

TREE DIVERSITY AS A DRIVER OF RESOURCE HETEROGENEITY AND ITS IMPACT ON AN UNDERSTOREY PLANT SPECIES

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DANKWOORD

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SUMMARY

Forests provide crucial ecosystem services to humanity and a habitat for many species. The identity of tree species is an important determinant of ecosystem functioning since trait differences between tree species cause species-specific impacts on the environment. The research field of Biodiversity – Ecosystem functioning (B-EF) found that most ecosystem functions are positively impacted by higher tree diversity, most notoriously, more diverse forests have a higher and more stable primary productivity than expected based on monoculture performance. Since tree species differentially affect resource conditions, a high tree diversity is often expected to create higher resource heterogeneity. This environmental heterogeneity is then anticipated to create a high niche diversity which enables coexistence and a high local diversity of associated species. This points at a win-win situation where high tree diversity leads simultaneously to high ecosystem functioning and high diversity of associated species. This PhD-thesis investigated to which degree tree diversity is able to drive diversity of understorey forest plants. In chapter 2 & 3 we studied how light and nutrient heterogeneity is affected by tree species diversity. In chapter 4 & 5 we determined which environmental factors shape understorey plant fitness, which is a first step in understanding how species could coexist. More specifically we evaluated the effect of light, nutrients and seed predation, either directly or indirectly via flowering phenology, on the fitness of our study species, *Geum urbanum*.

Light and soil nutrients are key resources for plant growth. Light is limited at the forest floor after leaf expansion in spring, due to light interception from the tree and shrub canopy. Nutrient availability at the forest floor and topsoil is driven by the quality and quantity of leaf litterfall. The research chapters 2 & 3 were performed in the TREEWEB project (fig. 2). The platform consists of 53 plots consisting of six to eight replicates of each of the seven possible species combination of the three focal tree species *Quercus robur*, *Quercus rubra* and *Fagus sylvatica*. These focal tree species differ in crown properties such as crown shape and density and therefore we expect differences in the quantity and heterogeneity of light they transmit. There is also interspecific variation in the chemical composition of the tree leaves and therefore we expect differences in the chemical properties of the leaf litterfall, forest floor and to a lesser extent topsoil. In contrast to the general assumption that tree diversity is positively related to heterogeneity, we expected decreases in both the quantity and spatial heterogeneity of light transmittance in mixed stands relative to

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monocultures, due to crown complementarity effects and niche filling. For nutrient availability in the litterfall, forest floor and topsoil we expected higher heterogeneity in more diverse plots since limited knowledge is available on the effect of tree mixtures on heterogeneity of nutrients. We additionally looked into the effect of spatial clustering on heterogeneity of nutrient availability.

In chapter 2, we quantified light transmittance to the forest floor with hemispherical photography. We tested the degree to which tree species identity and diversity affected, via differences in tree and shrub cover, the spatiotemporal variation in light availability before, during, and after leaf expansion.

Increased tree diversity led to increased canopy packing and decreased spatial light heterogeneity at the forest floor in all of the time periods. During leaf expansion, light transmittance did differ between the different tree species and timing of leaf expansion might thus be an important source of variation in light regimes for understory plant species. Although light transmittance at the canopy level after leaf expansion was not measured directly, it most likely differed between tree species and decreased in mixtures due to canopy packing. A complementary shrub layer led, however, to similar light levels at the forest floor in all species combinations in our plots. Light availability at the forest floor is thus ultimately determined by the combined effect of the tree and shrub layer.

In chapter 3 we derived spatial heterogeneity of key characteristics of the leaf litterfall, the forest floor and the mineral topsoil (C, N and base cation concentration, C:N ratio and mass) at three locations within each of the 53 plots. We found that spatial heterogeneity increased from the leaf litterfall, through the forest floor down to the mineral topsoil. Mixing tree species did not lead to an increased heterogeneity in the forest floor and topsoil compared to monocultures. However, we did find that mixed plots where trees are clustered per species are more heterogeneous than plots where species are intimately mixed.

The results from chapter 2 & 3 demonstrate that variability of soil nutrients and light availability did not increase with tree diversity. This implies that plots with high tree diversity are not likely to support higher levels of associated biodiversity via light or soil nutrients than monocultures. We found that, for nutrient availability within mixed stands, the spatial organization of tree species mattered for the within-plot heterogeneity and should be considered in relation to the scale at which heterogeneity is desired.

In the second part of this PhD we wanted to gain a better understanding of the environmental factors and spatial variability therein as drivers of understorey plant diversity. Testing which environmental factors impact reproductive fitness is a first step in evaluating its role in species coexistence. Since correct timing of the different life history events is essential, we expected that flowering phenology would be an essential trait mediating the effect of environmental factors on reproductive performance.

We used the forest understorey plant *Geum urbanum* as a study species and monitored during four consecutive years the phenology, predation rate and reproductive fitness of more than 60 individuals spread over ten locations with different environmental conditions in the Aelmoeseneie forest. In chapter 4, we explored the relative importance of the direct and indirect effects of the abiotic environment, plant performance and phenology on reproductive output with structural equation models. In chapter 5, we studied the effect of pre-dispersal seed predation by the beetle *Byturus ochraceus*, a specialist seed herbivore, on the flowering phenology of *Geum urbanum*. We tested the hypotheses that pre-dispersal seed predation exerts a selection pressure leading to within-season compensatory flowering as well as to phenological avoidance in the succeeding season.

We found that flowering within *Geum urbanum* individuals was spread over a long period from June to October. Reproductive output of individual flowers, measured as total seed mass per flower, declined during the season, most likely because of a lack of resources. Reproductive output at the plant level was higher in larger plants and was positively correlated with light availability and soil nutrient availability. Variation in phenology between plants was partially driven by environmental factors and plant size, but did not impact the reproductive output. We found no indication that seed predators induced compensatory flowering within a growing season, but plants that experienced predation shifted their flowers to the end of the flowering season the subsequent year. We suggest that this induced delayed phenological avoidance is an adaptive plastic response to pre-dispersal seed predation.

In conclusion, this PhD-thesis showed that an intimate mixture of tree species does not necessarily result in a higher light and nutrient heterogeneity at the level of the forest understorey. While a more clustered spatial configuration of tree species led to higher resource heterogeneity, variation in resource availability appeared to be mainly driven by other factors than tree species identity. We believe that consideration of the spatial scale of heterogeneity should be an important aspect of future studies about resource heterogeneity in forests. In the second part of the PhD-

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thesis we found that the variation in light, nutrient and seed predation available in our study did not lead to different ecological selection pressures since plant fitness seemed to be little impacted by these variables. Moreover, we identified other factors such as plant age which might be important determinants of plant fitness. Within the same study system we provided evidence for induced delayed phenological avoidance as an adaptive plastic response to pre-dispersal seed predation. Seed predation seems to be an evolutionary selection pressure for plasticity in flowering phenology in *Geum urbanum*.

SAMENVATTING

Bossen voorzien de samenleving van essentiële ecosysteemdiensten en zijn een habitat voor veel soorten. De identiteit van boomsoorten is een belangrijke sturende factor van ecosysteemfuncties aangezien eigenschappen verschillen tussen boomsoorten en er dus soortspecifieke effecten zijn op de omgeving. Onderzoek binnen het onderzoeks domein “Biodiversiteit – Ecosysteem functioneren” toonde aan dat de meeste ecosysteemfuncties positief beïnvloed worden door een hogere boomsoortdiversiteit. Het best gekende effect is de hogere en stabielere primaire productie in diverse bossen dan verwacht op basis van de productie in monoculturen. Aangezien boomsoorten de beschikbaarheid van natuurlijke hulpbronnen op een verschillende manier beïnvloeden wordt vaak verwacht dat een hogere boomsoortdiversiteit tot een hogere heterogeniteit van hulpbronnen zal leiden. We verwachten dat deze omgevingsheterogeniteit op zijn beurt een hoge verscheidenheid aan niches veroorzaakt die toelaat dat soorten lokaal kunnen samenleven en er een hoge diversiteit van geassocieerde soorten bereikt wordt. In dat geval zou er een win-winsituatie zijn waarbij hoge boomsoortdiversiteit zowel een positief effect heeft op ecosysteemfunctioneren als tot een hoge diversiteit van geassocieerde soorten leidt. In deze doctoraatsverhandeling onderzoeken we in welke mate boomsoortdiversiteit de diversiteit van bosplanten in de ondergroei kan bepalen. In hoofdstuk 2 & 3 bestuderen we hoe licht en bodemvoedingsstoffen door de boomsoortdiversiteit worden beïnvloed. In hoofdstuk 4 & 5 bepalen we welke omgevingsvariabelen de fitness van planten in de ondergroei bepalen, wat een eerste stap is in het begrijpen hoe soorten kunnen samenleven. Meer specifiek evalueren we het directe effect, en indirecte effect via bloeifysiologie, van licht, voedingsstoffen en zaadpredatie op de fitness van onze studiesoort, geel nagelkruid (*Geum urbanum*).

Licht en voedingsstoffen in de bodem zijn essentiële natuurlijke hulpbronnen voor plantengroei. De lichttoevoer op het niveau van de bosvloer is beperkt na de bladontplooiing in de lente doordat het licht wordt tegengehouden door de boomkruinen en de struiklaag. De beschikbaarheid van voedingsstoffen in de strooisellaag, humuslaag en de bovenste bodemlaag wordt bepaald door de kwaliteit en kwantiteit van de bladval. De onderzoekshoofdstukken 2 & 3 werden uitgevoerd binnen het TREEWEB project. Dit platform bevat 53 onderzoekspcelen (30 m x 30 m) bestaande uit zes tot acht replicaten van elk van de zeven mogelijke combinaties van de drie onderzochte boomsoorten, zomereik (*Quercus robur*), Amerikaanse eik (*Quercus rubra*) en beuk (*Fagus sylvatica*). De kruin van deze drie boomsoorten

verschilt in vorm en geslotenheid waardoor we verwachten dat de hoeveelheid en heterogeniteit van het doorgelaten licht verschilt tussen soorten. De chemische eigenschappen van de bladen verschilt ook tussen boomsoorten, waardoor we verschillen verwachten in de strooisellaag en in mindere mate in de humuslaag en de bovenste bodemlaag. In tegenstelling tot de algemene verwachting dat boomdiversiteit positief gerelateerd is aan heterogeniteit van grondstoffen, verwachtten we dat heterogeniteit van de lichtcondities in gemengde bosbestanden lager is dan in monoculturen. De oorzaak hiervan is dat gemengde bosbestanden een denser kroondak hebben door complementariteit tussen soorten wat zou betekenen dat er gemiddeld minder licht tot op de bosvloer doordringt. We verwachten wel een hogere heterogeniteit van voedingsstoffen in de strooisellaag, de humuslaag en de bodem ondanks dat er weinig geweten is over het effect van boomsoortmengingen op de variatie in voedingsstoffen. Daarnaast bekeken we hoe de ruimtelijke clustering van boomsoorten de heterogeniteit van voedingsstoffen beïnvloedde.

In hoofdstuk 2 werd de lichthoeveelheid op de bosbodem bepaald via hemisferische fotografie. We testten in welke mate boomsoortidentiteit en – diversiteit de ruimtelijke variatie in lichtbeschikbaarheid voor, tijdens en na de bladontplooiing bepaalde via verschillen in bedekking van de struiklaag en de boomkruinen. Een hogere boomsoortdiversiteit leidde tot een hogere samenpakking van de boomkruinen en een lagere heterogeniteit aan lichtcondities op de bosvloer in alle tijdsperiodes. Tijdens de bladontplooiing verschilde de lichtpenetratie tussen monoculturen van de verschillende boomsoorten. Het moment van bladontplooiing is daarbij mogelijk een belangrijke bron van variatie in lichtregimes voor planten in de ondergroei. Ondanks het feit dat we geen rechtstreekse meting hebben van de lichtpenetratie op het niveau van de boomkruinen, kunnen we aannemen dat lichtpenetratie verschilde tussen boomsoorten door de verschillen in de samenpakking van de boomkruinen die we observeerden. Een complementaire struiklaag leidde echter tot een gelijkaardige lichthoeveelheid op de bosvloer in alle boomsoortcombinaties. Lichtbeschikbaarheid op de bosvloer wordt dus bepaald door het gecombineerde effect van de boom en de struiklaag.

In hoofdstuk 3 werd de heterogeniteit van chemische kenmerken (C, N en base kation concentratie, C:N ratio en massa) van de strooisellaag, de humuslaag en de minerale bodem op basis van metingen op drie locaties binnen elk van de 53 onderzoekspcelen berekend. We vonden dat ruimtelijke heterogeniteit steeg naarmate we afdaalden in de bosvloerlagen van strooisellaag via humuslaag naar de minerale bodem. De voedingsstoffen in de humuslaag en de bodem in gemengde bosbestanden was niet heterogener dan in de monoculturen. We vonden echter dat

gemengde bosbestanden waar bomen geclusterd stonden per soort een hogere heterogeniteit vertoonden dan bosbestanden waar de soorten intiem gemixt waren.

De resultaten van hoofdstuk 2 & 3 toonden aan dat variatie in voedingsstoffen en lichtbeschikbaarheid niet hoger werd met stijgende boomsoortdiversiteit. Dit betekent dat percelen met een hoge boomdiversiteit waarschijnlijk niet tot hogere diversiteit van geassocieerde soorten leidt via licht en bodemvoedingsstoffen dan monoculturen. We vonden dat heterogeniteit in de beschikbaarheid van voedingsstoffen in gemengde bosbestanden afhankelijk was van de ruimtelijke structuur van de boomsoorten. Ruimtelijke structuur van boomsoorten moet bekeken worden in relatie tot de ruimtelijke schaal waarop heterogeniteit gewenst is.

In het tweede deel van deze doctoraatsverhandeling wilden we beter begrijpen hoe omgevingsfactoren, en de ruimtelijke variatie ervan, bosplantdiversiteit kan sturen. Een eerste stap in de evaluatie van het belang van omgevingsfactoren voor het samenleven van soorten is testen welke omgevingsvariabelen het voortplantingssucces beïnvloeden. Aangezien een correcte timing van de verschillende levensstadia en in het bijzonder het bloeitijdstip verwachtten we dat de bloeifenologie een essentiële eigenschap zou zijn die tussenkomt in de effecten van omgevingsvariabelen op het voortplantingssucces.

We gebruikten de bosplant 'geel nagelkruid' (*Geum urbanum*) als studiesoort en volgden gedurende 4 opeenvolgende jaren de fenologie, zaadpredatie en het voortplantingssucces van meer dan 60 individuen verspreid over 10 locaties met uiteenlopende omgevingscondities in het Aelmoeseneiebos op. In hoofdstuk 4 onderzochten we het relatieve belang van directe en indirecte effecten van abiotische variabelen (licht en voedingsstoffen), plantgrootte en fenologie op de zaadproductie met 'structural equation' modellen. In hoofdstuk 5 bestudeerden we het effect van predispersie zaadpredatie door een kever, *Byturus ochraceus*, op de bloeifenologie van geel nagelkruid. We testten de hypothese dat predispersie zaadpredatie een selectiedruk uitoefent die leidt tot compensatorische bloei binnen een seizoen en tot fenologische ontwijking in het daaropvolgende groeiseizoen.

We vonden dat de bloei binnen geel nagelkruid planten gespreid was over een lange periode van juni tot oktober. Voortplantingssucces van individuele bloemen, gemeten als het totale zaadgewicht per bloem, nam af gedurende het bloeiseizoen, hoogstwaarschijnlijk door een gebrek aan natuurlijke hulpbronnen later in het seizoen. Zaadproductie op plantniveau was hoger in grote planten en was positief

gecorrleerd met beschikbaarheid van licht en voedingsstoffen in de bodem. Variatie in fenologie tussen planten was deels gestuurd door omgevingsvariabelen en plantgrootte maar had geen impact op zaadproductie. We hebben geen indicatie gevonden dat zaadpredatoren voor compensatiebloei zorgden binnen hetzelfde groeiseizoen maar planten die gepredeerd werden bloeiden gemiddeld later tijdens het bloeiseizoen van het jaar volgend op de predatie. We suggereren dat deze 'geïnduceerde uitgestelde fenologische ontwikkeling' een adaptieve plastische respons is op predispersie zaadpredatie.

We kunnen uit deze PhD thesis concluderen dat een intieme mix van boomsoorten niet noodzakelijk leidt tot een hogere heterogeniteit van licht en voedingsstoffen op het niveau van de kruidlaag. Alhoewel meer gegroepeerde ruimtelijke configuraties van boomsoorten leidden tot een hogere heterogeniteit van grondstoffen was variatie in grondstofbeschikbaarheid blijkbaar voornamelijk gestuurd door andere factoren dan boomsoortidentiteit. We geloven dat het in volgende studies over heterogeniteit van grondstoffen in bossen belangrijk zal zijn om de ruimtelijke schaal van heterogeniteit in acht te nemen. In het tweede deel van de doctoraatsverhandeling vonden we dat de beschikbare variatie in licht, voedingsstoffen en zaadpredatie in onze studie niet leidde tot verschillen in ecologische selectiedruk aangezien voortplantingssucces weinig beïnvloed leek door deze variabelen. Mogelijks zijn er andere factoren zoals de leeftijd van de plant die belangrijk voor het bepalen van voortplantingssucces. Binnen hetzelfde studiesysteem vonden we bewijs voor geïnduceerde uitgestelde fenologische ontwikkeling als een adaptieve plastische respons op predispersie zaadpredatie. Zaadpredatie lijkt een evolutionaire selectiedruk te zijn die zorgt voor plasticiteit in bloeifenologie in geel nagelkruid.

CHAPTER 1 – GENERAL INTRODUCTION

BRAM K. SERCU

1.1 FOREST DIVERSITY AND FUNCTIONING

The human impact on ecosystems is so profound that we as human species are well on our way to irreversibly move the hospitable environment, in which we originated, outside the ‘safe operating space for humanity’ (Steffen et al. 2015). We have an especially large impact on biodiversity. The current extinction rate is manifold the background extinction rate and literature suggests that a 6th mass extinction event in the history of the planet earth is underway (Ceballos et al. 2015). The loss of biodiversity has profound consequences since biodiversity is considered as an important driver for the functioning of ecosystems both on a local and global scale (Chapin et al. 2000, Mace et al. 2014). The major direct drivers of biodiversity loss are land-use change, climate change, pollution (especially nitrogen and phosphorus), overexploitation and invasive species. Among these, the most important driver of biodiversity loss and associated ecosystem services is land-use change, mainly conversion to and intensification of agriculture and forestry at the expense of natural habitats or traditional land use (Duraiappah et al. 2005, IPBES 2018, Newbold et al. 2015).

Loss of quantity and quality of habitat was especially severe in forest ecosystems in western Europe. Millennia of human interference, especially during the industrial revolution, reduced the total forested area, to the current cover of 35 – 40% and led to fragmentation and isolation of forest fragments (Bastrup-Birk et al. 2016). From the remaining forests in Central-Europe, only 0.2% was classified by Hannah et al. (1995) as ‘undisturbed’ or primary forest. More than 95% of Europe’s forests are managed and modified by human activities, and more than 80% of European forests are managed for production purposes with potential of timber extraction (Bastrup-Birk et al. 2016). While conservation of the still remaining natural fragments (primary and old-growth forests) should be a priority of governments worldwide, the restoration and management of secondary habitats (newly planted forests, production forests, managed forests) will be an essential element in the safeguarding of a diverse and functional ecosystem locally and globally (Perring et al. 2018). Afforestation and adapted forest management can be important tools in the provisioning of ecosystem services and safeguarding forest biodiversity, when done correctly (Paquette & Messier 2010). The question ‘how to restore and manage forest habitats?’ is thus a pertinent one. Of course, the answer depends on the goal

of the management. The framework of ecosystem services that has been developed in the past decades is a helpful tool to identify the different services that an ecosystem is expected to deliver (Brockerhoff et al. 2017). Often, the same habitat patch can and is expected to deliver several services simultaneous to a diverse group of stakeholders (Allan et al. 2015), e.g. 30% of European forests are managed as multiple-use forest (Bastrup-Birk et al. 2016). A good understanding of the functioning of ecosystems is essential to purposefully restore or manage a habitat in order to deliver a predetermined set of services to society.

One of the key attributes of a forest stand, is the diversity and composition of tree species. Trees can be considered as ecosystem engineers, because their tall physical appearance provides a habitat for many species and has a large impact on the abiotic conditions below the canopy (Jones et al. 1997). Tree species differ in traits and the way they affect ecosystem functions (Leuschner & Meier 2018), e.g. light transmission (Canham et al. 1994), chemical composition of leaves, Carbon, Nitrogen, Phosphorus and base-cations content in the litter layer (Carnol & Bazgir 2013) and mineral topsoil (Vesterdal et al. 2008, Finzi et al. 1998, Augusto et al. 2002). Tree species diversity and composition is therefore considered as one of the driving factors behind ecosystem functioning (Ratcliffe et al. 2017) and the diversity of associated species, e.g. herb and shrub layer (Augusto et al. 2003), leaf herbivores (Claridge & Wilson 1982) and birds and bats (Charbonnier et al. 2016). The research field of Biodiversity – ecosystem functioning (B-EF) aims at unraveling the relationship between species diversity and ecosystem functioning. Many studies find that more diverse system have a higher and more stable primary production than expected based on the performance of their respective monoculture (Tilman et al. 2014). This positive relationship between diversity and ecosystem functioning is found across many systems (grasslands, microbes, fresh water, forests) and for several functions (Balvanera et al. 2006, Allan et al. 2013). Moreover diverse systems are better at delivering multiple functions simultaneously i.e. a ‘higher ecosystem multifunctionality’ (Van Der Plas et al. 2017, Gamfeldt et al. 2013, Hector & Bagchi 2007) but see (Gamfeldt & Roger 2017). Srivastava & Vellend (2005) point out that the results of B-EF research are most relevant and applicable in humanly managed forests and forest restoration where managers decide on the tree species that will be planted. Based on the results of B-EF research, planting diverse forest stands seems advisable both for forests with production purpose (80% of European forests) and forest with multiple use function (30% of European forests) (Brockerhoff et al. 2017, Paquette & Messier 2010).

From a biodiversity conservation point of view this seems a win-win situation where diverse forests are also best at delivering ecosystem functions. As (Tilman et al. 2014) writes in his paper reviewing the history and current state of the B-EF research field: “it was the concern about biodiversity loss that revived the interest to study the effect of biodiversity on ecosystem functioning and ecosystem services”. However, trees are only a very limited set of all species in a forest food web. The relationship between tree diversity and other organism groups varies across taxa, geographic regions and studies. Species richness of arthropods across all trophic levels was well predicted by that of woody plants in the tropics (Basset et al. 2012). Also in sub-tropic forests in Asia woody plant diversity was a good predictor for diversity of other species groups across spatial scales although relationships were highly non-linear relationships across taxa (Schuldt et al. 2015). Some B-EF studies found positive relationships between tree diversity and herb diversity (Gamfeldt et al. 2013) , however, other studies found no such effect (Ampoorter et al. 2016, Ampoorter et al. 2014, Gazol & Ibáñez 2009, Ewald 2002, Houle 2007, Thomsen et al. 2005, Both et al. 2011). A mechanistic understanding of the effect of tree diversity on the diversity of associated species is essential to maximize the win-win situation in forest restoration and conservation projects.

1.2 POTENTIAL MECHANISMS BEHIND THE POSITIVE TREE DIVERSITY – ASSOCIATED SPECIES DIVERSITY RELATIONSHIP.

Questions about patterns and mechanisms of species diversity are essentially research topics within community ecology. To identify potential mechanisms behind the relationship between tree diversity and diversity of other organism groups, we searched for relevant information within the field of community ecology. We adhere to the theoretical framework of Vellend (2010) who defines four high level processes that play a role in community composition. The framework of Vellend brings structure to the messy field of community ecology and encompasses all possible processes that drive community composition. This will help to summarize and classify the processes that are supposed to drive the tree diversity – associated species diversity relationship and identify processes that are ignored or undervalued. In analogy to the modern Evolutionary Synthesis, processes in community ecology fall in one of the four categories: selection, drift, speciation and dispersal. Speciation and dispersal are the two ways to gain species in a community while selection and drift are the two processes to lose species. **Speciation** is mostly relevant on large spatial and temporal scales and can be safely ignored in our context. **Dispersal** is the movement of organisms from one place of residence to another. **Ecological drift** occurs when demographic events occur at random with respect to the species identity or traits, ecological drift does not require an assumption of neutrality— that is, an assumption that individuals of different species are precisely equivalent demographically. However, the influence of drift on community dynamics is greatest and easiest to think through in the purely neutral scenario. Finally, **ecological selection** results from deterministic fitness differences between individuals of different species. Ecological selection consists in three relevant forms according to Vellend (2010). a) Constant selection is equivalent to the principle of competitive exclusion, which states that stable coexistence between competing species requires them to occupy different niches and remains one of the most fundamental principles in ecology today Chase & Leibold (2003, 2010). Constant selection occurs if relative fitness is constant in space and time. Independent of species' densities but variable across species, the species with the highest fitness will exclude all others. b) Density dependent selection is more complex and occurs when individual fitness in a given species depends at least in part on the density of that species, as well as the densities of other species. For two species, negative density-dependent selection favors species when they are at low density and is thus capable of maintaining stable coexistence, whereas positive density-dependent selection favors species at high density and cannot maintain stable coexistence. c) Selection, whether constant or

density-dependent, may vary over space (or time). Models accounting for spatiotemporal variation in selection where relative fitness of species changes over space or time can allow for coexistence of species that is otherwise not possible (Vellend 2010, Chesson 2000a, Fox 2013). In addition we want to point out that ecological selection, in contrast to evolutionary selection, applies to species in a community rather than to alleles in a species' population. The phenotype of interest, which may be under selection, is most often just the species identity (Vellend 2016).

We reviewed a selection of the literature to find out which of the four high level processes were mentioned in B-EF articles and other articles investigating the effect of tree diversity on diversity of associated groups. While generally a positive effect of tree diversity on diversity of other organism groups is expected, discussions of the potential mechanisms behind the relationship between tree diversity and associated diversity are rather scarce and discuss low level processes. This makes it hard to summarize the literature and leaves room for interpretation when classifying the low-level processes in one of the high-level processes of Vellend's framework. Nevertheless, based on the selection of literature that was investigated, almost all mechanisms that are discussed can be classified as ecological selection processes. Essentially, the explanation of the relationship between tree diversity and diversity of other groups seems to be that tree diversity creates a heterogeneous patchwork of biotic and abiotic environmental conditions, i.e. high variation in niches, leading to species coexistence in the area of the research plot.

The components of the selection process within forest B-EF research can be summarized as follows 1) tree species differentially impact the environmental variables on the scale of a research plot, 2) associated species differ in their requirements, 3) the environmental variables that are affected by trees are important variables determining the fitness of the species within the group under study. In other words, it is expected that, within a research plot, high tree diversity creates high environmental heterogeneity which enables coexistence and a high local diversity of associated species. Understanding how the heterogeneity of resources is affected by tree diversity is an important step in the mechanistic understanding of the positive relationship between tree diversity and diversity of associated species. Almost all B-EF studies measure and report mean values for environmental conditions and ecosystem functions, but they fail to quantify and discuss how the variance of environmental conditions is impacted by tree identity and diversity.

1.3 DOES TREE DIVERSITY INDUCE RESOURCE DIVERSITY AT THE FOREST FLOOR?

This section aims to review the evidence that tree diversity can induce environmental heterogeneity on a small spatial scale. The selection process can be readily understood for aboveground herbivores in the tree canopy where coexisting species exhibit different diet or foraging patterns. The tree canopy constitutes a diverse food source with different nutritional quality and species-specific toxic compounds (Bennett & Wallsgrave 1994, Lämke & Unsicker 2018, Wright et al. 2004) enabling different species to thrive on the different trees. A higher tree diversity in a plot constitutes a higher heterogeneity of food sources for herbivorous animals. For example, specialist herbivores such as leafminers and leaf-hoppers will occur in a plot only when their host species is present. A mixed species plot will therefore have approximately the sum of the specialist herbivores occurring on each constituent species (Claridge & Wilson 1982). However also in cases where associated species are less specialized, trait differences between trees will lead to a specific set of species that coexist on each tree species and a higher diversity in diverse forests (Stamps & Linit 1997, Futuyma & Agrawal 2009, Castagneyrol & Jactel 2012).

Since trees have species-specific impacts on environmental conditions at the forest floor, it seems likely that a high tree diversity in a stand will cause high environmental heterogeneity. Studies about soil microbial community, earthworm community, herb community etc. expect that diversity and composition of these organism groups is influenced by tree diversity via litter composition and root exudates, however, the results did not always confirm the expectations. (Ampoorter et al. 2015, De Wandeler et al. 2018, Thoms et al. 2010, Schwarz et al. 2015). The conditions at the forest floor do not necessarily show the same spatial pattern as the aboveground canopy. Light and nutrient conditions at a certain spot at the forest floor are not necessarily determined by only one species. The amount of light will be determined by the combined interception of the surrounding trees and nutrient conditions and the litter layer may consists of a mix of leaves of the surrounding trees (Jonard et al. 2006, Rothe & Binkley 2001). Therefore, a diverse forest where species are intermixed might not just be the small-scale equivalent of different adjacent monocultures, instead, positive or negative interaction effects are likely to arise.

Before discussing the specific patterns of heterogeneity for light and soil nutrients in mixed forests, let me break down the hypothesis about the way heterogeneity in mixtures originates (fig. 1.1). First, assume that every spot at the forest floor is

influenced by purely one species (fig. 1.1 a), that is, the value of every spot is a random draw from the monoculture distribution of one of the species (monospecific effect scenario) (fig. 1.1 d). In this scenario, heterogeneity in a mixture is determined by the distance between the mean values of the composing monocultures and by the variability within each monoculture. If the mean value of two species are the same, variability of the mixture will be intermediate between the variability of the two composing species. If the mean values of two species are very different then variability will mainly be determined by the distance between the means of the composing species. Second, if we assume that a spot at the forest floor can be under influence of different species, e.g. litter contains leaves of different species, but effects are additive, the value of every spot will be the sum of the proportional effect of a random value drawn from the composing species distributions (additive effect scenario; fig 1.1 b & e). The variability in this scenario will depend on the level of relative influence of the species. In the extreme case where every spot on the forest floor is equally influenced by every composing species (e.g. in a two species mixture, every spot on the forest floor contains 50% leaves of each species; fig 1.1 c & f), the variability will be a function of the variability of the composing species. Values for variability will thus lie somewhere in between this and the monospecific influence scenario. Third, if we consider that effects are not purely additive, but interaction effects might occur, variability could deviate from that expected from the purely additive scenario and be either lower or higher (diversity effect scenario). We can conclude that spatial position, the extent of the influence of individual trees and interactions between species will combinedly determine heterogeneity of resources.

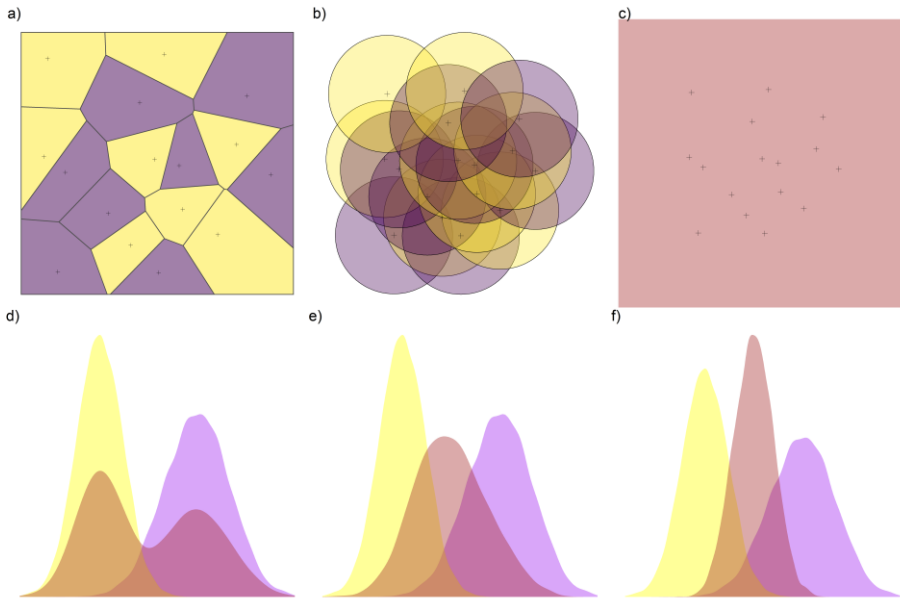


Fig1.1 - The relative influence of trees (crosses) in a mixed species plot with two different tree species, each with a different influence on the environment. The top row shows the zone of influence of each tree on its environment. a) is the monospecific effect scenario where every point at the forest floor is influenced by only one species, b) is a scenario where the relative influence of each species varies across the forest floor due to spatial overlapping of the zone of influence of individual trees and c) is a scenario where every point at the forest floor is influenced equally by every species. At the bottom row, the influence of the two tree species on the environment is shown as a distribution, for instance the amount of light that is transmitted to the forest floor (expressed as a percentage of total incident light), sampled at random locations in a forest plot. The yellow and purple curve represent the values from the monocultures of two different species: yellow has a mean = 7% (sd = 0.7) and purple has a mean = 10% (sd = 1). The brown distribution is the distribution of values in the mixed plot. The distribution of the mixed plot is based on purely additive effects (identity effects sensu (Kirwan et al. 2009)) and is derived by randomly drawing values from the monoculture distributions and summing a proportion of each value $p \times \text{yellow} + (1 - p) \times \text{purple}$. Proportions are d) either 1 or 0, e) uniformly distributed values between 0 and 1 and f) 0.5. Mean and standard deviation of the different scenarios are d) $8.50\% \pm 1.73$, e) $8.53\% \pm 1.12$, f) $8.52\% \pm 0.61$. The distribution in case of a diversity effects (sensu (Kirwan et al. 2009)) is not represented here, but would depend on the type and strength of the diversity effect.

Light transmission to the forest floor is characterized by a high temporal and spatial variability. The angle of the sun that changes during the day and over seasons will lead to temporal variability in light transmission within a day and over the seasons. In this PhD thesis we are, however, mainly interested in the spatial heterogeneity of the total light transmission over a certain period (e.g. total light transmission during the period from total canopy closure in May until leaf senescence in October). Spatial variability will be driven by the spatial configuration of canopies and canopy gaps and the properties of the light interception of the tree species. The monospecific effect scenario described in the previous paragraph is per definition impossible due to the nature of light transmission in forests. During the sun's daily trajectory over the sky, the irradiation will be intercepted by several of the surrounding trees. In mixed species plots, light transmission to a certain point on the forest floor will therefore always be impacted by several tree species. The differences in light transmission between tree species (Ellenberg 1988, Leuschner & Meier 2018), might lead to more heterogeneous conditions at the forest floor in mixed forests compared with monocultures. However, we do not expect that the effect of the different tree species on light transmission will be purely additive, since diversity effects are likely to arise. We know from B-EF research in forests that mixed plots more fully exploit the incident light due to different and complementary crown morphology which leads to a more tightly packed canopy (Williams et al. 2017, Jucker et al. 2015). This reduction in mean light conditions might reduce heterogeneity of light transmission in more diverse plots beyond the additive species effects, which is a negative diversity effect.

A similar set of expectations can be formulated for soil nutrients. Since chemical composition and abundance of leaf litter differs between tree species (Leuschner & Meier 2018), one could expect a higher heterogeneity of soil nutrients across the different soil horizons in tree mixtures compared with monocultures. To be more specific, when only additive effects are considered, one could expect heterogeneity values somewhere between the monospecific effect scenario and the scenario where there is a homogeneous distribution of litter of every species across the plot (fig 1b). The spatial distribution of leaf litter of different species across the plot would be the main determinant. Straight under a certain tree, the litter layer might be composed of almost 100% leaves of one species but intimate mixing will most likely lead to mixed leaf litter at the forest floor, since leaves do not fall straight to the ground (Jonard et al. 2006, Rothe & Binkley 2001). However, based on the current literature, it is difficult to make clear predictions about how mixed leaf litter will

affect nutrient conditions in the forest floor layer and the topsoil. Diversity effect leading to nutrient conditions that are higher or lower than expected from the monocultures could arise. The quantity of leaf litter might increase due to canopy packing and higher leaf production in mixed plots (Jucker et al. 2015). In addition, the chemical and nutritional properties of leaves of a certain tree species might depend on the identity and diversity of neighborhood trees (Nickmans et al. 2015). Finally, decomposer community and decomposition rate can differ from expectations based on additive effects (Jonard et al. 2008, Joly et al. 2017, Setiawan et al. 2016, Hättenschwiler et al. 2005). Due to the complexity of these diversity effects we have no well-grounded expectations about the effect of tree diversity on heterogeneity of nutrient conditions in the leaf litterfall, the forest floor and the topsoil.

Clearly, while there are good reasons to assume higher niche diversity at the forest floor in mixed species plots compared with monocultures, this is not always the case and hardly any study aimed at testing this basic assumption in forest ecosystems at the scale of the local research plot. Most studies are focused on mean values, and although this information is valuable, in the face of diversity relationships, variability or heterogeneity at the plot-level is the most important metric. These observations and expectations form the basis of **Chapters 2 and 3** of this work, where we investigated the effect of tree identity and diversity on the heterogeneity of light and nutrients.

1.4 DRIVERS OF PLANT FITNESS

A positive relationship between tree diversity and associated group diversity caused by the ecological selection process not only requires heterogeneous conditions in mixed forests, it also requires that fitness of species changes along the environmental gradients present at the forest floor. In the dual definition of the niche by Chase & Leibold (2003), niche is both the impact of a species on its surrounding as well as the requirements of a species to survive and reproduce. In the previous part of the introduction, we were occupied with the first aspect: the impact of tree species diversity on the creation of heterogeneous environmental conditions. In the following part, we look at the requirements of an understorey species to attain high fitness. Testing which environmental variables impact reproductive fitness is a first step in evaluating their importance in species coexistence (Chase & Leibold 2003).

To narrow the scope of the introduction, we will consider the effect of heterogeneous conditions on the fitness of herbaceous forest plant species. Light, nutrients and water are the abiotic resources that are essential for plant growth and reproduction. Since tree species are expected to act differentially upon light and nutrient availability at the forest floor, we specifically focus on the effect of differences in these variables on plant fitness in **chapter 4**. There is high variation in nutrient and light conditions between forest types. Nutrient conditions can vary from relative low (e.g. ancient forest on sandy soils) to very high (post-agricultural forest). One study found that experimental manipulation of Phosphorus and light levels showed increased fitness in some but not all understorey plant species (Baeten et al. 2010a). Plant fitness is not only determined by abiotic niche axes determined by resource availability. Interactions with biota, also at other trophic levels such as herbivorous animals, might impact plant fitness as well. In **chapter 5** we look whether pre-dispersal seed predators have a significant effect on plant reproductive output.

Plants are not merely passive receivers of their environmental conditions. Although they cannot move in space to find optimal conditions for growth and reproduction, they can move in time. Heterogeneity in resource availability might have a temporal component (see box 1) and therefore we hypothesized that flowering phenology is an essential trait mediating the effect of environmental factors on reproductive output. On the level of a population, we expect that the mean phenology of understorey plants is in equilibrium with the mean prevailing growth place conditions in the forest. However, intraspecific trait variation, either via phenotypic

plasticity or genetic variability, might enable plant species to maintain high fitness under different environmental conditions. Accounting for intraspecific trait variation in models of coexistence yields different results compared to models that only include mean trait values (Barabás & D’Andrea 2016, Hart et al. 2016, Clark et al. 2010, Violle et al. 2012, Yamauchi & Miki 2009). In **chapter 4 and 5** we specifically quantify the variation in flowering phenology and its impact on plant reproductive fitness.

BOX 1. Light availability during canopy closure

Light transmission to the forest floor is affected by the density of the tree canopy. Moreover differences in timing of leaf expansion might drastically impact the amount of light plants receive during a growing season. During spring, the photoperiod increases when days lengthen until leaf expansion in the canopy which causes the light transmission to drop to a few percentages of the incoming radiation. Phenological differences in leaf expansion of a few days or weeks can induce significant differences in total light transmission to the forest floor over a season (Augspurger et al. 2005). It is well established that species differ in the amount of light they intercept when the canopy is fully closed (Ellenberg 1988, Leuschner & Meier 2018). However it has never been studied whether phenological differences in leaf expansion induces differences in the total amount of light that is received on the forest floor during the growing season, nor whether this is an important factor determining fitness of herbaceous plants. In almost all studies, light is measured once, after the canopy is fully closed. This value does not take into account the effect of timing of canopy closure however. In the next research chapters, we measured light transmittance throughout the season starting before leaf expansion until leaves were fully expanded. This gives us a detailed dataset enabling us to calculate very precisely how much light was transmitted at a certain point throughout the entire growing season.

1.5 OBJECTIVES AND OUTLINE OF THE THESIS

Increasing tree diversity in production forests and restoration projects is considered as a win-win strategy for the simultaneous delivery of multiple ecosystem services to society while conserving or restoring biodiversity at all levels of the foodweb. The premise of this win-win situation is that high tree diversity leads to high heterogeneity in resources and other niche axes. While this seems to be the case for some trophic groups, e.g. specialist leafminers, it is much less clear how tree composition affects light and nutrient availability at the forest floor, which are essential resources for herbaceous forest species and shapes the habitat of forest floor organisms. Moreover, it is not sure whether differences in these resources at the forest floor are a major driver of plant fitness and species coexistence. Testing whether different environmental conditions (light, nutrient and seed predation) impact forest herb fitness is a possible way to gain insight in this question (Chase & Leibold 2003).

Our overarching objective was twofold: 1) evaluate the effect of tree identity and diversity on the heterogeneity of resources (light and nutrients) on the forest floor, 2) investigate the impact of abiotic (light and nutrients) and biotic (pre-dispersal seed predator) factors on the fitness of a herbaceous forest plant via its flowering phenology.

We tried to answer these two overarching questions in four separate chapters

Chapter 2 evaluates the effect of tree identity and diversity on the mean and heterogeneity of light transmission to the forest floor

Chapter 3 evaluates the effect of tree identity and diversity on the heterogeneity of nutrient content of the leaf litterfall, forest floor and topsoil.

Chapter 4 investigates the impact of light and nutrient availability on the fitness of *G. urbanum*, a herbaceous forest plant, via its flowering phenology.

Chapter 5 investigate the impact of pre-dispersal seed predation on the fitness of *G. urbanum* via its flowering phenology.

In **chapter 6**, the general discussion, we link the results of the different chapters and discuss the results in an integrated manner.

In the **appendix** we concisely present the results of a small additional research project that investigated the hypothesis that late leaf expansion in trees is a means to escape leaf herbivory.

We performed the fieldwork of chapter 2, 3 and the appendix within the TREEWEB platform which investigates the impact of tree diversity and forest fragmentation on the functioning of different trophic levels. The study system consists of fifty three plots with different combinations of three locally important tree species, *Fagus sylvatica*, *Quercus robur* and *Quercus rubra* across a gradient of fragmentation. The set-up of the TREEWEB platform is perfectly suited to study the effect of tree diversity on resource heterogeneity because of its balanced set-up. For every possible species combination (three monocultures, three two-species mixtures and one three-species mixture) 7 or 8 replicate plots were installed. The plots are located in ancient forest fragments with similar soil conditions in a region of 30 x 20 km below Ghent, Belgium. This enabled us to identify light and nutrient conditions under monoculture conditions and evaluate whether conditions in tree species mixtures show additive or interactive effects.

For the research chapters 4 and 5 we performed the reseach in one of the forests where part of the TREEWEB plots are located: the Aelmoeseneie forest in Gontrode, Belgium. The forest consists of stands with different tree species and with different land-use history (ancient and post-agricultural). We attempted to maximize light and nutrient gradients during the selection of populations of our study species. This allowed us to detect the effects of resource conditions on the fitness of our study species in its natural range of occurrence. As a study species we used *Geum urbanum* (Box 2), a forest understorey species whose seeds are subjected to predation by a specialist seed predator *Byturus ochraceus*. Focusing on a single plant species enabled us to investigate in detail the effect of light availability, soil nutrient content and seed predation on the phenology and fitness of *G. urbanum*.

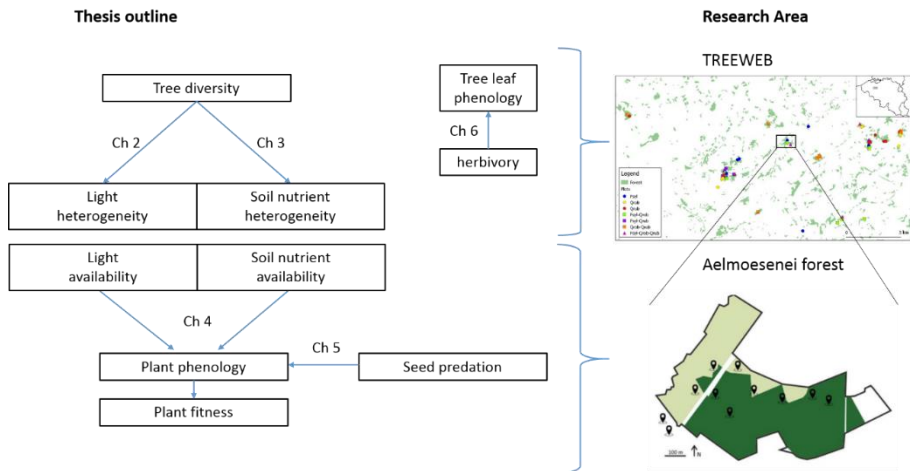


Fig. 1.2 - A conceptual representation of the four main chapters of the thesis with indication of the chapter numbers in the figure. Chapter 1 & 2 are performed in the plots of the TREEWEB exploratory while fieldwork for Chapter 4 & 5 was done in the Aelmoesenei forest, one of the forest of the TREEWEB exploratory. The location of the 53 plots of the TREEWEB project and of the 11 sampled populations of *G. urbanum* in the Aelmoesenei forest are indicated in the maps (See fig SI 1.1 for a larger map of the TREEWEB project). Light and nutrient heterogeneity in chapter 1 & 2 was quantified at the plot level. Soil nutrient availability was quantified at the level of the population and light availability was quantified at the level of the individual plant in chapter 4. Flowering phenology, seed predation and seed output was quantified at the level of each individual flower. For chapter 6, leaf phenology and herbivory was quantified on the level of individual trees.

BOX 2. *Geum urbanum*

Geum urbanum is a perennial semi-rosette hemicryptophyte; basal rosettes overwinter in the vegetative state. Photosynthetic capacity of leaves is highest in winter and early spring, when the overwintering rosette leaves make up the bulk of the leaf area. The species is an early summer bloomer (Kudo et al. 2008) that can take advantage of high levels of irradiance just prior to tree canopy closure through a combination of high photosynthetic capacity, the ability to maintain photosynthesis at relatively low temperatures and the presence of overwintering leaves. Rhizomes, rosette leaves and leaves from

seedlings are moderately frost resistant. (Taylor 1997, Graves 1990). *G. urbanum* has a short rhizome which is little developed but supports the production of new rosette leaves in spring and autumn and flowering stems in March-April. Plants flower every year and flowering takes place from the end of May to October. Within a plant, flowering is generally spread over a longer time and the number of flowers flowering simultaneously is mostly a small proportion of total number of flowers during an entire season of a plant. Inflorescences consist of 2-5 flowers on long stalks in an open cyme. Flowers are mainly self-pollinated and populations show no inbreeding depression (Ruhsam et al. 2010, Vandepitte et al. 2010). A single flower produces c. 100 achenes. Seeds are hairy and have a 5–7-mm long hook, facilitating exozoochorous seed dispersal. Seed is set from July to September, germinates in spring and does not form a persistent seed bank. Plants possess little capacity for vegetative spread so that reproduction by means of seeds germinating is far more important for regeneration (Taylor 1997).



CHAPTER 2 - HOW TREE SPECIES IDENTITY AND DIVERSITY AFFECTS LIGHT TRANSMITTANCE TO THE UNDERSTORY IN MATURE TEMPERATE FORESTS

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2.1 ABSTRACT

Light is a key resource for plant growth and is of particular importance in forest ecosystems, because of the strong vertical structure leading to successive light interception from canopy to forest floor. Tree species differ in the quantity and heterogeneity of light they transmit. We expect decreases in both the quantity and spatial heterogeneity of light transmittance in mixed stands relative to monocultures, due to complementarity effects and niche filling.

We tested the degree to which tree species identity and diversity affected, via differences in tree and shrub cover, the spatiotemporal variation in light availability before, during and after leaf expansion. Plots with different combinations of three tree species with contrasting light transmittance were selected to obtain a diversity gradient from monocultures to three species mixtures. Light transmittance to the forest floor was measured with hemispherical photography.

Increased tree diversity led to increased canopy packing and decreased spatial light heterogeneity at the forest floor in all of the time periods. During leaf expansion, light transmittance did differ between the different tree species and timing of leaf expansion might thus be an important source of variation in light regimes for understory plant species. Although light transmittance at the canopy level after leaf expansion was not measured directly, it most likely differed between tree species and decreased in mixtures due to canopy packing. A complementary shrub layer led, however, to similar light levels at the forest floor in all species combinations in our plots.

Synthesis We find that a complementary shrub layer exploits the higher light availability in particular tree species combinations. Resources at the forest floor are thus ultimately determined by the combined effect of the tree and shrub layer. Mixing species led to less heterogeneity in the amount of light, reducing abiotic niche variability.

2.2 INTRODUCTION

Light availability at the forest floor is of central importance to many forest ecosystem processes. Light is an important resource affecting the performance and diversity of understory plants (Jelaska et al. 2006, Bartels & Chen 2010, Bartemucci et al. 2006). It also has a large impact on the microclimatic conditions at the forest floor such as soil and air temperature and soil water content (Gray et al. 2002, Ritter et al. 2005). This microclimate shapes the diversity and composition of several organism groups, especially endothermic animals (Niemelä et al. 1996, Richards & Windsor 2007) and influences ecosystem processes such as litter decomposition and both directly and indirectly tree regeneration (Hobbie et al. 2006, Tingstad et al. 2015, Lin et al. 2014).

Light transmittance to the forest floor is spatially and temporally variable and is largely determined by tree species composition, stand density, stand structure and canopy patterns, including the spatial arrangement of tree crowns and canopy gaps (Tinya & Ódor 2016, Martens et al. 2000). These aspects of forest structure change with developmental stage. Old-growth forests with natural tree fall dynamics often show a high quantity and spatial heterogeneity of light transmittance (Tinya & Ódor 2016, Canham et al. 1994), but only few of the temperate forests are actually in the old-growth stage (Hannah et al. 1995, Bengtsson et al. 2000). The more prevalent mature forests in the understory reinitiation stage generally have continuous closed canopies and light is expected to be primarily determined by species composition under similar stand basal area (Canham et al. 1994, Ligot et al. 2016). For these systems it is therefore important to understand how the tree community composition (here identity and diversity) affects the light availability at the forest floor.

Several studies found that mixed stands had a denser canopy than monocultures because of complementary crown architecture and plasticity (Jucker et al. 2015, Pretzsch 2014, Sapijanskas et al. 2014, Williams et al. 2017). Such increased canopy packing allows the trees to pre-empt the light resource more effectively and leads to lower light availability below the canopy (Ligot et al. 2016, Forrester et al. 2017). This is in accordance with the more general prediction of functional biodiversity research that more diverse systems use resources more efficiently due to complementarity between species (Tilman 1999, Forrester 2014, Loreau & Hector 2001). Two experimental studies from tropic regions concluded that mixed stands of young trees had a higher light interception than any of the monocultures (Sapijanskas et al. 2014, le Maire et al. 2013). Two different studies using a computer model of broadleaf and coniferous trees and found a complementarity effect. Light interception in mixed

stands was intermediate between the interception values in monocultures but higher than expected if the effects would be purely additive (Forrester et al. 2017, Ligot et al. 2016). The three mentioned studies focus on forests in the stem exclusion phase, however, evidence for higher light interception in more diverse forests from field studies in mature forests in the understory reinitiation stage is lacking. In these systems, light interception might not occur solely by tree crowns but also on shrub level (Messier et al. 1998, Bartemucci et al. 2006).

While mean light transmittance is predicted to decrease in mixed stands, it is unclear whether spatial heterogeneity of light transmittance will increase or decrease. More diverse forest stands are often assumed to create a more heterogeneous environment, because the trees create species specific conditions below their canopies (Vockenhuber et al. 2011, Barbier et al. 2008, Ampoorter et al. 2014). Reich et al. (2012) found that heterogeneity increased with decreasing light quantity on the stand level. Other studies found that variability in understory light peaked at 40% canopy cover (Martens et al. 2000) and decreased with increasing canopy cover (Dupré et al. 2002, Ligot et al. 2016). Variability of light transmittance would in this case decrease in mixed stands if canopy density is increased due to complementarity between species. Ligot et al. (2016) explicitly studied the effect of species mixtures on light heterogeneity and found mixed results depending on which species were used in the two models with highest stand basal area. Mixing pine and fir increased heterogeneity compared to pure stands, whereas other mixtures had intermediary levels of heterogeneity.

The light environment at the forest floor shows not only spatial but also temporal variability. Light conditions change dramatically throughout the season, especially in temperate deciduous forests. The total light transmittance throughout a year will be determined by the position of the sun, the amount and position of gaps before and after leaf expansion and the timing of bud burst and leaf senescence. In temperate regions, the period in spring before leaf expansion can contribute disproportionately to the total biological relevant light reception at the forest floor. For example, tree saplings can receive more than 90% of the total annual irradiance before leaf expansion of adult trees (Augsburger et al. 2005). Substantial inter- and intraspecific variation in the timing of tree leaf expansion exists (Lechowicz 1984, Bobinac et al. 2012, Wesolowski & Rowinski 2006), which could create large differences in the yearly biological relevant light transmitted to the forest floor between species compositions. This has never been studied in temperate forests as far as we know.

Here, we investigated how the quantity and spatiotemporal heterogeneity of light on the forest floor in mature temperate forests varies with tree identity and diversity. We used a tree diversity-oriented research platform composed of deciduous tree species with different light transmittance characteristics. The tree species used in this study were (in order of increasing light transmittance) *Fagus sylvatica* L. (beech), *Quercus rubra* L. (red oak), and *Quercus robur* L. (pedunculate oak) (Canham et al. 1994, Ellenberg 1988, Rebbeck et al. 2011). We quantified the shrub and canopy cover and measured light transmittance before, during and after leaf expansion to answer the following questions: (1) how do tree identity and diversity determine tree canopy cover and shrub cover; (2) is the light quantity at the forest floor different between species and is it lower in mixtures than expected from monoculture values; (3) do monocultures differ in light heterogeneity and do mixtures decrease heterogeneity in light transmittance; (4) do the patterns in light quantity and heterogeneity differ between key phenological periods: before, during and after leaf expansion?

2.3 MATERIALS AND METHODS

2.3.1 SITE AND EXPERIMENTAL SETUP

The research was conducted across 53 plots (30 m x 30 m) located in mature forests in the region of Ghent, Belgium (the 'TREEWEB' platform; Fig. SI 1.1; De Groote et al., 2017). All forests have been historically continuously forested. The plots were selected to vary principally in tree species identity and diversity, while minimizing the variation in other environmental variables. The plots have similar soil texture and are mature, extensively managed forests that showed no signs of recent management. With a species pool of three regionally common tree species (*Quercus robur*, *Quercus rubra* and *Fagus sylvatica*) a diversity gradient from monocultures to three species mixtures was created. Each of the seven possible species combinations was included in the design, with seven or eight realizations for each combination. During plot selection, the admixture of non-target tree species was minimized ($\leq 5\%$ of the basal area) and the evenness of the target tree species in mixtures was maximized ($> 60\%$ of maximum evenness based on basal area) (Baeten et al. 2013). The study plots had a mean stem number of 16 trees per plot (178 trees/ha; range: 100 – 333 trees/ha) and a mean basal area of 38.58 m²/ha (range: 25.09 - 52.48 m²/ha).

The three selected focal tree species represent different light strategies. They strongly differ in their light transmittance and shade tolerance, two tree characteristics that are generally inversely related (Canham et al. 1994). Based on studies about light transmittance and the shade tolerance ranks we can assume that *F. sylvatica* has the lowest and *Q. robur* the highest light transmittance, while *Q. rubra* has an intermediate transmittance (Ellenberg 1988, Niinemets & Valladares 2006). *Q. rubra* is an exotic species but is abundant and economically important in the region, which makes it relevant to study. The status as exotic species is, however, expected to have no influence on light transmittance.

2.3.2 TREE AND SHRUB COVER

For each 900 m² plot, we mapped the position of each tree with a diameter at breast height (DBH) larger than 15 cm using the Field-Map system (www.field-map.com). For all the trees of which the crown covered part of the plot, we measured the dbh and crown projection to four directions. Each plot was subdivided into four 15 m x 15 m squares. Five subplots of 5 m x 5 m were established, one in the center of each square and one in the center of the plot. Shrub cover was visually estimated for each

of the five subplots as the vertical projection of the shrub layer (Fig. SI 1.2). The shrub layer was defined as all shrub and subcanopy woody species between 1.5 and 7 meter high. To analyze the data, the mean shrub cover per plot was calculated from the five subplot values. Total crown area per plot, the sum of all individual tree crown projections that fell within the plot area, was calculated based on crown projections in the Field-Map system using QGIS (QGIS Development Team 2009).

2.3.3 QUANTIFYING LIGHT TRANSMITTANCE

Light transmittance was measured with hemispherical images before and after leaf expansion at four locations in each of the 53 plots. The locations of the images were equally distributed over the plot, at least 5 m away from the edge of the plot and *ca.* 10 m of each other, avoiding spatial autocorrelation (Lin et al. 2003). The first series of images was captured between 18 – 20 March 2016, that is, well before budburst of any of the tree or shrub species in these communities. The second series of images was captured between 22 – 24 May 2016, that is, two weeks after full leaf expansion of all trees in the plots. For a subset of two randomly selected plots within each possible species combination along the diversity gradient ($N = 14$), we additionally took images at the four locations per plot weekly, between the aforementioned dates. With this sampling, we obtained a time series of ten images per location covering the entire leafing-out period. Hemispherical images were taken at 1.5 m height, with the top of the camera orientated north. We used a Nikon D5200 (24.1 megapixels, dynamic range of 12.5 Ev at ISO 200) with a circular fisheye lens (Sigma EX 4.5 mm) fixed in a self-leveling mount on a tripod to obtain a horizontal position of the lens. Images (6000 x 4000 pixels, 14 bit/color, ISO 200) were taken when sky illumination was homogeneous, that is, during overcast days or during an interval of 90 minutes centered around sunrise or sunset. Histogram selection based on Beckschäfer et al. (2013) was too time consuming to perform in the field. Instead, three images with different underexposure (-3 ± 1.3 stops) using matrix light measurement were taken at each location using the bracketing function (Brusa & Bunker 2014, Beckschäfer et al. 2013). We automatically selected the image with the highest exposure value but with fewest overexposed pixels for further analysis. Binarization of the images was done with the K-means clustering algorithm (Lloyd's algorithm) from the 'scikit-learn.cluster' package in python (Pedregosa et al. 2011) since this was one of the best performing algorithms according to the review by Jonckheere et al. (2005). We used two clusters, other parameters were kept at default values. We used the free software CIMES (Gonsamo et al. 2011) to calculate total transmitted PAR to the forest floor, both direct and diffuse radiation, based on

the binarized images. We calculated total transmitted PAR for standard overcast conditions (SOC) and for clear sky condition (CLEAR) (Fig. 2.1). Based on these estimates we calculated the gap light index (GLI), which is the total transmitted PAR to the forest floor as a percentage of total incident PAR above the canopy (Canham 1988)

$$GLI = [(Tsoc * Psoc) + (Tclear * Pclear)] * 100 \quad (\text{eq. 1})$$

Psoc and Pclear are the proportions of days with overcast sky and clear sky conditions, respectively. Tsoc and Tclear are the proportions of diffuse and direct-beam radiation that are transmitted through the canopy to the understory under overcast sky and clear sky conditions, respectively. A GLI of 0 indicates that there is no light in the understory, while a GLI of 100 indicates a totally open site.

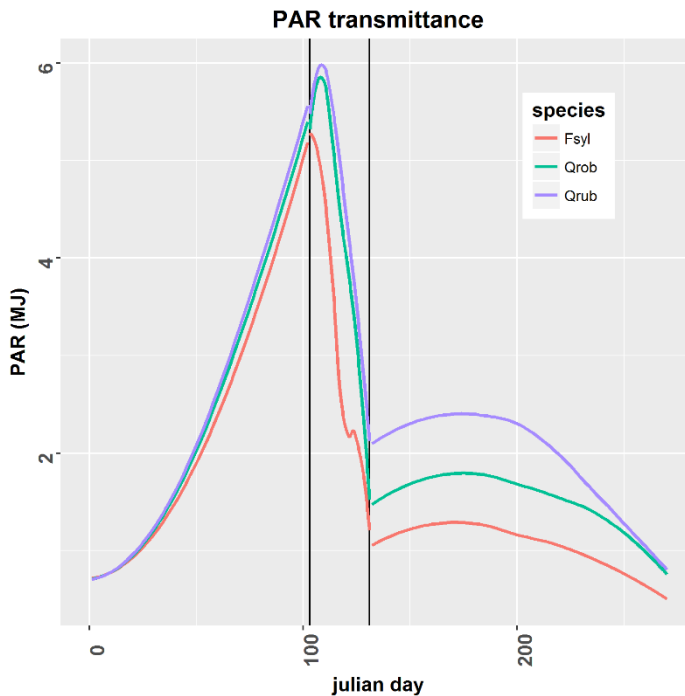


Figure 2.1. - Loess smoother for the mean transmitted PAR for the three monocultures from 1 January until 12 October. The vertical lines at Day of the year 103 and 131 enclose the period of leaf expansion.

For the calculation of GLI, the parameters P_{soc} and P_{clear} were estimated as 0.5 which is an average value for Western Europe for the entire growing season that approximates our local conditions (Gendron et al. 1998). T_{soc} and T_{clear} are calculated for each day using CIMES based on the appropriate hemispherical image. We calculated one GLI for each of the three periods: before (01 January – 12 April), during (13 April – 9 May) and after (10 May – 12 Octobre) leaf expansion based on the images captured in the respective time periods. The GLI during leaf expansion was only calculated for the fourteen plots that were measured weekly. Day of the year 286, 12 October, was considered as the end of the growing season and this date coincides with the onset of leaf coloration in trees (Gressler et al. 2015).

2.3.4 STATISTICAL ANALYSES

To investigate how the identity and diversity of the tree species affect the tree and shrub cover, we calculated a set of diversity-interaction models with either shrub cover per plot or total crown area per plot as dependent variable (Kirwan et al. 2009). A first null model (M0) assumes that all tree species have a similar, non-interactive effect on the dependent variable. The dependent variable is modelled in function of total basal area of all trees in the plot and one intercept is estimated. The species identity model (M1) models the dependent variable as a function of total basal area and the relative abundance (based on basal area) of each focal tree species. This model estimates a species-specific intercept, but does not allow for species interaction effects in mixtures, assuming purely additive effects. The species interaction model (M2) extends M1 by adding the two-way and three-way species interactions between the species' relative abundances. The model thus estimates a species-specific intercept (identity effect) and the interactions between species' relative abundances (diversity effect), while accounting for total basal area. The diversity effect (interaction) is then the difference between the actual performance of a mixture and the performance expected from the monoculture performances. The total crown area was modeled with a Gaussian distribution and shrub cover, which was bound between 0 and 1, was modeled with a beta distribution. All analyses were performed in the probabilistic programming language Stan, called from R using the package *brms* in R 3.3.0 (Bürkner 2016, R Core Team 2016). We used the widely applicable information criterion, WAIC (Vehtari et al. 2017) to compare the models and identify the most parsimonious model that best explains the data.

To study the intertwined effect of species identity and species diversity on the spatial and temporal variation in light availability, we modeled the GLI with a species

interaction model similar to M2 specified above. We extended the model proposed by Kirwan et al. (2009) by including 'plot' as a random effect to account for the spatial dependence of the variables measured at the four locations within plots. Furthermore, rather than having a single residual variance term to quantify the within-plot variation in GLI, this variance was allowed to be different for each species composition level. This implies that the within plot variance is a relative measure for heterogeneity for each composition, an important variable in addition to the mean light quantity to understand the light environment in forests. Such model thus allows quantifying differences in within-plot light variation between the different species combinations and diversity levels. Models were again fitted using brms. We ran the model for the three light variables: GLI before, during and after leaf expansion. All three dependent variables are bound between 0 and 1, therefore, a beta distribution was used¹.

¹ For all Bayesian analyses reported in the text we used default sampling settings with 4 chains, 2000 iterations per chains and a burn-in of 1000 iterations leading to posterior samples of 4000 iterations. As divergence appeared in some models, the adapt delta parameter was set to 0.99 for problematic models. Default prior distributions were used for all parameters: all slope parameters got unbounded uniform priors, the variation in the random effects followed a t-distribution with 3 degrees of freedom and a non-centrality parameter of 10. All model parameters converged (Rhat values < 1.1) and sufficient effective number of posterior samples were available from the posterior distributions (Neff- ratio > 0.1). In addition posterior predictive checks of the mean and standard deviation showed that all models provided a relatively good fit.

2.4 RESULTS

2.4.1 EFFECT OF TREE IDENTITY AND DIVERSITY ON TREE CROWN AND SHRUB COVER

The model that best explained the total crown area was the species interaction model (M2) which means that there were significant interaction effects when species grow in mixtures (Table 2.1.). Total tree crown area was significantly higher in all mixtures than expected based on monoculture values. The null model had a slightly lower WAIC-value than the identity model which means that the monoculture values for total crown area were similar. Although the *Q. robur* monoculture had the lowest and *Q. rubra* the highest total crown area, these differences were not significant since the parameter estimates for the monoculture values have a large overlap. (Fig. 2.2; Table SI 2.1 in Supplementary Information).

Shrub cover was best explained by the more parsimonious species identity model (M1) (Table 2.1.) because it differed between tree species. The shrub cover under *F. sylvatica* monocultures of *Q. rubra* and *Q. robur*. The shrub cover in mixtures did not differ from the expected cover based on monocultures, interactions were not significant, which means that shrub cover was explained only by additive effects (Fig. 2.2; Table SI 2.1.). Overall, we observed a large variation in shrub cover, plot values ranged from 0% to 82%.

Table 2.1 - WAIC (widely applicable information criterion) values for the three nested models for the two dependent variables. Total crown area is the sum of all tree crown areas, shrub cover is the mean of the estimated shrub cover for the 5 subplots. The lowest WAIC, thus the best model, is indicated in bold.

	Total crown area	Shrub cover
M0: nullmodel	477.09	-27.47
M1: species identity model	478.74	-56.15
M2: species interaction model	463.67	-52.55

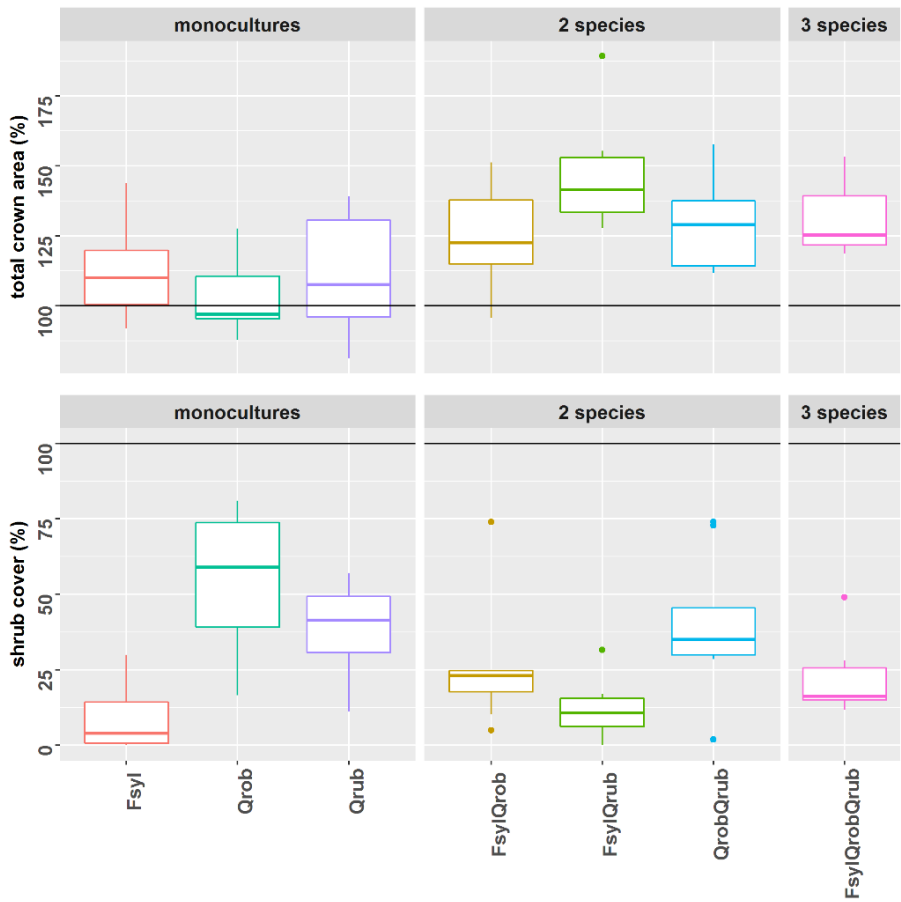


Figure - 2.2. Boxplots of a) total crown area and b) shrub cover as a percentage of the plot area for each species combination. The horizontal black line indicates 100%.

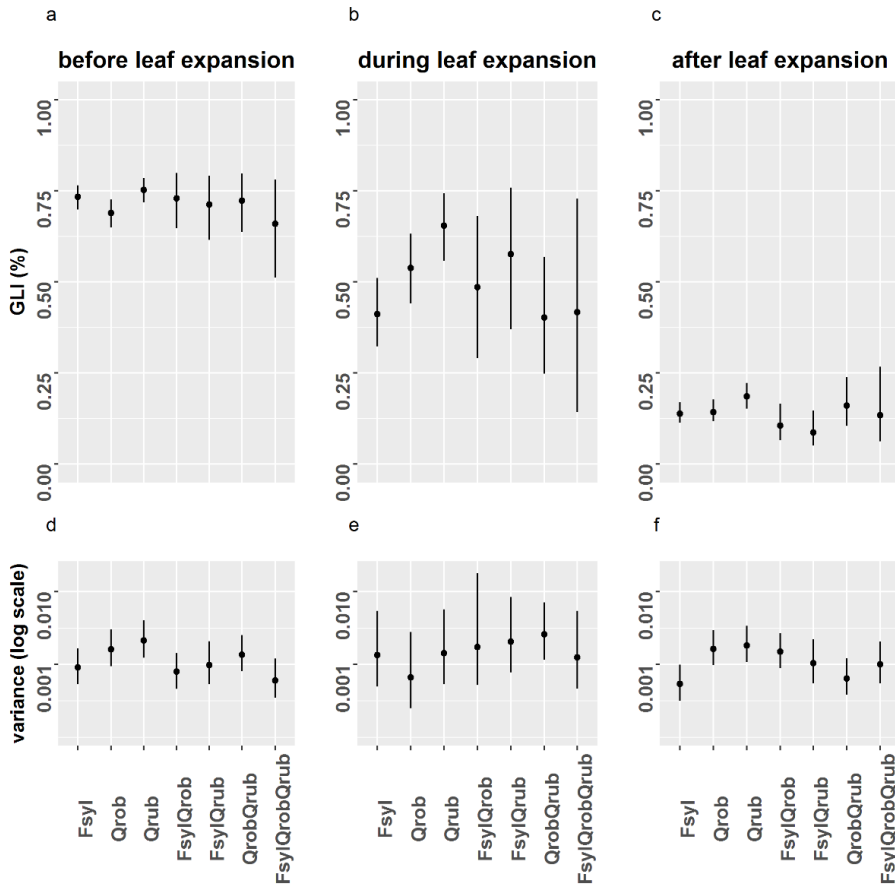


Figure - 2.3. Estimates of the light transmittance (GLI) with 95% credible intervals for monocultures and the different mixtures (based on posterior parameter estimates of the species interaction model). Equal basal area of the species is assumed. Variances are calculated using the estimate for the GLI for each species combination. a) Mean GLI before, b) mean GLI during, c) mean GLI after leaf expansion. d) variance of GLI before, e) variance of GLI during, f) variance of GLI after leaf expansion.

2.4.2 IDENTITY EFFECT ON LIGHT TRANSMITTANCE BEFORE, DURING AND AFTER LEAF EXPANSION

Mean light transmittance in monocultures did not significantly differ between any of the species neither before nor after leaf expansion. Mean GLI in the monocultures ranged from 68.90% (*Q. robur*) to 75.29% (*Q. rubra*) before leaf expansion and decreased after leaf expansion to a range from 14.57% (*F. sylvatica*) to 18.91% (*Q. rubra*). The GLI during leaf expansion did differ significantly between all monocultures (Fig. 2.3; Table SI 2.2).

Before leaf expansion, the three monocultures had a similar within-plot variance of GLI although variance of *F. sylvatica* tends to be somewhat lower. During leaf expansion, within-plot variance is similar for all species combinations. The posterior values have large credibility intervals due to the low sample size during leaf expansion. After leaf expansion, the within-plot variance in *F. sylvatica* monocultures was significantly lower compared to *Q. robur* and *Q. rubra* monocultures (Fig. 2.3; Table SI 2.3).

2.4.3 DIVERSITY EFFECT ON LIGHT TRANSMITTANCE BEFORE, DURING AND AFTER LEAF EXPANSION

Most mixtures showed no diversity effects in any of the time periods and had a GLI which was purely determined by additive effects, i.e. intermediate between the monoculture values. Diversity effects on mean GLI were only observed in mixtures of *Q. robur* – *Q. rubra* during leaf expansion and *F. sylvatica* - *Q. rubra* after leaf expansion. These mixtures had a significantly lower light transmittance than expected based on monocultures (Fig. 2.3; Table SI 2.2).

The within-plot variance of GLI before, during and after leaf expansion was always intermediate between or lower than the constituent monocultures. The three species mixtures before leaf expansion showed lower within-plot variance than we would estimate from the monocultures. Within-plot variance during leaf expansion is similar for all species combinations. The *Q. robur* – *Q. rubra* mixture after leaf expansion had a lower within-plot variance than we would estimate from the monocultures. Within-plot variance in mixtures before and after leaf expansion was never higher than highest within-plot variance in monocultures (Fig. 2.3; Table SI 2.3).

2.5 DISCUSSION

2.5.1 GENERAL DISCUSSION

We measured light transmittance across a tree diversity gradient of mature forest plots to study how light quantity and heterogeneity at the forest floor are influenced by the identity and diversity of the trees throughout the year. Light conditions will ultimately be determined by the combined interception of tree and shrub cover. Therefore, we additionally measured tree identity and diversity effects on the tree and shrub cover. While the three different tree species showed similar total crown area, this crown area increased in mixtures. This positive diversity effect is in agreement with earlier studies (Jucker et al. 2015, Pretzsch 2014, Williams et al. 2017). Contrary to the total crown area, however, we observed a clear identity effect but no diversity effects on shrub cover. *F. sylvatica* had a lower shrub cover compared with both *Q. robur* and *Q. rubra*. This is in line with the lower cover of the herb layer community below beech found in other studies. (Mölder et al. 2008, Wulf & Naaf 2009).

We found that the quantity of light transmitted before and after leaf expansion is similar in all species compositions and thus little impacted by identity or diversity effects. During the period of leaf expansion we found, however, a clear identity effect and all three monocultures differed significantly in the quantity of transmitted light. These differences in spring light transmittance are anticipated to have a great impact on yearly biological relevant light availability for understory plants.

In terms of light heterogeneity after leaf expansion, we found that the level of variation is species-dependent: in *F. sylvatica* monocultures light is much more homogeneously distributed compared with the two *Quercus* species. Increased tree diversity does not lead to an increased heterogeneity and heterogeneity is actually intermediate or lower than that of the constituent species. While mixtures have an increased tree crown cover compared to monocultures, this did not lead to a lower light transmittance to the forest floor in mixtures, most likely due to complementary light interception by the shrub layer and a high variability in GLI within plots.

2.5.2 IDENTITY EFFECT ON LIGHT QUANTITY AND HETEROGENEITY

The light transmission before leaf expansion was similar across species combinations which is not surprising since light interception of stems and branches is expected to be similar between tree species. We did find that monocultures significantly differ

from each other in light transmittance during the three weeks of leaf expansion. Light transmittance was lowest in *F. sylvatica* monocultures, highest in *Q. rubra* monocultures and intermediate in *Q. robur*. These differences might be due to differences in timing of leaf expansion between the species. The dense shrub layer of the early leafing out *Coryllus avellana* under *Q. robur* caused lower light transmittance then would be expected based solely on the tree layer.

Contrary to our expectations and to other studies (Canham et al. 1994, Härdtle et al. 2003, Vockenhuber et al. 2011) we did not find differences in light transmittance to the forest floor after leaf expansion between *Q. robur*, *F. sylvatica* and *Q. rubra* monocultures. We attribute this lack of tree identity signal on light transmittance to the varying contribution of the shrub layer. In monocultures of *F. sylvatica*, there was almost no shrub cover while monocultures of *Q. robur* had a very high shrub cover and monocultures of *Q. rubra* had an intermediate shrub cover. In the virtual absence of a shrub layer, the low light transmittance in *F. sylvatica* was purely determined by the dense tree canopy. In *Q. robur* stands, on the other hand, the combined light interception by the tree and the abundant shrub layer was similar to *F. sylvatica*, which implies that interception by the *Q. robur* canopy was lower than that of *F. sylvatica*. These results are similar to the results of Bartemucci et al. (2006) who found uniformly low light levels at the lower understory and forest floor level despite clear differences in light transmittance above the shrub layer at a height of 4 m.

Almost all studies looking at tree species effect on understory processes solely measure light transmittance after leaf expansion. Light transmittance in early spring, before and during leaf expansion is, however, as important or even more important for understory plant growth (Baeten et al. 2015, Augspurger et al. 2005, Augspurger & Salk 2017). Augspurger et al. (2005) found that seedlings of different tree species received between 33 - 97.6% of their total irradiance before 100% canopy closure. Small differences in timing of leaf expansion between trees could therefore lead to large differences in total irradiance. Although it is generally acknowledged that canopy avoidance in forest herbs and seedlings is ubiquitous, differences in light transmittance during this period are almost never accounted for when studying the effect of tree species on understory cover, diversity and other light dependent processes.

Despite the similar mean light quantity after leaf expansion in the different species compositions, the light heterogeneity in *F. sylvatica* is significantly lower than in the two *Quercus* monocultures. Our observation that *F. sylvatica* has a homogeneous

low light transmittance and an early leaf expansion, while the shrub layer is almost absent, confirms other observations that find low light transmittance in *F. sylvatica* stands (Härdtle et al. 2003, Mölder et al. 2008, Vockenhuber et al. 2011).

2.5.3 DIVERSITY EFFECT ON LIGHT QUANTITY AND HETEROGENEITY

Our expectation that mixtures would show an increased canopy packing due to complementarity effects was confirmed since total tree crown area was higher than expected based on the monoculture values for all mixtures. The mixture of *F. sylvatica* - *Q. rubra* showed the highest increase in crown area

An increased tree diversity has no effect on light transmittance before canopy closure and leads to intermediate light transmittance during leaf expansion for *F. sylvatica* – *Q. rubra* and *F. sylvatica* – *Q. robur* mixtures. The mixture *Q. robur* – *Q. rubra* had a lower light transmittance during the period of leaf expansion than would be expected based on monoculture values. The lower light transmittance in the mixture of *Q. robur* – *Q. rubra* is probably an artifact due to a relative low light transmittance before leaf expansion and an early leaf expansion in this particular subset of two plots that was used to determine light transmittance during leaf expansion.

Based on the increased canopy packing, we expected that light transmittance through the canopy will decrease in all mixtures after leafing-out. At the forest floor, however, there was only a significant diversity effect in the *F. sylvatica* - *Q. rubra* mixture. This mixture had a high increase in crown area. Moreover, differences in light transmittance between this mixture and the monocultures are purely the effect of tree canopy density since shrub cover is very low in the mixture and monocultures of *F. sylvatica* and *Q. rubra*. The other two mixtures had a similar light transmittance after leaf expansion at the forest floor compared to the monocultures. This is most likely due to the complementary shrub layer that was high in plots with a rather open canopy and lower under closed canopies. In conclusion we do find indication for a higher light interception at the tree canopy level if tree diversity increases (Ligot et al. 2016, Jucker et al. 2015), but the complementary shrub layer effectively homogenizes light transmittance to the forest floor across species combinations.

Higher tree diversity decreased within-plot heterogeneity to levels intermediate or lower than expected based on monoculture values. This contradicts with the assumption that tree diversity will create more heterogeneous conditions on the forest floor which is implicitly or explicitly made in many studies (Ampoorter et al.

2016, Reich et al. 2012, Dupré et al. 2002). From the perspective of *F. sylvatica* monocultures, however, adding other species breaks the homogeneous light transmittance and increases heterogeneity (Mölder et al. 2008, Härdtle et al. 2003, Vockenhuber et al. 2011).

2.5.4 IMPACTS FOR UNDERSTORY PLANTS

The importance of light quantity and heterogeneity for processes at the forest floor is well studied with regard to plant understory diversity and cover (Thomsen et al. 2005, Mölder et al. 2008, Tinya & Ódor 2016, Tinya et al. 2009). Increased tree diversity in stands is generally assumed to create a higher heterogeneity of abiotic conditions at the forest floor (Thomsen et al. 2005, Canham et al. 1994, Dupré et al. 2002, Ampoorter et al. 2016) and the resulting higher number of niches in the mixed stand are expected to promote the coexistence of more understory species (Levine & HilleRisLambers 2009, Reich et al. 2012, Barbier et al. 2008). In young and mature stands that do not exhibit strong canopy dynamics, average light quantity also is an important factor in governing understory diversity and species composition (Tinya et al. 2009, Reich et al. 2012, Bartels & Chen 2010).

Our results indicate that increasing tree diversity by intermixing species leads to lower heterogeneity within in forests in the understory reinitiation stage. This might partially explain why several studies find no effect of overstorey diversity on herbaceous diversity (Ampoorter et al. 2016, Ampoorter et al. 2014, Gazol & Ibáñez 2009, Ewald 2002, Houle 2007, Thomsen et al. 2005, Both et al. 2011). Thomsen et al. (2005) suggests that the fine-grained mixture of tree species attenuates the differential impact of the tree species on any given area on the forest floor by causing a mixing of their light and litter effects. Additionally, our results show that the shrub layer is able to attenuate the differential impact of the tree species on the forest floor leading to similar light quantities across different species combinations. Studies focusing on the effect of tree species identity and diversity on plant understory dynamics should thus include those components of the forest ecosystem that respond to tree species composition and determine the ultimate availability of resources.

2.6 CONCLUSION

Our results show that light transmittance to the forest floor after leaf expansion is similar across species combinations. Although light transmittance is expected to differ between tree species and to decrease in mixtures, a complementary shrub layer exploits the higher light availability in particular tree species combinations so that the ultimate light levels at the forest floor is similar across all species combinations in our plots. We found evidence that light transmittance during the three weeks of leaf expansion does, however, differ significantly between species. This could be a major source of variation in light transmittance between tree species with a large impact on the performance of understory plants. Finally, we show that, in the case of light, higher tree diversity does not lead to higher heterogeneity of resources at the forest floor.

CHAPTER 3 - SOIL HETEROGENEITY IN TREE MIXTURES DEPENDS ON SPATIAL CLUSTERING OF TREE SPECIES

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After Sercu, B. K., Hertzog, L. R., De Groote, S.R.E., Baeten, L., Lens, L., Martel, A., Bonte, D., Verheyen, K. (submitted). Soil heterogeneity in tree mixtures depends on spatial clustering of tree species. *Basic and Applied Ecology*

3.1 ABSTRACT

Heterogeneity in soil characteristics promotes and maintains coexistence between a diverse set of species. In forests, trees have species-specific impacts on soil abiotic characteristics and mixing of tree species is being promoted as a tool to ensure high levels of diversity and functioning. Yet, limited knowledge is available on the effect of tree species composition and spatial clustering on heterogeneity in soil characteristics. In this paper we derived heterogeneity of key characteristics of the leaf litterfall, the forest floor and the mineral topsoil (C, N and base cation concentration, C:N ratio and mass) in 53 plots of 7 different tree species compositions. We found that heterogeneity increased from the leaf litterfall, through the forest floor down to the mineral topsoil. Mixing tree species did not lead to an increased heterogeneity in the forest floor and topsoil compared to monocultures. However, we did find that mixed plots where trees are clustered per species are more heterogeneous than plots where species are intimately mixed. Our results imply that heterogeneity in soil characteristics does not necessarily increase with tree diversity, but that within mixed stands the spatial organization of tree species should be considered in relation to the scale at which heterogeneity is desired.

3.2 INTRODUCTION

Heterogeneity in resource supply is key to the dynamics, interactions and coexistence of species (Chase & Leibold 2003, Chesson 2000a). Spatial and temporal variation in resources can promote coexistence between a diverse set of organisms by increasing available niche resource space (Baldrian 2017, Nielsen et al. 2010, Silvertown 2004). At the same time, species also impact environmental conditions through their functional traits (Hooper et al. 2005), so that functionally diverse communities may create and maintain resource heterogeneity (Cadotte et al. 2011).

Forests are a good model system to study the role of biodiversity in creating spatial resource heterogeneity, since trees generally have strong and species-specific effects on their neighboring environment (Jones et al. 1997). When trees shed leaves in autumn, the leaf litterfall layer is formed which gradually decomposes into the forest floor which eventually leaches nutrients to the topsoil. Each of these compartments provides a habitat for many species groups. Several studies have compared monocultures of different tree species and showed that they differ in litter, forest floor and mineral soil properties (Vesterdal et al. 2008). These differences in turn determine the composition, diversity and abundance of other biotic groups such as soil microbial community (Baldrian 2017), earthworms (Schelfhout et al. 2017), oribatid mite community (Wardle et al. 2006) and the herb community (Augusto et al. 2003). As a result, it is often explicitly or implicitly assumed in the literature that mixed forest stands will have a higher niche diversity and spatial resource heterogeneity compared with monocultures, so that they can harbor a higher soil biota and herb diversity (Ampoorter et al. 2016, Reich et al. 2012).

This assumption relies on the spatially limited and species-specific effects of tree individuals on soil characteristics. This can be conceptualized as a belowground zone of influence of individual trees (May et al. 2009). However, there is little to no information on the spatial extent of the influence of an individual tree in determining soil properties. In a simplified scenario, each point of the forest soil is influenced purely by one species (Fig. 3.1A). Some studies find that spatial patterns of some soil properties converges with the canopy projection of tree species (Lechowicz & Bell 1991, Rodriguez et al. 2009). However, other litter, forest floor and topsoil variables in these studies show spatial patterns that seem independent of the spatial position of trees. Differences in nutrient stocks and ratios in the forest floor and topsoil are mainly driven by leaf litterfall according to Augusto et al. (2002). The spatial positioning of the trees, the prevailing wind direction, wind speed, and the species

leaf shedding attributes, all might lead to spatial patterns of leaf litterfall in mixtures that differ from the spatial pattern of tree crowns (Jonard et al. 2006).

At small spatial scale (i.e., stand-level), the nature of the spatial mixing of tree species (intimate versus clustered mixtures) could have consequences for variability in litter, forest floor and topsoil characteristics. Stands where individual trees are clustered per species could lead to high stand-level heterogeneity with distinct zones of monoculture influence and a mixed zone (Fig. 3.1C). On the other hand, intimate mixing of tree species could lead to lower heterogeneity via the creation of homogeneous conditions that are intermediate between monocultures as is illustrated in Fig. 3.1B (Ampoorter et al. 2016, Thomsen et al. 2005). This could explain why most studies investigating the relationship between overstory and understory diversity found no evidence for a positive relationship between tree and understory diversity (Ampoorter et al. 2016, Thomsen et al. 2005). Therefore, the implicit assumption that mixing tree species increases environmental heterogeneity needs empirical testing as the effect of intimate mixing on variation may be more complex than commonly expected.

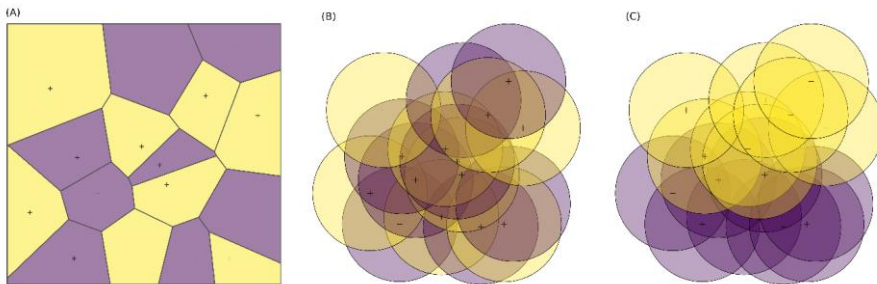


Figure - 3.1: Three different hypothetical scenarios of the effect of tree individuals from two different species (differing colors in the figure) growing in a mixture on soil characteristics. Crosses represent the position of the stems. (A) Soil characteristics at any point is affected by only one tree species, this is a simplified scenario enabling the derivation of expected mixture heterogeneity from monoculture values. (B) The two species are an intimate mixture as in (A) but the overlapping zone of influence from different species create overall more homogeneous soil characteristics when compared to the other scenarios. (C) Individual trees are clustered by species, creating more heterogeneous conditions at the level of the plot.

Here we studied the effect of tree species composition and spatial clustering on the spatial heterogeneity of mass and nutrient concentration in the litter, forest floor and top soil in order to test whether spatial heterogeneity increases in tree mixtures relative to monocultures. The study was performed in a tree diversity research platform (TREEWEB), where we sampled three tree species and all their potential combinations: (i) beech (*Fagus sylvatica*), (ii) pedunculate oak (*Quercus robur*) and (iii) red oak (*Quercus rubra*). These three species have different leaf nutrient concentration (De Groote et al. 2017) but all belong to the low-end of the leaf quality spectrum (Vesterdal et al. 2008). In this paper, we ask the following questions: What is the level of spatial nutrient heterogeneity in the leaf litter, forest floor and mineral topsoil? Is within-plot spatial nutrient heterogeneity of leaf litter, forest floor and topsoil higher in tree mixtures compared with monocultures? What is the expected spatial nutrient heterogeneity in mixtures based on monoculture information? What is the effect of tree clustering on within-plot spatial nutrient heterogeneity?

3.3 METHODS

3.3.1 STUDY AREA

Data were collected across 53 plots (30 m × 30 m) located in 19 forest fragments (fragment size ranges from 1.31 until 90.36 ha with an average of 28.13 ha) in northern Belgium (the “TREEWEB” platform; Fig. SI 1.1. in supplementary information; De Groote et al., 2017). The climate is temperate and characterized by a mean annual temperature of 9.5°C and an annual precipitation of 726 mm evenly distributed during the year (1980-2010, Royal Meteorological Institute of Belgium). As land use legacy effects can influence soil conditions in forests (eg. high Phosphorus content in post-agricultural soil), all plots have been selected in forest stands that have been continuously forested for at least the last 150 years. The plots were selected to vary principally in tree species composition, while minimizing the variation in other environmental variables such as soil texture. Plots have dry, sandy loam soil and are located in mature, extensively managed forest stands that showed no signs of management for the last 10-15 years. The tree species pool was formed of three regionally common tree species (*Quercus robur*, *Quercus rubra*, and *Fagus sylvatica*). *Q. rubra* is locally invasive and thus of concern to both forest managers and policy makers. Each of the seven possible species combinations (1. *Q. robur* ; 2. *Q. rubra*; 3. *F. sylvatica*; 4. *Q. robur* – *Q. rubra*; 5. *Q. robur* – *F. sylvatica*; 6. *Q. rubra* – *F. sylvatica* ; 7. *Q. robur* – *Q. rubra* – *F. sylvatica*) was included in the design, with six to eight replicates for each possible combination. The exact location of the stems was mapped and total basal area of the tree stems was calculated using a Field-Map system (www.field-map.com). Plots were selected so that the proportion of nontarget tree species was minimized ($\leq 5\%$ of the basal area) and the evenness of the target tree species in mixtures was maximized ($> 60\%$ of maximum Shannon’s evenness based on basal area) (Baeten et al. 2013). The study plots had a mean stem number of 16 trees per plot (stem density: 178 trees/ha; range: 100–333 trees/ha, standard error: 7.85 trees/ha) and a mean basal area of 38.58 m²/ha (range: 25.09–52.48 m²/ha, standard error: 0.85 m²/ha). Each plot was subdivided into four 15 m x 15 m squares. Five subplots of 5 m x 5 m were established, one in the center of each square and one in the center of the plot, that were used for standardized collection of litterfall, forest floor and topsoil samples (Fig SI 3.2 in supplementary information).

3.3.2 LITTERFALL, FOREST FLOOR AND TOPSOIL SAMPLE COLLECTION

Full details on the data collection are given in De Groote et al. (2018), the most important points are outlined below. We refer the interested reader to the above-mentioned publication for further information. As we aimed to generate generalizations across gradients of tree diversity, rather than to document detailed fine-scale patterns in few locations, we derived the spatial heterogeneity from a restricted number of samples per plot (N=3), but maximized replication of these local heterogeneity indices across forest plots (N=53).

3.3.2.1 LEAF LITTERFALL

Leaf litterfall was sampled via 1 m tall litterfall traps with a surface area of 0.24 m². Litterfall traps were placed in three of the five subplots in all plots: one was placed in the center of the plot, one northeast of the center and one either northwest or southeast of the center. Litterfall was collected from all 159 traps every two weeks from September 2014 until January 2015, resulting in eight collection dates. Litterfall samples were pooled later on in the processing (see ‘derived variables’) to obtain one value per litterfall trap. The litterfall samples were oven-dried at 65° C. Litterfall material was weighed to the nearest 0.01 g and samples for chemical analysis were ground, keeping the leaves of each target tree species separately. Leaf litterfall from non-target species (i.e. shrub species such as *Corylus avellana*) was pooled together and was ignored in the analysis, if they accounted for less than 5% of the total litter biomass (in 27 out of 53 plots, See Table 2 in De Groote et al., 2018), heterogeneity levels in the 27 plots where non-target species litter was ignored did not differ from the other 26 plots (Fig. SI 3.3 in supplementary information).

3.3.2.2 FOREST FLOOR

The forest floor was sampled once using 25 cm x 25 cm wooden frames at the same three subplots as the litterfall traps in March 2016. The recently fallen rather intact leaves were removed and the fragmentation and humification layer, containing the fragmented and (partly) decomposed leaves was processed further. Forest floor samples were oven-dried at 65° C twigs, fruits and non-litter material (e.g. moss) were removed and the remaining fraction was weighed to the nearest 0.01 g, samples for chemical analysis were ground.

3.3.2.3 TOPSOIL

The mineral soil was sampled once using a soil core (diameter: 3 cm) at three random locations per subplot in the same three subplots as the leaf litterfall and forest floor

collection between August and September 2014. The three replicates per subplot were pooled in subsequent processing. Soil sampling was done at the same subplots of the litterfall and forest floor sampling and was confined to the upper 10 cm of soil. All soil samples were dried at 40° C until of constant mass, samples for chemical analysis were crushed and sieved through a 1 mm mesh.

3.3.2.4 CHEMICAL ANALYSIS

Leaf litterfall chemical characteristics were analyzed per species and per plot pooling together the leaf materials over the 8 collecting dates and over the three subplots. Total nitrogen and carbon concentration was measured by dry combustion using an elemental analyser (Vario MAX CNS, Elementar, Germany). Exchangeable potassium, calcium and magnesium concentration was measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS) after extraction in BaCl₂ (NEN 5738:1996).

3.3.2.5 DERIVED VARIABLES

Three types of response variables were derived: (i) mass, (ii) elemental concentration and (iii) stoichiometric ratios. We used leaf litterfall mass and forest floor mass. For each leaf litterfall trap, the dried leaf mass was summed across the temporal replicates. For the forest floor, the sum of the dried fragmentation and humification layer was computed for each subplot. The concentration variables used were the carbon, nitrogen and base cations concentration. These were derived per subplot for the litterfall, forest floor and topsoil. The base cation concentration was calculated as summing the atomic equivalents (mmolc / kg) of potassium, calcium and magnesium taking into account the valence and the atomic mass of the elements. Finally one stoichiometric ratio was used: the C:N ratio also derived for each subplot in the litterfall, forest floor and topsoil. In the leaf litterfall, the concentrations were derived per species and per plot. We calculated the values per leaf litterfall trap as the weighted average of the concentration per tree species, with the weights being the dry mass of leaf litterfall from the tree species from each trap.

As an additional explanatory variable, a clustering index was derived for each plot based on the locations of the tree stems from the Field-Map. This index represents the proportion of trees in the plot that have a conspecific as the nearest tree neighbor. Low values indicate that most trees in the plot have another species as nearest neighbor and form an intimate mixture. High values mean that most trees have as nearest neighbor an individual of the same species, which indicates clustering by tree species.

3.3.3 DATA ANALYSIS

Full detail of the data analysis is given in Supplementary information SI 3.1 Extended Analysis section, we give here only the core information. For each response variable, (i) mass (ii) carbon concentration, (iii) nitrogen concentration, (iv) C:N ratio and (v) base cation concentration within the (a) leaf litter, (b) forest floor and (c) topsoil, we built a separate model (14 in total as mass was not relevant for topsoil). These models were Gaussian with two parameters: the mean and the residual deviation. The mean was regressed against tree basal area and species composition with a random intercept per plot. The residual deviation was modelled with a log link and was regressed against species composition with a random intercept per plot. For the purpose of this paper we represent within-plot variability or heterogeneity with the coefficient of variation (CV). The CVs were calculated per species composition as the estimated residual deviation divided by the estimated mean. We chose to rely on model-estimated CVs due to the limited spatial replication (3 subplots per plot) in the dataset that prevented us to directly compute within-plot heterogeneity from the observed values. This partial-pooling approach (Gelman & Shalizi 2013, Gelman & Hill 2007) capitalizes on the relatively large number of sampled plots to provide more reliable estimates of average within-plot heterogeneity by shrinking extreme within-plot values towards the mean. We compared those model-derived CV values for two-species and three-species mixtures with expected values based on a simplified or null scenario that assumes a monospecific impact and only additive effects in mixtures (see Fig. 1A). Under these assumptions the calculation of the expected CV values in the mixtures can be estimated from the statistical properties of the monoculture distributions. Basically, these expectations were obtained by dividing the average of the estimated standard deviations by the average of the estimated means from monocultures of the tree species present in the mixtures. More details and caveats of this approach are given in Supplementary information SI 3.1.

In a second step, we added the plot-level clustering value as a covariate in the regression on the residual variation to explore the effect of clustering vs intimate mixing on the CVs. The dataset used for this analysis included only the species combinations with two or three species.

All models were fitted with a Bayesian approach, using the Stan probabilistic language called from R v3.3 (R Core Team 2016) with the package ‘brms’ v1.10 (Bürkner 2016).

3.4 RESULTS

Observed mean and variation of the explored variables in the leaf litterfall, forest floor and topsoil and for the nutrient and mass variables are shown in Table 3.1. The overall within-plot variation (quantified with the coefficient of variation CV) generally increased from the leaf litterfall to the topsoil (Fig. 3.2). This pattern was less marked for the C:N ratio than for the other variables. The highest CV was observed for the mass of the forest floor which was considerably higher (median 0.5) than CV of leaf litterfall mass (median 0.09).

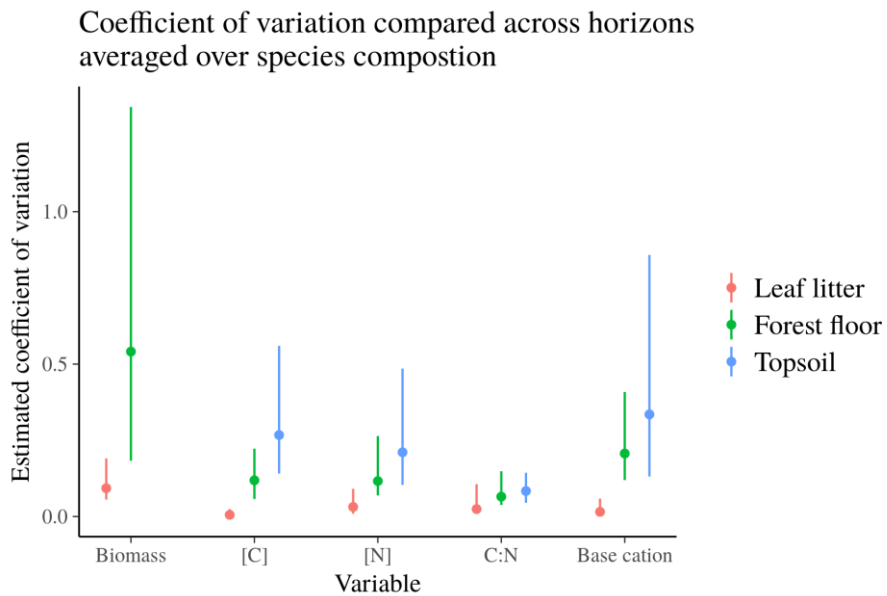


Figure 3.2 - Within-plot heterogeneity (represented by coefficient of variation) across species compositions for the five different variables in the litter, forest floor and topsoil. The dots are the posterior medians together with 95% credible intervals.

Table 3.1 - Average and standard deviation values (in brackets) of all modelled variables (Mass, C: Carbon concentration, N: Nitrogen concentration, CN: Carbon:Nitrogen ratio, BC: base cation concentration) for the different tree species composition (FsyI: *Fagus sylvatica*, Qrob: *Quercus robur*, Qrub: *Quercus rubra*) in the leaf litterfall, forest floor and topsoil.

Horizon	Variable	FsyI	Qrob	Qrub	FsyIQrob	FsyIQrub	QrobQrub	FsyIQrobQrub
Leaf litter	Mass (kg/m ²)	88.43 (11.44)	112.3 (12.22)	99.19 (17.92)	99.05 (11.36)	104.46 (11.71)	105.43 (15.53)	107.34 (11.16)
	C (%)	49.96 (1.55)	51.11 (1.31)	52.17 (1.73)	49.47 (1.34)	51 (1.26)	50.95 (2.01)	50.17 (1.61)
	N (%)	1.14 (0.1)	1.39 (0.13)	0.94 (0.16)	1.19 (0.07)	1.09 (0.09)	1.21 (0.12)	1.1 (0.1)
	CN	43.79 (2.74)	37.22 (3.66)	57.31 (7.51)	41.43 (2.83)	48.05 (4.5)	44.3 (5.08)	47.19 (5.19)
Forest floor	BC (cmol/kg)	563.67 (80.91)	730.53 (92.19)	589.11 (60.01)	703.32 (91.98)	525.98 (58.05)	632.29 (81.85)	643.62 (161.75)
	Mass (kg/m ²)	47.24 (26.31)	36.94 (24.47)	24.18 (21.22)	35.88 (21.49)	32.73 (15.07)	22.04 (17.52)	38.99 (24.37)
	C (%)	46.73 (3.82)	42.38 (5.55)	44.15 (8.65)	43.81 (5.86)	44.54 (8.44)	42.35 (5.77)	45.58 (5.22)
	N (%)	2.05 (0.18)	2.1 (0.23)	1.77 (0.39)	2 (0.23)	1.94 (0.42)	1.88 (0.25)	2.01 (0.23)
Topsoil	CN	22.8 (1.5)	20.16 (1.26)	25.25 (3.24)	21.93 (1.27)	23.1 (1.66)	22.53 (1.98)	22.75 (1.35)
	BC (cmol/kg)	315.4 (100.22)	395.4 (95.25)	368.44 (118.46)	396.5 (98.44)	301.56 (81.96)	391.52 (80.87)	319.69 (88.87)
	C (%)	3.05 (0.87)	4.16 (2.01)	2.7 (0.79)	5.03 (2.42)	3.83 (1.81)	4.78 (1.3)	3.85 (1.35)
	N (%)	0.19 (0.04)	0.27 (0.11)	0.18 (0.06)	0.3 (0.14)	0.21 (0.08)	0.3 (0.07)	0.24 (0.06)
	CN	16.1 (2.06)	15.19 (1.4)	15.41 (1.83)	16.93 (1.25)	17.55 (2.41)	16.07 (1.32)	15.95 (1.94)
	BC (cmol/kg)	5.98 (3.31)	15.83 (8.06)	4.84 (2.86)	18.56 (17.88)	6.42 (3.66)	12.61 (5.44)	10.31 (5.46)

3.4.1 TREE COMPOSITION EFFECT ON HETEROGENEITY

When comparing monoculture plots, we found that in the leaf litterfall, *Q. rubra* had a higher CV of litterfall mass, nitrogen concentration and C:N ratio than the other monocultures (Fig. 3.3). This pattern was also in part present in the forest floor where *Q. rubra* had a higher CV of nitrogen concentration and C:N ratio than the other monocultures. In the topsoil *Q. robur* had a higher CV of carbon and nitrogen concentration than the other monocultures.

In the mixture plots, for the leaf litterfall the mixture of *Q. robur* - *Q. rubra* tended to have higher CV of nitrogen concentration and C:N ratio than other 2-species mixtures. Interestingly, litterfall mass did not have a higher CV in 2 and 3-species mixtures compared to monocultures. Few consistent patterns emerged from the forest floor heterogeneity for the mixtures, but 3-species mixtures tended to have lower CV than 2-species mixtures. In the topsoil, the only marking feature was the very low CV for C:N ratio for *Q. robur* - *Q. rubra* mixtures. While all other species composition had CV around 0.08, *Q. robur* - *Q. rubra* mixtures had a CV of 0.05.

3.4.2 OBSERVED VS EXPECTED VARIATION IN THE MIXTURES

A general pattern found across tree species mixtures for mass and nutrients in the litterfall, forest floor and topsoil, was that the observed CV was lower than expected based on the monocultures (Fig. 3.3). In other words, expecting that the heterogeneity of a mixture is based on an additive combination of monoculture effect of the individual species (Fig. 3.1a) led to a general overestimation of the observed heterogeneity. Some deviation from this general pattern emerged for instance in the forest floor where the observed CV of carbon and nitrogen was identical or even larger than expected in *F. sylvatica* - *Q. rubra* and *F. sylvatica* - *Q. robur* mixtures. In addition, the CV of forest floor mass in *F. sylvatica* - *Q. robur* and *Q. robur* - *Q. rubra* mixtures was similar to expected values. This pattern was also found in the topsoil where mixtures of *F. sylvatica* - *Q. rubra* and *F. sylvatica* - *Q. robur* had similar observed and expected CV for carbon and nitrogen. Overall, *F. sylvatica* - *Q. rubra* and *F. sylvatica* - *Q. robur* mixtures led to a high CV that was closer to the expected CV.

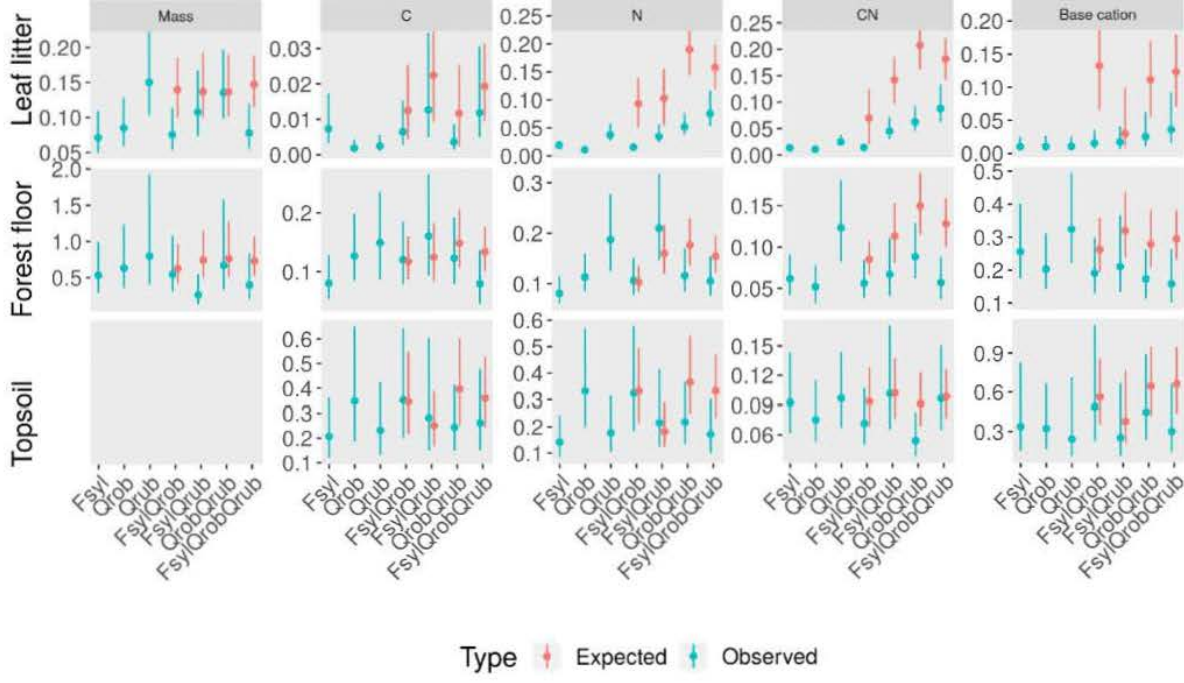


Figure 3.3 - Observed vs expected within-plot heterogeneity (represented by coefficient of variation) for the different tree species composition (Fsy: *Fagus sylvatica*, Qrob: *Quercus robur*, Qrub: *Quercus rubra*). The plots are ordered per layer (in rows) and per soil variable (in columns).

3.4.3 NEAREST NEIGHBOR EFFECT

The spatial clustering of tree species in the mixtures had an effect on the soil heterogeneity. Generally, high species clustering levels led to higher CV of nutrients in litterfall, forest floor and topsoil and mass of the forest floor (Figure 3.4). Only one variable departed from this pattern: litterfall mass CV was highest in plots where tree species are intimate mixtures (i.e. low clustering).

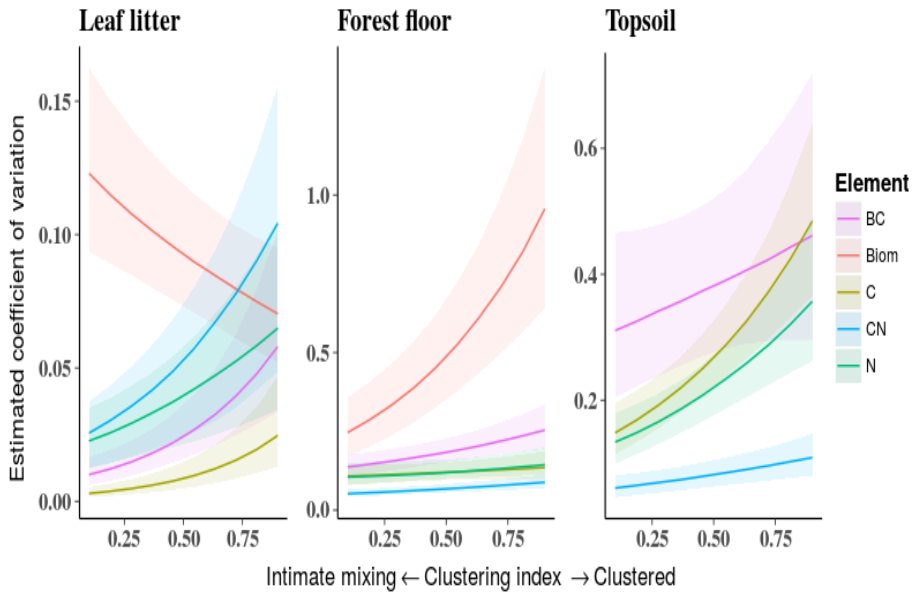


Figure 3.4 - Effect of spatial clustering of species (measured as the proportion of trees whose nearest neighbor is of the same species) on the within-plot heterogeneity (represented by coefficient of variation) for litterfall, forest floor and topsoil.

3.5 DISCUSSION

3.5.1 GENERAL DISCUSSION

Four general patterns emerged from our results: (i) heterogeneity increased from leaf litterfall to topsoil, (ii) within-plot heterogeneity in forest floor and topsoil characteristics did not increase in tree mixtures compared to monocultures, (iii) the observed heterogeneity in mixtures was generally lower than expected based on a simple scenario and (iv) spatial clustering of tree species affected within-plot heterogeneity, in the litterfall clustering reduced heterogeneity while in the forest floor and topsoil clustering increased heterogeneity.

3.5.2 INCREASE IN HETEROGENEITY FROM LITTERFALL OVER FOREST FLOOR TO TOPSOIL

Leaf litterfall mass and chemical composition was relatively homogeneous throughout the plots, even in mixtures. This may be due to the similarity in litterfall properties of the three species: they are broad-leaved tree species with relatively similar and low leaf litterfall quality (Vesterdal et al. 2008). Despite the homogeneous leaf litterfall, the forest floor and topsoil showed a high heterogeneity. The forest floor mass was the variable showing the highest heterogeneity but also concentration of the nutrients were more variable in the forest floor and topsoil compared to the leaf litter. We need to be cautious with the comparison of variability in litterfall, forest floor and topsoil since the sampling area and protocol differed between the layers. Specifically, the sampling area was highest for the leaf litterfall (0.24m²), lower for the forest floor (0.0625 m²) and lowest for the topsoil (0.0027 m²). Nevertheless, we believe that the large variability in the topsoil and forest floor clearly indicates that other processes than tree litterfall composition drive variation in the forest floor and topsoil, despite the interference of the different sampling areas.

Other studies also find high stand-level spatial heterogeneity of soil nutrients and microbial biomass in temperate forests (Gömöryová 2004, Baldrian et al. 2010). Climatic variables such as humidity, light and temperature are capital drivers of decomposition rates on broad geographical scales (Bradford et al. 2016), but they can also differ on a scale of meters and create different microclimates below the canopy. For instance due to the structure and position of canopy, seasonal precipitation can be double as high only few meters apart (Staelens et al. 2006). These microclimatic differences might create consistent differences in decomposer

activity and decomposition rates which in turns leads to high heterogeneity in the mass and nutrient concentration of the forest floor. Subsequently organic matter and nutrients are gradually incorporated into the topsoil horizon via bioturbation and leaching. Since differences accumulate over time, spatial heterogeneity of the nutrients in the topsoil horizon increases even further (Trum et al. 2011).

3.5.3 DO MIXTURES HAVE HIGHER HETEROGENEITY THAN MONOCULTURES?

We found no support for the assumption that mixtures of tree species generally harbor higher heterogeneity in the forest floor and topsoil than monocultures. The leaf litterfall was the only layer where we observe an increase in heterogeneity of the nutrient concentration in mixtures, but observed values were always lower than the expected values. This could be due to the fact that 1) the leaf litterfall at a certain location belonged not purely to one species (simplified scenario, fig 1A) but was a more homogeneous mix of litter of the different tree species present in the plot (fig 1B); or 2) that phenotypic plasticity in leaf nutrient concentration led to lower variation in the mixtures than in the monocultures (Pérez-Barrales et al. 2013). During leaf shedding, although most leaves end up close to the tree of origin, part of the leaves are carried up till 40 m away from the tree (Rothe & Binkley 2001, Jonard et al. 2006). The litterfall thus is a mix of leaves of several of the surrounding trees. In mixed plots, the relative influence of each species varies spatially, some locations have a litterfall dominated by one species while other locations have equal proportions of leaves of different species. Despite that all three tree species in our study have poor leaf quality (Vesterdal et al. 2008), they are significantly different from each other (see Table 3.1 and De Groote et al., 2017). Leaf litterfall quality is a driving force in determining decomposition rate (Bradford et al. 2016) and other studies found that our three study species differ significantly in decomposition rate and biomass loss (Hobbie et al. 2006, Jonard et al. 2008). From the spatial variation in leaf litterfall quality in mixtures and the differences in decomposition rate, we can expect a higher heterogeneity of mass and nutrient concentration of the forest floor and topsoil in mixtures compared to monocultures. However, both in the forest floor and topsoil horizons, the heterogeneity of mass and nutrient concentration in mixtures was equal to monoculture values or only slightly higher. Similar to the litterfall, the observed heterogeneity in the forest floor and topsoil was generally lower than the expectations from the simplified scenario which assumes that each point of the forest soil was influenced purely by one species. The similar levels of heterogeneity in mixtures and monocultures may indicate: that litterfall quality is

not a driving force in decomposition in our system that non-additive effects are present (Gartner & Cardon 2004, Lummer et al. 2012), or that the studied tree species had too similar litter properties to establish strong patterns (Vesterdal et al. 2008). Micro-environmental differences might be more important than chemical quality of the leaves in the creation of heterogeneous conditions in the forest floor and topsoil for our study system. Moreover, belowground factors such as root distribution or root functional traits have also been shown to mediate the effect of tree species on topsoil nutrient distribution via changes in root chemistry such as C/N ratio (Hishi 2007). Further studies encompassing a broader gradient of litterfall quality and accounting for belowground effects of trees would improve our knowledge on the effect of tree mixtures on heterogeneity in the forest floor and topsoil.

3.5.4 TREE CLUSTERING AFFECTS WITHIN-PLOT HETEROGENEITY.

The effect of individual trees on soil characteristics was spatially constrained. Spatial organization of tree species will therefore affect heterogeneity within a stand. We find higher within-plot heterogeneity when trees are clustered instead of intimately mixed. Intimate mixing of tree species leads to homogenization in environmental conditions. This might explain why many studies don't find a positive relationship between tree diversity and understory diversity (Thomsen et al. 2005, Ampoorter et al. 2016). As a result, the relationships between biodiversity and ecosystem functions from experiments may be affected by the specific planting regime and the resulting levels of heterogeneity. Care should be taken when considering such plots as a model for processes that respond to environmental conditions at small spatial scales such as microbial or plant communities. However, it is important to note that observed heterogeneity levels depend on the spatial scale of the measurements. Studies should be carefully tailored to assess heterogeneity at the relevant scale for the process under investigation. Our results imply that tree species clustering might increase soil heterogeneity on a scale of meters but this is not necessarily true on smaller scales of decimeters, which may be relevant for microbial diversity (Saetre 1999), or on larger scale in landscapes.

3.6 CONCLUSION

We did not find strong evidence for the assumption that mixed forest stands will have a higher niche diversity and spatial resource heterogeneity compared with monocultures, and therefore could harbor a higher soil biota and herb diversity. Species-specific impacts were present in our study and more clustered spatial configurations of tree species led to higher heterogeneity than intimately mixed plots. However, in general, variances were similar across species combinations in the forest floor and topsoil which indicates that tree species characteristics are not the main drivers of heterogeneity for the studied species. In all species combinations, the forest floor and the topsoil had a high spatial heterogeneity in nutrient conditions. Further research would be needed to confirm our suggestion that micro-environmental differences that are unrelated to tree identity such as precipitation, temperature and light might be the major drivers of nutrient heterogeneity in the forest floor and topsoil via differences in decomposition.

CHAPTER 4 - FLOWERING PHENOLOGY AND REPRODUCTION OF A FOREST UNDERSTOREY PLANT SPECIES IN RESPONSE TO THE LOCAL ENVIRONMENT

BRAM K. SERCU, IRIS MOENECLAHEY, BIRGIT GOEMINNE, DRIES BONTE, LANDER BAETEN

After Sercu, B. K., Moeneclaey, I., Goeminne, B., Bonte, D., Baeten, L. (submitted). Flowering phenology of a forest understorey plant species in response to the local environment. *Plant Ecology*

4.1 ABSTRACT

Temperate forest understorey plants are subjected to a strong seasonality in their optimal growing conditions. In winter and early spring, low temperatures are suboptimal for plant growth while light becomes limited later in spring season. We can thus expect that differences in plant phenology in relation to spatiotemporal environmental variation will lead to differences in reproductive output, and hence selection. We specifically studied whether early flowering, a paradoxical pattern that is observed in many plant species, is an adaptive strategy and whether selection for early flowering was confounded with selection for flower duration or was attributable to environmental variables. We used *Geum urbanum* as a study species to investigate the effect of relevant environmental factors on the species' flowering phenology and the consequences for plant reproductive output. We monitored the phenology of four to six plants in ten locations in a temperate deciduous forest (Belgium). We first quantified variation in flowering time within individuals and related this temporal variation to individual flower reproductive output. Then, we studied inter-individual variation here-in and linked this to reproduction at the plant-level, hence studying the selection differential. We found that flowering within individual plants of *Geum urbanum* was spread over a long period from June to October. Reproductive output of individual flowers, measured as total seed mass per flower, declined during the season. We found no indication for selection for early flowering, but rather for longer flower duration. Larger plants had an earlier flowering onset and a higher seed mass, which suggests that these factors covary and are condition dependent. None of the studied environmental variables could explain plant size, although soil pH and to a lesser extent light availability had a positive direct effect on seed mass per plant. Finally, we suggest that the high intra-

individual variation in flowering time, which might be a risk spreading strategy of the plant in the presence of seed predation, limits the potential for selection on flowering phenology.

4.2 INTRODUCTION

Phenology or the seasonal timing of life events is a fundamental life history trait allowing plants to adapt to the environmental conditions they experience (Ehrlén 2015, Donohue 2005). Timing of reproduction is one of the key events in a plant's life cycle, because it has a large impact on reproductive success and, hence, plant fitness. It is therefore crucial that the timing of flowering and fruiting is matched with environmental conditions to allow maximization of fitness, for instance by optimizing pollination, minimizing predation pressure or allowing maximal resource uptake prior to seed set (Ehrlén 2015, Elzinga et al. 2007). While we could expect that, on average, flowering phenology is in balance with local conditions (Ehrlén 2015, Austen et al. 2017), selection for early flowering is found to be consistent and strong across different plant species (Munguia-Rosas et al. 2011).

The start of flowering implies a switch from vegetative growth to reproduction, which is classically considered a trade-off between timing of reproduction and size at reproduction (Forrest & Miller-Rushing 2010). However, contrary to this expectation, in many cases large individuals are found to flower earlier than smaller ones (Forrest 2014). This is typically explained by condition dependence: individuals in better condition – whether because of microhabitat quality, age, or stored resources – are able to flower earlier, and at a larger size (Austen et al. 2017). The observed selection for earlier flowering might be the result of that both size and timing of reproduction are influenced by the environment (Ehrlén 2015, Austen et al. 2017).

In addition, the flower duration and the temporal pattern of flowering within a plant might interfere with the flowering onset. By continuing resource acquisition and prolonging sequential flower formation, trade-off between onset and size/resource status becomes weak, and might lead to a decoupling of the two aspects of flowering timing (Austen et al. 2017). In reality, however, a strong correlation between onset of flowering and flower duration is prevalent. Observed selection for early flowering might actually be driven by a selection for longer flower periods (Austen et al. 2017). Therefore, only by quantifying intra-individual distribution of flowers over time relative to the inter-individual variation in timing of flowers, we are able to understand how temporal patterns of flowering within individuals determines whole plant reproductive success and how selection can act upon this (Forrest & Thomson 2010).

Understorey plants in temperate forests are subjected to a strong environmental seasonality, with a relatively narrow time window in spring for plants to develop.

During winter and early spring, cold temperatures inhibit the onset of plant growth (Augspurger & Salk 2017). Interannual and regional differences in climatic factors, such as temperature and precipitation, lead to broad variation in the start of the growing season conditions (Kudo et al. 2008, Jeong et al. 2011). Once temperatures are favorable, understorey plants can initially develop in high light conditions. However, in late spring, leaf expansion of the tree canopy preludes the end of this favorable high-light period. Light at the forest floor drops to only a few percent of the total incoming irradiation after canopy closure in forests with a dense canopy (Canham et al. 1994, Augspurger et al. 2005). This means that understorey plants receive a substantial part of their total irradiation during the short period of high light availability in spring (Kudo et al. 2008, Lopez et al. 2008, Rothstein & Zak 2001). Augspurger et al. (2005) found, for example, that tree saplings species received between 33 and 98% of total irradiance prior to 100% canopy closure in spring depending on the species and its leaf-out phenology. Spatio-temporal variation in irradiation, caused by differences in canopy density and timing of canopy closure, is hence a potentially important driver of understorey plant phenology in addition to climatic differences (Baeten et al. 2015, Augspurger et al. 2005, Sercu et al. 2017, Canham et al. 1994).

We used *Geum urbanum*, a forest understorey plant, as a study species to investigate the effect of environmental factors on the species' flowering phenology and the consequences for plant reproductive output – here both seed production and seed germination. Since *G. urbanum* individuals start their growth before canopy closure and their flowering during or right after canopy closure it could be strongly affected by small differences in canopy closure timing and is therefore well suited to investigate the effect of light availability on understorey plant phenology. *G. urbanum* populations have a long flowering period from June to October, which enables us to investigate the correlation between flower duration and flowering onset. An extended flowering duration of the species, may result from both variation within and among individuals. We quantified this variation in relation to the relevant local environmental conditions (light availability and soil conditions) and individual performance. The phenology of four to six plants within each of ten locations in a temperate deciduous forest was recorded in great detail. In addition to phenology metrics at the plant level (i.e. date of first flowering), we focused on flower-level variation in timing and the consequences for reproductive output. We hence linked the phenology of individual flowers to flower performance as characterized by the number of seeds, seed mass and germination capacity of the seeds.

We specifically addressed the following questions:

- 1) How much variation in flower-level phenology can be attributed to intra-individual variation between flowers within plants, individual variation between plants within the plots, and spatial variation between plots?
- 2) Is the phenology of individual flowers related to their reproductive output?
- 3) Do plants with an early flowering onset have a higher reproductive output than plants with a later flowering onset, that is, do we find indication for selection for early flowering.
- 4) Is flowering duration correlated with date of first flower and does it explain plant reproductive output better than date of first flower?
- 5) Do we observe a trade-off between plant size and timing of flowering or do we find evidence that plant size, flowering phenology and reproductive output are influenced by the environment (light availability and soil chemical composition).

4.3 MATERIAL AND METHODS

4.3.1 STUDY SITE AND SPECIES

The research was conducted in the Aelmoeseneie forest, located south of Ghent (Gontrode, Belgium). This ca. 30 ha forested area comprises a mix of ancient and post-agricultural forest and has been managed as high forest since 1950 (Vanhellemont & Verheyen 2011). The soil consists of sand and sandy loam with alluvial ash forest on the more humid parts and acidophilous beech forest on the drier sandy parts. Within the forest, we selected ten groups of *Geum urbanum* plants in locations with different abiotic conditions; i.e. canopy openness, canopy phenology and chemical soil conditions. A group of individuals located close to each other, within a radius of 15 m, was considered a plot. In 2014, four to six plants were marked in each of the ten plots, 59 plants in total, for detailed monitoring and in 2015 an additional five plants were marked to replace the plants that died during winter of 2014-2015.

Geum urbanum is a perennial semi-rosette hemicryptophyte; basal rosettes overwinter in the vegetative state. Photosynthetic capacity of leaves is highest in winter and early spring, when the overwintering rosette leaves make up the bulk of the leaf area. The species can take advantage of high levels of irradiance just prior to tree canopy closure, through a combination of the presence of overwintering leaves, high photosynthetic capacity, and the ability to maintain photosynthesis at relatively low temperatures. Rhizomes, rosette leaves and leaves from seedlings are moderately frost resistant (Taylor 1997, Graves 1990). *G. urbanum* has a short rhizome which is little developed but supports the production of new rosette leaves in spring and autumn and flowering stems in March-April. Plants flower every year and flowering takes place from the end of May to October. Inflorescences consist of two to five flowers on long stalks in an open cyme. Flowers are mainly self-pollinated and populations show no inbreeding depression (Ruhsam et al. 2010, Vandepitte et al. 2010). A single flower produces c. 100 achenes. Seed is set from July to September, germinates in spring and does not form a persistent seed bank. As plants possess little capacity for vegetative spread, reproduction by means of seeds germinating is far more important for regeneration (Taylor 1997).

4.3.2 PLANT PERFORMANCE AND PHENOLOGY METRICS

Inflorescence length was measured once or twice a week during inflorescence elongation in the spring of 2014 and 2015. The sum of the final inflorescence lengths

was used as a proxy for plant size. Individual flowers were marked and monitored twice a week in 2014 and once a week in 2015 from May until October. We observed two peaks with high numbers of flowers in June (around day of the year 150) and in August (around day of the year 225) and a period with fewer flowers in between on the level of the population (Fig. 4.1), but also within individual plants (see first analysis). Based on these monitoring data, we derived the date of first flower, the date of the median flower and the flower period (number of days between first and last flower) for each plant.

In 2015, flowers were collected at regular intervals when seeds were fully ripe. Flowers that senesced on the plant and never produced ripe seeds were not collected. At the end of the season, all flowers with seed production had been collected. Some seeds showed herbivory damage caused by the larvae of *Byturus ochraceus* that burry through the seeds. We did not detect significant loss of flowers or seeds due to physical damage or predation by other organisms than *B. ochraceus*. The seeds from every flower were counted and weighed. As a measure of reproductive output per flower, total seed mass per flower was calculated as the sum of the mass of predated and unpredated seeds. Total seed mass per plant by was used as a measure of reproductive output per plant. Mean mass per seed was derived from the unpredated seeds. The effect of predation on reproductive output and flowering phenology is the subject of a separate study. Since predation was spread over plots and plants and seemed not to be systematically related to plant size (SI 4.1), we choose not to include this variable in the analyses of the current paper.

From one flower per plant per collection date, two subsamples of maximum 25 unpredated seeds were weighed and distributed in petri dishes lined with moist filter paper. If there were fewer than 25 unpredated seeds in a flower only one petri-dish was used. For every petri-dish, the number of seeds was recorded, total seed mass was measured and the mean mass per seed was calculated. The dishes were immediately placed in warm incubation chambers (22°C) under a 16 hour light-8 hour dark regime (see also Baeten et al. 2010b). The filter paper was moistened every week with non-distilled water. The germinated seeds were counted and removed every two weeks during 6 months until germination virtually ceased in all petri dishes. Germination was marked by the extrusion of the radicle. We calculated the proportion of seeds that germinated.

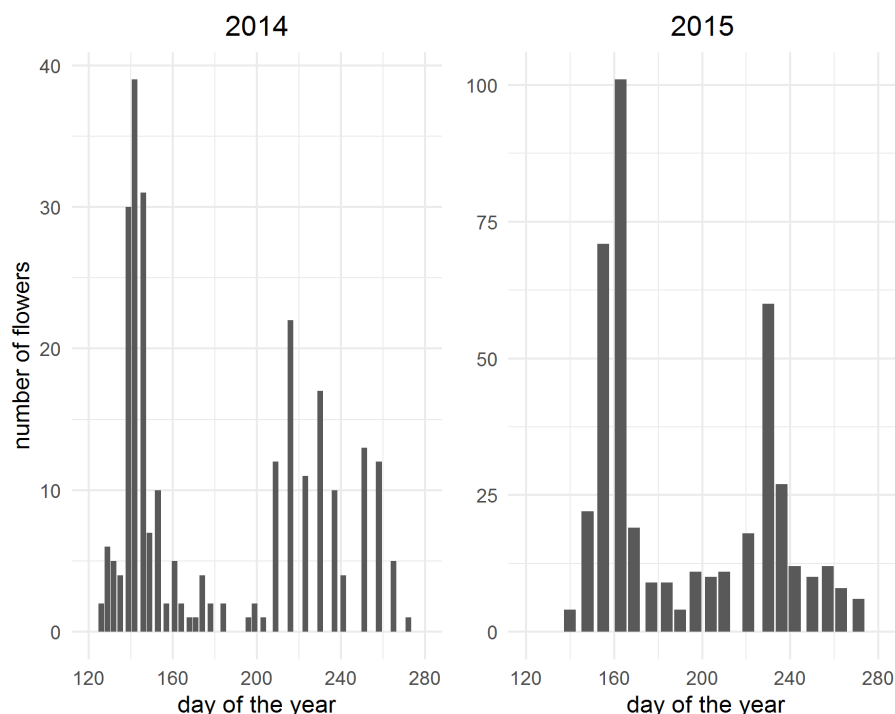


Fig 4.1. The bimodal flowering pattern of *Geum urbanum*. Number of flowers per measurement in 2014 (left) and 2015 (right).

4.3.3 ABIOTIC VARIABLES

Abiotic conditions were measured at the plot level (soil characteristics) and the plant level (light availability). In autumn 2013, four 10 cm deep mineral soil samples (after removing the litter) were taken randomly from each plot, dried at 40 °C for at least 78 h, ground and sieved with a 2 mm mesh sieve. The same was done in autumn 2014 for an additional plot. Total nitrogen (N), carbon (C) and phosphorus (P) content, plant-available phosphorus (Olsen P) and pH were measured. Total N and C concentrations were measured with a vario MACRO cube (Elementar) CNS analyser. The sample is first flushed with Argon carrier gas to remove all atmospheric nitrogen. Then, the sample is combusted under very high temperature (>1200 °C) and converted to gaseous products. The combustion gases are reduced on hot copper and the gases (N₂, CO₂ and SO₂) are separated by purge and trap chromatography and detected by a thermal conductivity detector (TCD). Total P concentrations were measured after a destruction with HClO₄ (70%), HNO₃ (65%) and H₂SO₄ (95%) in

Teflon bombs for 4 h at 150 °C. Olsen P concentrations, a measure of plant available P within one growing season, were measured after extraction in NaHCO_3 (according to ISO 11263:1994(E)). Both P fractions were measured colorimetrically according to the malachite green procedure. The soil pH was measured using 14 mL of soil in water and KCl (1M) with an Orion pH-electrode (model Ross sure-flow 8172).

A principal component analysis was performed to reduce the dimensionality of the soil data by transforming correlated variables into a set of orthogonal axes of variation, with the first representing the largest between-plot variation in soil properties. The first axis explained 66% of the variation and was positively correlated with both nitrogen and carbon concentrations and negatively with phosphorus concentration. The second axis explained 21% of the variation and was positively correlated with pH. We refer to the first axis as the soil nutrient axis and the second axis as the pH axis.

Light transmittance was measured with hemispherical images before, during and after leaf expansion above every plant. Images were captured weekly between 18 March and 22 May (2014) and 18 March and 20 May (2015), that is, from well before budburst until several weeks after full leaf expansion of all trees in the forest. With this sampling, we obtained a time series of ten images per location covering the entire leafing-out period. Full technical details on the camera-lens combination and the camera positioning and settings can be found in Sercu et al. (2017). Post processing started with binarization of the images to black and white in order to separate sky from leaf and branches with the K-means clustering algorithm (Lloyd's algorithm) from the "scikit-learn.cluster" package in python (Pedregosa et al. 2011). We used two clusters; other parameters were kept at default values. We used the free software CIMES (Gonsamo et al. 2011) to calculate total transmitted PAR to the forest floor, both direct and diffuse radiation, based on the binarized images. We calculated total transmitted PAR for each day based on the image taken closest to the respective day. We calculated PAR transmission for standard overcast conditions (SOC) and for clear sky condition (CLEAR). We assumed that each day experienced 50% overcast conditions (SOC) and 50% clear sky (CLEAR) and PAR transmission for each day was calculated as the mean of the daily SOC and CLEAR values. This is in line with the local conditions (own observations) and the estimated values for Western Europe for the entire growing season where 50% clouded and 50% open days are observed (Gendron et al. 1998). To obtain the total PAR transmission for a certain period, we summed the daily PAR transmission for the given period. We calculated total PAR transmission for a location for each of the three time periods: before (2014: day of the year 1-95; 2015 day of the year 1-103), during (2014: day of

the year 96 - 131 ;2015: day of the year 104 – 131) and after leaf expansion (2014 & 2015: day of the year 132 -286).

4.3.4 DATA ANALYSES

4.3.4.1 PARTITIONING SPATIAL VARIATION IN FLOWER-LEVEL PHENOLOGY

We used multilevel analysis of variance (Gelman & Hill 2007, Qian & Shen 2007) to partition the variation in flower-level phenology between the different spatial levels of sampling: between flowers within plants, between plants within the plots, and between the plots (first research question). The timing of flowering of every individual flower, expressed in day of the year, was used as a measure of phenology. The model included an intercept, group-level effects for plot and plant within plot, and a residual variance term accounting for the within-plant variation. The finite-population standard deviations of the effects are used as variance components for each effect (Gelman & Hill 2007, Qian & Shen 2007). We used a Bayesian approach and fitted the models with the package ‘*brms*’ with a Gaussian distribution family. We did this analysis for the data of 2014 and 2015 separately.

4.3.4.2 INTRA-INDIVIDUAL VARIATION IN FLOWERING PHENOLOGY AND FLOWER REPRODUCTIVE OUTPUT

To determine the effect of the phenology of individual flowers on their reproductive output (second research question), we regressed three variables that relate to output - that is, number of seeds (Poisson distribution), mean seed mass (Gaussian distribution) and total seed mass (Gaussian distribution) - against flowering time (day of the year) as explanatory variable. A random intercept per plant accounted for the dependency of flowers growing on the same plant. In a second analysis, we then tested whether mean seed mass is a good predictor of germination success. The probability of germination of the individual seeds of a flower (binomial distribution) was used as response variable, the mean individual seed mass of the flower as explanatory variable and we included a random intercept per plant. We used the package ‘*lme4*’ (Bates et al. 2014) to fit the models.

4.3.4.3 SELECTION FOR EARLY FLOWERING AND FLOWERING DURATION

To detect selection for early flowering and account for the role of flower duration, that is, testing the third and fourth research question, we fitted different linear selection gradients, (Austen et al. 2017, Lande & Arnold 1983). For the linear selection gradients we used the logarithm of total seed mass per plant as response

variable and included a random effect for plot in all models. As explanatory variable we used the date of first flower in the first model, the date of first flower and the flower period in the second model and the date of first flower, the flower period and total number of flowers in the third model.

4.3.4.4 CONDITION DEPENDENCY OF PLANT SIZE, FLOWERING PHENOLOGY AND PLANT REPRODUCTIVE OUTPUT

We used piecewise structural equation modelling (SEM) to test the fifth research question, that is, effects of the abiotic factors on reproductive plant performance (i.e., flowering phenology, number of flowers and seed mass), either directly or via indirect effects on vegetative plant performance (i.e. plant size). We used a SEM model to test for the alternative effects on phenology, based on the different relations the models assume. The connections and directionality in our path diagram were based on a pilot study conducted in 2013 and the knowledge within this field of research as summarized in the introduction. The piecewise SEM analyses consisted of four component multilevel regression models with plot as random effect in every model. The response variables are the plant performance variables: (i) plant size measured as total inflorescence length (Gaussian distribution), (ii) flowering phenology measured as day of the year of first flower (Gaussian distribution), (iii) number of flowers (Poisson distribution) and (iv) logarithm of the total seed mass (Gaussian distribution). The SEM tests the effect of four abiotic variables (soil nutrient axis, soil pH axis, light before canopy closure and light after canopy closure) on each response variable; the effect of plant size on flowering phenology and the number of flowers per plant, and the effect of flowering phenology and number of flowers on total seed mass. Piecewise SEM analyses were performed with the '*piecewiseSEM*' package (Lefcheck 2015).

In the supplementary information (SI 4.2) we present for completeness the same model where we interchanged the 'day of the year of first flower' with two different phenology metrics: flower duration and median flower date. We also included the analyses for 2014, where a measure for total seed mass was lacking. We excluded datapoints with missing values for any of the variables from our dataset leading to a sample size of 51 plants in 2015 and 50 plants in 2014. All analyses were performed in R Version 3.3.2 (R Core Team 2016).

4.4 RESULTS

4.4.1 INTRODUCTORY RESULTS

The vegetative growth of *G. urbanum* started at the end of March and was finished before canopy closure was completed. Flowering started when the tree canopy was about fully expanded in May. The date (day of the year) of the first flower per plant ranged from 122 to 161 in 2014 and from 140 to 242 in 2015. Plants that flowered early in one year tended to be early the next year (correlation coefficient: 0.41). *Geum urbanum* has an elongated flowering period that stretches from late spring until early autumn with two distinct peaks: during the end of June and mid-August (Fig. 4.1). The mean flower date in 2014 was day of the year 182 and in 2015 day of the year 188. A total of 424 flowers were monitored with on average of 7.9 flowers per plant (min=0, max= 47) in 2015. In 2014, a total of 575 flowers were monitored with on average 11.2 flowers per plant (min=0, max= 35).

4.4.2 PARTITIONING SPATIAL VARIATION IN FLOWER-LEVEL PHENOLOGY

Variance partitioning of the phenology of individual flowers in both years showed that most of the variation in flowering time is present within a plant (2014: 57.4%, 2015: 55.5%). Variation between plants within plots (2014: 12.5%, 2015: 26.1%) and between plots (2014: 30.2%, 2015: 18.4%) was clearly lower. This means that individuals plants show a wide range of flowering time (early as well as late flowers), rather than that there are plants or groups of plants in plots with overall early or late flowering.

4.4.3 INTRA-INDIVIDUAL VARIATION IN FLOWERING PHENOLOGY AND FLOWER REPRODUCTIVE OUTPUT

The timing of a flower within a plant was highly correlated with the total seed mass of a flower (Fig. 4.2). Flowers that emerged later in the season had a significantly lower seed mass per flower (estimate of the slope: $p < 0.001$, $R^2_m = 0.18$, $R^2_c = 0.44$). This low seed mass per flower is attributable to the significant lower seed numbers per flower (estimate of the slope: $p < 0.001$, $R^2_m = 0.68$, $R^2_c = 0.94$) as well as the significantly lower mean seed mass (estimate of the slope: $p < 0.001$, $R^2_m = 0.10$, $R^2_c = 0.46$). Finally, seeds with a higher mass were more likely to germinate (estimate of the slope: $p < 0.001$, $R^2_m = 0.19$, $R^2_c = 0.33$).

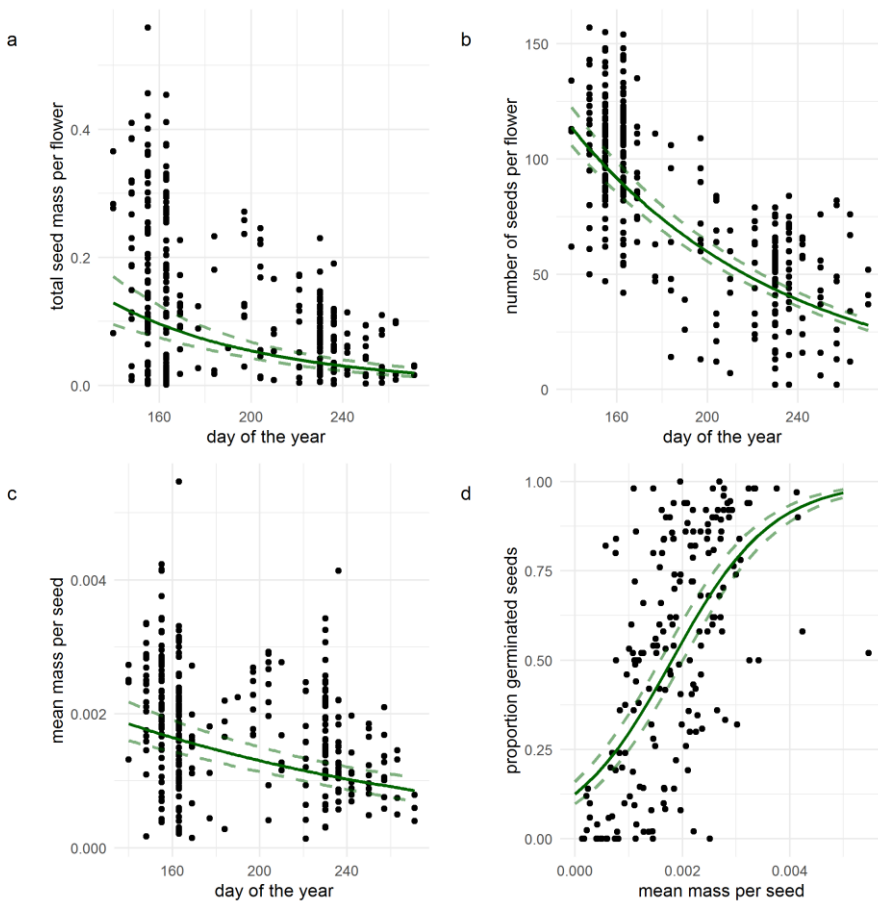


Fig. 4.2. Relationship between the timing of flowering and total seed mass (a), number of seeds (b) and mean seed mass (c) of *Geum urbanum* plants. Points are observed data for individual flowers with the model predictions (full line) and 95% confidence interval for the fixed effect (dashed lines). The last panel shows the relationship between the mean seed mass and the percentage of germinated seeds (d).

4.4.4 SELECTION FOR EARLY FLOWERING AND FLOWERING DURATION

The date of first flower was a non-significant predictor of the logarithm of seed mass per plant in all three models. Flower period was a positively related and significant predictor of the logarithm of seed mass per plant in the second model and nearly significant in the third model. The number of flowers was positive and significantly related to the logarithm of total real seed mass in the third model. Date of first flower

was not significantly related to the logarithm of total real seed mass and flower duration was positively and nearly significant related to the logarithm of total real seed mass. The correlation between the model estimates of flower period and the date of first flower was positive but rather low. In other words, plants with a large numbers of flowers and a longer flower period had a higher total seed mass. The timing of first flower seemed to have no effect on total seed mass per plant and was not correlated to the flower period. Model estimates and significances are summarized in table 4.1.

Table 4.1 : Estimates of the intercept and the fixed effects, the marginal and conditional R-squared values (R^2_m & R^2_c) and the correlation between flower duration and number of flowers of the selection gradients modeling the relation between total seed mass per plant and date of first flower (M1,M2,M3), flower duration (M2,M3) and number of flowers (M3). The significance codes indicate p-values: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < § < 0.1.

	Model 1	Model 2	Model 3
Intercept	0.51 ± 2.66	-1.73 ± 2.83	-4.81 ± 2.60 §
Date of first flower	-0.011 ± 0.017	-0.001 ± 0.017	0.015 ± 0.016
Flower duration	/	0.011 ± 0.005 *	0.008 ± 0.004 §
Number of flowers	/	/	0.083 ± 0.019 ***
R^2_m / R^2_c	0.005 / 0.525	0.100 / 0.482	0.375 / 0.545
Correlation between flower duration and number of flowers	/	0.328	0.266

4.4.5 CONDITION DEPENDENCY OF PLANT SIZE, FLOWERING PHENOLOGY AND PLANT REPRODUCTIVE OUTPUT

In the SEM analysis of 2015, the environmental variables had significant direct relationships with the three reproductive plant performance measures. The PCA-axis corresponding best to soil pH was significantly and positively related to the number of flowers and total seed mass and negatively to the flowering onset, that is, higher pH led to higher number of flowers, higher seed mass and earlier flower onset. Higher light after canopy closure and high soil nutrient content both were marginally significant and respectively positively and negatively related to total flowering onset, that is, high nutrient content and low light availability after canopy closure correlated with early flowering onset.

Larger plants produced significantly more flowers and a higher flower number was significantly related to a greater total seed mass. Larger plants also had a significant earlier flowering onset (Fig. 4.3). The SEM with flowering duration instead of flowering onset showed that none of the environmental variables correlated with flowering duration. Flower duration was however marginally significant and positively related to total seed mass (SI 4.2). All other relationships stayed unaltered. These results were supported by the analysis of the 2014 data (SI 4.2).

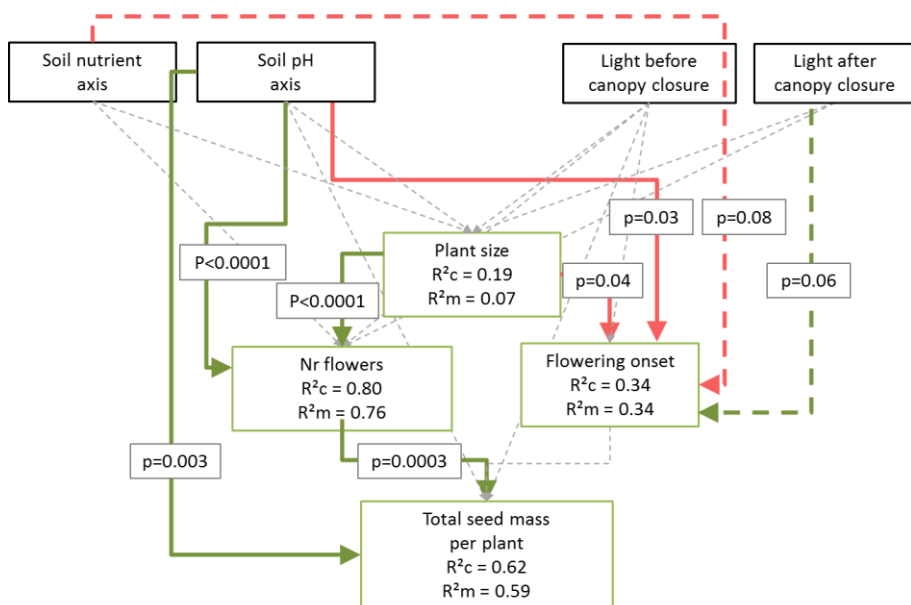


Fig. 4.3. Effects of light and soil characteristics on vegetative and reproductive output of *Geum urbanum* plants in 2015. Relationships were modeled with a piecewise SEM approach. Boxes represent the measured variables and arrows represent directional relationship among variables. The green arrows represent positive relationships, red arrows represent negative relationships. The line style indicates the significance of the relationships: full lines represent significant relationships ($p < 0.05$), thick dashed lines are marginally significant ($0.05 < p < 0.10$), thin dashed lines are not significant ($p > 0.10$) significances are given for parameter estimates with $p < 0.1$. The conditional R^2_c and marginal R^2_m values of the component models are given in the boxes of the endogeneous (response) variables.

4.5 DISCUSSION

Plants cannot move in space to find optimal environmental conditions but they can move in time by adjusting their timing of growth and flowering. We found that flowering within individual plants of *Geum urbanum* was spread over a long period from June to October with two distinct peaks, one in June-July and one in August-September and a period of few flowers at the end of July – beginning of August in between. Variation in timing of flowering between plants was low compared to the within-plant variation. Nevertheless the date of the first flower varied up to 30 days between *G. urbanum* individuals within a year.

Intra-individual variation in reproductive output of flowers showed a clear temporal trend as the number of seeds and mean seed mass were higher in early flowers compared with flowers that emerged later in the season. Heavier seeds produced by the early flowers also showed higher germination success, consistent with previous germination experiments with *G. urbanum* (Baeten et al. 2010b). Most of the reproductive output of a plant is thus realized by flowers that emerge in the early flowering peak, while flowers that emerge later in the season contribute relatively little to the overall reproductive output. Since flowering starts when the canopy is about fully expanded, flowering and seed ripening takes place under shaded conditions. Early flowers most likely still benefit from the resources that were taken up during the period of high light availability. We could thus expect that it is beneficial for *G. urbanum* individuals to flower as much as possible early in the season and that the plant has nothing to lose by extending its flowering period using the lower amount of resources available after canopy closure.

A meta-analysis by Munguia-Rosas et al. (2011) found phenotypic selection for early flowering across species. Selection was found to be stronger in more temperate regions and in species that are highly selfing and thus less dependent on pollinators for their reproduction. *G. urbanum*, a selfing, temperate understorey species with high reproductive output early in the season that declines over time seems therefore the perfect candidate to detect selection for early flowering. However, Austen et al. (2017) pointed out that this consistent selection for early flowering is rather paradoxical and could be due to (1) the fact that date of early flower is highly correlated with flowering duration and that selection for early flowering truly is selection for longer flowering period and (2) condition dependency of flowering time, plant size and fitness in which case there would be no trade-off between plant size and flowering time and thus large plants would flower early.

We found no indication for selection for early flowering, that is, we found no significant relationship between date of first flower and total reproductive output per plant in our study. The date of first flower was not correlated with the flower duration, although we found that plants with a longer flower period had a higher total seed mass. This is a logical consequence of the fact that plants with a longer flower period produce on average more flowers. But also when we controlled for the number of flowers on a plant, plants with a longer flower period tended to have a higher seed mass. The presence of seed predation is a potential factor that could explain the lack of selection for early flowering and the longer flowering period. Flowers of *G. urbanum* in the first flower peak were subject to predation from a specialist beetle, *Byturus ochraceus* (32 % of the flowers in the first peak showed seed predation and 0 % in the second peak). This seed predation could favour off-peak or later flowering and long flowering duration (Elzinga et al. 2007, Ehrlén 2015). Secondly, the long flowering period with a bimodal flowering pattern, which might be a risk spreading strategy, induces greater variation in phenology within than among individuals which limits the potential for selection on the onset of flowering (Starrfelt & Kokko 2012).

If plant fitness is condition-dependent, and related to flowering timing, then individuals growing in the most favorable micro-site conditions will be larger and flower earlier than their conspecifics (Ehrlén 2015, Forrest & Thomson 2010). We expected under this premise a clear correlation between environmental variables, phenology, plant size and plant fitness. Indeed, we found a significant relationship between plant size and flowering phenology, with earlier flowering in large plants. This agrees with other studies (Munguia-Rosas et al. 2011, Forrest & Miller-Rushing 2010). Hence, because plant size was strongly correlated with the number of flowers, it also appeared to be the main driver of total seed mass per plant. As discussed in the previous paragraph, no relationship was found between the date of first flowering and total seed mass. In addition, we found that several of the environmental variables were related with the flowering onset and with reproductive output. Low light transmittance after canopy closure and high nutrient availability and pH led to earlier flowering. There was also a direct positive relation between soil pH and the total seed mass effect and a positive indirect relation between both via the number of flowers. While these results suggest that plant size, flowering time and fitness are condition dependent, our SEM model found no correlation between the environmental variables and plant size. We explain this surprising result by the fact that plant size is likely to be principally determined by age in perennial plants; older plants might have a larger rosette and a thicker root

than younger plants. Unfortunately, we were not able to determine plant age based on the roots and we had no prior information about the age of the plants since this was an observational study. Secondly, while we expected that most environmental variation would come from soil nutrients and light availability, driven by canopy differences, we might simply have not measured those environmental variables that drive plant size in our study species (e.g. Staelens et al. 2006).

4.6 CONCLUSION

In conclusion, individual *G. urbanum* plants have a long flowering season with a bimodal pattern. Reproductive output of flowers within a plant, measured as total seed mass per flower, declines during the season, possibly because of a resource limitation (notably light). Selection for early flowering, a pattern that is commonly found in other studies (Munguia-Rosas et al. 2011), was not observed in our study. Instead, we found that a long flowering period tended to lead to higher total seed mass per plant. We also observed that larger plants had an earlier flowering onset and a higher seed mass. This suggests that plant size, timing and reproduction are condition-dependent. While light and soil nutrients were related to the flowering onset, they could not explain plant size. We suggest that plant age or an unmeasured environmental variable is the main determinant of plant size and thus reproductive output. These results provide, to a certain extent, evidence for two of the four mechanisms that Austen et al. (2017) suggested to underly the pattern of phenotypic selection for early flowering, namely selection for longer flowering duration and condition-dependency of flowering time, size and fitness.

CHAPTER 5 - INDUCED DELAYED PHENOLOGICAL AVOIDANCE: A NEGLECTED DEFENSE MECHANISM AGAINST SEED PREDATION IN PLANTS.

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After Sercu, B. K., Moeneclaey, I., Bonte, D., Baeten, L. (submitted). Induced delayed phenological avoidance: a neglected defense mechanism against seed predation in plants. *Journal of Ecology*

5.1 ABSTRACT

Flowering phenology is an important life history trait affecting plant reproductive performance and is influenced by various abiotic and biotic factors. Pre-dispersal seed predation and pollination are expected to impose counteracting selection pressure on flowering phenology, with pre-dispersal seed predation expected to favor off-peak flowering and pollination to favor synchronous flowering.

Here we studied the effect of pre-dispersal seed predation by the beetle *Byturus ochraceus*, a specialist seed herbivore, on the flowering phenology of *Geum urbanum*. This forest understorey plant species is self-pollinating so that the influence of seed predation can be studied independent from pollination. We measured in detail the timing and predation rate of individual flowers during three consecutive years in more than 60 individuals. We tested the hypotheses that pre-dispersal seed predation exerts a selection pressure leading to within-season compensatory flowering as well as to delayed phenological avoidance in the succeeding season.

We found no indication for compensatory flowering within a growing season, but plants that experienced predation shifted their flowers to the end of the flowering season the subsequent year. This induced delayed phenological avoidance points at an adaptive plastic response to pre-dispersal seed predation. Importantly, the delay in flower production came at a cost, since flowers later in the season had a reduced seed output because of increasing light limitation following forest canopy closure.

Synthesis: We found *Geum urbanum* to display a defense strategy against pre-dispersal seed predation by means of induced delayed phenological avoidance, that is, plants partially shift flowering to a later moment in the growing season succeeding a predation event.

5.2 INTRODUCTION

Plants cannot move in space to find optimal conditions for growth and reproduction, but they can move in time by adapting the phenology of their life history stages. The timing of flowering is important in this context as it directly affects reproductive success. Plant flowering phenology is determined by a multitude of biotic and abiotic factors. The impact of abiotic factors is relatively well studied: temperature, precipitation and photoperiod are constraints as well as cues for the phenology of plants (Forrest & Miller-Rushing 2010). However, not only abiotic forces play a role in shaping a plant's flowering phenology, selection on flowering phenology can also be modulated by biotic interactions (Elzinga et al. 2007). In general, selection for early flowering is found to be consistent and strong across different plant species (Munguia-Rosas et al. 2011, Geber & Griffen 2003).

Pre-dispersal seed predators and pollinators are the main biotic selective agents acting on flowering phenology (Elzinga et al. 2007, Munguia-Rosas et al. 2011). In a review study, Kolb et al. (2007) found that pre-dispersal seed predators act as selective agents on flowering phenology in more than 80% of all tested species. Hence, plants that shift their timing of reproduction and flower off-peak in order to avoid the moment of predation could experience a fitness advantage (Elzinga et al. 2007). On the other hand, plants that depend on pollinators need to flower preferably during the peak of flower availability in the population to ensure successful fertilization. Hence, pollination and pre-dispersal seed predators are two counteracting selective agents (Atlan et al. 2010).

Off-peak flowering is a commonly observed response to pre-dispersal seed predation (Elzinga et al. 2007, Kolb et al. 2007). However, different defense mechanisms and adaptive responses could lead to off-peak flowering. First, pre-dispersal seed predation could impose disruptive selection on the date of first flowering, and/or directional selection for longer flowering periods (Elzinga et al. 2007), resulting in changes in the mean and variance of flower phenology at the population-level. The defense mechanism deployed in this scenario can be classified as phenological avoidance. Secondly, pre-dispersal seed predation might also select for adaptive phenotypic plasticity (Grenier et al. 2016), which is considered as induced defenses in the plant defense literature. On the one hand, seed predation early in the season can induce the growth of extra flowers within the same growing season, i.e. compensatory flowering, a tolerance trait. Compensatory flowering has been observed as a response to herbivory of inflorescences early in the season, before flowering, but not yet as a response to seed-predation (Eriksson 1995, Pettersson

1994). For instance, *Ipomopsis aggregata* plants that experienced damage to the apical meristem early in the season produced significantly more inflorescences, but there was a delay in flowering time and fewer flowers were produced in total (Freeman et al. 2003, Brody & Irwin 2012). The delay in flowering reduced, however, the rate of seed predation because the overlap in time between the flowers and seed predators was smaller. Similarly, *Lythrum salicaria* plants produced more inflorescences and more flowers after clipping and this had no effect on the rate of pollen limitation (Thomsen & Sargent 2017). On the other hand, pre-dispersal seed predation might lead to phenotypic plasticity in the form of an induced delayed response in the growing season succeeding the predation event. It has been shown that perennial plants can plastically adapt in response to environmental pressures they experienced the year before (Stam et al. 2014, Karban 2008). The timing of growth and flower development are traits expressed over an entire growing season and the onset of flowering cannot be adjusted within one season. But if the risk of seed predation is predictable based on the predation experienced the previous year, we can expect that plants respond to seed predation by delaying the flowering phenology in the next growing season. We refer to this defense mechanism as an induced delayed phenological avoidance.

Here we studied the effect of pre-dispersal seed predation on flowering phenology during three consecutive years using *Geum urbanum* L. as a study system. As this forest understorey plant species is mainly self-pollinating and does not require pollinators to reproduce successfully (Ruhsam et al. 2010, Vandepitte et al. 2010), the selective pressure of pre-dispersal seed predation is not counteracted by pollinators. Furthermore, *G. urbanum* has a long flowering period from the end of May until October and seed predation only occurs on the seeds of the early flowers, because adult predators are only active from the end of June till the end of July. The pre-dispersal seed predator are the larvae of the widespread and specialized beetle *Byturus ochraceus*, which exclusively feed on *G. urbanum* (Taylor 1997). We collected detailed data on the flowering phenology of individual flowers to test the following hypotheses related to the consequences of predation for timing of flowering and plant fitness (here reproductive output):

- i) The reproductive output of individual flowers within a plant decreases with later flowering phenology, so that early flowers produce, on average, the highest seed mass.
- ii) In plants that experience pre-dispersal seed predation, there is a substantial reduction of reproductive output in the early flowers, so that, on average, late flowers have the highest seed mass.

- iii) Pre-dispersal seed predation changes the selection gradient between plant level flowering phenology and total seed mass from directional selection for early flowering in unpredated plants to directional selection for later flowering in predated plants.
- iv) Pre-dispersal seed predation induces compensatory flowering within a growing season and/or delayed phenological avoidance, with the phenological response in the year after the predation event.

5.3 MATERIAL AND METHODS

5.3.1 STUDY SITE AND SPECIES

The research was conducted in the Aelmoeseneie forest, located south of Ghent (Gontrode, Belgium). This ca. 30 ha forested area entails a mix of ancient and post-agricultural forest and has been managed as high forest since 1950 (Vanhellemont & Verheyen 2011). The soil consists of sand and sandy loam with alluvial ash forest on the more humid parts and acidophilous beech forest on the drier sandy parts. Within the forest, we selected ten groups of *Geum urbanum* plants in different locations spread over the forest. Differences in abiotic conditions, i.e. canopy openness, canopy phenology and chemical soil conditions were measured and controlled for but none of these variables were correlated with flowering phenology, reproductive output or presence of seed predators and could therefore be ignored in this study. A group of individuals located close to each other, within a radius of 15 m, was considered a 'plot' (N = 10 plots). In 2014, 59 plants were marked for detailed monitoring and in 2015 an additional five plants were marked to replace the plants that died during winter of 2014-2015. In 2016, the set of monitored plants was expanded with another 50 plants. In each plot, a minimum of four and maximum of six plants were marked.

Geum urbanum is a perennial semi-rosette hemicryptophyte; basal rosettes overwinter in the vegetative state. Photosynthetic capacity of leaves is highest in winter and early spring, when the overwintering rosette leaves make up the bulk of the leaf area. The species is an early summer bloomer (Kudo et al. 2008) that can take advantage of high levels of irradiance just prior to tree canopy closure through a combination of high photosynthetic capacity, the ability to maintain photosynthesis at relatively low temperatures and the presence of overwintering leaves. Rhizomes, rosette leaves and leaves from seedlings are moderately frost resistant (Graves 1990, Taylor 1997). *G. urbanum* has a short rhizome which is little developed but supports production of flowering stems in March-April and new rosette leaves in spring and autumn. Plants flower every year and flowering takes place from the end of May to September. Inflorescences consist of 2-5 flowers on long stalks in an open cyme. Flowers are mainly self-pollinated and populations show no inbreeding depression (Ruhsam et al. 2010, Vandepitte et al. 2010). A single flower produces ca 100 achenes. Seeds are hairy and have a 5–7-mm long hook, facilitating exozoochorous seed dispersal. Seed is set from July to September, germinates in spring and does not form a persistent seed bank. Plants possess little

capacity for vegetative spread so that reproduction by means of seeds germinating is far more important for regeneration (Taylor 1997).

Byturus ochraceus (Byturidae, Scriba 1790) is a small beetle which emerges about three weeks before the flowering of *Geum urbanum* (Pellmyr 1985). During this interim period, the beetles are found feeding on pollen and nectar in the flowers of at least 30 different plant species such as *Taraxacum officinale* and *Ranunculus acris*, and the forest understorey species *Anemona nemerosa*, *Geranium sylvaticum* and *Fragaria vesca* (Pellmyr 1985, Bohem 2015). The beetles will start to feed and mate exclusively on flowers of their host plant, *Geum urbanum*, as soon as it starts flowering. Oviposition takes place from mid-June to mid-July. After 14 days, the first larvae emerge and begin feeding on the fruit, leaves and seeds according to Springer & Goodrich (1986). In our study, leave and fruit herbivory was negligible and *byturus ochraceus* predated almost exclusively on seeds. The larvae are present from the end of June till the end of August (Springer & Goodrich 1986).

5.3.2 PLANT PHENOLOGY AND PREDATION METRICS

Plants were monitored twice a week in 2014 and once a week in 2015 from May until October. Timing of flower emergence was recorded as the day of the year and all open unmarked flowers were marked with a unique tag to enable identification of individual flowers. Total number of flowers per plant could be derived from this data at the end of the season. Based on the data of 2014 and 2015, flowers were monitored on fewer occasions in 2016 (on day of the year 186, 194, 202, 209, 225, 251) and 2017 (day of the year 194, 262, 291). We observed two flowering peaks per season during the four consecutive years of observations: high number of flowers in June (around day of the year 150) and in August (around day of the year 225) and a period with less flowers in between (around day of the year 194) (SI 5.1 in the Supplementary Information). This period of fewer flowers coincides with the end of predator infection. Because of the bimodality of the flowering phenology of *G. urbanum* it was hard to define one general flowering peak and, therefore, to derive a measure of off-peak flowering. During the first flowering peak most flowers emerged and seed predation took place, so that we considered a higher proportion of flowers in the second peak to represent 'off-peak' flowering. We calculated for each plant the proportion of flowers that appeared in the second peak (from day of the year 194 until the end of the season) relative to the total number of flowers.

In 2014 – 2016, flowers were collected throughout the season when seeds were ripe, that is, for each plant we collected flowers on different occasions. In 2014 and 2015, few flowers contained beetle larvae, which means that seed herbivory was still ongoing. In 2016, flowers were collected slightly later in order to ensure that seed herbivory was completed and no larvae were found in the flowers anymore. The seeds from all flowers were checked for herbivory by *Byturus ochraceus*. The number of seeds and seed mass was determined separately for predated and unpredated seeds for each flower. From this data, we calculated the mean mass per seed for unpredated seeds. As a measure of reproductive output per flower, total seed mass per flower was calculated as the sum of the mass of predated and unpredated seeds. As a measure of reproductive output per plant, total seed mass per plant was calculated as the sum of the seed mass of all flowers on that plant.

5.3.3 ANALYSIS

5.3.3.1 REPRODUCTIVE OUTPUT PER FLOWER OVER TIME IN PREDATED AND UNPREDATED PLANTS

To test the first two hypotheses, we quantified the change in reproductive output per flower with the timing of flowering in predated and unpredated plants. More specifically, we analyzed whether, within a plant, seed mass per flower decreased linearly over time or was best predicted by a quadratic function and whether this pattern was different in plants that experienced predation compared with unpredated plants. The first model regressed seed mass per flower as response variable against flowering date as explanatory variable, including plant identity as random effect. The second model was an expansion of the first model with the second order polynomial of flowering date as an extra explanatory variable. To ensure that the estimate of the intercept from the model is an estimation of the seed mass for the earliest possible flower we rescaled the flowering date. Instead of using the day of the year, which would estimate an intercept for the 1th of January, we set the date of the earliest flower in the population to zero. Both models were fitted on the subset of the data including all flowers from plants that experienced no predation and on the subset of the data including all flowers from plants that experience predation in at least one flower. We compared the first and the second model using their AIC values and considered the model with the lowest AIC to be most consistent with the data and represented these in the result section. If the difference between AIC values was lower than two, the simplest model was preferred (Burnham & Anderson 2004). We fitted the models with the 'lmer' function from the lme4 package in R.

5.3.3.2 SELECTION GRADIENTS OF FLOWERING PHENOLOGY IN PREDATED AND UNPREDATED PLANTS

To quantify the effect of predation on the selection gradient of plant flowering phenology on plant-level fitness, that is, testing the third hypothesis, we fitted linear and quadratic selection gradients, (Lande & Arnold 1983). The logarithm of total seed mass per plant was used as response variable and the total number of flowers and the first (and second in the quadratic selection gradient) polynomials of the proportion of flowers in the second peak were used as explanatory variables. The models included a random effect for plot identity. We fitted these models for 2015 and 2016 data separately. To infer how predation influences the fitness gradients, we created a model based on data from the plants that experienced no predation and a model based on data from the plants where at least one flower was predated. We used the logarithm of total seed mass per plant as response variable since the seed mass per plant showed a non-normal distribution without transformation.

5.3.3.3 COMPENSATORY FLOWERING AND DELAYED PHENOLOGICAL AVOIDANCE

According to the fourth hypothesis, we expected plants to use tolerance as a defense mechanism and show compensatory flowering, that is, plants that experienced predation early in the growing season compensated the loss in reproductive output by increasing the number of flowers later in the growing season in the same year. To test this expectation, we regressed the proportion of flowers per plant in the second peak against the percentage of flowers per plant in the first peak that experienced predation. Plot identity was included as a random effect. This analysis was done on the 2015 and 2016 data separately. We used a Bayesian approach and fitted the models with the package ‘*brms*’ (Bürkner 2016). We used a zero inflated binomial distribution to account for the high number of zero’s for plants that did not have flowers in the second peak.

The fourth hypothesis also led to the expectation that plants use delayed phenological avoidance as a defense mechanism. We analyzed the effect of predation events on the flowering phenology the year after the flowering event. As a measure of off-peak flowering, the proportion of flowers per plant in the second peak compared to the total number of flowers per plant was used as response variable. The explanatory variable was the proportion of flowers from the first peak that were predated the previous year and we included plot as a random effect. Two separate models were fitted for two subsequent years: the effect of predation in 2015 on the temporal distribution of flowers in 2016 and the effect of predation in

2016 on the temporal distribution of flowers in 2017. Models were fitted again with *brms*; for the analysis of 2015-2016 we used a binomial distribution and for the analysis of 2016-2017 a zero inflated binomial distribution after checking the posterior predictions. All analysis were performed in R (R Core Team 2016).

5.4 RESULTS

5.4.1 INTRODUCTORY RESULTS

Geum urbanum has an elongated flowering period that stretches from June to October with two distinct peaks during the end of June and mid-august. Predation took place almost exclusively during the first flowering peak in June and the beginning of July (Fig. SI 5.1 a,b,c). More than 50% of the plants experienced predation (2014: 88%, 2015: 65%, 2016: 56%, 2017:56%). Both large and small plants were predated (table SI 5.1) and the severity of predation differed from plant to plant, between 2% and 100 % of all flowers of a plant were predated (Fig. SI 5.1,d,e,f & table SI 5.1). From the flowers that were predated, on average about half of the seeds were eaten (2014: 44%, 2015: 60%, 2016: 65%).

5.4.2 REPRODUCTIVE OUTPUT PER FLOWER OVER TIME IN PREDATED AND UNPREDATED PLANTS

The reproductive output of flowers, measured as total seed mass per flower, decreased throughout the growing season of 2015 within a plant (first hypothesis). For unpredated plants, the first model with only the first order polynomial was most consistent with the data (AIC of the first model = -235; AIC of the second model = 277) and had an intercept of 0.29 g (SE= 0.021), i.e. seed mass of earliest flowers for an average unpredated plant, and a significant slope of -0.0028 (SE = 0.0002, $p < 0.001$), i.e. the decrease in seed mass per flower per day for an average unpredated plant. For predated plants, we also found that the linear model fitted the data better than the quadratic model (AIC of the linear model = -346; AIC of the quadratic model = 486). The linear model had an intercept of 0.15 (SE = 0.013) and a significant slope of -0.0013 (SE = 0.0002, $p < 0.01$). In sum, predation caused the seed mass of early flowers to be lower than in an average unpredated plant, so that late flowers are relatively more important for reproductive output in predated plants than in unpredated plants (second hypothesis). Predation was not so severe that the sign of the slope changed, that is, that seed mass increased over time or showed an optimum at an intermediate timing, instead, the average seed mass of early flowers was still higher than that of late flowers in predated plants. (Fig. 5.1).

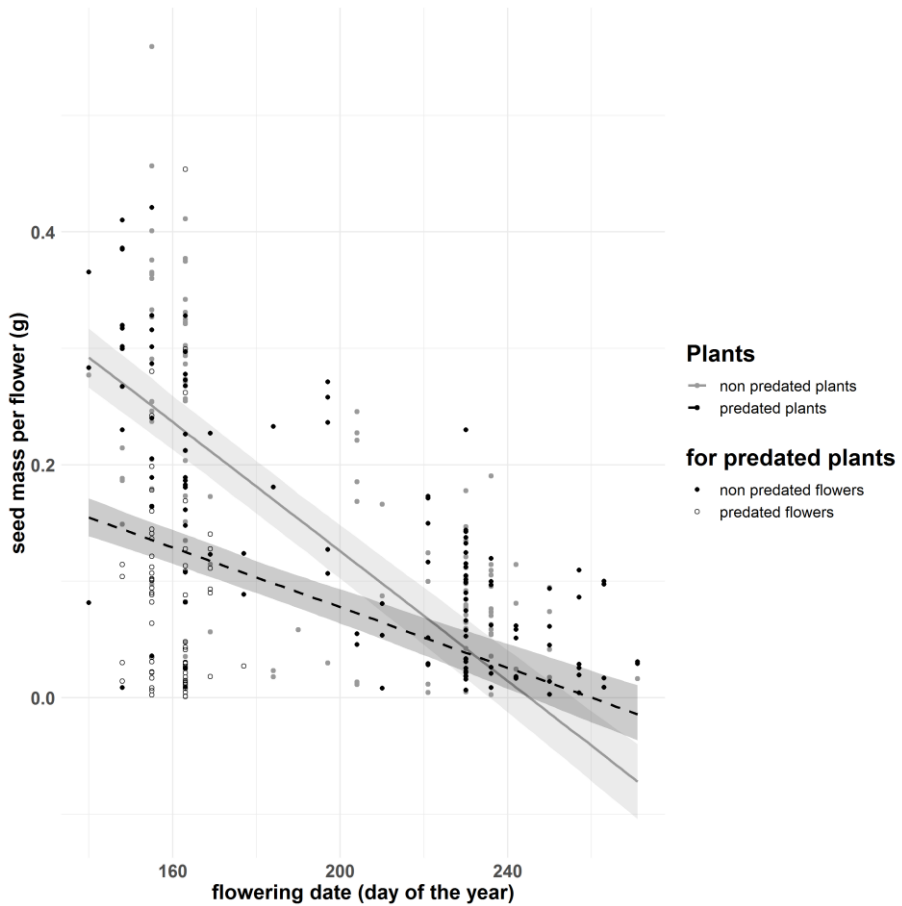


Fig. 5.1 - Change in seed mass per flower within *G. urbanum* plants over time. The seed mass per flower of predated and unpredated plants was regressed against the flowering date (day of the year). The lines show the fits of linear models $\pm$ 95% confidence intervals accounting for the fixed effects. For both predated (dashed, black) and unpredated plants (solid, gray), the mean seed mass per flower decreases linearly over time. For predated plants, the mean seed mass of early flowers is lower than in unpredated plants, therefore, late flowers are relatively more important for reproductive output in predated plants than in unpredated plants.

5.4.3 SELECTION GRADIENTS OF FLOWERING PHENOLOGY IN PREDATED AND UNPREDATED PLANTS

The total plant fitness, measured as total seed mass per plant, was mostly determined by the total number of flowers of plants, since it was significant in all models (see SI 5.2 for model parameters). The more flowers, the higher the total plant-level seed mass. The effect of flower phenology on total seed mass per plant varied. For unpredated plants, no significant relationship between proportion of flowers in the second peak and total seed mass per plant was found. For predated plants in 2016, the total seed mass of predated plants showed a quadratic relationship, with significant parameter estimates, so that plants with an intermediate degree of off-peak flowering (36% of flowers in the second peak) had the highest seed mass per plant (Fig. 5.2) (third hypothesis). For predated plants in 2015, the quadratic model was most consistent with the data showing a similar pattern as in 2016 however the first and second polynomial of the proportion of flowers in the second peak were respectively nearly and not significantly related to seed mass (SI 5.2). Plants with an intermediate degree of off-peak flowering (43% of flowers in the second peak) had the highest seed mass per plant in 2015.

5.4.4 COMPENSATORY FLOWERING AND DELAYED PHENOLOGICAL AVOIDANCE

We found no indication for compensatory flowering within a single year. In both years, the slope of the relationship between plant-level predation and the proportion of flowers per plant that flowered in the second peak in the same year did not differ significantly from zero (95% credible interval (CI) of slope in 2015: -0.0040 – 0.0129 ; 2016 CI: -0.0094 – 0.0029). The sign of the mean effect even differed between years (Fig. 5.3 a, b). Plants did show delayed phenological avoidance, that is, plants that experienced high predation rates in 2015 had a higher proportion of their flowers in the second peak in 2016 (95% CI: 0.0021 – 0.0241). A similar relationship between predation in 2016 and flowering phenology in 2017 (95% CI: -0.0051 – 0.0182) was observed, however the relationship was less strong than in 2015 since the tail of the 95% credible interval contained zero (Fig. 5.3: c, d).

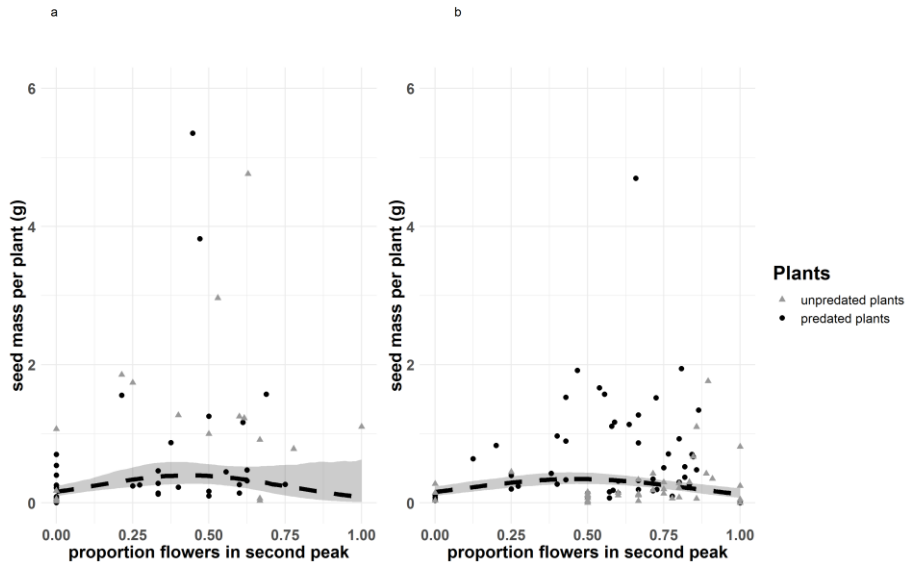


Fig. 5.2 - Selection gradients for total seed mass per plant (reproductive output) against the proportion of flowers in the second peak in (a) 2015 and (b) 2016. Points represent data, lines represent fitted values from models for predated plants (black circles, black dashed line) and unpredated plants (gray triangles) separately and shaded areas are the 95% credible intervals of the fitted relationships. Fitted values are derived for plants with an average number of flowers and are only shown for significant relationships ($P < 0.1$), that is, only for predated plants in 2015 and 2016. No significant relationship was found for unpredated plants in 2015 and 2016.

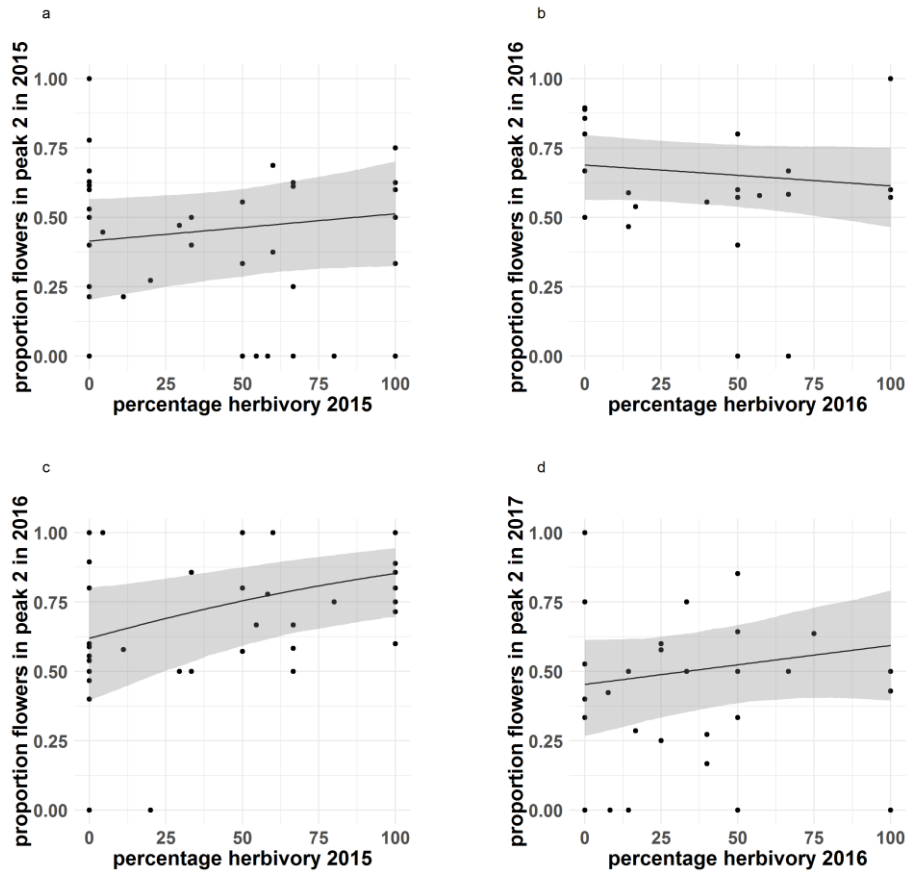


Fig. 5.3 - Panel a & b: The effect of the proportion of seed predation experienced (x-axis) on the proportion of flowers flowering in the second peak within the same year (y-axis), that is, compensatory flowering. Panel b & d: The effect of the proportion of seed predation experienced (x-axis) on the proportion of flowers flowering in the second peak the year after the predation event (y-axis), that is, delayed phenological avoidance. The left panels (a & c) shows the effect of seed predation in 2015, the right panels (b & d) show the effect of seed predation in 2016. 95% credible Intervals are indicated in gray.

5.5 DISCUSSION

Pre-dispersal seed predation can exert a selection pressure on the flowering phenology of plants, favoring off-peak flowering. However, in many plants this selection pressure is counteracted by the selection pressure for flowering in synchrony with the population to attract pollinators. The choice of *Geum urbanum*, a selfing understory forest plant, as study species enabled us to study the effect of pre-dispersal seed predation without the counteracting effect of pollinators. We found that the species shows a long flowering duration and two distinct flowering peaks. In absence of predation, most of the reproductive output of the species is realized in the first flowering peak and seed mass per flower declines in later flowers. Seed predation by the specialized seed herbivore *Byturus ochraceus* is also restricted to this first flowering peak and predation varied from 0 up to 100% of all flowers from the first flowering peak per plant and significantly reduced the average reproductive output of early flowers. Looking at plant fitness measured as total seed mass per plant, there was no indication for selection for early flowering in unpredated plants, but there was evidence that the pre-dispersal seed predation imposes selection for moderate off-peak flowering. This indicates that predation might exert a selective pressure on flowering phenology. We found no indication for compensatory flowering within the same year, but pre-dispersal seed predation induced delayed phenological avoidance: a high predation rate led to a shift in flowering phenology to the second flowering peak the year after the predation event.

Selective pressures can only impose evolutionary changes in trait means and variances when they have a high heritability, i.e. a high amount of additive genetic variation. Organisms can, however, also adapt to environmental changes by plastic responses, i.e. by adjusting trait means and variances during development, or across generation through induced maternal effects (Donohue 2005). While genetic adaptation works over several generations, phenotypic plasticity can take place within one or two generations and promotes rapid adjustment to the environment (Grenier et al. 2016). We found pre-dispersal seed predation in *G. urbanum* to be unrelated to spatial variation in a set of environmental variables (soil pH, soil nitrogen, carbon and phosphorus content, light availability) and to plant characteristics (plant size, number of flowers) (Moenneclaey et al. unpublished data). In the context of our study system, where pre-dispersal seed predation is variable in space and likely equally subjected to variation on longer time frames, phenotypic plasticity is anticipated to be more adaptive than a genetically fixed phenology (Grenier et al. 2016, Karban 2011). On a short timescale, the level of predation in one

year is a good predictor for the level of predation the year after, as larvae hibernate and pupate in the forest floor litter close to the locations of the infected plants they developed in (Burgess & Marshall 2014). Furthermore, a prerequisite for the expression of a plastic response to predation is that plants can detect one way or another that they suffer predation. Plants are known to possess sophisticated perceptual abilities that allow them to monitor and respond to a wide range of changing biotic and abiotic conditions. This sensory perception system can play a role in plant-animal interactions including the perception of herbivory and even oviposition (Mescher & De Moraes 2014).

Plants that experienced pre-dispersal seed predation did not compensate the fitness loss via the production of more flowers in the second flowering peak within the same growing season. The lack of compensatory flowering might be the consequence of resource limitation (Sercu et al. submitted), restrictive architectural and developmental pathways of the plant (Prusinkiewicz et al. 2007, Diggle 1999) or a combination of both. In the few studies that found compensatory flowering after predation, predation or clipping happened before bud formation or flowering. All three studies found a higher number of inflorescences after the apical meristem had been damaged, but also a delayed flowering phenology (Brody & Irwin 2012, Freeman et al. 2003, Thomsen & Sargent 2017). However the time frame to plastically respond to herbivory is much smaller in the case of pre-dispersal seed predation compared with vegetative damage to the inflorescences before bud formation. From an architectural or developmental point of view, *G. urbanum* plants could compensate for damage in the first peak since the flowers from the second peak mostly originate from branches that grow on the main inflorescence branch and form extra flowers (personal observations). However, the elongation of lateral branches and the formation of new buds consumes time and resources leaving little opportunity for compensatory flowering later in the season. We can conclude that *G. urbanum* does not use tolerance traits as a defense strategy against pre-dispersal seed predation. Such tolerance strategies were neither found in other species with a similar bimodal flowering pattern that suffered predation in the first peak (Pettersson 1994, Eriksson 1995).

We found evidence that *G. urbanum* uses delayed phenological avoidance as a defense mechanism against pre-dispersal seed predation. Plants that experienced seed predation partly shifted their flowering to the second flowering peak the year after the predation event. While phenological avoidance of pre-dispersal seed predation might lead to lower herbivory damage, it entails a fitness cost because flowering and seed ripening might happen at moments that are sub-optimal due to

unfavorable abiotic conditions, especially limited light availability. To our knowledge, delayed induced phenological avoidance in a perennial plant species has only rarely been reported. Adaptive shifts in timing are thus so far a neglected strategy of delayed induced resistance, which adds to the more frequently reported morphological and biochemical mechanisms, as for instance increased production of spines (Alves-Silva & Del-Claro 2016) or defense metabolites (Kaitaniemi et al. 1998, Korndörfer & Del-Claro 2006, Zvereva et al. 1997, Martemyanov et al. 2012) during seasons succeeding substantial herbivory damage.

CHAPTER 6 – GENERAL DISCUSSION

BRAM K. SERCU

In this PhD thesis we aimed to detect whether high tree diversity creates more heterogeneous conditions, with a focus on light transmission to the forest floor (Chapter 2) and soil nutrients (Chapter 3). In a second step we determined whether differences in resource quantity had a detectable effect on plant fitness (chapter 4) and whether interactions with other trophic levels, in our case pre-dispersal seed predation, was an important driver of plant fitness (Chapter 5). For chapter 4 & 5, we used *Geum urbanum* as a study species and especially focused on the role of plasticity of flowering phenology as a mechanism to maintain high fitness under different environmental conditions.

In the first part of the discussion we critically discuss the spatial scale at which the research was conducted and the implications for the interpretation of the results. In the second part we discuss the results of chapter 2 & 3 about heterogeneity in resource conditions. We discuss the evidence from my research and from literature that tree diversity increases resource heterogeneity. In the third part of the discussion we discuss the results from chapter 4 & 5 in the light of chapter 2 & 3. After a more theoretical reflection we discuss the effect of light and nutrients on flowering phenology and reproductive output and the role of variability of flowering phenology as means to maintain high fitness under pre-dispersal seed predation. In a fourth part we point out some future research directions. More specifically we return to the question of spatial scales of heterogeneity and diversity, secondly we discuss possible ways to study the relative importance of ecological selection, dispersal and drift, the three relevant high level processes that shape herbaceous communities in forests.

6.1 SPATIAL SCALES OF HETEROGENEITY AND BIODIVERSITY

Spatial scale is a very important aspect of ecological studies and is increasingly considered in B-EF studies. The appropriate plot size for a study will depend on the process or pattern of interest. For example, the study of decomposition processes requires smaller plots than the study about how forest diversity affects diversity of large associated species such as birds (Scherer-Lorenzen et al. 2005). Moreover, conditions within the plot may be affected by the plot's surrounding, for example, soil chemical properties can be affected by litter input of surrounding trees over distances of several tens of meters (Rothe & Binkley 2001) and edge effects can alter microclimatic conditions and nutrient cycling up to 125 m (Schmidt et al. 2017). To eliminate edge effects, plot sizes of several hectares would be required, which is practically and financially unfeasible (Scherer-Lorenzen et al. 2005). Plot size in the tree diversity experiments typically range from 10 m² to 40 000 m², with most study plots below 900 m² (a rectangle of 30 m × 30 m) (Verheyen et al. 2015, Leuschner et al. 2009). The plot size recommended by Scherer-Lorenzen et al. (2005) is to use double the height of final tree height as side length of a study plot, i.e. 5000 – 10 000 m² for trees reaching 30m in height (Leuschner et al. 2009). However in mature forests it is hard to find such large plots that have homogeneous abiotic conditions and don't have admixture of non-target species. Studies about the effect of tree diversity and composition on the herb layer in mature forests used circular plots with a radius of 20 m (1256 m²) (Vockenhuber et al. 2011), square plots of 50 m × 50 m (2500m²) (Mölder et al. 2008), 10 m × 10 m (100 m²) or 20 m × 20 m (400 m²) (Wulf & Naaf 2009). The tree diversity-oriented exploratory platforms FundivEurope and TREEWEB plot size was 900 m² (Baeten et al. 2013, De Groote et al. 2017). This plot size was the optimal balance between size and avoiding non-target species (Baeten et al. 2013). During the selection of the TREEWEB plots we ensured that the trees adjacent to the plot were the same species as the trees within the plot to reduce effects of the surrounding environment. It has to be noted that the plots in the TREEWEB exploratory were located in forest fragments with different degree of fragmentation and isolation since the TREEWEB project explicitly quantified the interactive effects of tree diversity and fragmentation on ecosystem functioning. Distance to the closest edge was defined as the shortest Euclidian distance from the center of the plot to the closest forest edge and ranged between 7.0 m and 215.5 m. This means that plots were differentially exposed to edge effects. There is evidence that distance to the forest edge might affect mean resource conditions (Schmidt et al. 2017), this means that edge effects might have created additional heterogeneity in the plots with strong edge effects in the parts of the plot close to the edge and

weaker effects in the area of the plot further away. We did not correct for these edge effects in our analyses since plots of every species combination were located along a similar fragmentation gradient leading to similar between-plot differences in every species combination, while we were mainly interested in ‘within-plot’ variability. Moreover, the distance to the closest edge was not significant in explaining mean nutrient values in the plots (S.R.E. Degroote personal communication).

Another important factor affecting heterogeneity of resources within a plot is the spatial configuration of the trees within the research plot. In tree diversity experiments a regular spatial distribution of tree individuals and tree species and equal distances between tree individuals are established in the plots, although more recently, clumped tree distribution patterns were also established in experiments (Leuschner et al. 2009). Also during the set-up of the plots in TREEWEB an intimately mixed configuration was sought after and clustered species configurations were avoided, still a gradient in the level of clustering was present between the plots of each species combination. For B-EF research, it is logical to plant or select intimate mixtures and avoid clustered patterns, since most diversity effects are to be expected at the contact zone between different tree species. For example, an intimate mixture of tree species is the perfect spatial configuration to enable crown complementarity, which leads to a higher primary productivity. However, according to the results in chapter 2 & 3, an intimate mixture does not increase spatial heterogeneity of light and soil nutrients. In chapter 3 we found that on the small spatial scale of a plot and despite the small effect of tree species identity, a more clustered spatial configuration led to higher heterogeneity of soil nutrients compared to a more intermixed configuration. This was most likely caused by the fact that the litter layer in intimate mixtures was more homogeneously mixed throughout the plot whereas higher clustering led to patches within the plot with monospecific litter composition. This means that highest heterogeneity within a 900 m² plot will be obtained when tree species are clustered rather than intermixed.

Finally we can ask ourselves at which spatial level we want to maximize resource heterogeneity and thus biodiversity (in the assumption that resource heterogeneity is the main driver of biodiversity). It is not because 900 m² is the most practical plot size to do research that we want to maximize heterogeneity at this level. Conservation goals are generally expressed at the level of a forest patch comprising several forest stands or even at the landscape level comprising several forest patches. Attempting to maximize biodiversity at a small spatial scale (i.e. α -diversity) could even lead to biotic homogenization, where all communities resemble each other and differences between local communities (i.e. β -diversity) is reduced. This

biotic homogenization is potentially negative for total associated diversity as well as ecosystem multifunctionality in a larger area (Mori et al. 2018, van der Plas et al. 2016, Vellend et al. 2017).

6.2 TREE DIVERSITY EFFECTS ON RESOURCE HETEROGENEITY

The tree species in the TREEWEB exploratory (*Fagus sylvatica*, *Quercus robur* and *Quercus rubra*), have species-specific impacts on the environment via differences in light transmittance and leaf nutrient concentrations (Canham et al. 1994, Leuschner & Meier 2018, De Groot et al. 2018). Therefore mixed stands are supposed to have more heterogeneous environmental conditions, which can enable coexistence of associated species. However, rigorous investigations of the effect of tree diversity on heterogeneity of environmental conditions on the scale of a research plot is very scarce. The research chapters 2 & 3 are therefore a first step to fill in this knowledge gap. A main outcome of these chapters investigating resource heterogeneity was that, despite the presence of interspecific differences in light transmittance and leaf nutrient concentrations in our study species, the heterogeneity of light transmission to the forest floor and nutrients in the forest floor and topsoil was not driven by tree diversity. Mixed species stands had in general a variance similar in magnitude compared to the monocultures. The exception was nutrient content in the leaf litter which was more heterogeneous in diverse stands. The mechanisms behind the similar variance in monocultures and mixed stands are different for light and nutrients. In the next section we discuss the patterns and processes for each resource separately. In concordance with the introduction (fig 1.1), we first look at the distribution of resource values (mean and variance) for each monoculture, secondly we formulate the expectations for the case where species are mixed and heterogeneity is determined by additive effects or diversity effects both for the monospecific fingerprint scenario and the complete mixing scenario (fig 1.1 a & c).

6.2.1 LIGHT TRANSMISSION

In most studies, light transmission is measured after canopy closure. However, most forest understorey plants start the development of photosynthesizing tissue before the canopy is fully developed. We therefore measured light transmittance in three periods, 1) before bud burst, 2) during canopy opening, i.e. between bud burst and full leaf expansion and 3) after all trees have fully expanded leaves.

Monoculture distribution: The mean and variance of light transmission before canopy closure was similar across monocultures although variance of *F.sylvatica* monocultures tended to be slightly lower than in *Q. robur* and *Q. rubra* monocultures. We expected differences in mean light transmission during and after canopy closure between monocultures of *F. sylvatica*, *Q. robur* and *Q. rubra* since it is known from literature that their crowns have a different light transmission

coefficient and different phenology (Canham et al. 1994, Leuschner & Meier 2018). We found indeed interspecific differences in mean light transmission during canopy closure but no differences in mean light transmission were observed after canopy closure in the monocultures of the three different species. The variance of light transmission during canopy closure was similar in all monocultures and after canopy closure we found that *F. sylvatica* had a lower variance than *Q. rubra* and *Q. robur*.

Mixed stands: In the case of light transmission, we expected diversity effects, most notably a denser canopy due to canopy packing and crown plasticity (Jucker et al. 2015, Williams et al. 2017), to reduce mean light transmission across the plot in all periods but most strongly after canopy closure. This reduction in mean light transmission was expected to reduce the variability in light transmission. Despite the fact that crown area was higher in mixtures than in monocultures, mean light transmission after canopy closure in mixtures was not systematically lower compared to monocultures in our study. A diversity effect was only present for the variance of light transmission after canopy closure in the *Q. rubra* – *Q. robur* mixture, where it was lower than would be expected from monoculture values. The lack of differences in mean and variance of light transmission across tree species compositions is most likely caused by the shrub layer, which forms a complementary layer reducing light transmission to similar levels within the different monocultures and mixtures. The species causing the complementary light reduction were mainly *Coryllus avellana* in the *Q. robur* plots and immature *Q. rubra* trees in the *Q. rubra* plots. Shrubs had the highest cover under the relatively open *Q. robur* canopy. Shrub cover was very low under *F. sylvatica* monocultures which are known to have a high light interception at the canopy level. The observation that the shrub layer attenuates any difference in light transmittance at the canopy level is found in other studies as well. Bartemucci et al. (2006) found that the upper understorey layer tended to homogenize light levels at the forest floor in different forest types. However, without the interference of a shrub layer our data suggests that heterogeneity of light availability at the forest floor in mixtures would rather be lower instead of higher compared to monocultures due to the effect of canopy packing in mixtures.

6.2.2 LITTERFALL, FOREST FLOOR AND TOPSOIL NUTRIENTS

The availability and distribution of nutrients in the forest floor and topsoil heavily depends on the decomposition of the leaf litter. Recent studies found that decomposition is mainly driven by species-specific litter traits and through local environmental variation (Joly et al. 2017, Bradford et al. 2016, Chomel et al. 2016).

Monoculture distribution: De Groote et al. (2018) found that in the TREEWEB plots, monocultures of *Q. robur*, *Q. rubra* and *F. sylvatica* have significant differences among each other in carbon, nitrogen and base cation stock and C/N ratio within the leaf litterfall and forest floor and in base cation stock in the topsoil. Despite the fact that all three species can be classified as trees with low quality litter, they differentially impact the nutrients in the leaf litterfall and topsoil.

Mixed stands: Plot mean nutrient values in mixed plots were intermediate between the constituting monocultures, that is, only additive but no diversity effects were detected (De Groote et al. 2018). Based on these observations we expected that the variance would be higher in mixtures than in monocultures wherever there are interspecific differences in nutrient content between monocultures. Only in the leaf litterfall, we found higher variance in mixtures than monocultures. In the forest floor and topsoil, heterogeneity of nutrient conditions was similar in all monocultures and mixtures. The similar variance across species combinations in the forest floor and topsoil might be due to the fact that tree species characteristics are not the main drivers of heterogeneity. Heterogeneity increased when going from the litter over the forest floor to the topsoil. In all species combinations, the forest floor and the topsoil had a high spatial heterogeneity in nutrient conditions. Although these results need to be interpreted with some caution since the sampling area differed between litter, forest floor and topsoil we believe the results represent the real situation. We suggest that micro-environmental differences that are to a large extent unrelated to tree identity such as precipitation, temperature and light are the major drivers of nutrient heterogeneity in the forest floor and topsoil via differences in decomposition. Also in other studies resource heterogeneity at the forest floor is found to be highly variable at small spatial scales (Bell et al. 1993) (Farley & Fitter 1999). Lechowicz & Bell (1991) measured pH, K^+ and NO_3^- concentration in a mixed forest with two tree species at different spatial scales. They found that all three factors varied significantly at the 0.1 m scale within a 1m² area. None of the three soil measures is very predictable at distances >2m. Although they did not relate the measurements to the overstorey, their results indicate that environmental factors vary at a spatial scale much smaller than the spatial configuration of tree canopies. Staelens et al. (2006) found high variation in rain troughfall below the canopy of a single tree. Also microbial activity was found to be equally variable over space and not to converge with crown projection patterns (Gömöryová 2004, Baldrian et al. 2010).

Table 6.1 summary of the results of chapter 2&3

		mean		variance	
		difference between monocultures	Diversity effects	difference between monocultures	Higher heterogeneity in mixtures
Nutrient - leaf litter	C	Yes	No	No	Yes
Nutrient - leaf litter	N	Yes	No	Yes	Yes
Nutrient - leaf litter	C/N	Yes	No	Yes	Yes
Nutrient - leaf litter	BC	Yes	No	No	Yes
Nutrient - leaf litter	Mass	Yes	No	Yes	No
Nutrient - forest floor	C	Yes	No	No	No
Nutrient - forest floor	N	Yes	No	Yes	No
Nutrient - forest floor	C/N	Yes	No	Yes	No
Nutrient - forest floor	BC	Yes	No	No	No
Nutrient - topsoil	C	No	No	Yes	No
Nutrient - topsoil	N	No	No	Yes	No
Nutrient - topsoil	C/N	No	No	No	No
Nutrient - topsoil	BC	Yes	No	No	No
Light	before canopy closure	No	No	No	No
Light	during canopy closure	Yes	Yes - negative	No	No
Light	after canopy closure	No	Yes - negative	Yes	No

6.2.3 CONCLUSION

We can conclude that tree species diversity is, in our study and in several other cases, not the main driver of heterogeneity of resources at the forest floor. We found that heterogeneity both for light and soil nutrients is strongly determined by factors that are largely independent of the tree species identity. Heterogeneity was lower in a mixed stand where species are intimately mixed compared to more clustered configuration, which indicates that intimate mixing further homogenizes the already weak tree identity effects. The absence of higher heterogeneity in light and soil variables in mixed species compared to monoculture plots can help to explain why so many studies do not find a relationship between tree diversity and herb species diversity (Ampoorter et al. 2016, Ampoorter et al. 2014, Augusto et al. 2003, Bartels & Chen 2010, Bartemucci et al. 2006, Dupré et al. 2002, Reich et al. 2012, Tinya & Ódor 2016).

6.3 RESOURCE AVAILABILITY AND PREDATION AS DRIVERS OF PLANT FITNESS AND THE ROLE OF FLOWERING PHENOLOGY TO PLASTICALLY RESPOND TO THESE DRIVERS.

In the next section we aim to interpret the results of Chapter 4 and 5 in the light of Chapter 2 & 3. We concluded from chapter 2 & 3 that tree species identity and diversity are not main drivers of resource heterogeneity at the forest floor, and therefore, are unlikely to be a driver of forest herb diversity, at least not for the functionally relatively similar tree species studied in these chapters. However, we found that resource conditions within a plot show considerable variation in monoculture as well as mixed species plots. Moreover, as we discussed in the section about spatial scales in this discussion, from a biodiversity conservation point of view we are not necessarily interested in resource heterogeneity at the spatial scale of a research plot but rather at the larger spatial scale of a forest or a landscape. At this spatial scale, resources might vary due to differences in, for example: land-use history, management practices or stand structure (Chen et al. 1999). The question therefore remains whether these resources constitute important niche axes for forest understorey plants. Spatial variability in resource conditions, regardless of the origin of this variation, could then lead to spatially variable ecological selection enabling species to coexist locally (Vellend 2010, Chesson 2000a). We use the term spatially variable selection as defined by Vellend (2016): “Spatially variable selection is, to a large extent, the manifestation of different strengths and directions of constant selection (i.e., selection favoring particular species, regardless of their frequencies) within sites. A key assumption here is that some aspects of the abiotic or biotic environment is the cause of ecological selection, favoring different species in different sites” (Vellend 2016).

A first step in determining whether gradients in resource conditions at the forest floor are driving understorey species coexistence via spatial variable ecological selection is finding out how resource gradients impact plant fitness (Chase & Leibold 2003), in our case, fitness of *Geum urbanum*.

6.3.1 FITNESS CONSEQUENCES OF VARIATION IN LIGHT AND NUTRIENT CONDITIONS.

We found evidence that differences in light and nutrient availability have an impact on fitness of *G. urbanum*. Other studies also found effects of light availability and nutrient conditions on plant vegetative and regenerative fitness in cases where the differences were large e.g. (Baeten et al. 2010a, Joner et al. 2012). However, the

effect of these resources is rather limited. Plant size was the most important factor determining total seed mass per plant, but plant size was unrelated to light or nutrients. We suggest three explanations for the absence of a correlation between plant size and resource availability: 1) plant size is a function of plant age, a factor we could not quantify. We supposed that the root of *G. urbanum*, although it has some capacity to store nutrients, is too small to impact plant size and fitness and therefore plant size and fitness is mainly a function of resource availability within the same season. However if this assumption is wrong and the nutrients stored in the root of *G. urbanum* can impact plant growth and fitness, plant performance in one year would be dependent on the age and life history of the plant. In that case plant reproductive output should be measured over the entire lifespan of a plant or somehow account for previous life history events; 2) other environmental variables (e.g. water availability, competition with other species, belowground herbivory) impacted plant size. It has to be noted that our studies did not take in account water availability because of practical reasons and because there is little evidence about species specific effects on water availability. However, throughfall water from precipitation is impacted by canopy architecture and some studies identified differences between tree species (Schume et al. 2003, Staelens et al. 2006). 3) while light values were measured for each plant separately, nutrient values were measured per plot and therefore within plot variation is not accounted for. As discussed above, there can be considerable spatial variation in nutrient availability even within one m² (Lechowicz & Bell 1991). More detailed measurements of nutrient availability might change the magnitude of the correlation between nutrient availability and total seed mass per plant, either directly or via plant size.

It has to be mentioned that the *G. urbanum* individuals that we measured were selected in the field. This means that we only selected plants at places where they grew naturally. If there is very strong ecological selection either allowing plants to grow and have high reproductive fitness or not to grow in a location at all we would not be able to observe the gradual effect of environmental factors on reproductive output. We do not think this is likely since *G. urbanum* plants occurred and were measured in stands with different light and nutrient conditions. However, maybe, light and nutrient conditions were rather similar between our study plots compared to the range of these variables throughout the entire forest. Finally, we cannot exclude that ecological selection via light, nutrients or any of the unmeasured environmental variables is weak, which would leave room for stochastic processes to play an important role in determining herbaceous plant species fitness at the forest floor.

6.3.2 FLOWERING PHENOLOGY AS A PLASTIC TRAIT

In the introduction and in chapter 4 and 5, we discussed the importance of flowering phenology as a central trait of life history that is therefore expected to be under strong selection. Flowering phenology is a complex trait: onset of flowering, mean flowering date and flowering period are different aspects of flowering phenology. Overall, *G. urbanum* plants have a long flowering period but most reproductive output is realized early in the season when resource availability is high. The elongated flowering period throughout summer and fall might be positive for plant fitness in absence of predation since it produces viable seeds despite the low light availability. In addition the long tail of flowering can serve as a mechanism to 1) maintain some reproductive output in case of predation and 2) enable plastic responses to herbivory the year after the predation event. We did not detect selection for early flowering, a pattern which is found in many studies and plant species (Munguia-Rosas et al. 2011, Geber & Griffen 2003). This widespread observation of selection for early flowering is in itself rather paradoxical as Austen et al. (2017) discusses in his article. He points out 4 mechanisms that can explain this apparent selection for early flowering, two of which we observed for *G. urbanum*, namely selection for longer flowering duration and condition-dependency of flowering time, size and fitness. We observed that larger plants had an earlier flowering onset and a higher seed mass. In addition, we found that a long flowering period tended to lead to higher total seed mass per plant, even after correction for the number of flowers.

From our results it appears that flowering phenology, although constrained by several factors, displays quite some intraspecific variation. Intraspecific trait variation can stem from fixed genetic differences or phenotypically plastic responses to different environmental conditions. Clearly some of the variation in flowering phenology in *Geum urbanum* is inducible phenotypic plasticity, as has been shown in chapter 5, allowing individuals to change their phenology from one year to the other based on predation events. However, the onset of flowering appeared to be rather conservative over the years within individuals. The fact that onset of flowering varies across individuals but is conservative within an individual over the years can be due either to genetic or environmental differences between individuals (Austen & Weis 2015). Our set up did not allow us to disentangle the effect from gene vs environment effects. Nevertheless, we found that light, soil pH and soil nutrients and plant size, which might reflect plant age, were related to date of onset of flowering. This suggests that at least some of the variation in flowering timing is driven by the environment.

6.3.3 CONCLUSION

Although light, soil nutrients and pre-dispersal seed predation, the environmental variables studied in chapter 4 & 5, all had an effect on seed output of *G. urbanum*, none of these variables was an important direct or indirect driver of plant reproductive output. Seed output was strongly correlated with plant size but we could not identify the set of environmental variables shaping plant size. Amongst the environmental variables that could drive plant size and reproductive output are several unmeasured variables: plant age, hydrology and soil nutrients which was measured at a coarse grain in this study.

While onset of flowering seemed to be rather constrained we found considerable intraspecific trait variability in the distribution of flowers throughout the season. Flowering phenology was not related to light and nutrients nor did it impact seed output. It seems that flowering phenology does not play a role in maintaining high fitness under different resource conditions. However we found that phenotypic plasticity of flowering phenology functions as an inducible defense mechanism. We found that predated plants shifted their flowering partially to a later moment in the season the year after a predation event. To our knowledge this is the first study showing delayed induced phenological avoidance as a defense strategy in a perennial plant species.

6.4 FUTURE RESEARCH DIRECTIONS

The current thesis is a first contribution to gain a better understanding whether and how forest restoration and management agencies can achieve high associated diversity by manipulating species richness and spatial configuration of planted species. The research was focused on herbaceous plant performance in response to the main resources for plant growth, light and nutrients. Clearly, an interesting future research direction is to study the same questions for other tree species mixtures and other associated species groups. However, in this section we will keep the focus on herbaceous plant species richness. Even for this well studied trophic level, still many questions are left unanswered.

6.4.1 SPATIAL SCALE OF BIODIVERSITY, HETEROGENEITY AND THE ROLE OF TREE SPECIES DIVERSITY

A pertinent aspect of all related research is the scale at which biodiversity needs to be established. Current studies mostly consider within plot diversity in plots with a size below 900 m² (a rectangle of 30 m x 30 m) (Verheyen et al. 2015, Leuschner et al. 2009). However, high diversity is mainly aimed for on the scale of an entire forest or even at the landscape level. This requires studies to look at alpha, beta and gamma diversity simultaneously (van der Plas et al. 2016, Ulrich et al. 2017, Mori et al. 2018).

The first question is whether and in what spatial configuration tree species lead to high resource heterogeneity on different spatial scales. In the current PhD thesis we investigated three tree species that were rather similar in leaf nutrient content. Choosing functionally less similar tree species could maybe lead to different or more pronounced patterns in resource heterogeneity in tree mixtures and monocultures. It would be interesting to take a very applied perspective and try to find what planting scheme and species combinations should be used in forest restoration if the goal is to achieve high resource heterogeneity at the forest floor level. Our results suggested that a more clustered species occurrence pattern might create higher heterogeneity in soil nutrient resources than intimate mixing in the area of a study plot. An area consisting of adjacent monocultures might therefore be a more heterogeneous environment than the same area consisting of an intimate mix of species (Ampoorter et al. 2014). However, on the landscape level a more complex configuration could be possible. Gamfeldt et al. (2013) proposes adjacent stands, each with multiple species but in different combinations, as the best way to provide multiple ecosystem services at the landscape scale. A landscape with a combination of monocultures and mixtures would be especially interesting if resource conditions

in a mixed plot form a ‘novel environment’ that is different from the conditions in the constituting monoculture species (Ampoorter et al. 2016).

A second question is whether and at what spatial scale the environmental heterogeneity created by tree species leads to higher associated diversity. The theoretical work of Chesson (2000a) about competitive coexistence in spatially variable environments suggests that spatial variation promotes coexistence on all spatial scales. The author states the location parameter ‘ x ’ in his equations, “could be a particular patch or cell in the landscape supporting a local population, a particular point in continuous space, or a point on a lattice able to support just a single individual organism”. In several review studies evaluating whether environmental heterogeneity is a driver of species richness across spatial scales, generally a positive relation is found (Stein et al. 2014, Tews et al. 2004, Lundholm 2009). However, Stein et al. (2014) found that this relationship is stronger when the grain size increases and Lundholm (2009) found more studies with no significant effect at smaller grain size. Some papers suggested that this is not an artefact but that environmental heterogeneity on a very small spatial scale might reduce species diversity due to microfragmentation (Laanisto et al. 2013, Tamme et al. 2010, Tews et al. 2004). We believe forest ecosystems provide an interesting study system to perform experimental and observational studies investigating the effect of spatial environmental variability on biodiversity. An important aspect of such study would be to define the scale of spatial variability. A workable definition of small spatial scales is given in Richardson et al. (2014) relative to the dispersal neighborhood of the species.

6.4.2 OTHER HIGH LEVEL PROCESSES IN FOREST UNDERSTOREY COMMUNITY COMPOSITION: DRIFT AND DISPERSAL

The expectation that tree diversity is positively related to herb diversity is based on the premise that the selection process is the driver of herb species diversity in forests at the scale of a research plot. So far there is little evidence that this is the case. We found that differences in light and nutrient conditions were only very weak drivers of plant fitness. Moreover, tree species composition had no effect on the heterogeneity of resource conditions at the forest floor. Light and nutrients were equally variable under monocultures and tree mixtures. Most studies investigating the relationship between overstorey diversity and understorey diversity at the plot scale do not find a positive relationship (Ampoorter et al. 2016, Ampoorter et al. 2014, Augusto et al. 2003, Bartels & Chen 2010, Bartemucci et al. 2006, Dupré et al. 2002, Reich et al. 2012, Tinya & Ódor 2016).

If ecological selection is weak, and relative fitness and demographic parameters of individuals of different species are very similar, drift, i.e. stochastic processes, rather than deterministic processes could play an important role in community composition (Vellend 2010, Chesson 2000b). In addition, the research plot is not a closed system but species from outside the plot might disperse into the research plot. Therefore diversity within a research plot might not just be dependent on processes within the plot but also from the plant community in the larger forest.

Clearly, considering simultaneously drift, dispersal and ecological selection enables a vast amount of interactions and outcomes as is discussed by Vellend (Vellend 2010, Vellend 2016). Moreover, all kind of confounding factors are possible. Nevertheless, we believe several more specific research questions could be posed in an attempt to understand how understorey plant communities are formed and what the role of tree species is in this.

A first research focus is to further investigate whether resource gradients in the forest constitute an constant ecological selection pressure. By planting or sowing individuals of *G. urbanum* and other forest understorey species with the same age in different environmental conditions it is possible to determine the effect of resource differences on plant fitness (reproduction and survival). This approach would overcome two limitations in our study : 1) the fact that plant age or previous life history might impact the performance of an individual and obscure the effect of instantaneous environmental conditions, 2) the fact that we selected individuals in their natural environment and therefore might not span the available resource gradients in the forest. If time was no constraint, the study could simultaneously comprise a part where resources were experimentally manipulated with addition of fertilizer, shade cloths and water treatments extending the natural range of resource conditions (e.g. Baeten et al. 2010a) and an *in situ* experiment in several pre-determined locations in an existing forest with known resource conditions. By planting enough replicates a natural gradient in the amount of herbivory could occur and be included in the measurements.

This study could be expanded by planting several individuals of different species together under different resource conditions to additionally quantify the effect of direct competition on plant fitness. Another expansion would be to plant species in different densities in order to try to detect density-dependent processes, an ecological selection mechanism that has been ignored in this PhD thesis thus far.

If constant or density dependent selection is detected as an important determinant of plant fitness, it would be interesting to further investigate whether and how spatially variable selection is important in forest plots. However if ecological selection is not detected this would indicate that drift potentially plays an important role in community composition. However finding conclusive proof for drift as an important mechanism is very hard since there is always the possibility that plant fitness and community composition is driven by a factor we did not measure or account for.

Finally based on the experiments described above and the pre-existing knowledge in literature about dispersal and colonization capacity of forest herbs (Verheyen et al. 2003, Baeten et al. 2014) it would be possible to make predictions about the distribution patterns of particular species within a forest where resource conditions are mapped. For example, species with high dispersal capacity that experience negative density-dependent selection are expected to be homogeneously dispersed throughout the forest. Species with low dispersal capacity that are subjected to strong constant ecological selection will occur in high densities locally and probably will not occur in remote places that would be suitable.

APPENDIX 1 - LATE LEAF EXPANSION, A STRATEGY TO ESCAPE LEAF HERBIVORY?

BRAM K. SERCU, VAN SCHROJENSTEIN LANTMAN, I.

APP. 1.1 INTRODUCTION

In temperate deciduous tree species, the phenology of leaf expansion is considered to be a trade-off between a longer growing season and frost damage (Bennie et al. 2010, Lenz et al. 2015, Augspurger 2013). Trees use a combination of day length and temperature (often measured as growing degree days) as cues to time their leaf expansion. Different tree species have a different sensitivity to these cues and differ in the exact conditions they require to expand their leaves (Lechowicz 1984, Vitasse & Basler 2013, Morin et al. 2010, Gerst et al. 2017, Zohner et al. 2017). Leaf-out timing therefore differs between species and the order in which trees expand their leaves in spring remains relatively constant over the years (Wesolowski & Rowinski 2006). Due to interannual variation in mainly climatic factors, the exact timing of leaf expansion of different trees varies slightly between years (Lechowicz 1984, Bennie et al. 2010).

Since the selection pressures driving leaf expansion phenology in trees seem fairly simple and strong, we would expect relatively low intraspecific variation in this trait (Zohner et al. 2017). However, in addition to the interspecific differences in the timing of leaf expansion, intraspecific differences occur as well (Wesolowski & Rowinski 2006, Cole & Sheldon 2017, Lechowicz 1984). And also within a species, the relative order of timing of leaf expansion is conservative between years, early trees will be consistently early across years (Wesolowski & Rowinski 2006). These intraspecific differences in timing of leaf expansion can amount to several weeks between individuals of the same species growing in the same location (Wesolowski & Rowinski 2008, Bobinac et al. 2012). While these differences might be partly due to differences in the local environmental conditions, it is likely that high variation in leaf expansion phenology is adaptive within a population (Dantec et al. 2015, Fenner 1998).

Fenner (1998) identifies herbivory as an important selective force shaping leaf expansion phenology. He suggests that leaf herbivory might be avoided by either synchronous leaf expansion leading to predator satiation or by off-peak flowering. Wesolowski & Rowinski (2008) found that late leaf expansion can be an anti-herbivore defense. While late leaf expansion is supposed to be disadvantageous due

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to a lower photosynthetic gain in this period of high light availability, trees with an early leaf expansion experience higher levels of herbivory. The lower photosynthetic gain in trees with late leaf expansion is then compensated by low levels of herbivory.

We tested the hypothesis that trees with late leaf expansion were less subjected to herbivorous damage. We did this for three tree species, *Quercus robur*, *Quercus rubra* and *Fagus sylvatica*. in the TREEWEB research platform in Belgium.

APP. 1.2 MATERIAL AND METHODS

APP. 1.2.1 STUDY AREA AND SAMPLING DESIGN

We scored the leaf expansion of every tree within the 53 plots of the TREEWEB exploratory, for a total of 1358 trees. Scoring was done once a week during 5 consecutive weeks on 8 april, 13 april, 20 april, 27 april and 6 may. We scored the trees according to their level of bud burst and leaf expansion on a scale from 1 to 5, where 1 was no bud burst and 5 was full leaf expansion (supplementary information SI app1.1). Based on these scores we divided each tree in one of the four phenological categories. If a tree had fully expanded leafs at the scoring date of 20 april, 27 april, 6 may it was assigned the category 'very early', 'early' and 'late', respectively. If leafs were not yet fully expanded on 6 may it was assigned the category 'very late'. No trees had full leaf expansion before 20 april.

For each tree species and for each phenological category, 7 or 8 trees were selected (86 in total). From these trees, we collected 30 leaves using a catapult and a manual chain-saw. For each leaf, we estimated the percentage of the leaf that was eaten by herbivores and we counted the number of leaf miners present on the leaf. For each tree, we finally calculated the mean percentage of herbivory per leaf and the total number of leafminers in the 30 leaves to use in further analysis

APP. 1.2.2 ANALYSIS

We first tested whether the level of herbivory differed between species. We performed an analysis of variance (ANOVA) with post hoc Tukey HSD test for the two dependent variables: logarithm of 'mean percentage herbivory' (Gaussian distribution) and 'total number of leafminers' (poisson distribution) with 'tree species' as explanatory variable and a random effect for plot identity. Since tree species differed significantly from each other in the level of herbivory and leafminers we performed the subsequent analysis per tree species separately.

To test whether phenological categories differed between each other in susceptibility to herbivory we performed an analysis of variance (ANOVA) with post hoc Tukey HSD test for each species. We performed the analysis for the two dependent variables: logarithm of 'mean percentage herbivory' (Gaussian distribution) and 'total number of leafminers' (poisson distribution) with 'phenological category' as explanatory variable and a random effect for plot identity.

APP. 1.3 RESULTS & DISCUSSION

Timing of leaf expansion varied up to 3 or 4 weeks between individuals of the same tree species in our plot (fig app. 1.1). Herbivory and number of leaf miners differed significantly between tree species. The percentage of herbivory was significantly lower in *F. sylvatica* than in *Q. robur* and *Q. rubra* whereas the number of leafminers was significantly higher in *Q. robur* than in *F. sylvatica* and *Q. rubra* according to the post hoc test. These results are similar to the findings of van Schrojenstein Lantman et al. (2018). However, we found no indication that late leaf expansion led to lower levels of herbivory in *Q. robur*, *Q. rubra* or *F. sylvatica* in our plots. The amount of herbivory and the number of leafminers did not differ significantly between the different phenological categories in none of the tree species. The only trend in the data (p -value < 0.1 in the ANOVA) was that herbivory on very late *Q. rubra* trees was lower than early (p -value = 0.13 in the post-hoc pairwise comparison) *Q. rubra* trees (fig. app. 1.2).

The intraspecific phenological differences in leaf expansion in our plots are similar to the variation observed in Wesolowski & Rowinski (2008). The potential for equally large differences in herbivory is therefore present in our plots. While our results suggest that herbivory is most probably not a driver of leaf phenology in our plots, our study cannot be considered conclusive disproof of the hypothesis that trees with late leaf expansion escape herbivory. First of all, the phenological scoring of the trees was rather coarse and by scoring trees more frequently, a finer temporal resolution could be obtained. This might enable us to see more subtle effects of leaf expansion phenology on herbivorous damage. Secondly, as was found by Wesolowski & Rowinski (2008), in some years the pattern may not be detected. Future studies could measure herbivory rates over multiple years, because the magnitude of differences is known to vary among years. Thirdly, we measured herbivory at the end of the season. Although we expect that the herbivory measured on the leaves that have been collected in September was mainly due to herbivory that took place early in the season, we cannot be certain of this. It is possible that differences in herbivory were present in early summer but have been reduced by herbivory later in the season.

Moreover, the selection pressure for late leaf expansion to avoid herbivory might not be the only selection pressure acting on leaf expansion phenology in trees. Other studies found a selection pressure for early leaf expansion to avoid mildew infection (Dantec et al. 2015). This article found a trade-off between frost damage and mildew infection. Early leaf expansion was beneficial in some circumstances since it led to

lower mildew infection rates. Moreover, other studies found higher incidence of leaf miner infection on trees with late leafing out (Mopper & Simberloff 1995).

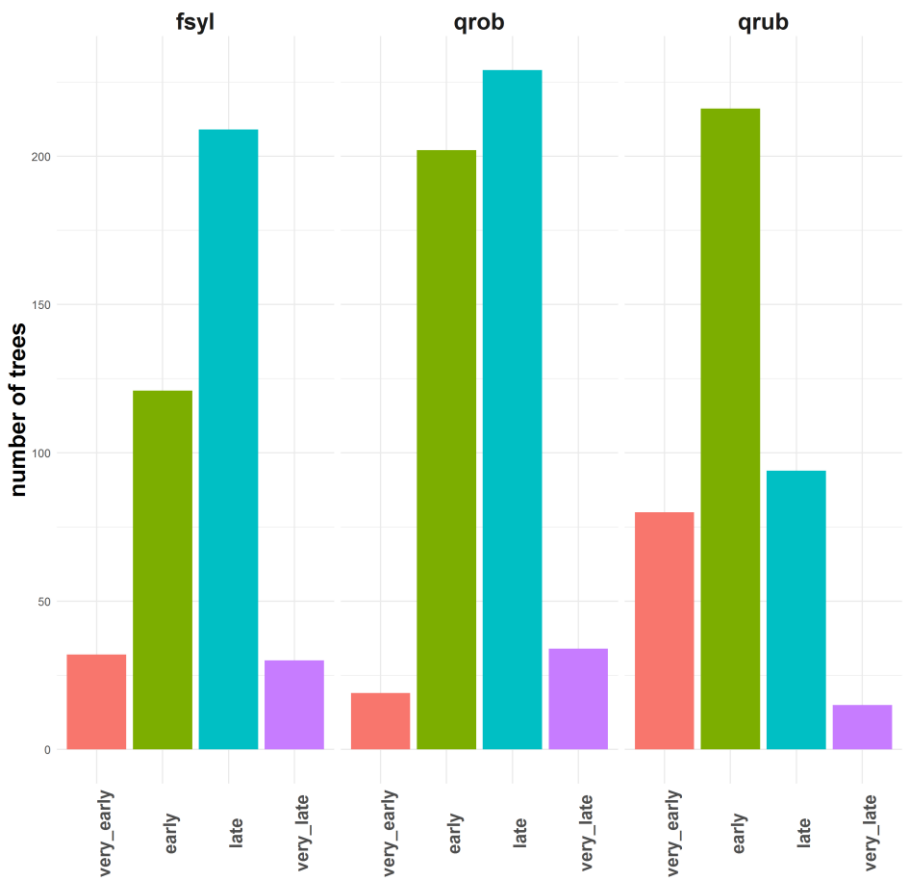


Fig. app. 1.1 The distribution of all measured trees in the TREEWEB project over the phenological categories. The measuring date at which tree leaves were found to be fully expanded determined the phenological category. Leafs fully expanded on: 20 april = 'very early', 27 april = 'early', 6 may = 'late', > 6 may = 'very late'.

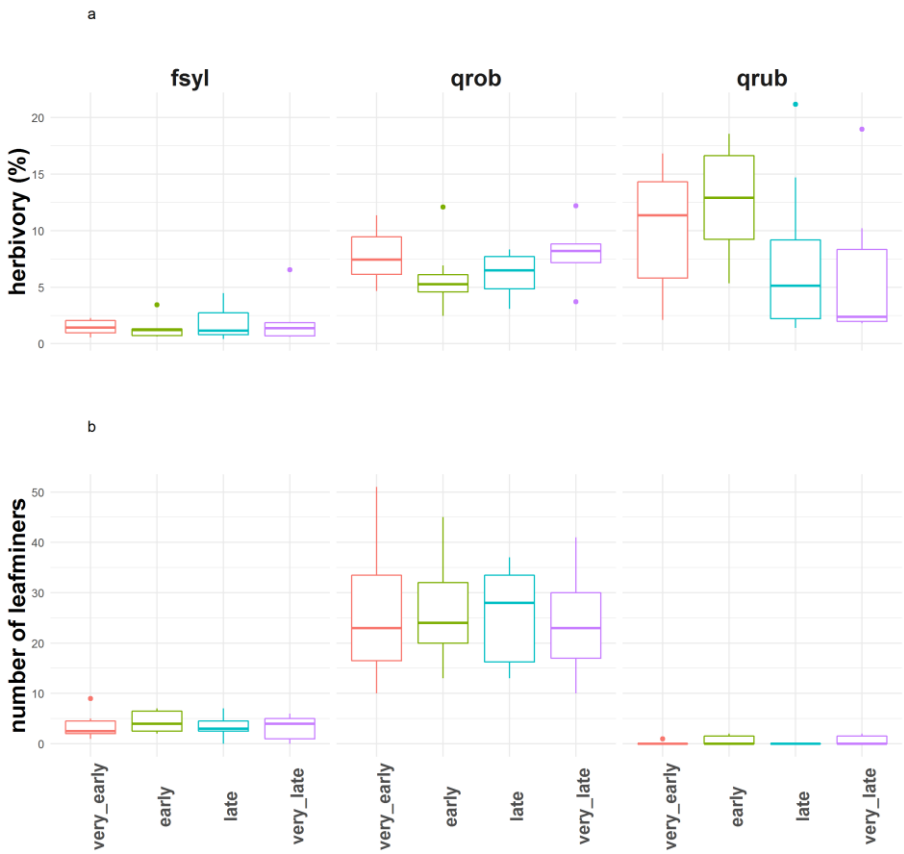


Figure app. 1.2. Difference in the percentage herbivory (panel a) and the number of leafminers (panel b) between the four phenological categories per tree species (fsyl = *Fagus sylvatica*, qrob= *Quercus robur*, qrub = *Quercus rubra*). None of the categories are significantly different for the two herbivory measures.

In conclusion, we found no evidence for the hypothesis that herbivorous damage was lower in trees with late leaf expansion in our study plots. To disprove the hypothesis conclusively, we would, however, need measurements for several years, ideally with a larger sample size and a higher temporal frequency. Moreover, it would be interesting to account for other biotic actors that could also exert selection pressures on leaf expansion phenology in trees.

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SUPPLEMENTARY INFORMATION

SI 1 SUPPLEMENTARY INFORMATION – GENERAL

SI 1.1 TREEWEB STUDY AREA

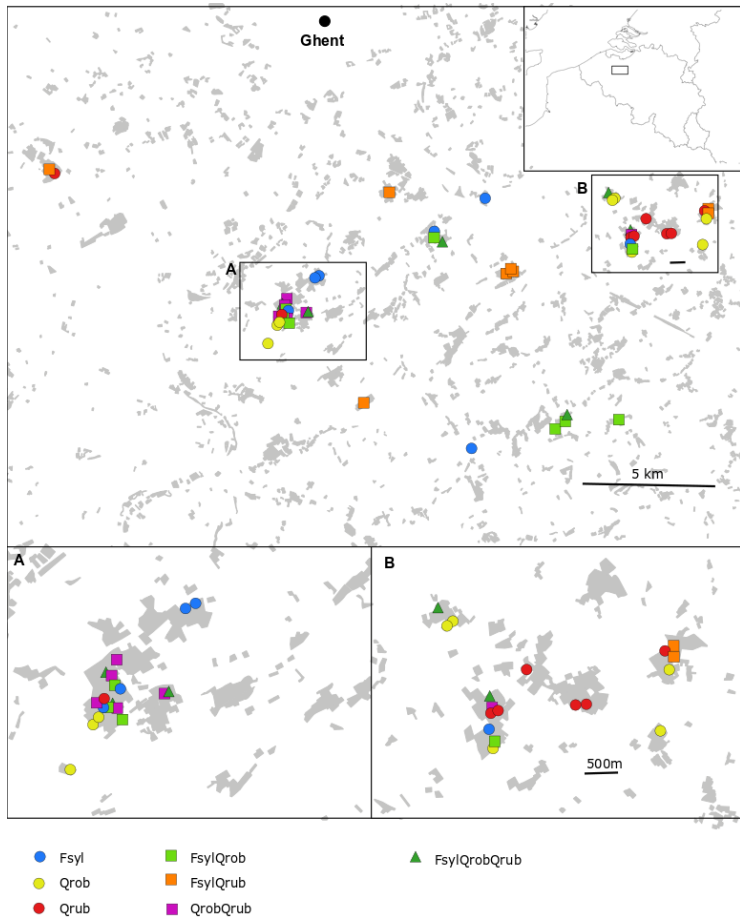


Figure SI 1.1 -- Map of the study area with an overview of the spatial distribution of the 53 TREEWEB plots in the 15 x 30km landscape window situated south of the city of Ghent in Belgium. Grey patches represent forest fragments, the 53 TREEWEB plots are indicated by coloured symbols, showing plot-level tree species diversity and composition (Quercus robur = Qrob, Fagus sylvatica = Fsyl and Quercus rubra = Qrub).

SI 1.2 TREEWEB PLOT

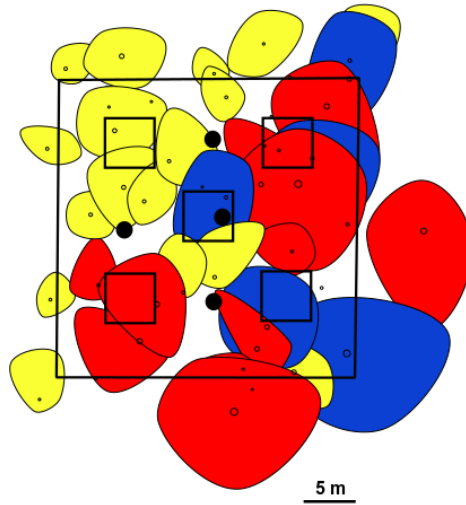


Figure SI 1.2 -- A detailed plot map. The big square is the 30 m x 30 m plot, open circles represent tree stems, polygons are tree crown projections (different colours for different tree species). Small squares indicate the 5 m x 5 m subplots in which shrub cover was estimated, full circles are the locations where hemispherical images were taken.

SI 2 SUPPLEMENTARY INFORMATION – CHAPTER 2

SI 2.1 MODEL PARAMETER ESTIMATES

Table SI 2.1 - Posterior distribution of the parameter estimates (mean and 95% credible intervals between brackets) for the best model for total crown area and shrub cover. The best model for total crown area was the species interaction model (M2) and the identity model (M1) for shrub cover (see table 2.1 in Chapter 2.). The colon between the species names indicates the interaction effect between relative basal area of Fsyl, Qrob and Qrub. Note that the parameter values for shrub cover are based on the logit transformed shrub cover values.

explanatory variable	total crown area	shrub cover
Fsyl	79.38 (43.07 - 115.90)	-2.38 (-4.27 - -0.43)
Qrob	75.02 (42.06 - 108.36)	0.28 (-1.52 - 2.02)
Qrub	85.58 (51.87 - 120.67)	-0.77 (-2.65 - 1.10)
Total basal area	8.40 (-1.16 - 17.82)	-0.01 (-0.52 - 0.50)
Fsyl:Qrob	76.55 (11.28 - 140.70)	
Fsyl:Qrub	142.71 (73.24 - 211.76)	
Qrob:Qrub	79.15 (16.05 - 144.24)	
Fsyl:Qrob:Qrub	-319.09 (-823.41 - 190.65)	

SI 2.2 MODEL PARAMETER ESTIMATES

Table SI 2.2: Posterior distribution of the parameter estimate (mean and 95% credible interval between brackets) for all explanatory variables in the species interaction model (M2) for GLI before, after and during leaf expansion. The colon between the species names indicates the interaction effect between relative basal area of Fsyl, Qrob and Qrub. Note that the parameter values for GLI are based on the logit transformed values of the GLI values.

explanatory variable	before leaf expansion	during leaf expansion	after leaf expansion
Fsyl	1.01 (0.84 - 1.18)	-0.35 (-0.74 - 0.04)	-1.82 (-2.05 - -1.59)
Qrob	0.79 (0.61 - 0.97)	0.16 (-0.24 - 0.54)	-1.79 (-2.02 - -1.53)
Qrub	1.12 (0.94 - 1.29)	0.64 (0.23 - 1.06)	-1.48 (-1.71 - -1.25)
Total basal area	0.02 (-0.05 - 0.09)	-0.14 (-0.32 - 0.04)	-0.03 (-0.13 - 0.06)
Fsyl:Qrob	0.17 (-0.72 - 1.08)	0.07 (-1.85 - 1.93)	-0.66 (-1.85 - 0.55)
Fsyl:Qrub	-0.33 (-1.29 - 0.65)	0.32 (-1.65 - 2.23)	-1.41 (-2.72 - -0.06)
Qrob:Qrub	0.02 (-0.9 - 0.96)	-1.59 (-3.25 - -0.01)	-0.04 (-1.2 - 1.12)
Fsyl:Qrob:Qrub	-2.55 (-9.52 - 4.25)	-2 (-16.81 - 12.59)	2.71 (-6.21 - 12.24)

SI 2.3

Table SI 2.3: Estimates of the within-plot variance of light transmittance (GLI), before, during and after leaf expansion, with 95% credible intervals for all species combinations. Variances are calculated using the estimate of the GLI for each species combination based on the posterior parameter estimates from the species interaction model (M2). Equal basal area of the species is assumed.

species combination	before leaf expansion	during leaf expansion	after leaf expansion
Fsyl	5.36 (4.77 - 5.90)	5.13 (3.79 - 6.19)	5.43 (4.84 - 5.97)
Qrob	4.88 (4.26 - 5.42)	5.87 (4.49 - 6.90)	4.33 (3.73 - 4.84)
Qrub	4.46 (3.83 - 5.02)	5.01 (3.68 - 6.04)	4.21 (3.60 - 4.74)
FsylQrob	5.51 (4.92 - 6.06)	4.77 (2.64 - 6.18)	4.41 (3.83 - 4.94)
FsylQrub	5.32 (4.59 - 5.95)	4.74 (3.37 - 5.75)	4.77 (4.02 - 5.41)
QrobQrub	4.98 (4.37 - 5.52)	4.50 (3.53 - 5.33)	5.25 (4.62 - 5.78)
FsylQrobQrub	5.90 (5.22 - 6.46)	5.21 (3.80 - 6.25)	4.80 (4.10 - 5.42)

SI 3 SUPPLEMENTARY INFORMATION – CHAPTER 3

SI 3.1 EXTENDED DATA ANALYSIS

SI 3.1.1 STATISTICAL MODELS

All fitted models were of the form:

$$y_i \sim N(\mu_i, \sigma_i)$$

With y being the response variable of interest, i indexing the rows, μ being the mean expectation and σ the residual standard deviation. Both the mean and the residual standard deviation were regressed against a set of predictors namely:

$$\mu_i = \text{Total basal area}_i + \text{Species composition}_i + \text{Plot_mean}_i$$

$$\log(\sigma_i) = \text{Species composition}_i + \text{Plot_sd}_i$$

The Plot_* values represent plot-level random effects that arose via:

$$\text{Plot_mean}_i \sim N(0; \sigma_{\text{mean}})$$

$$\text{Plot sd}_i \sim N(0; \sigma_{\text{sd}})$$

From the models we extracted the posterior draw of the mean and residual standard deviation per species composition (controlling for total basal area and plot effects).

For the purpose of this paper we represent within-plot variability or heterogeneity with the coefficient of variation (CV). The CVs were calculated per species composition as the estimated standard deviation divided by the estimated mean.

All models were fitted with a Bayesian approach, using the Stan probabilistic language called from R with the package “brms”. We used default sampling settings with 4 chains, 2000 iterations per chains and a burn-in of 1000 iterations leading to posterior samples of 4000 iterations. As divergence appeared in some models, the adapt delta parameter was set to 0.99 for problematic models. Default prior distributions were used for all parameters: all slope parameters got unbounded uniform priors, the variation in the random effects followed a t-distribution with 3 degrees of freedom and a non-centrality parameter of 10. All model parameters converged (Rhat values < 1.1) and sufficient effective number of posterior samples were available from the posterior distributions (Neff- ratio > 0.1). In addition

posterior predictive checks of the mean and standard deviation showed that all models provided a relatively good fit (Fig. SI 3.1).

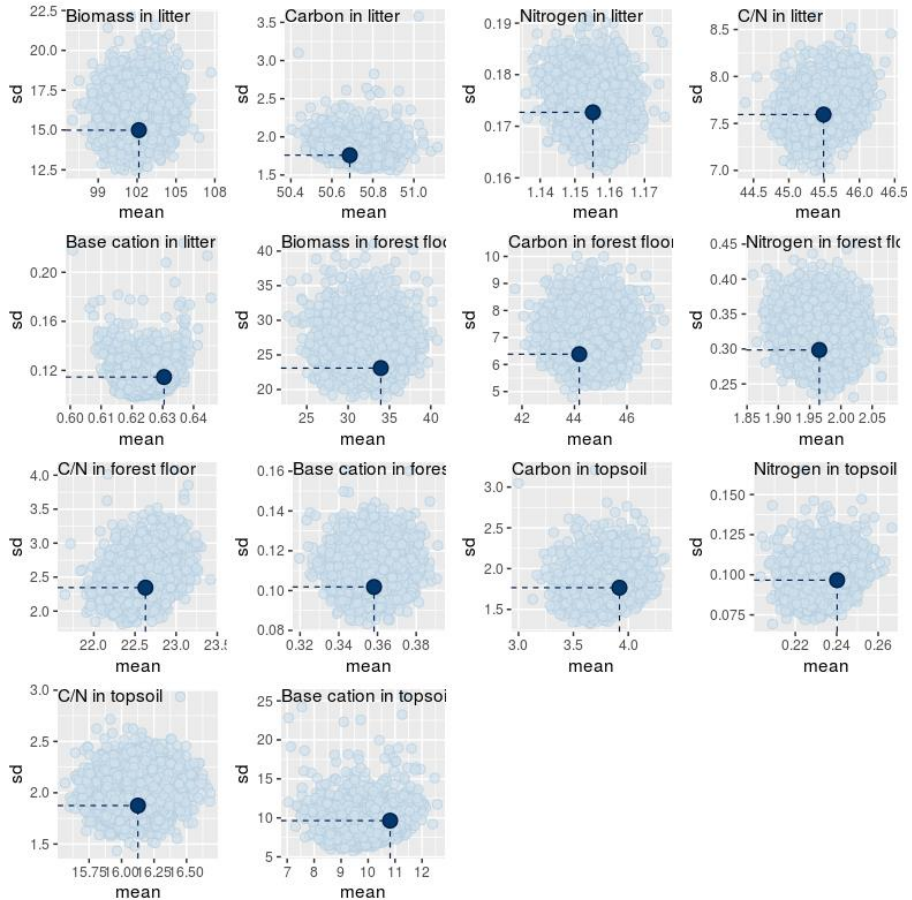


Figure SI 3.1.1 - Posterior predictive checks of the 14 `_tted` models with one panel per separate model. The dark blue dot indicates the observed mean and standard deviation while the lighter blue dots represent the 4000 posterior draws for the mean and the standard deviation.

SI 3.1.2 DERIVATION OF EXPECTED CV

We compared the observed CV values for two-species and three-species mixtures with expected values based on the simplified or null scenario (fig 3.1a). The aim of this comparison was to explore how much observed CV derived from null expectations. The expected values were derived as follow: assuming that each tree

species equally contributed to the litter, forest floor and soil condition in a mixture, and that only species identity effects were present (i.e. no species interaction present). The expected means in the mixtures were:

$$\mu_{exp} = \sum_{j=1}^N \left(\frac{1}{N}\right) * \mu_j$$

Where j index the species present in the mixture, N is the total number of species, and the μ_j are posterior draws for the respective monoculture mean.

The expected variance of the mixture was calculated as:

$$\sigma_{exp}^2 = \sum_{j=1}^N \left(\frac{1}{N}\right) * \sigma_j^2 + \sum_{j=1}^N \left(\frac{1}{N}\right) * \mu_j^2 - \mu_{exp}^2$$

Which has three components: the weighted average variance plus the average squared mean minus the square of the average mean. The σ_j are the posterior draws of the standard deviation for the respective monocultures. These formula are derived from the statistical properties of finite mixtures of normal distributions with known weights. Fig. SI 3.1.2 illustrates how expected CV changes with different monoculture distributions (varying mean and standard deviation).

SI 3.1.3 CAVEATS OF THE EXPECTED VALUES

The derivation of the expected values correspond to a simplified or null scenario where every point in space is purely influenced by one species and only additive effects occur. Therefore heterogeneity in mixtures (i.e. CVs) can be mathematically derived from monoculture values. If we relax the assumption about the spatial distribution of species influences and allow that a point in space can be simultaneously influenced by several species, the heterogeneity can, theoretically, still be simply calculated from the monoculture properties and the proportional influence of each species (fig 3.1b in chapter 3) but then cannot be linked to existing statistical properties. In reality additional interactive effects might occur if i) leaf litter properties of a species growing in a mixture deviates from that in a monoculture ii) decomposition or other processes lead to nutrient values that are not proportionate to the monoculture values.

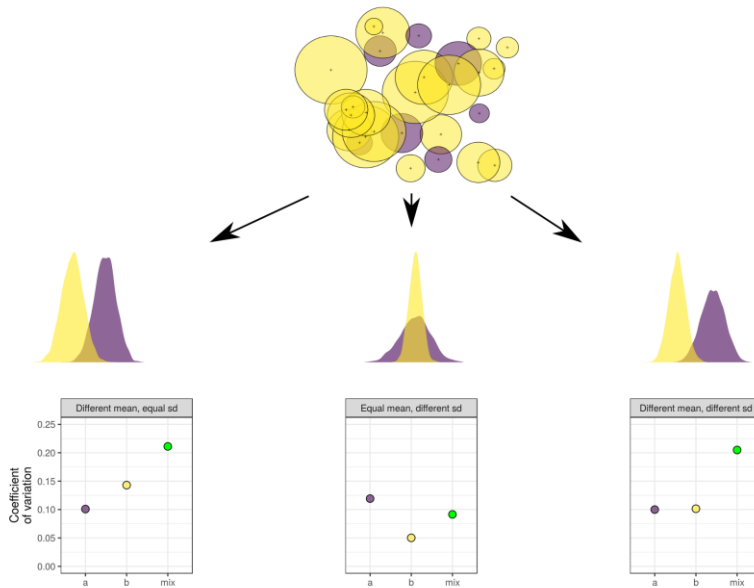


Figure SI 3.1.2 - Conceptual figure representing the logic behind the derivation of the expected coefficient of variation in the mixtures from the constituting monocultures. Top graph: hypothetical mixture of two tree species where each polygon represent the zone of influence of one tree species on soil conditions. Middle panel: distribution of soil conditions in some variables such as Carbon concentration for the two species under different scenarios of varying means and standard deviations. Bottom panel: the resulting coefficient of variation in monocultures and in the mixture. If the mean values of two species is the same then the heterogeneity of the two-species mixture will be intermediate between the heterogeneity of both species. However when the two species have very different mean values, the heterogeneity of the mixture will be mainly determined by the distance between the means and to a lesser extent by the variance of both species.

SI 3.2 EXTENDED SAMPLING AND BACKGROUND INFORMATIONS

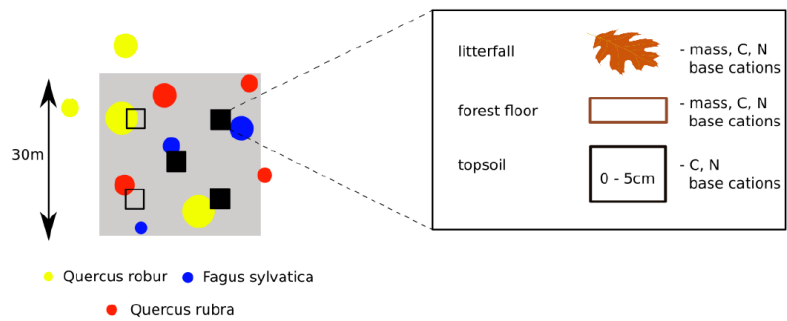


Figure SI 3.2 - A detailed plot map with the sampling scheme. The big square is the 30 m x 30 m plot, coloured dots represent trees (different colours for different tree species, different sizes for different diameter at breast height). The small squares indicate the five 5 m x 5 m subplots. In the three black squares, litterfall, forest floor and topsoil were sampled. Figures adapted from DeGroote et al (2018).

SI 3.3 EXTENDED FIGURES

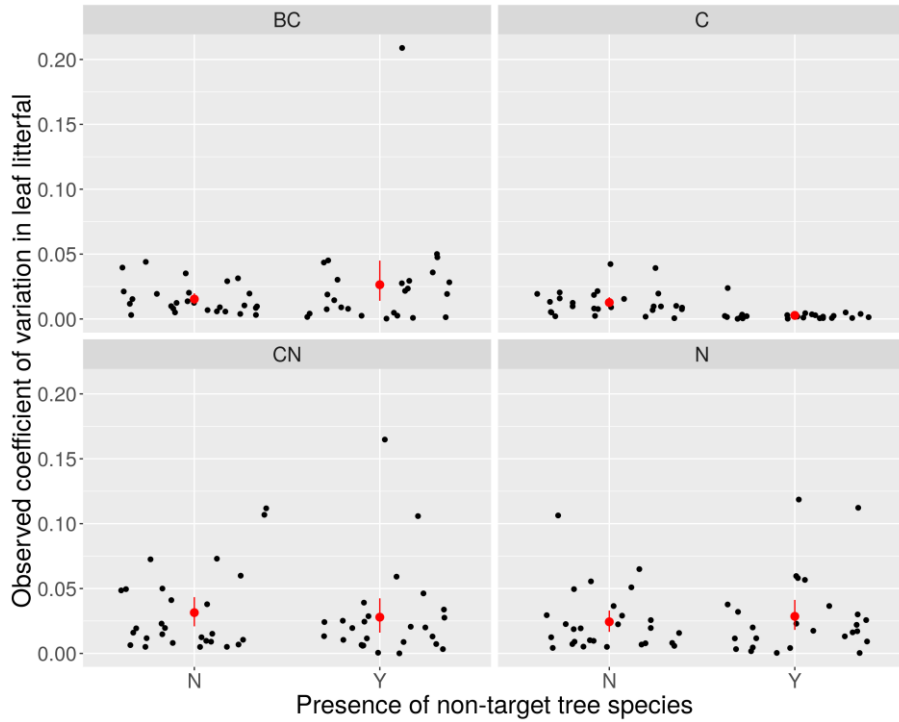


Figure SI 3.3: Observed coefficient of variations (per plot) for the leaf litterfall for the four chemical variables explored in the analysis (Carbon, Nitrogen, Base Cations and C/N ratio) in relation to the presence or not of non-target tree species leaf materials in the analysis. The red dot with the linerange represent the average value with the bootstrapped 95% confidence interval.

SI 3.4 EXTENDED BIBLIOGRAPHY

Baeten, L., Verheyen, K., Wirth, C., Brulheide, H., Bussotti, F., Finer, L., & Ampoorter, E. (2013). A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. *Perspectives in Plant Ecology, Evolution and Systematics*, 15(5), 281-291.

De Groote, S. R., van Schroyensteen, L., Irene, M., Sercu, B. K., Dekeukeleire, D., Boonyarittichaikij, R., ... & Matheve, H. (2017a). Tree species identity outweighs the effects of tree species diversity and forest fragmentation on understorey diversity and composition. *Plant Ecology and Evolution*, 150(3), 229-239.

De Groote, S. R., Vanhellemont, M., Baeten, L., De Schrijver, A., Martel, A., Bonte, D., & Verheyen, K. (2017b). Tree species diversity indirectly affects nutrient cycling through the shrub layer and its high-quality litter. *Plant and Soil*, 1-16.

Jabiol, B., Brêthes, A., Ponge, J. F., Toutain, F., & Brun, J. J. (2007). L'humus sous toutes ses formes (p. 68). Engref.

SI 4 SUPPLEMENTARY INFORMATION – CHAPTER 4

SI 4.1 RELATIONSHIP BETWEEN PREDATION AND PLANT SIZE

Below we show the results from the master thesis of Moeneclaey Iris (2017) investigating the relationship between predation prevalence and environmental factors. Predation was spread over plots and not explained by environmental variables or plant size.

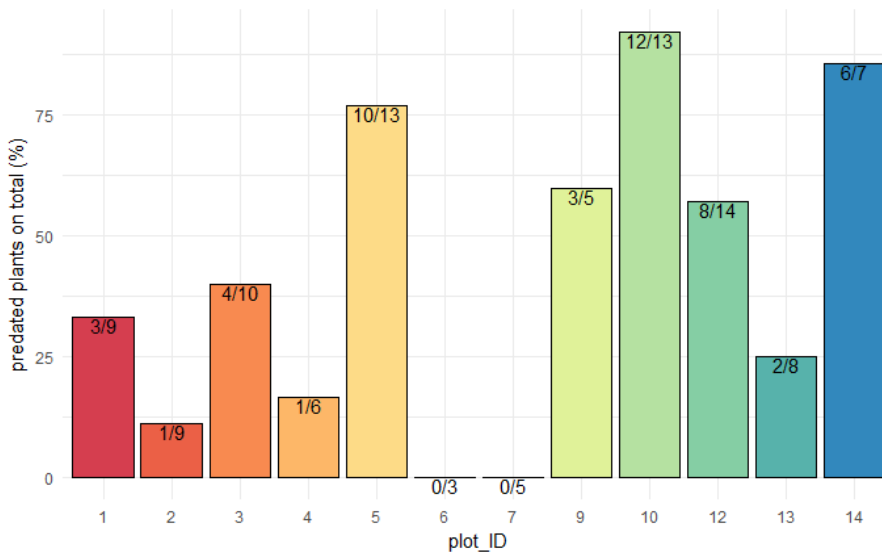
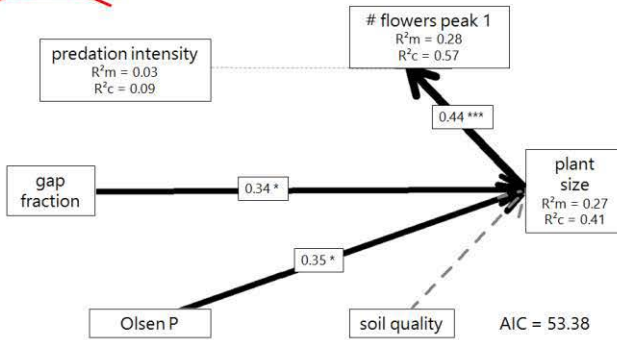


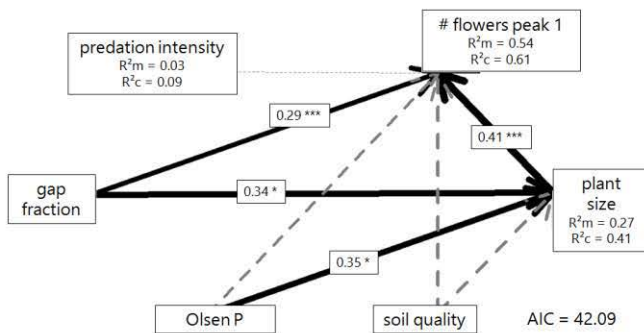
Figure SI 4.1.1 - Spatial variation in seed predation prevalence: the proportion of plants that suffered predation visualised per plot. The ratio in the bar indicates the number of predated plants on total number of plants in the plot.

SUPPLEMENTARY INFORMATION

~~Hypothesis 1~~



Hypothesis 2



Hypothesis 3

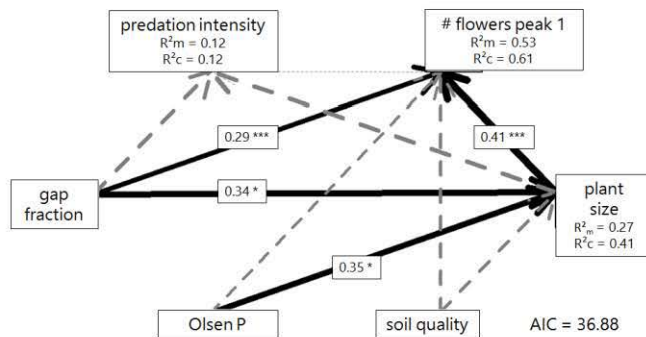


Figure SI 4.1.2 - Structural equation models (SEM) derived from the 3 hypotheses exploring the effect of the ecological context on seed predation intensity. Boxes represent measured variables. Arrows represent unidirectional relationships among variables. Dotted arrows are non-significant paths ($P \geq 0.05$). The thickness of the significant paths (full arrows) has been scaled based on the magnitude of the standardized regression coefficient (β), given in the associated box. Coefficients of determination for the component models are given in the boxes of response variables. Both marginal ($= R^2_m$, only based on the variance of the fixed effects) and conditional R^2 values ($= R^2_c$, based on the variance of both the fixed and random effects) are reported. For each model, the associated AIC-value that indicates the model quality is reported in the right corner.

Reference

Moenecleay, Iris (2017) To seed or not to seed? Seed predation and compensatory flowering in *Geum urbanum* (Master dissertation). Retrieved from <https://lib.ugent.be/nl/catalog/rug01:002349779?i=0&q=Moenecleay+Iris>

SI 4.2. ADDITIONAL SEM ANALYSES

The SEM analyses for the supplementary analyses were performed in the same way as explained in the main text.

SI 4.2.1 SEM ANALYSIS FOR FLOWERING DURATION IN 2015

We interchanged date of first flower per plant in 2015 with flowering duration in 2015 in the SEM analysis.

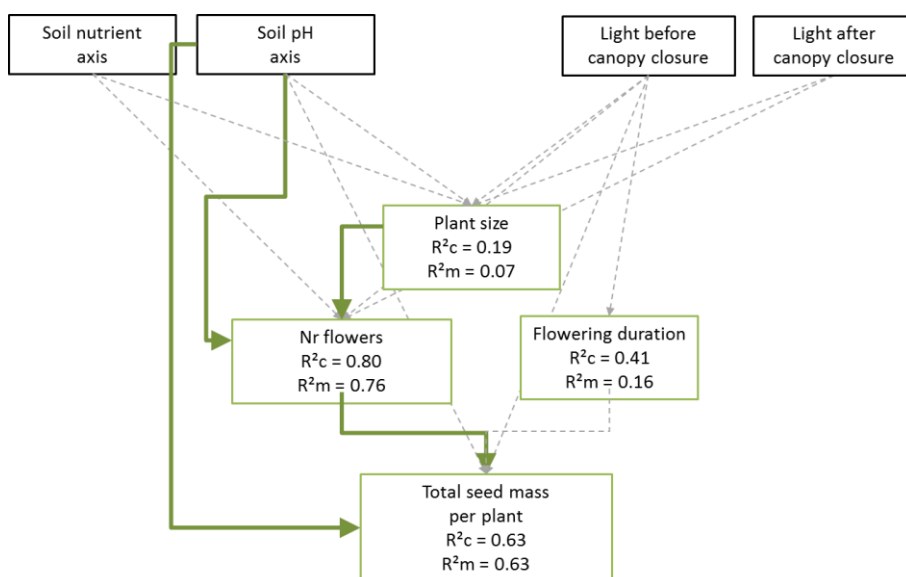


Figure SI 4.2.1 - Effects of light and soil characteristics on vegetative and reproductive performance of *Geum urbanum* plants in 2015. The metric for flower timing is the flower duration per plant. Relationships were modeled with a piecewise SEM approach. Boxes represent the measured variables and arrows represent directional relationship among variables. The green arrows represent positive relationships, red arrows represent negative relationships. The line style indicates the significance of the relationships: full lines represent significant relationships ($p < 0.05$), thick dashed lines are marginally significant ($0.05 < p < 0.10$), thin dashed lines are not significant ($p > 0.10$). The conditional R^2_c and marginal R^2_m values are given in the boxes of response variables

SI 4.2.2 SEM ANALYSIS FOR DATE OF MEDIAN FLOWER IN 2015

We interchanged date of first flower per plant in 2015 with date of median flower per plant in 2015 in the SEM analysis.

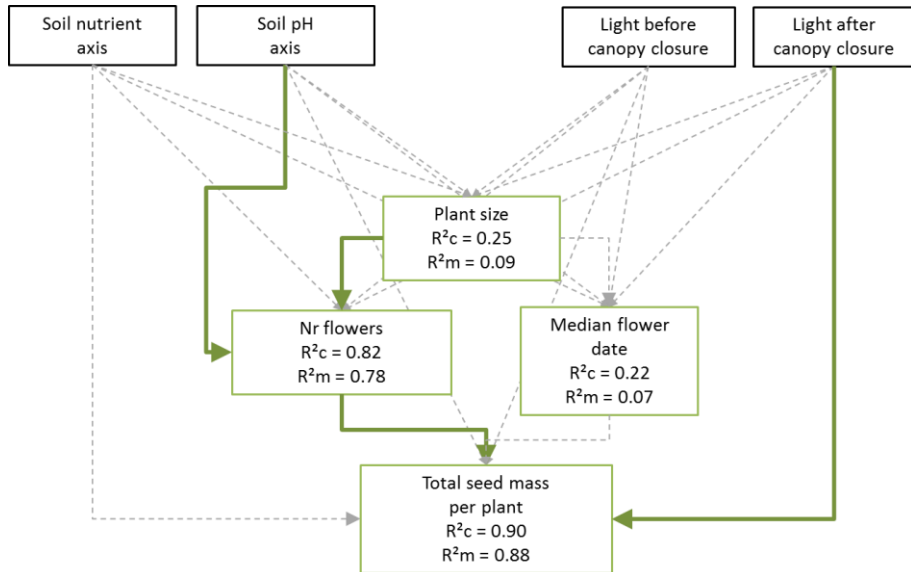


Fig. SI 4.2.2 - Effects of light and soil characteristics on vegetative and reproductive performance of *Geum urbanum* plants in 2015. The metric for flower timing is the date of median flower per plant. Relationships were modeled with a piecewise SEM approach. Boxes represent the measured variables and arrows represent directional relationship among variables. The green arrows represent positive relationships, red arrows represent negative relationships. The line style indicates the significance of the relationships: full lines represent significant relationships ($p < 0.05$), thick dashed lines are marginally significant ($0.05 < p < 0.10$), thin dashed lines are not significant ($p > 0.10$). The conditional R^2_c are based on the variance of both the fixed and random effects and are given in the boxes of response variables

SI 4.2.3 SEM ANALYSIS FOR DATE OF FIRST FLOWER IN 2014

Instead of the data of 2015, data of 2014 on plant size, number of flowers and date of first flowering were used in this analysis. Since we had no data about total seed mass per plant in 2014, the SEM contained only three component models with the response variables (i) plant size (Gaussian distribution), (ii) flowering phenology (Gaussian distribution), (iii) number of flowers (poisson distribution). The fourth component model of the 2015 model with total seed mass as response variable was absent for 2014.

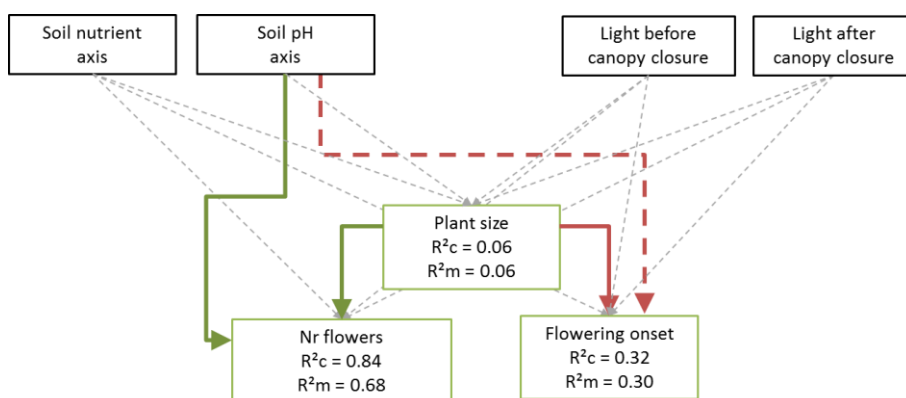


Figure – SI 4.2.3 - Effects of light and soil characteristics on vegetative and reproductive performance of *Geum urbanum* plants in 2014. The metric for flower timing is the date of the first flower per plant. Relationships were modeled with a piecewise SEM approach. Boxes represent the measured variables and arrows represent directional relationship among variables. The green arrows represent positive relationships, red arrows represent negative relationships. The line style indicates the significance of the relationships: full lines represent significant relationships ($p < 0.05$), thick dashed lines are marginally significant ($0.05 < p < 0.10$), thin dashed lines are not significant ($p > 0.10$). The conditional R^2_c are based on the variance of both the fixed and random effects and are given in the boxes of response variables

SI 4.2.4 SEM ANALYSIS FOR DATE OF MEDIAN FLOWER IN 2014

This analysis is identical to the previous analysis but date of median flower was used as metric for flowering phenology.

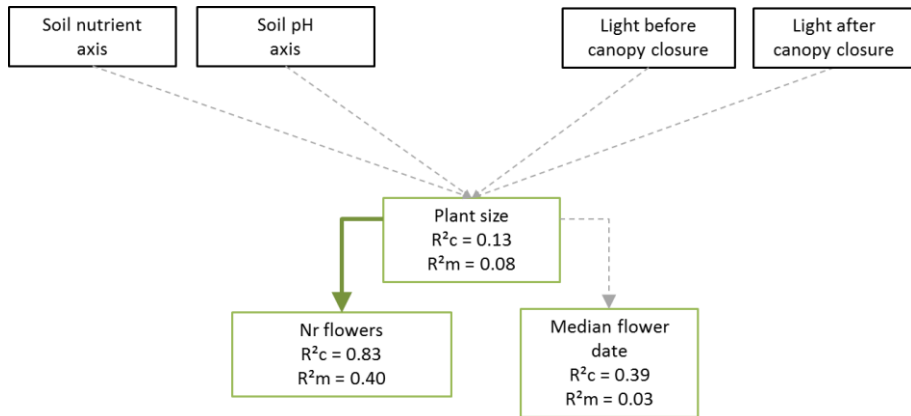


Figure SI 4.2.4 - Effects of light and soil characteristics on vegetative and reproductive performance of *Geum urbanum* plants in 2014. The metric for flower timing is the date of median flower per plant. Relationships were modeled with a piecewise SEM approach. Boxes represent the measured variables and arrows represent directional relationship among variables. The green arrows represent positive relationships, red arrows represent negative relationships. The line style indicates the significance of the relationships: full lines represent significant relationships ($p < 0.05$), thick dashed lines are marginally significant ($0.05 < p < 0.10$), thin dashed lines are not significant ($p > 0.10$). The conditional R^2_c are based on the variance of both the fixed and random effects and are given in the boxes of response variables

SI 4.2.5 INTERPRETATION

The results of the three additional analyses are in line with the SEM that is reported in the main text of the article. It has to be noted that only the flower timing metric differs between the two analyses of the same year. (i) The positive relationship between plant size and number of flowers is found in 2015 and 2014. (ii) The negative relationship between plant size and date of first flower is present in 2014 and 2015, however this relationship disappears in both years when date of median flower is used as flower timing metric instead of date of first flower. (iii) The positive relationship between pH and the number of flowers and the negative relationship between pH and flowering onset was also present in both years. (iv) The positive effect of soil nutrients and light after canopy closure on flowering onset was not present in 2014. (v) There was a significant positive effect of light after canopy closure on total seed mass when date of first flower was interchanged for date of median flower in 2015.

SI 5 SUPPLEMENTARY INFORMATION – CHAPTER 5

SI 5.1 OCCURRENCE OF PREDATION

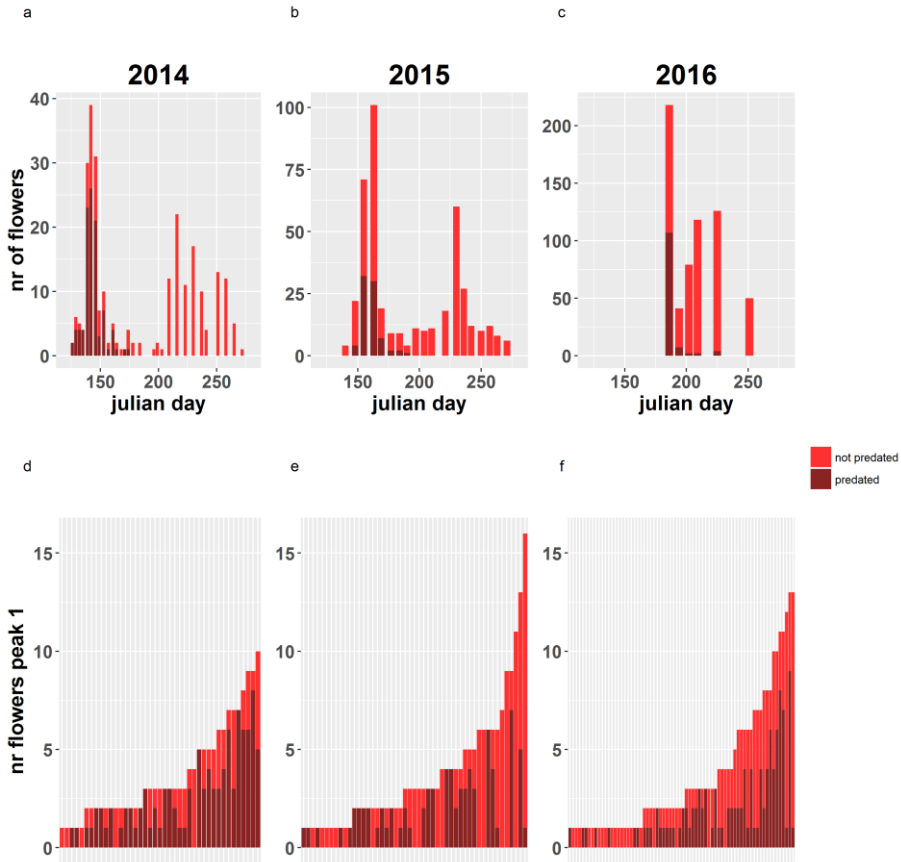


Figure SI 5.1 - number of flowers per collection moment in (a) 2014 , (b) 2015 and (c) 2016 . Number of flowers that were predated are marked in dark red. Total number of flowers per plant for (d) 2014 , (e) 2015 and (f) 2016, ordered from low to high number of flowers. Flowers that experienced predation are indicated in dark red. Every bar represents a plant.

Table SI 5.1 - Correlation between flower number in the first peak and proportion of flowers of the first peak that experienced predation. There is no correlation between flower number and proportion of predation.

	Correlation coefficient
2014	0.017
2015	-0.278
2016	0.100

SI 5.2 MODEL SUMMARY OF PLANT LEVEL SELECTION GRADIENTS.

Table SI 5.2: Estimate, standard error and significance code of each model parameter from the best model (i.e. the models with the lowest AIC values) to estimate the logarithm of the total seed mass for predated and unpredated plants in 2015 and 2016. The significance codes indicate between which values the p-value was located: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < . < 0.1.

	Intercept	Scaled total number of flowers	Proportion flowers in peak 2	Second polynomial of proportion flowers in peak 2
2015 unpredated	-1.01 (±0.76)	0.73 (±0.31) *	-3.61 (±2.70)	5.27 (±3.01)
2015 predated	-1.85 (±0.35) ***	0.76 (±0.17) ***	4.30 (±2.16) .	-4.98 (±3.34)
2016 unpredated	-1.08 (±0.44) *	1.31 (±0.24) ***	-0.49 (±0.56)	
2016 predated	-1.85 (±0.33) ***	0.84 (±0.09) ***	3.35 (±1.19) **	-3.58 (±1.26) **

SI APP1 SUPPLEMENTARY INFORMATION – APPENDIX

SI APP1.1 LEAF EXPANSION SCORING

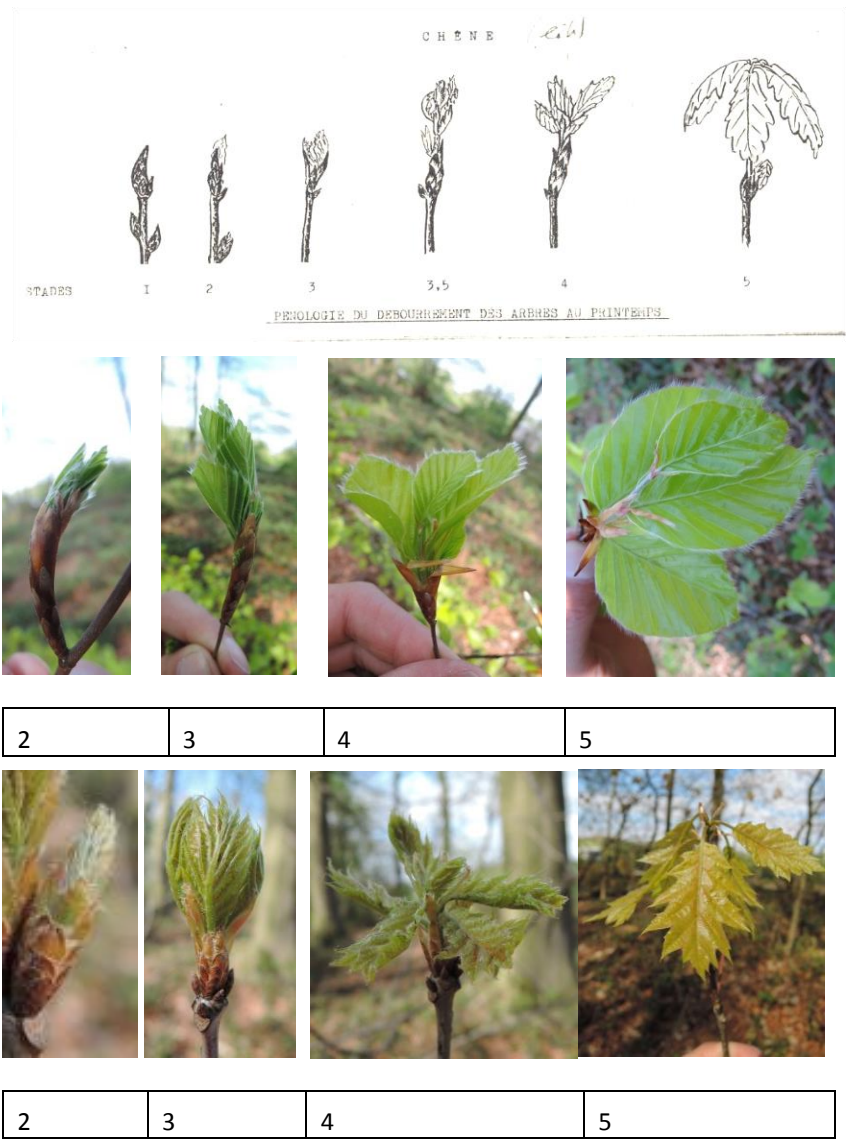


Figure SI app1.1. - Scores for the three different tree species *Quercus robur* (top) , *Fagus sylvatica* (middle) and *Quercus rubra* (bottom)

The Dandelion's pallid tube

The Dandelion's pallid tube
Astonishes the Grass,
And Winter instantly becomes
An infinite Alas --

The tube uplifts a signal Bud
And then a shouting Flower, --
The Proclamation of the Suns
That sepulture is o'er.

A phenology poem by Emily Dickinson