination. The study also underscores that despite the often underappreciated role of flies in pollination, they are one of the most abundant pollinator taxa in northern forests, and therefore we call for a broader inclusion of these often-overlooked insects in pollination research. However, since our study used artificial flowers, it is important that future research explore whether patterns in insect visitation differ when real flowers are used. The negative impact of dense forests on pollinator presence suggests that some management practices that increase canopy openness could enhance pollinator habitats.

This is especially relevant for maintaining or increasing biodiversity in managed forests. Additionally, understanding species-specific responses to forest composition and understory vegetation can guide the selection of tree species in reforestation or afforestation projects to support diverse pollinator communities. This holistic understanding of pollinator behaviour and ecology can inform more effective conservation strategies and forest management practices aimed at maintaining healthy pollinator populations and ecosystem resilience under a changing climate.

4.4 The effect of microclimate and forest structure on flower-visiting arthropods assessed through flower eDNA (Paper IV)

Paper IV assessed the effect of microclimate and forest density and overstory species composition on arthropod flower-visitors detected through flower eDNA. For this, 160 plants of wild strawberry (*F. vesca*) and red clover (*T. pratense*) were grown in a greenhouse, and translocated to a forest in southern Sweden once they were flowering.

The study demonstrated that a high diversity of flower-visiting arthropods occurs in this forest, and that many of these insects were recorded only once, resulting in highly specific communities in different samples. A total of 92 arthropod taxa were identified through flower eDNA: 34 in *F. vesca*, and 64 in *T. pratense*. Of these, only six taxa were shared between the two plant species. Of the 40 plots sampled, 23 yielded amplifiable eDNA in *F. vesca*, and 28 in *T. pratense*. The maximum number of taxa per plot was nine in *T. pratense* and six in *F. vesca*. Notably, 12 *F. vesca* plots contained only a single taxon, as did seven *T. pratense* plots. The mean number of taxa per plot was 1.025 for *F. vesca* and 2.05 for *T. pratense*. Of the 49 flowers that were sequenced individually for *F. vesca*, only 10 amplified arthropod DNA, and of the 42 individual flowers from *T. pratense*, 25 amplified DNA. Nevertheless, the number of taxa detected in our rather simplified system was relatively high compared to the 216 taxa found in a Danish grassland with many more plant species, following the same sampling procedure (Thomsen & Sigsgaard, 2019). Based on the asymptotic diversity estimates of single flowers, the expected number of flower-visiting taxa in *F. vesca* and in *T. pratense* across all plots was 24.40±15.39 and 341±154.38, respectively. Specifically, in the most open plots, 24.50±10.44 species were expected for *F. vesca*, and 67±27.22 for *T. pratense*, whilst in the densest plots, 12.5±5.88 species were expected for *F. vesca* and 140.26±63.44 for *T. pratense*.

The reasons behind the high turnover of flower visitor species across plots remain unclear. One possibility is that in a large and evenly distributed community of flower visitors, significant differences in species detected through flower eDNA could arise simply due to stochastic processes. Alternatively, it could be that eDNA does not accumulate on flower heads, indicating a short residence time for genetic material. An ecological explanation might be that these flower visitor communities are structured by factors we failed to measure. Another possibility is that the environment is so inhospitable or resource-poor that foraging for food becomes unviable, which could also explain our observations of zero flower visitors. The minimal overlap in flower-visiting species between *F. vesca* and *T. pratense* samples might result from the turnover of potential pollinators captured at different sampling times for these species.

Forest structure significantly affects the species richness of flower-visiting arthropods, while microclimate seems to play a minimal role. Open plots and broadleaf dominance were favorable for the number of taxa in *F. vesca*, while *T. pratense* did not show any clear patterns. One advantage of using eDNA is its ability to detect species interactions that may be overlooked by traditional methods, such as identifying nocturnal pollinators. Moths, which are key nocturnal pollinators, contribute to the seed set of *T. pratense* (Alison et al., 2022) and were more frequently observed on *T. pratense* flowers compared to those of *F. vesca* (see Table S1 in Appendix). The vertical structure of forests influences moth communities (De Smedt et al., 2019), which could explain the expectation of higher flower visitor richness in denser plots for *T. pratense*. It is possible that the balance between diurnal (more common in open plots) and nocturnal (potentially more common in denser plots) flower visitors confounded the impact of forest structure on the observed diversity of flower visitors for *T. pratense*.

Given our low species detectability in the eDNA samples, it could be that other ecological patterns might arise if more samples, PCR replicates or molecular markers were added to the experiment. In fact, it seems that some pollinator groups, such as Hymenoptera do not amplify optimally with COI primers, as they do with 16S (Thomsen & Sigsgaard, 2019), thus introducing some bias to PCR amplification (Clarke et al., 2014). Similarly, we only used the average, minimum or maximum microclimatic temperature values across the experimental period as predictors. Perhaps, increasing the resolution of microclimatic data and matching it with fine-scale flower-visitor data would shed different results for this predictor.

The difference in the number of taxa detected between flower species, with *T. pratense* attracting almost twice as many taxa as *F. vesca* might be due to the different sampling times between plant species, the different levels of attractiveness to pollinators, or the fact that flower heads were used in *T. prantense* instead of single flowers as in *F. vesca*. However, one may speculate that the flower structure of *T. pratense*, which forces a more intimate contact with pollinators, may result in more eDNA left behind. Additionally, this flower shape may also potentially prevent DNA degradation to some degree, as DNA inside flowers is less exposed to environmental degrading factors such as UV radiation. Future research should explore how flower shape affects the persistence of eDNA and its implications for biodiversity monitoring.

Amongst the other methodological insights obtained through this work, we show that the number of flowers pooled before DNA extraction positively influences the number of arthropod species detected, both in *T. pratense* and *F. vesca*. On the other hand, sequencing flowers separately and pooling them after seems to be an even better approach to detecting more diversity, although there is also an increased risk of not amplifying any eDNA from some samples, presumably due to lower eDNA concentrations. Increasing the number of samples from where the eDNA will be extracted typically yields higher amounts of genetic material (Hunter et al., 2019). By extracting and sequencing samples separately, each sample is processed in an independent PCR; hence the DNA is analyzed as many times as the number of samples, compared to a single pooled sample. Moreover, different species may amplify in different samples, while in pooled samples one species may dominate in abundance regardless of the number of PCRs conducted.

5. Conclusions of each paper

(I) The effect of climate change on boreal pollinators and their interactions have so far been largely neglected by science. Most research to date uses plant reproductive success as a proxy for pollinator effectiveness, which is blind to any potential changes in pollinator community composition that could be crucial for predicting the impacts of climate change. Moreover, despite the high potential relevance of flies to successful pollination in northern latitudes, there is limited knowledge about the role of flies in the pollination of boreal plants.

(II) Both forest density and overstory species composition play a role in determining understory microclimatic buffering. These two variables interact to drive understory temperatures. However, forest density is the main driver of microclimate, both in the warmest and coldest periods, and for air and soil temperature. Therefore, the same buffering capacity obtained through conifers can be obtained through broadleaved species, although the effectiveness in buffering temperatures of the latter is diminished in winter due to leaf loss. Forest overstory affects soil microclimatic temperature at a scale of around 6-7 m, and air temperature at a scale of at least 10 m.

(**III**) Macroclimate is the primary factor influencing pollinator foraging rate, whilst microclimate explained foraging time the best. As climate change leads to rising temperatures, significant shifts in pollinator activity are expected. Dense forests negatively affected pollinator visitation, and high wildflower abundance boosted pollinator presence but reduced foraging time. The increasing proportion of broadleaves reduced hoverfly foraging duration. Precipitation diminished pollinator activity, although post-rain periods increased flower visitation.

(IV) Forest structure, particularly forest density and the proportion of broadleaves, positively affected the species richness of flower visitors in *F. vesca*, while no significant effects were observed for *T. pratense*. The abundance of naturally occurring flowers negatively affected species detection in *F. vesca*. Microclimate had no significant effect on arthropod richness. Pooling flowers before DNA extraction and sequencing flowers separately both increased species detection, although the latter had a higher risk of failing to amplify DNA.

6. Implications for forest management and future research needs

The overall goal of this thesis was to address some of the knowledge gaps between climate, forest structure and boreal pollinators in order to develop effective forest management strategies that support biodiversity and ecosystem services in the face of ongoing climate change. The results of this thesis show that the tree layer plays a fundamental role in buffering the microclimate temperatures inside the forest, potentially mitigating some of the negative effects of climate change. These benefits can be equally achieved by both conifers and broadleaves (**II**). This aligns with current management strategies that focus on diversifying silviculture through the replacement of conifer monocultures with broadleaved tree species in order to adapt forests to climate change (Löf et al., 2012) and biodiversity conservation (Felton et al., 2010). Broadleaved trees allow more light in the forest understory than conifers, which benefits understory vegetation (Felton et al., 2016), flower abundance (Eckerter et al., 2019), and ultimately, pollinator activity (**III**) and diversity (**IV**). Our estimation of the scale at which the overstory affects understory temperatures (**II**) offer valuable guidance for climate-smart forest management. Specifically, to regulate understory soil microclimates, the overstory should be managed within a radius of approximately 6-7 meters around the targeted area. In contrast, to impact understory air temperature, management should extend to a scale of at least 10 meters. Nevertheless, in addition to microclimatic considerations, different management strategies should be developed separately for conifer and broadleaf forests to support pollinators, given the distinct ecological characteristics of these forest types (Ulyshen et al., 2024). In conifer forests, the dense canopy and lower light penetration typically result in fewer flowering plants, which are essential for pollinators. Therefore, managing these forests to create gaps or increase the diversity of understory vegetation can provide more resources for pollinators. On the other hand, broadleaf forests often have a richer diversity of flowering plants and a more open canopy, making them naturally more beneficial to supporting pollinators.

Forest density is also an important driver of microclimate that likewise affects light availability in the understory. Increasing forest density improves microclimate temperature buffering (**II**), however, it reduces pollinator activity (**III**) and diversity (**IV**). Therefore, management strategies that aim at reducing basal area might benefit pollinators (Hanula et al., 2016), as these increase canopy openness and light availability (Tsai et al., 2018). Such measures could improve understory plant reproduction and berry yields, providing a cascade of benefits for biodiversity and the organisms that rely on these resources for food (Eckerter et al., 2019). However, as reducing basal area reduces the temperature buffering capacities of forests, perhaps the best scenario would be to increase spatial heterogeneity in forest structure, as this not only increases light availability, but also microclimate variability (Kovács et al., 2017; Menge et al., 2023). Future studies should explore the microclimatic implications of forest management decisions that aim to increase canopy openness in favour of pollinators. Additionally, these studies should also consider the trade-offs between promoting pollinator habitats by reducing forest density and other forest-related services such as wood production.

Flower visitation rates of forest pollinators depend largely on macroclimate, whilst microclimate influences how long insects forage on flowers (**III**). As insect flower visitation rates respond very steeply to a rather narrow macroclimatic temperature window (**III**), we expect significant changes in pollinator activity with rising temperatures, at least within a specific temperature range. Whether this will translate to higher or lower pollination rates needs to be further explored.

In addition to microclimatic effects, managing the forest overstory can also affect pollinators through the vegetation that is able to grow and flower in the understory, as well as by affecting how attractive this vegetation is to pollinators through visibility and other sensory cues (Figure 1). For example, when the overstory allows abundant understory flowers, pollinators are likely to spend less time on individual flowers, resulting in increased visitation rates (**III**) and potentially higher levels of pollination. Therefore, forest management not only needs to be climate-smart, but it also needs to consider the impact of decisions on understory vegetation, seeking to achieve microclimatic goals while also preserving biodiversity.

To ensure that management decisions that mitigate the impacts of climate change have a positive impact on pollinators, effective monitoring should be implemented. So far, research has overlooked many potential changes in boreal pollinator communities caused by climate change (**I**). Without such data, it is difficult to predict the impacts of climate change on specific plant-pollinator interactions, as well as to evaluate the benefits of climate-smart forestry on pollinator health. Some of the potential reasons for this lack of data include insufficient ecological knowledge about boreal pollinators, as well as the difficulty of recording them under cool weather conditions. In this thesis, we used two novel methodologies to record forest pollinators, overcoming these shortfalls: camera traps with image recognition (**III**) and flower eDNA metabarcoding (**IV**). Both approaches were successful, providing high-resolution data on flower visits (**III**) and uncovering a diverse range of flower visitors that would likely have been overlooked using traditional surveying techniques (**IV**). Although these methodologies should be further refined, we encourage their use to record plant-pollinator interactions and monitor pollinator populations in boreal forests over time. Establishing a baseline of information will help better predict the impacts of climate change on boreal pollinators. Additionally, it is also important to consider species-level responses to warming, as this information can be used to target management decisions to the conservation of particular species.

In conclusion, this thesis highlights the complex connections between climate, forest structure, and boreal pollinators. The findings underscore the critical role of the overstory in regulating understory microclimate temperatures, benefiting both vegetation and its pollinators. As forest management evolves, it will be essential to balance tree density and composition to create more heterogeneous forest structures that support pollinators while maintaining temperature buffering. By implementing innovative methodologies for monitoring pollinator populations and interactions, we can build a robust baseline of ecological knowledge that informs future conservation efforts. Ultimately, fostering resilient boreal ecosystems requires a proactive approach to management that prioritizes the needs of pollinators, ensuring their survival and the continued provision of the ecosystem services they provide in the face of current and future climate change.

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Popular science summary

The boreal biome, the world's largest contiguous forested region, is experiencing significant changes due to rising global temperatures. This warming is affecting the way trees, understory plants, and pollinators interact. These interactions are crucial for providing some of the benefits we humans get from such forests, like berry production or pest control. In science, we call these benefits *ecosystem services*.

Understanding how climate and trees interact is crucial for maintaining healthy ecosystems, especially in how these habitats support various plant and animal species. Moreover, as we humans use forests for wood production, we also need to consider how our management decisions affect the plants and animals that live there. However, predicting how shifts in climate and land use will impact these systems is challenging due to gaps in our current knowledge.

Addressing these gaps is crucial for developing forest management strategies that support biodiversity and ecosystem services in the face of climate change, as well as for the conservation of species affected by warming. The overall goal of this thesis is to provide some of the necessary information to tackle these challenges and inform management practices. Each chapter of the thesis focuses on different aspects of this complex relationship, aiming to shed light on these important issues and help create strategies that enhance the ability of both forests and their pollinators to bounce back from disturbances and keep on thriving.

Now, imagine that you are enjoying a day in the forest. The sun is shining, birds are chirping, and there is a gentle breeze rustling the leaves. You take a deep breath of fresh air, admire the blooming flowers that will become berries in a few weeks, and maybe even spot a moose in the distance. It is a beautiful scene, but let's increase the temperature a bit. The sun is beating down, and you start sweating. So, you would probably do what many other animals would do: find a nice spot in the shade under a tree to keep on enjoying your day. Even though you have only moved a few meters, the temperature changes considerably. This is what we refer to as *microclimate*. A microclimate is essentially a local zone where the climate differs from the surrounding area. These differences can be influenced by various factors, such as the density and the types of trees in a forest.

Forests, microclimate, understory vegetation and pollinators are interconnected in complex ways. Most pollinators are insects, and just like humans, they need to regulate their body temperature to function properly. However, as insects cannot produce their own heat, they rely on external sources of temperature. Therefore, as forests can shape the microclimate in the understory, forests will also determine how many and which insect pollinators we can find, as well as how insects move to find and collect food. Furthermore, as pollinators feed on pollen and nectar in flowers, the interaction between forest and microclimate will also determine the level of pollination of understory plants, and how many fruits (including berries!) and seeds are produced. In this regard, we found that ambient temperatures are in charge of regulating pollinator foraging activities, while the local microclimate determines how much time they spend on flowers.

Forests are therefore essential in moderating ambient temperatures and creating a wide array of microclimates, which, in some cases, can serve as *climatic refugia* for species affected by warming. Climatic refugia are places where the local climate remains relatively stable and favorable even when surrounding areas undergo significant climatic changes, such as during droughts, or extreme heat. With the work of this thesis, we noticed that denser forests are particularly effective at buffering ambient temperatures, and that both broadleaf and coniferous trees offer the same benefits. Broadleaf trees, in particular, besides achieving the same level of temperature regulation as conifers, let more light reach the forest understory, which is beneficial for many plants and associated organisms. However, the ideal forest for pollinators may be one in which there are different types of trees and shrubs, which allow for open areas where pollinators can bask and get warm, areas with flowers, and shaded areas where they can rest and take shelter from rain or if temperatures rise too much. Interestingly, we also identified that the number of insect species that visit flowers tends to be higher in forests with more diverse structures, especially those where broadleaf trees are more abundant, while the microclimate itself has a lesser impact on the variety of these insects.

Sadly, despite the promising potential of forests as climatic refugia, we found that many of the assessments of climate change impacts on boreal pollinators that have been done so far do not measure pollinators directly. Instead, they use indirect measures such as whether plants are being effectively pollinated or not, overlooking specific pollinator species or how many pollinators are needed for plants to produce seeds. This lack of detail limits our understanding of how particular species might respond to climate change, and therefore, limits our understanding of what to do to avoid their possible decline.

By managing forests with their microclimates in mind, and considering how both plants and pollinators use these areas, we can create better habitats for pollinators and help them adapt to climate change. However, we need more research to refine these management strategies and to understand how different species respond to changing environmental conditions.

Populärvetenskaplig sammanfattning

Det boreala biomet, världens största sammanhängande skogsregion, genomgår betydande förändringar på grund av stigande globala temperaturer. Uppvärmningen påverkar samspelet mellan träd, markvegetation och pollinatörer. Dessa interaktioner är avgörande för att tillhandahålla några av de fördelar som vi människor får från våra skogar, som bärproduktion eller kontroll av skadedjur. Inom vetenskapen kallar vi dessa fördelar för ekosystemtjänster.

Att förstå hur klimat och träd samverkar är avgörande för att upprätthålla hälsosamma ekosystem, särskilt när det gäller hur dessa fungerar som livsmiljöer för olika växt- och djurarter. Eftersom vi människor använder skogar för träproduktion måste vi också ta hänsyn till hur våra förvaltningsbeslut påverkar de växter och djur som lever där. Det är dock svårt att förutse hur förändringar i klimat och markanvändning kommer att påverka dessa system, eftersom det finns luckor i vår nuvarande kunskap.

Att åtgärda dessa luckor är avgörande för att utveckla skogsskötselstrategier som stöder biologisk mångfald och ekosystemtjänster inför klimatförändringarna, liksom för att bevara arter som påverkas av uppvärmningen. Det övergripande målet med denna avhandling är att tillhandahålla en del av den information som behövs för att ta itu med dessa utmaningar och förbättra skötselmetoder. Varje kapitel i avhandlingen fokuserar på olika aspekter av detta komplexa förhållande och syftar till att belysa dessa viktiga frågor och bidra till att skapa strategier som förbättrar både skogarnas och deras pollinatörers förmåga att återhämta sig från störningar och fortsätta att frodas.

Föreställ dig nu att du njuter av en dag i skogen. Solen skiner, fåglarna kvittrar och en svag bris prasslar i löven. Du tar ett djupt andetag av frisk luft, beundrar blommorna som kommer att bli bär om några veckor och kanske till och med ser en älg i fjärran. Det är en vacker scen, men låt oss höja temperaturen en aning. Solen gassar och du börjar svettas. Så du skulle förmodligen göra som många andra djur: hitta en skön plats i skuggan under ett träd för att fortsätta njuta av dagen. Trots att du bara har förflyttat dig några meter ändras temperaturen avsevärt. Det är detta som vi kallar mikroklimat. Ett mikroklimat är i princip en lokal zon där klimatet skiljer sig från det omgivande området. Dessa skillnader kan påverkas av olika faktorer, t.ex. tätheten och typen av träd i en skog.

Skogar, mikroklimat, markvegetation och pollinatörer är sammankopplade på ett komplext sätt. De flesta pollinatörer är insekter, och precis som människor måste de reglera sin kroppstemperatur för att fungera ordentligt. Men eftersom insekter inte kan producera sin egen värme är de beroende av externa temperaturkällor. Eftersom skogarna kan forma mikroklimatet i undervegetationen kommer skogarna därför också att avgöra hur många och vilka pollinerande insekter vi kan hitta, liksom hur insekterna förflyttar sig för att hitta och samla in mat. Eftersom pollinerare livnär sig på pollen och nektar i blommor kommer samspelet mellan skog och mikroklimat också att avgöra pollineringsnivån hos växter i undervegetationen och hur många frukter (inklusive bär!) och frön som produceras. I detta avseende fann vi att den storskaliga temperaturen är ansvarig för att reglera pollinatörernas födosöksaktiviteter, medan det lokala mikroklimatet avgör hur mycket tid de tillbringar på blommorna.

Skogar är därför viktiga för att dämpa omgivande temperaturer och skapa ett brett utbud av mikroklimat, som i vissa fall kan fungera som klimatrefugier för arter som påverkas av uppvärmningen. Klimatrefugier är platser där det lokala klimatet förblir relativt stabilt och gynnsamt även när omgivande områden genomgår betydande klimatförändringar, till exempel under torka eller extrem värme. I arbetet med denna avhandling har vi lagt märke till att tätare skogar är särskilt effektiva när det gäller att buffra omgivande temperaturer, och att både lövträd och barrträd erbjuder samma fördelar. Särskilt lövträd, förutom att de uppnår samma nivå av temperaturreglering som barrträd, låter mer ljus nå skogens undervegetation, vilket är fördelaktigt för många växter och tillhörande organismer. Den ideala skogen för pollinatörer kan dock vara en skog där det finns olika typer av träd och buskar, som möjliggör öppna områden där pollinatörer kan sola och värma sig, områden med blommor och skuggade områden där de kan vila och ta skydd mot regn eller om temperaturen stiger för mycket. Intressant nog identifierade vi också att antalet insektsarter som besöker blommor tenderar att vara högre i skogar med mer varierande strukturer, särskilt i skogar där det finns fler lövträd, medan mikroklimatet i sig har en mindre inverkan på mångfalden av insekter.

Trots skogarnas lovande potential som klimatrefugier fann vi tyvärr att många av de utvärderingar av klimatförändringarnas effekter på pollinatörer i boreala områden som hittills har gjorts inte artbestämmer pollinatörer. Istället använder de indirekta mått som huruvida växter pollineras effektivt eller inte, vilket förbiser specifika arter av pollinatörer och hur många pollinatörer som behövs för att växter ska producera frön. Denna brist på detaljkunskap begränsar vår förståelse för hur vissa arter kan komma att reagera på klimatförändringarna, och därmed också vår förståelse för vad vi ska göra för att undvika att de minskar.

Genom att sköta skogarna med mikroklimatet i åtanke och ta hänsyn till hur både växter och pollinatörer använder dessa områden kan vi skapa bättre livsmiljöer för pollinatörer och hjälpa dem att anpassa sig till klimatförändringarna. Vi behöver dock mer forskning för att förfina dessa förvaltningsstrategier och för att förstå hur olika arter reagerar på förändrade miljöförhållanden.

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I guess time flies when you are having fun. I sometimes forget that I started this thesis in the middle of a pandemic, how tricky it was to travel in between countries, and how I went directly from the airport to the forest to do fieldwork. Over these last four years, I have challenged myself a lot, learnt a lot, and driven a lot of kilometres in between forest stands.

I would like to start expressing how grateful I am to my main supervisor, Per-Ola Hedwall. Before starting the PhD, I was a little bit scared. One always hears stories about evil supervisors and the importance of choosing a topic you really like. Well, I have been lucky with both. PO, thanks for all your patience, your humour and your great support throughout this journey. I couldn't have asked for a better supervisor.

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Appendix

Table S1. List of arthropod flower visitors identified through flower eDNA. The columns *Trifolium* and *Fragaria* show whether a flower visitor was present (1) or absent (0) from the samples of each respective plant species.

Class	**Order**	**Family**	**Genus**	**Species**	***Trifolium***	***Fragaria***
Arachnida	Araneae	Clubionidae	*Clubiona*	*Clubiona comta*	1	0
Arachnida	Araneae	Salticidae	*Neon*	*Neon reticulatus*	1	0
Arachnida	Sarcoptiformes	Chamobatidae	*Chamobates*	*Chamobates borealis*	1	0
Arachnida	Sarcoptiformes	Chamobatidae	*Chamobates*	*Chamobates birulai*	1	0
Arachnida	Sarcoptiformes	Oppiidae	*Oppiella*	*Oppiella nova*	1	0
Arachnida	Trombidiformes	Anystidae	*Anystis*	*Anystis* sp.	1	0
Arachnida	Trombidiformes	Triophtydeidae	*Triophtydeus*	*Triophtydeus* sp.	1	0
Collembola	Entomobryomorpha	Entomobryidae	*Entomobrya*	*Entomobrya nivalis*	1	0
Insecta	Coleoptera	Brentidae	*Protapion*	*Protapion fulvipes*	1	0
Insecta	Coleoptera	Byturidae	*Byturus*	*Byturus* sp.	1	0
Insecta	Coleoptera	Carabidae	*Pterostichus*	*Pterostichus oblongopunctatus*	1	0
Insecta	Coleoptera	Carabidae	*Amara*	*Amara consularis*	1	0
Insecta	Coleoptera	Chrysomelidae	*Plagiodera*	*Plagiodera versicolora*	0	1
Insecta	Coleoptera	Chrysomelidae	*Altica*	*Altica ampelophaga*	0	1
Insecta	Coleoptera	Chrysomelidae	*Altica*	*Altica engstromi*	0	1
Insecta	Coleoptera	Cryptophagidae	*Micrambe*	*Micrambe abietis*	1	0
Insecta	Coleoptera	Curculionidae	*Tychius*	*Tychius picirostris*	1	0
Insecta	Coleoptera	Curculionidae	*Sitona*	*Sitona lineatus*	1	0
Insecta	Coleoptera	Curculionidae	*Pityogenes*	*Pityogenes chalcographus*	1	0
Insecta	Coleoptera	Melyridae	*Dasytes*	*Dasytes cyaneus*	1	1
Insecta	Coleoptera	Nitidulidae	*Meligethes*	*Meligethes denticulatus*	1	0

Insecta	Coleoptera	Nitidulidae	*Lamiogethes*	*Lamiogethes pedicularius*	0	1
Insecta	Coleoptera	Tenebrionidae	*Lagria*	*Lagria hirta*	1	0
Insecta	Coleoptera	Tenebrionidae	*Lagria*	*Lagria* sp.	1	0
Insecta	Diptera	Agromyzidae	*Liriomyza*	*Liriomyza* sp.	1	0
Insecta	Diptera	Asilidae	*Eudioctria*	*Eudioctria propinqua*	1	0
Insecta	Diptera	Brachystomatidae	*Trichopeza*	*Trichopeza longicornis*	0	1
Insecta	Diptera	Cecidomyiidae	*Massalongia*	*Massalongia* sp.	1	0
Insecta	Diptera	Cecidomyiidae	*Asynapta*	*Asynapta strobi*	1	0
Insecta	Diptera	Cecidomyiidae	*Contarinia*	*Contarinia* sp.	0	1
Insecta	Diptera	Cecidomyiidae	*Aphidoletes*	*Aphidoletes* sp.	0	1
Insecta	Diptera	Ceratopogonidae	*Atrichopogon*	*Atrichopogon brunnipes*	1	0
Insecta	Diptera	Ceratopogonidae	*Culicoides*	*Culicoides scoticus*	0	1
Insecta	Diptera	Chironomidae	*Halocladius*	*Halocladius varians*	1	0
Insecta	Diptera	Chironomidae	*Limnophyes*	*Limnophyes habilis*	1	1
Insecta	Diptera	Chironomidae	*Bryophaenocladius*	*Bryophaenocladius* sp.	1	0
Insecta	Diptera	Chironomidae	*Thienemannimyia*	*Thienemannimyia carnea*	1	0
Insecta	Diptera	Chironomidae	*Cricotopus*	*Cricotopus vierriensis*	0	1
Insecta	Diptera	Chironomidae	*Pseudosmittia*	*Pseudosmittia* sp.	0	1
Insecta	Diptera	Chironomidae	*Pseudosmittia*	*Pseudosmittia albipennis*	0	1
Insecta	Diptera	Chloropidae	*Dasyopa*	*Dasyopa triangulata*	1	0
Insecta	Diptera	Chloropidae	*Conioscinella*	*Conioscinella frontella*	0	1
Insecta	Diptera	Culicidae	*Aedes*	*Aedes communis*	1	0
Insecta	Diptera	Dolichopodidae	*Medetera*	*Medetera* sp.	0	1
Insecta	Diptera	Empididae	*Rhamphomyia*	*Rhamphomyia longipes*	0	1
Insecta	Diptera	Heleomyzidae	*Suillia*	*Suillia convergens*	1	0
Insecta	Diptera	Hybotidae	*Hybos*	*Hybos culiciformis*	1	0
Insecta	Diptera	Lauxaniidae	*Lauxania*	*Lauxania cylindricornis*	0	1

Insecta	Diptera	Limoniidae	*Dicranomyia*	*Dicranomyia* sp.	0	1
Insecta	Diptera	Limoniidae	*Molophilus*	*Molophilus bihamatus*	0	1
Insecta	Diptera	Lonchaeidae	*Priscoearomyia*	*Priscoearomyia withersi*	0	1
Insecta	Diptera	Muscidae	*Phaonia*	*Phaonia angelicae*	1	0
Insecta	Diptera	Muscidae	*Polietes*	*Polietes lardarius*	1	0
Insecta	Diptera	Rhagionidae	*Rhagio*	*Rhagio scolopaceus*	0	1
Insecta	Diptera	Sciaridae	*Scatopsciara*	*Scatopsciara atomaria*	1	0
Insecta	Diptera	Sciaridae	*Bradysia*	*Bradysia impatiens*	1	0
Insecta	Diptera	Sciaridae	*Ctenosciara*	*Ctenosciara alexanderkoenigi*	0	1
Insecta	Diptera	Tabanidae	*Tabanus*	*Tabanus maculicornis*	0	1
Insecta	Diptera	Tipulidae	*Tipula*	*Tipula scripta*	0	1
Insecta	Ephemeroptera	Baetidae	*Procloeon*	*Procloeon* sp.	0	1
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis ruborum*	1	1
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis fabae*	1	0
Insecta	Hemiptera	Aphididae	*Aphis*	*Aphis nasturtii*	1	0
Insecta	Hemiptera	Aphididae	*Macrosiphum*	*Macrosiphum parvifolii*	1	1
Insecta	Hemiptera	Aphididae	*Aulacorthum*	*Aulacorthum* sp.	1	0
Insecta	Hemiptera	Aphididae	*Aulacorthum*	*Aulacorthum solani*	1	1
Insecta	Hemiptera	Aphididae	*Myzus*	*Myzus persicae*	1	0
Insecta	Hemiptera	Aphididae	*Acyrthosiphon*	*Acyrthosiphon pisum*	1	0
Insecta	Hemiptera	Aphididae	*Nearctaphis*	*Nearctaphis* sp.	1	0
Insecta	Hemiptera	Miridae	*Pinalitus*	*Pinalitus rubricatus*	1	0
Insecta	Hemiptera	Tingidae	*Stephanitis*	*Stephanitis oberti*	0	1
Insecta	Hymenoptera	Braconidae	*Aphidius*	*Aphidius ervi*	1	0
Insecta	Lepidoptera	Coleophoridae	*Coleophora*	*Coleophora glitzella*	0	1
Insecta	Lepidoptera	Endromidae	*Endromis*	*Endromis versicolora*	1	0
Insecta	Lepidoptera	Erebidae	*Atolmis*	*Atolmis rubricollis*	1	0
Insecta	Lepidoptera	Erebidae	*Catocala*	*Catocala fraxini*	1	0
Insecta	Lepidoptera	Geometridae	*Macaria*	*Macaria liturata*	1	0
Insecta	Lepidoptera	Geometridae	*Macaria*	*Macaria signaria*	1	0
Insecta	Lepidoptera	Hesperiidae	*Ochlodes*	*Ochlodes sylvanus*	0	1

Insecta	Lepidoptera	Incurvariidae	*Incurvaria*	*Incurvaria pectinea*	0	1
Insecta	Lepidoptera	Noctuidae	*Apamea*	*Apamea crenata*	0	1
Insecta	Lepidoptera	Pieridae	*Pieris*	*Pieris napi*	1	0
Insecta	Lepidoptera	Pyralidae	*Dioryctria*	*Dioryctria abietella*	1	0
Insecta	Lepidoptera	Tortricidae	*Epinotia*	*Epinotia tedella*	1	0
Insecta	Lepidoptera	Tortricidae	*Epinotia*	*Epinotia tetraquetrana*	1	0
Insecta	Lepidoptera	Tortricidae	*Zeiraphera*	*Zeiraphera ratzeburgiana*	1	0
Insecta	Lepidoptera	Tortricidae	*Cydia*	*Cydia strobilella*	1	0
Insecta	Lepidoptera	Tortricidae	*Gypsonoma*	*Gypsonoma oppressana*	1	0
Insecta	Lepidoptera	Yponomeutidae	*Yponomeuta*	*Yponomeuta evonymella*	1	0
Insecta	Psocodea	Peripsocidae	*Peripsocus*	*Peripsocus subfasciatus*	1	1
Insecta	Psocodea	Psocidae	*Metylophorus*	*Metylophorus* sp.	1	0
Insecta	Thysanoptera	Thripidae	*Thrips*	*Thrips tabaci*	1	0

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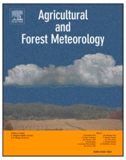

From broadleaves to conifers: The effect of tree composition and density on understory microclimate across latitudes

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ABSTRACT

Forest canopies buffer the macroclimate and thus play an important role in mitigating climate-warming impacts on forest ecosystems. Despite the importance of the tree layer for understory microclimate buffering, our knowledge about the effects of forest structure, composition and their interactions with macroclimate is limited, especially in mixtures of conifers and broadleaves. Here we studied five mixed forest stands along a 1800 km latitudinal gradient covering a 7°C span in mean annual temperature. In each of these forests we established 40 plots (200 in total), in which air and soil temperatures were measured continuously for at least one year. The plots were located across gradients of forest density and broadleaved proportions (i.e. from open to closed canopies, and from 100% conifer to 100% broadleaved tree dominance). Air minimum, mean and maximum temperature offsets (i.e. difference between macroclimate and microclimate) and soil mean temperature offsets were calculated for the coldest and warmest months. Forest structure, and especially forest density, was the key determinant of understory temperatures. However, the absolute and relative importance of the proportion of broadleaves and forest density differed largely between response variables. Forest density ranged from being independent of, to interacting with, tree species composition. The effect of these two variables was independent of the macroclimate along our latitudinal gradient. Temperature, precipitation, snow depth and wind outside forests affected understory temperature buffering. Finally, we found that the scale at which the overstory affects soil microclimate approximated 6-7 m, whereas for air microclimate this was at least 10 m. These findings have implications for biodiversity conservation and forest management in a changing climate, as they facilitate the projection of understory temperatures in scenarios where both forest structure and macroclimate are dynamic. This is especially relevant given the global importance of ongoing forest conversion from conifers to broadleaves, and vice versa.

1. Introduction

Temperature is an important driver of biodiversity at different scales. For instance, it affects the development of animals (Gillooly and Dodson, 2000) and plants (Porter and Delecolle, 1988), phenology (Zohner and Renner, 2014; MacCannell and Staples, 2021), reproductive success (Monasterio et al., 2013) and behavior (Caraco et al., 1990; Angiulli et al., 2020). These responses to temperature, in turn, influence species interactions (Kordas et al., 2011) and shape species diversity (Condamine et al., 2012; Zhou et al., 2016), composition (Macek et al., 2019) and distribution (Woodward, 1988; Repasky, 1991), which ultimately affects ecosystem functioning (García et al., 2018). Since pre-industrial

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times, the world has warmed 1.1°C on average with some areas, such as Europe, warming up considerably faster (IPCC, 2021). This temperature rise is causing largely negative effects on biodiversity (Arneth et al., 2020) and ecosystems (Ito et al., 2020). With global warming, species are expected to shift their native distribution ranges pole-wards (Thomas, 2010), or to higher altitudes (Frei et al., 2010), towards cooler, more suitable environments. Therefore, as a response to warming, species community composition can change, favoring warm-affinity species over cold-affinity species, leading to a process known as "thermophilization" (De Frenne et al., 2013).

Temperatures and other meteorological variables can be distinctive for very different spatial scales, from large regional scales to just a few centimeters. Variations in temperature and other meteorological variables found within a small or restricted area (centimeters to meters) that differ considerably from the macroclimatic conditions in a given geographical region are referred to as "microclimate" (De Frenne et al., 2021). Microclimatic conditions can shape the physiology, presence, distribution and development of species (e.g. Ulrey et al., 2016). For instance, local soil temperature and moisture conditions are important for seed germination and seedling establishment (Egley, 1995; Greenwood et al., 2015), as well as for plant growth, both directly and via their effect on nutrient availability (Paul et al., 2002; Onwuka, 2018). Similarly, development, occurrence and habitat selection of saproxylic beetles is influenced by microclimatic conditions in dead wood found in forests (Lindman et al., 2022). These microclimatic conditions can be affected both by understory plant cover (Pierson and Wight, 1991) and the forest overstory (Paul et al., 2002; De Frenne et al., 2019).

Trees are able to modulate the microclimate of their surroundings near the forest floor (De Frenne et al., 2019). For example, forest canopies buffer ambient temperatures (i.e. decrease high temperatures and increase low temperatures), creating microclimatic conditions that affect understory vegetation and ecosystem processes (De Frenne et al., 2013; Xu et al., 2022). Under forest canopies, maximum and mean temperatures are generally cooler than the macroclimate, whereas minimum temperatures are warmer compared to more open habitats (De Frenne et al., 2019). Therefore, forests can act as climatic microrefugia, allowing the presence of species that could not survive under the ambient macroclimate, and thereby slow down or mitigate the thermophilization of understory communities (De Frenne et al., 2013; Zellweger et al., 2020). In the microrefugia of boreal forests, cold-adapted plants are favored by lower summer and autumn maximum temperatures, late snow melt and higher climatic stability (Greiser et al., 2020). As temperature is considered the main driver of spring phenology in temperate and boreal ecosystems (Kramer et al., 2000), these microrefugia may be of utmost importance to mitigating the effects of phenological mismatches caused by climate change.

In many countries, the past widespread prioritization of conifer monocultures within production forests is being challenged by growing awareness of the potential benefits for biodiversity and ecosystem services derived from diversifying silviculture to include practices that better match natural forest disturbance regimes and tree species composition (Bauhus et al., 2017; Felton et al., 2020; Berglund and Kuuluvainen, 2021). This often involves the replacement of conifers with broadleaved tree species, as motivated by the need to adapt forests to climate change (Löf et al., 2012), current and future pathogen attacks (Thom et al., 2017), biodiversity conservation (Felton et al., 2010), and decreased soil acidification (Oostra et al., 2006). However, the microclimate implications from replacing the overstory species from conifer to broadleaves are still largely unclear (but see Ellison et al., 2005).

Understanding the relationship between forest structure (e.g. the interaction between forest density and composition) and macroclimate temperature is essential in adapting forests and forest management to climate change and for mitigating the impacts of rising temperatures on understory communities. Forest structural complexity is a key determinant of understory microclimates and structural differences among forest stands (e.g. as caused by management practices or stand age) that may explain differences in microclimatic conditions (Kovács et al., 2017; Ehbrecht et al., 2019; Lindenmayer et al., 2022). For instance, forests with denser canopies are better able to buffer macroclimate warming during the growing season (De Frenne et al., 2013). However, high forest densities may hamper biodiversity by reducing light availability (Hedwall et al., 2019b). Increasing spatial heterogeneity in forest structure (and thus in light availability) is a preferable option to increasing forest density, as it can increase microclimate variability (Kovács et al., 2017; Menge et al., 2023). Greiser et al. (2020) showed that the boreal forest patches in which cold-adapted understory species occur have higher basal area values and lower amount of incoming radiation reaching the understory layer in spring and autumn, and are thereby acting as microrefugia. Besides forest density, tree species composition can also affect macroclimate buffering in forests (Zhang et al., 2022). For example, higher tree species diversity in the overstory can create a more diverse set of microclimates, with unique microhabitats that can enhance understory biodiversity (Cavard et al., 2011) and even reduce human thermal stress (Gillerot et al., 2022).

In forests managed for wood production, stand density and tree species composition is largely governed by silvicultural activities. Therefore, forest management decisions can affect forest microclimate dynamics and hence forest efficacy as microclimatic refugia. This not only occurs when choosing the overstory species mixture and stand density, but also through thinning, harvesting or causing other disturbances that increase forest canopy openness. For instance, after a clear-cut, thermophilization of the understory plant community regularly takes place (Stevens et al., 2015; Christiansen et al., 2022), as there are only a few trees left to buffer extreme temperatures. Subsequently, when forest regeneration progresses and the overstory becomes dense enough to buffer macroclimatic temperatures, the proportion of cold-adapted species may increase again (Christiansen et al., 2022).

Despite the recognized importance of the tree layer for understory microclimate, our knowledge about the interacting effects of forest density, tree species composition, tree spatial arrangement, temperature variation, and season remains limited. Here we used a large-scale natural experiment along a 1800 km latitudinal gradient to evaluate the absolute and relative importance of the proportion of broadleaved trees and forest density for understory temperature. We used 200 plots with temperature measurements in five mixed forests along a macroclimatic gradient spanning *ca.* 7°C difference in mean annual temperature, with forest density and tree species composition (broadleaves-conifer) gradients within each of these forests. Specifically, we proposed the following hypotheses: (1) Forest density and the proportion of broadleaves interact in their microclimatic buffering effects (i.e. the buffering effect from conifers is stronger than that one from broadleaves at a given density), (2) The effect of forest density and the share of broadleaves on the understory microclimate is independent of latitudinal temperature differences in macroclimate, (3) Meteorological variables outside the forest, such as the macroclimate temperature, precipitation and wind affect the amount of climate buffering. Additionally (4), we hypothesized that it is possible to quantify the area of influence at which trees affect soil and air microclimate buffering, and thus the scale to be considered when managing forests for macroclimate buffering.

2. Material and methods

2.1. Study sites and experimental setup

To increase the generality of our findings and to test whether the understory temperature buffering effect varies depending on macroclimate, five mixed forest areas (further referred to as "sites") were selected along a latitudinal gradient from 49° N to 64° N in North-Western Europe. These sites all had a large within-site variation in tree species composition and forest density, small altitudinal differences and relatively homogeneous soil conditions. Three sites were located in Sweden: spanning the north (NS), mid (MS) and south (SS) of the country. Additional sites were located in western Germany (G) and eastern Belgium (B). These last two sites, albeit close in proximity, presented rather different macroclimates (Fig. 1A, Table 1).

The forests in G, SS, MS and NS had similar overstory species composition: the dominant conifer species was Norway spruce (*Picea abies* H. Karst), and the dominant broadleaved species was birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.). The overstory species composition in B differed to some extent, with oaks (*Quercus robur* L. and *Q. petraea* (Matt.) Liebl.) as the most abundant broadleaved species. All forest stands at the study sites originate from natural or artificial regeneration of conifers and broadleaves following clear-cutting or salvage harvesting after windthrow. We selected these species in our mixtures as representatives of tree species with different characteristics: Norway spruce as an evergreen, late-successional, shade-tolerant species, birch (and to some extent oak) as a deciduous, early successional, shade-intolerant species (Niinemets and Valladares, 2006). We believe that our results can also be valid for other tree species in these groups. In addition to spruce, birch and oak, other tree species were found to a lesser extent (< 4% of each stand basal area across all plots; Table 1). A shrub layer was generally lacking in all forest stands.

In all sites except B, we established 40 ten-meter radius plots. In site B, as trees were larger and tree separation higher, a 20 m radius was used instead. Plots were located along gradients of forest density and tree species composition, i.e. ranging from evergreen conifer to deciduous broadleaf dominance and from open to closed canopy, to maximize variability across these two overstory variables (Fig. 1C). In the center of each plot, two HOBO Pendant® MX Water Temperature Data Loggers (Onset Computer Corp., Bourne, MA, USA) were placed (80 in total per site): one recording air temperature at *ca.* 1.2 m height and the other recording temperature in the top soil layer (0–5 cm). No soil loggers were installed in site B due to frequent wild boar rooting at this site. Soil loggers were placed inside hermetic plastic jars to protect them from direct contact with the soil. Additionally, air loggers were protected by a well-ventilated plastic radiation shield (Fig. 1B). Both air and soil temperature measurements were taken every 15 min.

2.2. Overstory measurements

For all trees within a 10 m radius (20 m in site B) from the temperature loggers, we recorded the species identity, diameter at breast height (DBH), and the distance and azimuth angle (circular degrees, with north as 0° and south as 180°) to the logger. These data were used to calculate the total basal area (i.e. forest density) per plot, as well as the percent basal area of broadleaved trees per plot (i.e. proportion of broadleaves from here on). To determine at which distance from the data logger overstory tree species composition and basal area best accounted for microclimate measurements, all tree species composition (i.e. proportion of broadleaves) and basal area measurements were calculated at different radii between 4 m and 10 m (4 m and 20 m in B), meter by meter.

Hemispherical pictures were taken in each plot in summer to calculate canopy cover, using a Nikon 5300 camera with a fish-eye lens and tripod. All pictures were taken from above the air temperature logger, at an approximate height of 1.75 m. Pictures were then processed and analyzed in R as in ter Steege (2018). The blue channel in the RGB channel was selected due to its high absorption by chlorophyl in green leaves, resulting in a high contrast between the sky and tree foliage (Brusa and Bunker, 2014). Black and white images were created using a threshold of 0.55, from which canopy openness was calculated.

Besides basal area, canopy cover was also used as another proxy for forest density. The reason for this was twofold. Whereas basal area is a widely used metric in forestry and is used in decision support systems, canopy openness is more ecologically relevant, and in some cases outperforms basal area when predicting forest understory microclimate (Zellweger et al., 2019; Meeussen et al., 2021). Moreover, canopy cover data might be a more easily accessible explanatory variable due to remote sensing and open data policies. In our study, these two variables were correlated with an r = -0.44.

2.3. Microclimate and macroclimate temperature data

Microclimate temperature data recorded by our loggers during the warmest (July) and coldest (January) month were selected between 2020 and 2021 for each plot in each site (Table S1). For each plot, daily

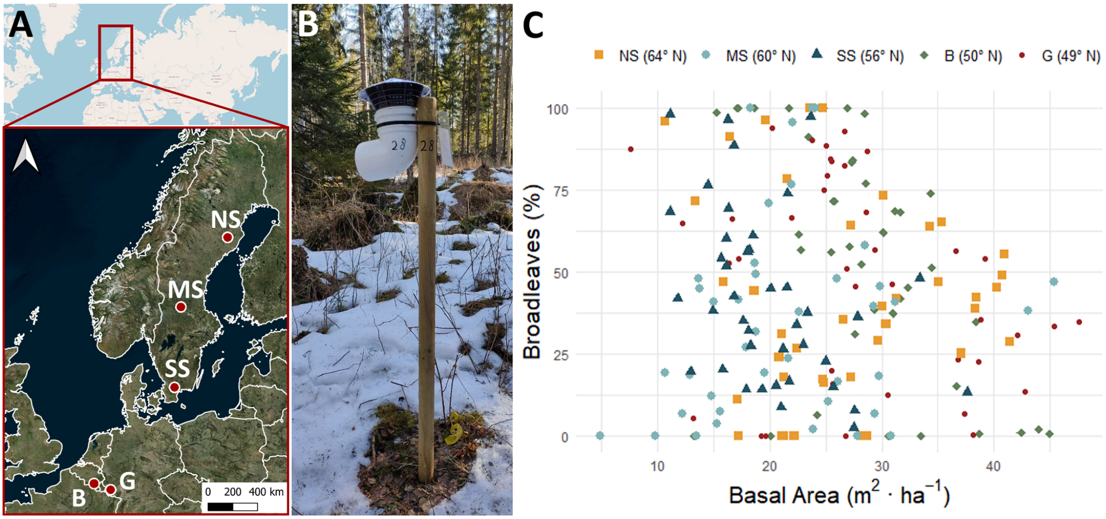

Fig. 1. A. Geographical location of forest stands used across Europe to discern how understory microclimate is affected by tree species composition and density. Stands were located in North Sweden (NS), Mid Sweden (MS), South Sweden (SS), Germany (G) and Belgium (B). **B.** Radiation shield used for air temperature loggers. **C.** Forest density estimated by basal area ($m^2 \cdot ha^{-1}$) and share (% of basal area) of broadleaved trees in 200 plots from the 5 sites (40 in each) included in the study.

Table 1

Description of the five forest sites across a 1800 km latitudinal gradient in which microclimate was measured. Tree species composition values represent the mean basal area ($m^2 \cdot ha^{-1}$) per species across all plots within site. Annual mean temperature and annual mean precipitation were extracted from the WorldClim version 2.1 climate data for 1970–2000 (Fick and Hijmans, 2017). Negative values for the elevational difference to the weather station indicate that the weather station was higher than the forest stands, and vice versa.

		Northern Sweden (NS)	Mid Sweden (MS)	Southern Sweden (SS)	Germany (G)	Belgium (B)
	Location	64°18′ N, 19°44′ E	60°50′ N, 14°40′ E	56°17′ N, 13°58′ E	49°36′ N, 7°01′ E	50°02′ N, 5°13′ E
	Mean annual temperature (°C)	1.50°C	4.52°C	6.79°C	7.51°C	8.58°C
	Total annual precipitation (mm)	599 mm	589 mm	738 mm	1029 mm	961 mm
	Distance to used weather station	~ 6 km	~ 15 km	~ 1 km	~ 17 km	~ 13 km
	Elevational difference to used weather station	35 m	-39 m	9 m	-33 m	-161 m
	Average tree diameter ± SD (cm)	16.7 ± 6.3	16.1 ± 5.3	12.9 ± 4.6	13.9 ± 6.4	35.7 ± 14.0
	Stem density (trees ha^{-1})	1061.56	946.17	1356.0	1522.17	248.57
	Average basal area ($m^2 \cdot ha^{-1}$)	26.8	21.6	19.9	27.9	28.7
Conifer species	*Picea abies*	14.8 $m^2 \cdot ha^{-1}$ (55.2%)	13.5 $m^2 \cdot ha^{-1}$ (62.4%)	12.1 $m^2 \cdot ha^{-1}$ (60.7%)	13.7 $m^2 \cdot ha^{-1}$ (48.8%)	14.6 $m^2 \cdot ha^{-1}$ (50.8%)
	Pinus sylvestris	1.0 $m^2 \cdot ha^{-1}$ (3.8%)	0.1 $m^2 \cdot ha^{-1}$ (0.6%)	-	<0.1 $m^2 \cdot ha^{-1}$ (0.3%)	-
	Pseudotsuga menziesii	-	-	-	0.2 $m^2 \cdot ha^{-1}$ (0.7%)	0.5 $m^2 \cdot ha^{-1}$ (1.8%)
	Larix spp.	-	-	-	1.1 $m^2 \cdot ha^{-1}$ (3.8%)	-
	Picea sitchensis	-	-	-	0.4 $m^2 \cdot ha^{-1}$ (1.5 %)	-
Broadleaf species	*Betula pubescens/pendula*	11.0 $m^2 \cdot ha^{-1}$ (41.0%)	8.0 $m^2 \cdot ha^{-1}$ (36.9%)	7.1 $m^2 \cdot ha^{-1}$ (35.6%)	11.4 $m^2 \cdot ha^{-1}$ (40.7%)	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)
	Alnus incana/glutinosa	-	-	0.4 $m^2 \cdot ha^{-1}$ (1.9%)	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)	-
	Fagus sylvatica	-	-	-	0.3 $m^2 \cdot ha^{-1}$ (1.1%)	0.3 $m^2 \cdot ha^{-1}$ (1.0%)
	Carpinus betulus	-	-	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)
	Sambucus spp.	-	-	-	<0.1 $m^2 \cdot ha^{-1}$ (0.2%)	-
	Quercus robur/petraea	-	-	0.3 $m^2 \cdot ha^{-1}$ (1.7%)	0.3 $m^2 \cdot ha^{-1}$ (1.1%)	13.2 $m^2 \cdot ha^{-1}$ (46.0%)
	Sorbus spp.	-	-	-	0.4 $m^2 \cdot ha^{-1}$ (1.5%)	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)
	Salix spp.	-	<0.1 $m^2 \cdot ha^{-1}$ (0.1%)	-	-	-

mean air and soil temperature were calculated. Additionally, daily maximum and minimum temperatures were calculated exclusively for air loggers, as we expected little variation in the daily soil temperature due to the additional insulating effect of the topsoil, litter layer and understory vegetation. In fact, the soil minimum and maximum temperatures were highly correlated with mean temperature. For loggers with less than 10% of data missing, absent values were filled with the mean of the temperatures recorded by the rest of the loggers from the same site for the respective period. Loggers missing more than 10% of data were removed from analyses (Table S1). Subsequently, daily temperature offsets (i.e. difference between macroclimate and microclimate) were calculated based on temperatures extracted from three different sources: the closest available weather stations to each site, the ERA5-Land climatic model (Hersbach et al., 2020) downloaded for reanalysis from the Copernicus Climate Data Source (cds.climate.copernicus.eu), and ERA5 data downloaded through the ‘mcera5’ R package (Klinges et al., 2022). The ‘mcera5’ R package applies an inverse distance weighting calculation and diurnal temperature range corrections (see Klinges et al. (2022) for details). Daily maximum and minimum temperatures for all these macroclimatic data sets were inferred from hourly mean temperature data. Temperature offsets were calculated by subtracting the macroclimate temperature from the microclimate temperature, so that negative offset values showed days in which the forest temperature was lower than the macroclimate, and positive values days in which temperature was higher. For a comparison of the air temperature offset values for each of the macroclimate temperature sources see Fig. S2. In the results, we refer to offsets calculated from nearby weather station data, as they followed microclimatic trends better than ERA5 and ‘mcera5’ data (Fig. S1). Results for offsets calculated with other macroclimatic sources can be found in the supplementary material, Table S3 and Figs. S6–9. Soil temperature offsets were calculated only from ERA5 data downloaded from the Copernicus Climate Data Source, as macroclimate soil temperature was not available from the other sources. Additionally, three meteorological variables were downloaded from the ERA5 climatic model and used as explanatory variables: wind gust, snow depth and precipitation. The maximum wind gust value per day was calculated. For snow depth data, values were averaged to obtain daily means. For precipitation, hourly data was summed to obtain daily values. Previous averaged macroclimatic temperature (PAT) and previous averaged precipitation (PAP), i.e. temperature and precipitation of the past 5 days, were calculated as a 5-day rolling mean. These two variables were always calculated from the same macroclimate data source as the response variable of each model, except for the models that used temperature offsets calculated from weather station data, as precipitation data were not available from all weather stations. In these cases, the previous precipitation was calculated from the ERA5 data instead.

2.4. Data analyses

To account for non-linearity and allow flexible response shapes, Generalized Additive Mixed Models (GAMMs) were used to model the effects of local forest structure and macroclimate on temperature offsets. A GAMM is a generalization of a Generalized Linear Mixed Model in which the assumption of a linear relationship between predictors and the response variable (on the scale of the link function) is relaxed. Daily mean, minimum and maximum offsets for air temperature and daily mean offsets for soil temperature were used as response variables. Forest density (i.e. canopy openness or basal area) and the proportion of broadleaves were used as predictors in Linear Mixed Models (LMM), both interactively and non-interactively for comparison with the GAMMs. In the GAMMs, these two predictors were used within a bidimensional smoother term, with and without interaction with site, or as separate smoother terms. All models were run with either canopy openness or basal area as proxies for forest density and for both the warmest (July) and coldest (January) months. To account for temporal autocorrelation, all models included date in a uni-dimensional smoother term. Finally, plots nested within site were used as a random intercept effect. Model selection was based on the Akaike's Information Criterion (AIC). Once the best smoother configuration was found, wind, PAT and PAP were added as covariates to the air models, and PAT and snow depth were added as covariates to the soil models, as these variables were assumed to affect the temperature offsets. See Table S2 for an overview of all the covariate and smoother term combinations used in the models and Table S3 for the models' details.

To assess the scale at which the overstory affects air and soil temperature offsets, GAMMs were run with basal area and proportion of broadleaves calculated at plot sizes of different radii. These two metrics were calculated at all possible combinations of radii between 4 and 10 m. Additionally, GAMMs with exclusively basal area or proportion of broadleaves were also run for each of these radii. This was only assessed for mean daily air and soil temperature. These models were run using basal area and proportion of broadleaves in a bidimensional smoother, and with date as a unidimensional smoother. In the cases where either basal area or the proportion of broadleaves were used alone, a unidimensional smoother was used instead. No covariates were used in these models. In site B, trees were measured within a 20 m radii and therefore, this site was analyzed independently from the other sites, including all data combinations from 4 to 20 m. A minimum radius of 4 m was selected in all sites based on the available data, as smaller radii resulted in a significant reduction of the total number of plots with trees. The GAMMs and LMMs were run using the 'gamm4' R package (Wood and Scheipl, 2020) in R version 4.2.2 (R Core Team, 2022). Refer to Tables S8 and S9 for further clarification of this analysis.

When reporting results we use the term "buffering" in relation to forest microclimates that operate within a narrower average range than macroclimate regardless of season (De Frenne et al., 2021). As such, a larger buffering capacity for maximum temperatures would involve cooler forest temperatures than the macroclimate, whereas a larger buffering capacity for minimum temperatures would involve warmer forest temperatures than the macroclimate.

2.5. Data and code availability

The data supporting this study are available upon request, and the code used for analyses is found at https://github.com/JDiazCalafat/ForestMicroclimate_Brodleaves_and_Density. For better reproduction of our results, we recommend using the R package "checkpoint" (Ooi et al., 2022) set to November 2022. This allows one to use the same version of the R packages that were applied when analyzing our data.

3. Results

3.1. Model selection

According to the AIC values, GAMMs outperformed LMMs (Table S3), indicating that non-linear models are needed to describe the effects of forest density and percentage of broadleaves (Table S3). Depending on the response variable and the season, either basal area or canopy openness produced the models with lowest AIC, yet differences in explained deviance were generally small (Table S3).

Generally, models with separate smoothers for forest density and percentage of broadleaves had a higher AIC than bidimensional smoothers, indicating an interactive effect of these two variables. Models without any site interaction had lower AICs than models where the effects of the overstory were allowed to vary between sites, which indicates that the effects of the overstory are consistent along our macroclimatic gradient. The only exception to this was for the mean air temperature models in the warmest month in which basal area was used as a proxy for forest density. In these, a bidimensional smoother with site interaction produced smaller AIC values. When canopy openness was used as a proxy for forest density, a bidimensional smoother with no site interaction was the best option in all cases, except in the coldest month for soil temperature models, in which separate unidimensional smoothers worked better (Table S3). The optimal models to predict the maximum, mean and minimum air and mean soil temperature can be found in Table 2. Output from the models can be found in Tables S4–S7.

Overall, differences between the AIC and R^2_{adj} values of the models with bidimensional smoothers without site interaction, and those with two separate individual smoothers, were rather small and thus we cannot assume that there were strong interactions between forest density and the proportion of broadleaves.

3.2. Air minimum temperature offset

Minimum air temperature offsets became larger with increasing basal area (i.e. they became smaller with decreasing canopy openness) in both the warmest and the coldest months. In other words, in both months, increasing basal area led to higher minimum air temperatures relative to the temperature outside the forest. The effect of the percentage of broadleaves, however, was different depending on the season: minimum temperature offsets became larger with a higher proportion of broadleaves in the warmest month (i.e. broadleaves held back longwave radiation released from the ground and understory plants more effectively than conifers), and became smaller in the coldest month (i.e. since broadleaves do not have leaves in winter, the energy loss from longwave radiation released from the ground was larger with an increasing proportion of broadleaves; Fig. 2A & B; Fig. S5A & B). Therefore, although forest minimum temperatures were always higher than those of the macroclimate ambient temperature, the maximum buffering capacity of the forest was obtained when both forest density and the proportion of broadleaves were high (in the warmest month) or when forest density was high and proportion of broadleaves low (in the coldest month, as broadleaves had no leaves). The amplitude of offsets predicted by our models within our double forest gradient was similar: 0.8°C for the warmest month and 0.7°C in the coldest month when assessing minimum temperature offsets through basal area and 0.5°C for the warmest month and 0.7°C in the coldest month when assessing temperature offsets with canopy openness.

3.3. Air mean temperature offset

Increasing forest density made mean air temperature offsets larger in both the warmest and in the coldest month (although with a relatively small effect, see below), making the forest cooler in summer and warmer in winter. Increasing the percentage of broadleaves made mean temperature offsets generally larger in the warmest month, while there was no clear effect in the coldest month. Therefore, the maximum buffering capacity of the forest with respect to mean air temperature was achieved when forest density was high in the coldest month and when both forest density and the proportion of broadleaves were intermediate or high in the warmest month (Fig. 2C & D; Fig. S5C & D). The effect of forest density on microclimate was consistently larger than that of the proportion of broadleaves. The amplitude of mean air temperature offsets predicted by the models was always higher in the warmest month than in the coldest month: in the warmest month it was 0.6°C both when considering basal area and canopy openness, and in the coldest month it was 0.3°C in the basal area models and 0.4°C in the canopy openness models. Therefore, despite our use of hemispherical pictures during the summer to calculate canopy openness, the predicted offset amplitudes during the coldest month were similar to those in the models where basal area was used.

Table 2
Models predicting the daily maximum, mean and minimum air and mean soil temperature offsets through forest and climate variables. An offset is the difference in temperature between the microclimate and the macroclimate (i.e. microclimate - macroclimate). Only the models with the lowest AIC values are shown. Air temperature offsets were calculated from nearby weather stations as macroclimatic data sources. Soil temperature offsets were calculated form ERA5 data. Wind and snow data was extracted from ERA5 data. Previous averaged precipitation in the last five days (PAP) was extracted with the 'mcera5' R package. Previous averaged temperature in the last five days (PAT) was calculated from weather station data (i.e. same macroclimate temperature source used for the offsets). Note that all models were conducted with a bidimensional smoother term including forest density (i.e. either basal area (BA) or canopy openness (CO)) and the percentage of basal area occupied by broadleaves. AIC values and R^2_{adj} are shown for the same models using basal area or canopy openness as a proxy for forest density. All models accounted for temporal autocorrelation.

				Basal area		Canopy openness	
Logger location	Response variable	Covariates	Period	R^2_{adj}	AIC	R^2_{adj}	AIC
Air	Min temp offset	site + wind + PAT + PAP	Coldest month	0.67	16482	0.67	16491
			Warmest month	0.48	17452	0.48	17477
Air	Mean temp offset	site + wind + PAT + PAP	Coldest month	0.64	13403	0.64	13395
			Warmest month	0.52	9658	0.53	9655
Air	Max temp offset	site + wind + PAT + PAP	Coldest month	0.60	13124	0.59	13141
			Warmest month	0.61	15168	0.60	15179
Soil	Mean temp offset	site + snow + PAT	Coldest month	0.53	13018	0.53	13028
		site + PAT	Warmest month	0.71	11191	0.71	11200

3.4. Air maximum temperature offset

Maximum air temperature offsets in the coldest month were affected mainly by species composition and to a lesser extent by forest density. The amplitude of this effect was overall rather small, though, of approximately 0.15°C (Fig. 2E; Fig. S5E). However, in the warmest month there was a linear response to density and a weak or absent response to proportion of broadleaves. Increasing forest density made maximum air temperature offsets larger (i.e. increased the cooling effect). In this month, the amplitude of the offsets predicted by our models was about 3°C (Fig. 2F; Fig. S5F). The buffering effect of maximum temperatures in the warmest month was maximal in high density plots largely independent of the proportion of broadleaves.

3.5. Soil mean temperature offset

The proportion of broadleaves had a much stronger effect on forest soil temperature in the warmest month than in the coldest month (Fig. 3). A higher proportion of broadleaves made the soil temperature offsets smaller in summer, but had little effect in winter (Fig. 3). When taking into account canopy openness in the coldest month, the percentage of broadleaves had a similar effect as in the warmest month: a higher proportion made the forest soil temperature offsets larger. Of these two models (basal area model and canopy openness model), the basal area model had the lowest AIC value (Table S3). In both the warmest and the coldest month, a higher basal area (or a lower canopy openness) made offsets larger (Fig. 3). Therefore, the temperature buffering capacity was highest when canopy openness was low and the proportion of broadleaves was low (in the coldest month) or when basal area was highest and the proportion of broadleaves was lowest (in the warmest month). The amplitude of the offsets predicted by our models for soil mean temperature was always higher in summer than in winter: in the basal area models it was 2°C, and in the canopy openness models 1.8°C, whilst in winter it was 1.1°C and 0.9°C, respectively.

3.6. Effects of macroclimate drivers on temperature offsets

Meteorological variables (averaged temperature and precipitation in the last five days, wind and snow) generally had consistent effects on our air temperature models regardless of the response variable (Table S4). In these models, increasing PAP and wind decreased the absolute values of the offsets and thereby reduced the temperature buffering effect of the forest in both the warmest and the coldest month (both $p < 0.001$). On the other hand, increasing PAT made the offsets smaller in the warmest month and larger in the coldest month ($p < 0.001$), increasing the general buffering effect of the forest in winter but reducing it in summer. This variable had no significant effect on the maximum air temperature offsets in the warmest month ($p = 0.84$).

Regarding soil temperature (Table S6), increasing snow depth made the offsets larger and thus increased the buffering effect of the forest canopy ($p < 0.001$) while higher PAT made the offsets larger in the warmest month and smaller in the coldest month (both $p < 0.001$), which is the opposite effect as on the air temperatures.

3.7. Distance analyses

These analyses aimed at clarifying the area of influence of the overstory upon mean air and soil temperature offsets. That is, the minimum radial distance from a temperature logger in which all trees should be measured in order to predict temperature offsets. The distance from the data loggers for which basal area and the proportion of broadleaves best predicted mean air temperature offsets in the warmest month was 10 m for basal area and 9 m for the proportion of broadleaves. However, the second best model included both variables at their maximum resolution (i.e. both 10 m) and the third best for basal area was a 9 m radius and the proportion of broadleaves within an 8 m radius. Differences in AIC values in these models were smaller than 0.6, and all models had the same R^2_{adj} (Table S8). For air mean temperature offsets in the coldest month, the best model included basal area calculated in an 8 m radius and proportion of broadleaves within a 9 m, whilst the second best model included broadleaves within 10 m and basal area within 8 m. The third best model included basal area calculated within 7 m and broadleaves in a 9 m radius (Table S8).

Regarding soil temperature offsets in the warmest month, the three best models included broadleaves at the maximum distance at which they were measured (i.e. 10 m) and basal area within these respective radii (from best to worst models): 8 m, 9 m and 7 m (Table S8). During the coldest month, the best models did not include percentage of broadleaves, and the distance at which basal area best predicted temperature offset was at 7, 8 and 6 m radius, respectively (Table S8).

In the case where only site B was considered in air temperature offset models, the lowest AIC value was obtained when including basal area calculated at the maximum radius (i.e. 20 m) and the proportion of broadleaves within 10 m. The two next best models were similar, with basal area calculated at 20 and 19 m, and broadleaves at 10 and 9 m, respectively (Table S9). For the coldest month, the results of the two best models were similar to those of the other sites: the percentage of broadleaves was not relevant, yet the radii at which basal area was calculated were 20 and 19 m, respectively. Contrastingly, the third best model did not include basal area but included the percentage of broadleaves within a 19 m radius (Table S9).

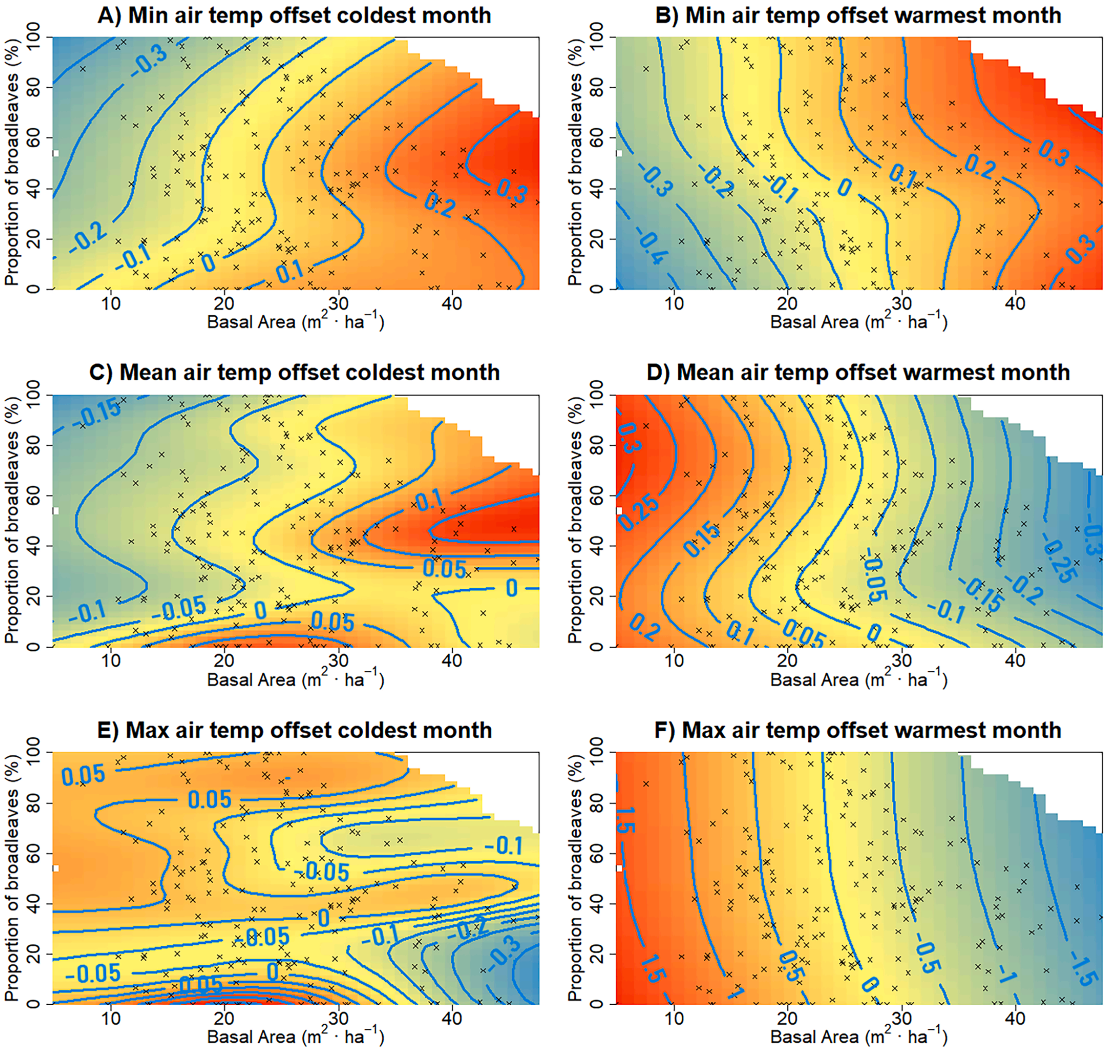

Fig. 2. Model predictions for the minimum, mean and maximum air temperature offsets (forest temperatures minus weather station data) for the warmest and coldest months in our study. In these predictions, basal area was used as a proxy for forest density. The numbers on the isolines indicate the predicted offsets in degrees Celsius. The predictions are centered at the mean and the zero isoline thus depicts the mean of the model predictions. The offsets of these models were calculated from weather station macroclimatic data. The colours represent a gradient from more positive (red) to more negative (blue) offsets. Gaps (i.e. white spaces) represent the model predictions that were too far from our data points. For component smooths with confidence intervals that include the uncertainty on the overall mean, see Fig. S3 in the supplementary material.

4. Discussion

Our results provide five important insights regarding the interaction between macroclimate and the tree canopy on forest understory temperatures. First, we found that both forest density and tree species composition play important roles in determining understory temperature. Second, forest density (i.e. basal area and canopy openness) can act independently or interact with the share of broadleaves to alter microclimate depending on the specific temperature metric assessed. Third, we show that the effect of forest density and percentage of broadleaves on microclimate seems to be consistent over a large latitudinal macroclimate gradient. Fourth, the temperature buffering capacity of forests is affected by meteorological variables such as the ambient temperature, precipitation and wind which thus may affect their local climate change mitigation potential. Finally, we found that the scale at which the tree overstory affects local soil temperature offsets approximated 6-7 m, whilst for air temperature offsets this range was close to 10 m or more.

The temperature buffering effect of forest density was always stronger than that provided by the proportion of broadleaves. According to our models, increasing forest basal area cooled down soil mean temperature and maximum and mean air temperatures in summer. In

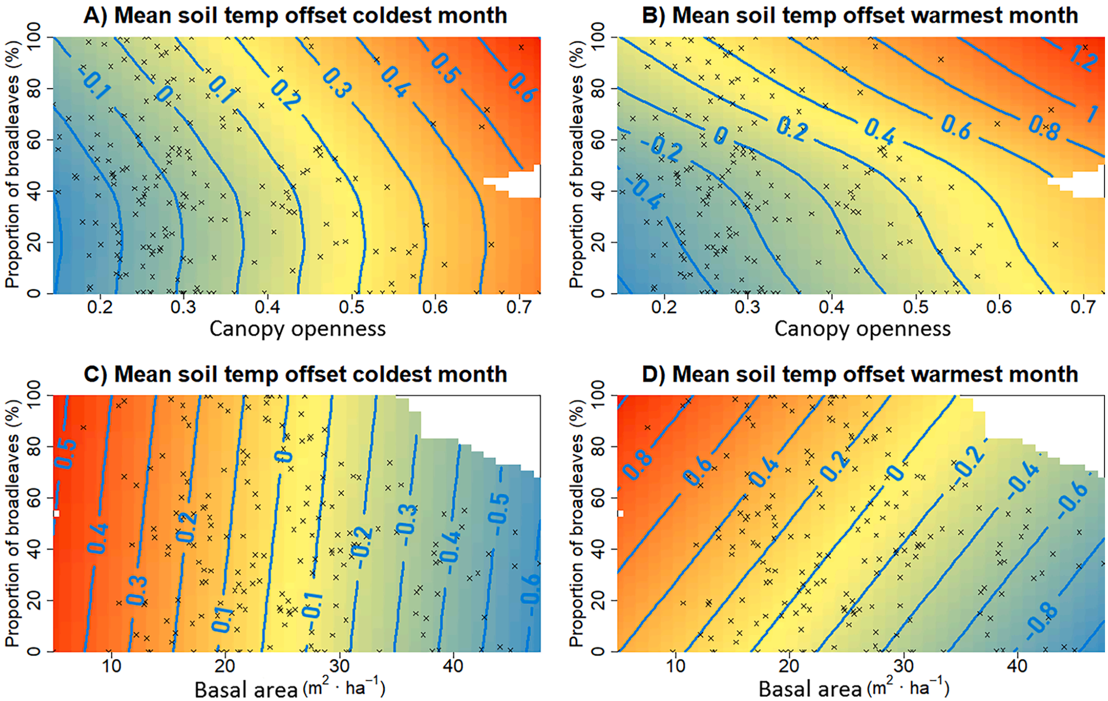

Fig. 3. Model outputs for the mean soil temperature offsets for the warmest and coldest months in our study, using canopy openness (A-B) and basal area (C-D) as a proxies for forest density. The numbers on the isolines indicate the predicted offsets in degrees Celsius where the zero isoline depicts the mean of the predictions of the model. The numbers on the isolines indicate the predicted offsets in degrees Celsius. The predictions are centered at the mean and the zero isoline thus depicts the mean of the model predictions. The offsets of these models were calculated from the ERA5 macroclimatic data. The colours represent a gradient from more positive (red) to more negative (blue) offsets. Gaps (i.e. white spaces) represent the model predictions that were too far from our data points. For component smooths with confidence intervals that include the uncertainty on the overall mean, see Fig. S4 in the supplementary material.

contrast, increasing basal area increased minimum air temperatures in both seasons, as well as mean air temperature in winter (Fig. 2). For instance, our models suggest that an average density forest stand (e.g. basal area of 25 $m^2 \cdot ha^{-1}$) with a share of broadleaves of 50% in northern Sweden would drop the maximum temperature in the warmest month by around 1.3°C, yet drop it by as much as 2.5°C if forest density was increased to a basal area of 40 $m^2 \cdot ha^{-1}$. If all trees were to be replaced by broadleaves, similar buffering effect would be achieved: a cooling of 1.4°C at a basal area of 25 $m^2 \cdot ha^{-1}$ and a cooling of 2.6°C at a basal area of 40 $m^2 \cdot ha^{-1}$. In the case of mean atmospheric temperature for the same site in the warmest month, a drop of around 0.4°C would be achieved at a basal area of 25 $m^2 \cdot ha^{-1}$ and 50% of broadleaves, reaching a cooling of 0.8°C in optimal conditions of forest density and broadleaves share. These projections of our models are similar to other observations in temperate deciduous Central European forests. For instance, Zellweger et al. (2019) found the average cooling effect of the overstory in summer to be of 2.1°C in deciduous forests with an average basal area of 33.2 $m^2 \cdot ha^{-1}$, and the average warming effect in winter of 0.4°C in the same forests. Similarly, in pine forests, Blumröder et al. (2021) showed a cooling effect of *ca.* 2°C between their densest and most open stands.

The range of temperature buffering we observed (which varies within an heterogenous stand), could potentially have an impact on phenology and voltinism of some ectothermic species, causing differences in their development rate compared to their counterparts outside the buffering effect of the forest. For instance, Greiser et al. (2022) found that the probability of diapause and the number of generations of *Pieris napi* (L., 1758) changed within the same forest landscape based on microclimatic variation, with potentially great impacts on survival and fitness. Our results are in accordance with those of De Frenne et al. (2019) and show the potential of forests to provide climate refugia to mitigate the negative effects of warming on species' fitness or phenology.

Often, production forests are kept within narrow ranges of basal area (although this depends on the applied silvicultural system) and therefore their potential to provide variation in microclimatic conditions is reduced. For example, the highest forest densities in our study are only occasionally found in production forests of this region, and by increasing basal area, this buffering potential can be exploited better. Our model predictions show that the buffering effect of the forest is reduced at low forest densities, which may be a result of higher insolation and wind shelter in openings (Baker et al., 2013). In fact, reducing canopy cover is shown to decrease the buffering effect of forest understory temperatures both in coniferous and broadleaves stands (Blumröder et al., 2021). This finding also has implications for choice of forest management system (e.g. single tree, gap, shelterwood, clear-cut; Kermavnar et al., 2020; Menge et al., 2023). For instance, Radler et al. (2010) observed that the maximum air and soil temperature were 2.5°C and 6°C higher (respectively) in a clearcut than in the surrounding spruce forest. However, increasing forest density also decreases light availability, reducing understory plant diversity, reproduction and abundance of many understory species. Consequently, increasing forest density is one of the main drivers behind species turnover and a decreasing understory plant cover in Swedish forests (Hedwall et al., 2019a, 2021). Specifically, Eckerter et al. (2019) showed that increasing canopy cover decreases the number of flowers, ovules, reproductive success and fruit quality of *Vaccinium myrtillus,* L., which is one of the most common and ecologically important understory shrubs in boreal systems, that also occurred in several of our sites. Therefore, even though increasing forest density might show some advantages when temperature buffering is pursued, it can also compromise resource availability for more light demanding understory species, regardless of whether the microclimatic conditions are more suitable.

Our results also indicate that the effect of forest density on the buffering of maximum temperatures in summer is rather independent of the proportion of broadleaves (e.g. Fig. 2F and example in the previous paragraph), which indicates that the same buffering capacity caused by the shade-tolerant Norway spruce is also achieved by shade-intolerant broadleaves (mostly birch and oak). These tree species categories differ largely in their effects on understory light environments, for which Norway spruce has a high leaf area index all year round (Verheyen et al., 2012) and thus has considerably stronger effects on the understory light environment (Hedwall et al., 2019b). Therefore, increasing the forest basal area by increasing the proportion of certain broadleaved tree species could reduce summer air temperatures without inducing the same extent of negative effects on light availability and biodiversity as shade tolerant conifers, while also providing a suite of additional biodiversity, ecosystem services and climate change adaptation benefits (Felton et al., 2010, 2016). Nevertheless, as deciduous trees do not have leaves in winter, their buffering capacity may be lower, potentially leaving frost-sensitive understory species less protected against late frost events than they would be beneath a conifer canopy. In a mixed forest scenario, however, fine-scale heterogeneity in light regimes, as provided by the addition of broadleaves to otherwise conifer dominated stands, benefits biodiversity in the forest understory (Helbach et al., 2022). Given the current trend of replacing conifers with broadleaved tree species (Löf et al., 2012), we recognize this temperature buffering as an added benefit of such changes to silvicultural practice.

The similar buffering effect of broadleaves and Norway spruce on summer maximum temperatures is surprising considering the generally larger radiation transmittance under broadleaves. The reason for this pattern is unknown but is perhaps related to differences in albedo and evaporative cooling (Geiger et al., 1995; Bonn et al., 2020). Species composition has a strong effect on albedo, with broadleaves generally having a higher albedo than most conifer species (Lukeš et al., 2013). Therefore, the higher albedo of broadleaves could dissipate radiation in a way that contributes to macroclimate temperature buffering (Bright et al., 2013). On the other hand, differences in evapotranspiration rates between these overstory species may alter water availability in the surroundings and also affect evaporative cooling in different ways (Moss et al., 2019). These differences in transpiration rate may result from different rooting depth between deep-rooting broadleaves such as oaks and the mostly shallow rooted Norway spruce. Ultimately, it may also be the case that such differences are tree species-specific and dependent on stand characteristics, as light transmittance can also be quite low beneath broadleaf stands.

If the tree species diversity of the overstory is to be increased, this should be done within reasonable limits if accompanied by concomitant increases in forest density. For example, Hedwall et al. (2019b) showed that stand density can override the benefits to understory biodiversity from increasing the broadleaf proportion in the overstory. Moreover, in the studied forests, the effect of the proportion of broadleaves on soil mean temperature offsets was far stronger during summer than winter, with a higher percentage of broadleaves warming up the forest soil significantly. Potential factors driving the contrastingly limited influence of the proportion of broadleaves during winter may stem from an overriding influence of snow and litter that insulated soil temperatures against radiation (Hennon et al., 2010; Fekete et al., 2016). However, as we did not measure differences in litter or snow between plots, this explanation remains speculative. We can however expect that increasing forest density through the increase of broadleaves would have distinctive effects on soil versus air microclimate. As our two primary overstory tree species (spruce and birch) were selected as representatives of late successional evergreen conifers and early successional broadleaves, respectively, our results also provide insights applicable to other forest systems with tree species possessing similar ecological and structural attributes. Furthermore, our results were largely consistent across a latitude gradient of *ca.* 7°C average annual temperature span, which further strengthens the potential for extrapolating our results beyond our specific sites. However, despite our similar results when using either canopy openness or basal area as proxies for forest density, the effect of these variables may differ in other forest systems in which their correlation is less strong.

Allowing for a greater variability of microclimatic conditions can be expected to benefit a greater range of understory biodiversity (Helbach et al., 2022). Microclimatic variation can be enhanced in forest understories by mixing overstory tree species with different structures and traits (Zhang et al., 2022), as well as by taking into account the spatial scale at which the individual trees are mixed (Felton et al., 2022) or allowing spatial coexistence of different forest developmental stages within the landscape (Menge et al., 2023). Our results also show that soil microclimate can be managed through the manipulation of forest density and the share of broadleaves at a scale of 6–9 m in radius (Table S8). These results could be highly relevant for large-scale conversions of conifer-dominated stands to broadleaf or mixed-broadleaf stands, as well as for conservation purposes. Baker et al. (2013) hypothesized that the re-establishment of species in harvested forest areas is in part influenced by microclimate, and distance to the forest edge, which in turn shapes temperature buffering. This was hypothesized to be especially important for vascular plants, bryophytes, lichens, amphibians and reptiles. Therefore, elucidating at what scales microclimate works opens a new door to microclimate management and microclimate restoration, which enables us to target specific microclimatic goals of expected benefit to a given set of species.

Our results were less clear with respect to air temperature, as we were often unable to identify the scale at which understory air microclimate operates. This was because the optimum distance selected by the models was at the limit of what we quantified in the field. This limitation applied to both the analyses that included all sites except B (NS, MS, SS and G; where trees were measured at 10 m; Table S8) and the analyses confined to site B (where trees were measured at 20 m; Table S9). We suggest that the key underlying difference is that soil temperature is more dependent on local radiation, whereas local air temperature is affected by air mixing from a larger surrounding area exposed to greater variation in radiation. Air mixing is also most likely the cause of temperature offsets reducing in size with increasing wind speed. In either regard, more research is needed to clarify the scale at which overstory structure affects understory microclimates. We also emphasize that the forest canopy metrics assessed here capture only a subset of potential influences on understory microclimates. In fact, Hedwall et al. (2019b) showed that the optimal plot size at which forest structure had the strongest influence on understory vegetation was well below 10 m. This contrast between the scale at which forest structure alters temperature (our results), and the scale at which forest structure influences understory vegetation (Hedwall et al., 2019b) would suggest that there are additional microclimatic or ecological factors affecting vegetation community structure at these scales (e.g. light availability; Tinya and Ódor, 2016). For example, in our forest stands an understory shrub layer was generally lacking, but in forests with high shrub cover, their presence can decrease air mixing and therefore reduce the scale at which the microclimate operates. In addition, tree crown dimensions and the height of the canopy itself may play an important role dictating microclimatic scales of influence (Souza et al., 2010; Ehbrecht et al., 2019). The taller the trees, the more evenly the radiation is spread, and thus the larger the scale at which the microclimate can be operating. Our site in B had a very high canopy compared to the other sites, but as we did not quantify this, we cannot draw any conclusions on the effect of this parameter on the scale at which microclimate operates in our sites. Additionally, soil characteristics may also be heterogeneous, and influence soil microclimate correspondingly. For instance, soil moisture is related to temperature differences between open and forested areas (von Arx et al., 2013). In our case, however, we do not expect large differences among our plots, as soil temperature loggers were only buried a few centimeters into the humus layer. In addition, the choice of forest overstory species may affect soil composition differently (e.g. by altering characteristics of the soil organic layer), and thereby change soil microclimate. Such effects were however beyond the scope of our study (Fekete et al., 2016). In conclusion, our results show that it is possible to make predictions of understory temperatures in scenarios where both forest structure and macroclimate are dynamic. This provides an opportunity to include these variables in forest management decision support systems to exploit the potential of forests to act as climatic refugia, and thereby mitigate potentially negative effects of climate change. Importantly however, our results also indicate that macroclimatic drivers like precipitation and wind may affect the buffering capacity of forest overstories, which has implications for the temporal and spatial consistency of these refugia in a changing climate. Moreover, our results can be considered in forest restoration plans and when choosing tree species for afforestation programs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2023.109684.

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The boreal biome, the world's largest contiguous forested region, faces significant changes due to rising global temperatures. These changes affect the interactions between trees, understory plants, and their pollinators, impacting essential ecosystem services. This thesis aims to enhance the understanding of the interplay between forest structure, microclimate, and pollinators. My findings highlight the crucial roles of forest density and overstory composition in regulating microclimates and influencing pollinator activity and diversity, emphasizing the need for further research to develop effective forest management strategies.

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