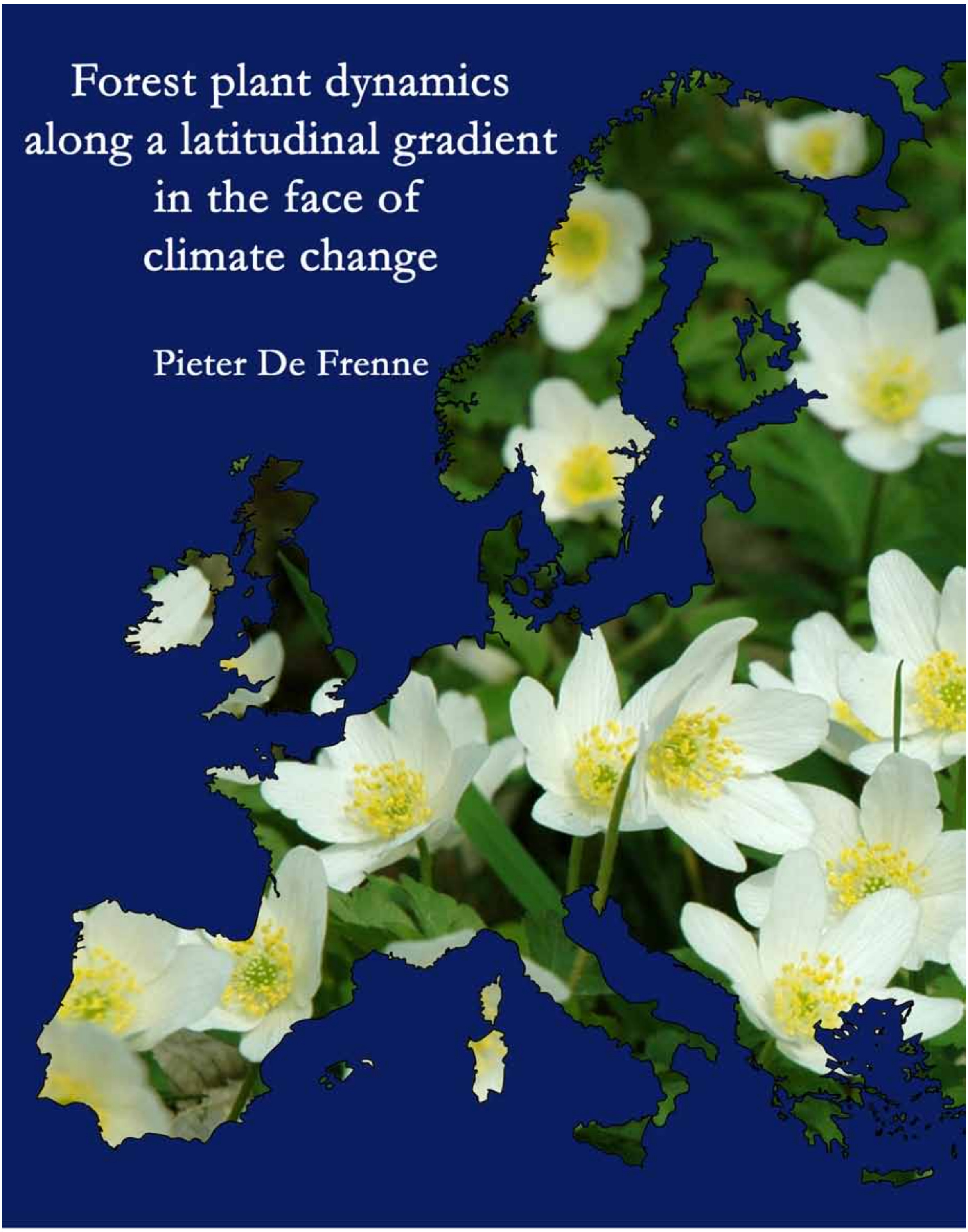

Forest plant dynamics along a latitudinal gradient in the face of climate change

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FOREST PLANT DYNAMICS ALONG A LATITUDINAL GRADIENT IN THE FACE OF CLIMATE CHANGE

Thesis submitted in fulfillment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences:
Land and Forest Management

Dutch translation of the title:
Dynamiek van bosplanten langsheen een latitudinale gradiënt in een veranderend klimaat

Illustrations on the cover:
Front: European map superimposed on an *Anemone nemorosa* population
Back: open-top chambers in a deciduous forest in Belgium to experimentally warm small vegetation plots (large image)
Anemone nemorosa, *Paris quadrifolia*, *Primula elatior*, seeds of *Anemone nemorosa* (small images in clockwise direction)
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> *Oh, the number one is not my favorite number*
> *'Cause one means only me and there's no you*

Pieter

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[1] Johnny Cash — One and one makes two [from the album: Children's Album]

Contents

Summary

Climate change is considered to be one of the major threats to biological diversity. The global average earth surface temperature has increased by 0.8 °C between 1956 and 2005 and is predicted to increase by an additional 1.8–4.0 °C by the end of this century. Biodiversity of temperate forests is mainly a function of the herbaceous understorey community. Many forest understorey plants, however, are probably not able to track the shifting climate due to their limited colonisation capacity. The acclimation potential to climate change within their occupied habitats will likely determine their short- and long-term persistence. Yet, it remains largely unclear to what extent forest understorey plants will be able to respond to climate change.

In this thesis we contributed to fill this knowledge gap by quantifying the response of growth and reproduction of a set of herbaceous forest understorey plants to temperature variation along a latitudinal gradient from northern France to northern Sweden and to experimentally elevated temperatures. We mainly focused on the spring flowering geophytic forb *Anemone nemorosa* and the early summer flowering grass *Milium effusum* due to their slow colonisation, distribution range, reproductive traits, phenology and growth form.

The plant height of *A. nemorosa* and *M. effusum* showed an increase in natural populations along a latitudinal gradient. Hence, plants grew taller in the north. This phenomenon could be attributed to northward increasing light availability due to increasing canopy openness and photoperiod in the growing season. Furthermore, by sampling *A. nemorosa* seeds in natural populations along the gradient, we were able to demonstrate a decline in the seed nitrogen concentration with increasing latitude. This can be caused by (i) the lower soil nitrogen (N) availability as a consequence of lower N depositions towards the north or (ii) a greater investment in clonal reproduction in northern populations. Since the degree of seed provisioning co-determines seedling survival, changes in the seed N concentration can affect the reproductive success along the gradient. Finally, we also showed that temperature and latitude explained most of the variation in reproductive output for early flowering understorey plants with a northerly distribution range edge (e.g. *A. nemorosa*, *Paris quadrifolia*, *Oxalis acetosella*). Reproduction of the more southerly distributed later flowering species (e.g. *Brachypodium sylvaticum*, *Circaea lutetiana*) was best explained by local environmental variables such as overstorey canopy cover.

Subsequently, experimental warming and transplant experiments have been used to examine the effects of temperature on phenology, growth and reproductive performance of the two selected understorey plants. Firstly, we validated the use of open-top chambers (OTCs) in deciduous forests for evaluating warming effects on understorey plants. OTCs are small greenhouses with inclined walls and an open top that passively heat a small vegetation plot. They raised average air and soil temperature with *ca.* 0.2 °C to 1.5 °C, mostly before overstorey canopy flush. Next, we used OTCs in combination with common garden experiments in three sites (Belgium, southern Sweden and northern Sweden). In a common garden, populations of the same species are transplanted in a common environment with different climate. We clearly demonstrated that vegetative growth and reproductive success of the vernal *A. nemorosa* benefited from higher temperatures. No such response could be noted for the early summer flowering *M. effusum*, probably because it mainly grows after canopy flush and was thus limited by light availability. We conclude that not all understorey plants respond equally to temperature variation, which may alter understorey community composition and dynamics in the future.

Since recruitment from seeds is an important phase in the life cycle of plants, we also assessed seedling establishment. We quantified the plasticity in growth of seedlings of the two species sampled along the latitudinal gradient in growth chambers. Seedling biomass of northern *M. effusum* populations responded stronger to increases in temperature than seedling biomass of southern populations. Seedlings of *A. nemorosa* did not show such a latitudinal cline in plasticity to temperature. Higher plasticity in *M. effusum* seedling growth in the north may thus offer an important way to cope with climatic changes.

Finally, we compiled distribution patterns of 90 understorey plants in both ancient (i.e. continuously forested since the first available maps) and post-agricultural forests (PAF) from 18 studies across Europe. We demonstrated that the colonisation of faster species (mainly short-lived herbs) into PAF increased with the amount of forest habitat within the landscape. Conversely, the characteristic slow colonising woodland flora (mainly small perennial herbs) was generally unsuccessful in colonising PAF — even in relatively densely forested landscapes. Variation in mean annual temperature across Europe had no effect on the recovery of PAF, which we attributed to long-term adaptations to the local environment, the larger significance of habitat loss and microclimatic heterogeneity within forests.

We conclude that climate change will likely have significant effects on forest understorey plant dynamics across Europe. We here clearly showed that growth and reproduction of understorey species can show divergent responses to temperature variation. Climate change can thus result in changed biotic interactions in understorey plant communities, and in enhanced regeneration from seed for some species. Clearly, more research is needed to further our understanding of possible changes in forest plant dynamics in a warmer climate.

Samenvatting

Klimaatverandering vormt één van de belangrijkste bedreigingen voor de biodiversiteit op aarde. De gemiddelde temperatuur van het aardoppervlak steeg tussen 1956 en 2005 met 0.8 °C. Bovendien voorspellen klimaatsmodellen een verdere opwarming van 1.8–4.0 °C tegen het einde van de 21^{e} eeuw. Het grootste deel van de biodiversiteit in gematigde bossen is te vinden in de kruidlaag. Van vele kruidachtige bosplanten wordt evenwel verwacht dat ze door hun beperkte kolonisatiecapaciteit onvoldoende snel zullen kunnen migreren. Daarom zal hun aanpassingsvermogen op de plaatsen waar ze nu al voorkomen uitermate belangrijk zijn voor hun voortbestaan. Tot op heden is er echter zeer weinig onderzoek uitgevoerd naar het effect van klimaatverandering op kruidachtige bosplanten.

In dit doctoraat gingen we na hoe de groei en reproductie van een set bosplanten reageerde enerzijds op temperatuursveranderingen langsheen een latitudinale gradiënt van noord-Frankrijk tot noord-Zweden en anderzijds op experimentele opwarming. We focusten vooral op de lentesoort *Anemone nemorosa* (bosanemoon) en de vroege zomersoort *Milium effusum* (bosgierstgras) omwille van hun trage kolonisatiesnelheid, algemeen voorkomen, reproductieve kenmerken, fenologie en groeivorm.

A. nemorosa en *M. effusum* individuen uit natuurlijke populaties werden groter in het noorden dan in het zuiden langsheen de latitudinale gradiënt. Dit is vermoedelijk te wijten aan noordwaarts toenemende lichthoeveelheden ten gevolge van een dalende bedekkingsgraad van de bomen en een stijgende fotoperiode in het groeiseizoen. We vonden ook een noordwaarts dalende stikstofconcentratie in de zaden van *A. nemorosa*. Waarschijnlijk wordt dit patroon veroorzaakt door (i) dalende stikstofdeposities die leiden tot een lagere hoeveelheid plantbeschikbaar stikstof en (ii) grotere investeringen in vegetatieve groei door de planten uit noordelijke populaties. Aangezien de hoeveelheid reservestoffen in zaden mee de overleving van de zaailingen bepaalt, kan een verandering in de stikstofconcentratie een impact hebben op de reproductie langsheen de gradiënt. Ten slotte toonden we aan dat de temperatuur en de breedtegraad het grootste deel van de variatie in reproductie van de lentesoorten met een noordelijk areaal verklaarden (bv. *A. nemorosa*, *Paris quadrifolia*, *Oxalis acetosella*). De reproductie van meer zuidelijke soorten met een latere fenologie (bv. *Brachypodium sylvaticum*, *Circaea lutetiana*) was daarentegen vooral gerelateerd aan lokale omgevingsfactoren zoals de bedekkingsgraad van de bomen.

Vervolgens pasten we experimentele opwarming en een *common garden* transplantatie-experiment toe om de effecten van temperatuur op fenologie, groei en reproductie van de twee geselecteerde bosplanten nader te onderzoeken. Eerst valideerden we het gebruik van *open-top chambers* (OTCs) in loofbos om het effect van klimaatopwarming op bosplanten te bestuderen. OTCs zijn kleine serres met schuin oplopende wanden en een open dakje. Ze kunnen passief een kleine vegetatie-plot met ongeveer 0.2 ℃ tot 1.5 ℃ opwarmen. Dit opwarmend effect is het grootst vóór de bladontluiking van de bomen. Deze OTCs werden dan gebruikt in combinatie met drie *common gardens* in België, zuid- en noord-Zweden. In een *common garden* groeien populaties van dezelfde plantensoort op een gemeenschappelijke plaats met ander klimaat. De vegetatieve groei en het reproductief succes van de lentesoort *A. nemorosa* namen duidelijk toe met stijgende temperatuur. De vroege zomersoort *M. effusum* daarentegen groeide voornamelijk na de bladontluiking van de bomen en was dus waarschijnlijk meer gelimiteerd door een beperkte lichthoeveelheid. Samenvattend kunnen we stellen dat niet alle kruidachtige bosplanten in dezelfde mate reageren op temperatuursvariaties, wat kan leiden tot een veranderde samenstelling en dynamiek in bosplantengemeenschappen.

We onderzochten ook het vestigingproces van zaailingen, aangezien rekrutering via zaden een belangrijke fase is in de levenscyclus van planten. Daartoe kwantificeerden we de groei van zaailingen van de twee soorten geoogst langsheen de latitudinale gradient in groeikamers. De biomassagroei van zaailingen uit de noordelijke *M. effusum* populaties reageerde veel sterker op temperatuursstijgingen dan de zaailingengroei van de zuidelijke populaties. Zaailingen van *A. nemorosa* vertoonden geen latitudinale gradiënt in plasticiteit. Meer plasticiteit in groei van *M. effusum* zaailingen in het noorden kan dus een belangrijke strategie zijn om klimaatverandering het hoofd te bieden.

Ten slotte voerden we een meta-analyse uit op 18 Europese studies, die de verspreiding van 90 bosplanten in oude bossen (i.e. altijd bebost sinds het oudst beschikbare kaartmateriaal) en in bossen op voormalige landbouwgrond onderzochten. Kolonisatie van de snelle soorten (voornamelijk éénjarige kruidachtigen) in bossen op voormalige landbouwgrond ging sneller als er meer bossen aanwezig waren in het landschap. De karakteristieke traag koloniserende bosplanten (vooral kleine meerjarige kruidachtigen) slaagden er meestal niet in bossen op voormalige landbouwgrond te bereiken — zelfs in dicht beboste regio's. Variatie in de gemiddelde jaarlijkse temperatuur door heel Europa had geen effect op het herstel van bossen op voormalige landbouwgrond, vermoedelijk hoofdzakelijk ten gevolge van langetermijn adaptatie aan de lokale omgeving, het grotere belang van habitatfragmentatie en de heterogeniteit in het microklimaat in bossen.

We concluderen dat de klimaatopwarming hoogstwaarschijnlijk significante effecten zal hebben op de dynamiek van bosplanten in Europa. We toonden aan dat groei en reproductie van kruidachtige bosplanten uiteenlopend kan reageren op temperatuursvariaties. Klimaatverandering kan dus de biotische interacties in bosplantengemeenschappen veranderen en het rekruteringssucces via zaden van bepaalde soorten doen toenemen. Vanzelfsprekend is meer onderzoek naar mogelijke veranderingen in de dynamiek van bosplanten ten gevolge van het opwarmende klimaat noodzakelijk.

List of abbreviations and symbols

AF	ancient forest
C	carbon
Ca	calcium
CH_4	methane
CO_2	carbon dioxide
CV	coefficient of variation
χ^2	likelihood ratio test statistic
d.f.	degrees of freedom
E	effect size
F	test statistic (F-value)
GDH	growing degree hours
Germ%	germination percentage
GF	permeable polypropylene garden fleece
GSO	germinable seed output
IPCC	Intergovernmental Panel on Climate Change (`http://www.ipcc.ch`)
IR	infrared
K	potassium
LA	leaf area
LDM	leaf dry matter
LHS	leaf-height-seed
LM	linear model
MAP	mean annual precipitation
MAT	mean annual temperature
MaxGerm%	maximum germination percentage
Mg	magnesium
MM	mixed-effect model
n	sample size
N	nitrogen

N_2O	nitrous oxide
ns	non significant ($p > 0.1$)
OTC	open-top chamber
OTC+GF	open-top chamber covered with garden fleece
OTC–GF	open-top chamber without garden fleece
p	significance of statistical test (p-value)
P	phosphorus
PAF	post-agricultural forest
PAR	photosynthetically active radiation
PCA	principal component analysis
PET	potential evapotranspiration
P.E.	parameter estimate in statistical models
pH	measure of acidity
r	Pearson correlation coefficient
$\mathrm{RDPI_S}$	simplified relative distance plasticity index
RIR	resource investment in reproduction
RH	relative humidity
RR	recovery rate
r_s	Spearman rank correlation coefficient
SCD	seed collection date
S.D.	standard deviation
S.E.	standard error
SLA	specific leaf area (LA/LDM)
t	test statistic (t-value)
TADM	total aboveground dry matter biomass
TF	total forest cover (ancient & post-agricultural)
UV	ultraviolet
VIF	variance inflation factors
(*)	$0.05 < p < 0.1$
*	$0.01 < p < 0.05$
**	$0.001 < p < 0.01$
***	$p < 0.001$

1

Introduction

1.1 Climate change

Climate change is a hot topic nowadays. The Intergovernmental Panel on Climate Change (IPCC), the leading international body for the scientific assessment of climate change and honoured with the 2007 Nobel Peace Prize, stated that *warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level* (IPCC 2007). Also the Millennium Ecosystem Assessment (2005), Butchart *et al.* (2010) and Rands *et al.* (2010) listed climate change amongst the most important drivers of biodiversity loss and changes of ecosystem services. Searching for *climate change* in Google[1] generates about 122 million results, leaving other major environmental issues such as *deforestation* (12.4 million), *water pollution* (6.5 million), *ozone depletion* (2.97 million), *nitrogen deposition* (0.55 million) and *habitat fragmentation* (0.42 million) far behind. Although this observation is clearly an oversimplification and certainly does not imply that these other issues are of minor importance, it does indicate that climate change is preoccupying the minds of many people today.

The IPCC defined climate change as *a change in the state of the climate that can be identified by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer. It refers to any change in climate over time,*

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[1] Accessed 19 November 2010, using `www.google.com`

whether due to natural variability or as a result of human activity (IPCC 2007). Climate change typically includes changing temperatures, precipitation and extreme events. According to the IPCC (2007) it is ***very likely*** (more than 90 % certainty) that most of the observed increase in global average temperatures since 1950 is due to an increase in anthropogenic greenhouse gas emissions such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and halocarbons brought about by human activities (see also Lovejoy & Hannah 2005). Moreover, it is ***extremely unlikely*** ($< 5\,\%$ probability of occurrence) that global climate change of the past 50 years can be explained by known natural variability alone (IPCC 2007).

The mean surface temperature of our planet has warmed discernibly over the past century, and very strikingly over the past decades (Lovejoy & Hannah 2005): the global average increase in mean annual surface temperature was 0.45 ℃ between 2000–2009 vs. 1961–1990 (**Fig. 1.1**; NASA 2010). The temperature anomaly increases with latitude, at least in the northern hemisphere (**Fig. 1.1**). The mean global surface temperature in 2010 just surpassed 2005 as the warmest year on record since 1880 (Hansen *et al.* 2010). The next warmest years since 1880 are 1998, 2002, 2003, 2006, 2007 and 2009 (Hansen *et al.* 2010). Hansen *et al.* (2006) calculated the resulting northward migration rate of the isotherms as $4\,\mathrm{km.year}^{-1}$ observed between 1975–2005 in the northern hemisphere. Moreover, the global average earth surface temperature is expected to increase by an additional 1.8–4.0 ℃ until 2090–2099 relative to 1980–1999 (IPCC 2007). In addition, precipitation has changed significantly in many regions across the globe with widespread declines in the Mediterranean, the Sahel and southern Africa and increases in northern Europe, eastern North America and central Asia (IPCC 2007). Precipitation is expected to further increase during winter and decrease during summer in northwestern Europe specifically in the coming decades (IPCC 2007). Finally, an increase in the frequency of extreme weather and climate events such as heavy precipitation, heat waves, tropical cyclone activity, and extreme high sea level are to be expected (IPCC 2007). We here only focus on a major aspect of climate change, i.e. changing temperatures.

1.2 Forest understorey plants

Temperature is one of the most significant factors affecting plant physiology, performance and distributions at broad spatial scales (e.g. Woodward 1987; Saetersdal *et al.* 1998; Larcher 2003). Climate change will directly and indirectly affect individuals, populations and species of contrasting plant taxa and subsequently also biological diversity in ecosystems worldwide (reviews: Thomas *et al.* 2004; Walther 2010). Processes with direct temperature effects include, for instance, growth (e.g. direct effects on potential growth rates through alterations of photosynthesis and respiration) and reproduction (e.g. seed dormancy break disruptions). Possible indirect effects consist of, e.g. changes in mineralization rates that affect nutrient availability (Rustad *et al.* 2001), modifications of (competitive) plant-plant interactions

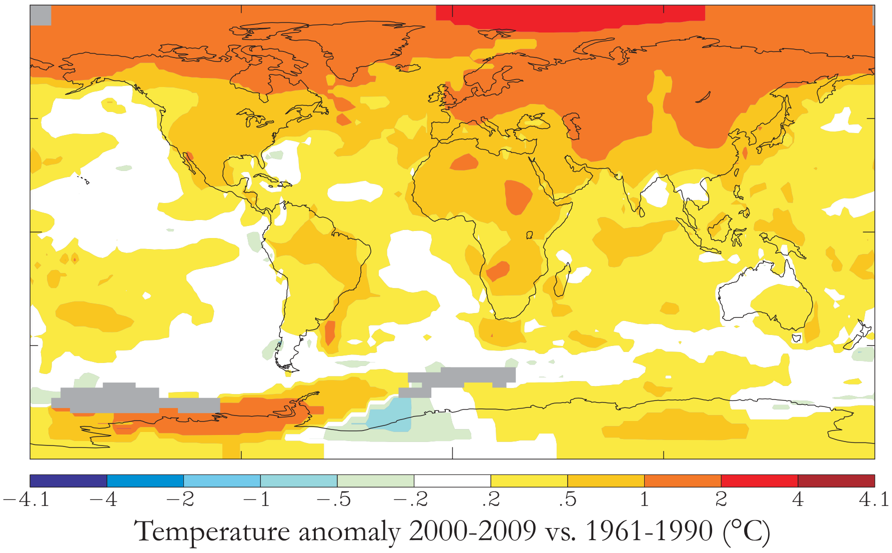


Figure 1.1: Evidence of the currently observed global warming trend. The temperature anomaly is calculated as the difference in mean surface temperatures between 2000–2009 and 1961–1990. The global average increase was 0.45 °C while the increase in northwestern Europe amounted to more than 1 °C. Image made using a smoothing radius of 1200 km through the online mapping tool of NASA (2010) which combines land station data with ship, buoy and satellite data.

(Klanderud 2005) or temporal plant-animal mismatches related to pollination, dispersal or herbivory (Lovejoy & Hannah 2005; Tylianakis *et al.* 2008; Berg *et al.* 2010).

Climate change is expected to be particularly challenging for sessile organisms such as plants since these are limited in their abilities to respond to environmental variation. More specifically, plants only have two options in a future, warmer world to avoid extinction: (i) migrate and escape the new unfavorable environmental conditions, and (ii) adapt to the new conditions by phenotypic plasticity or genetic changes (Matesanz *et al.* 2010; Parmesan & Yohe 2003; Lovejoy & Hannah 2005; Leimu *et al.* 2010).

Many plant species are expected to be unable to track climate change as the currently observed migration rates of many species are several orders of magnitude lower than the isotherm shift (Malcolm *et al.* 2002; Thomas *et al.* 2004; Hansen *et al.* 2006). Moreover, colonisation into new habitats is strongly limited for many plant species due to the large-scale habitat fragmentation in contemporary landscapes (Honnay *et al.* 2002b; Travis 2003; Leimu *et al.* 2010). In this respect are forest understorey plants convenient examples of spatially limited plants (Matlack 2005). Forest understorey species are those plants that can bear the shade of a closed canopy, create the canopy itself or that prefer forest conditions *sensu lato*, for instance, edge species (Peterken 1974). However, we here only consider herbaceous forest understorey species and not woody species (trees and shrubs). Large parts of the present-day forest area in Europe and eastern North America have been cleared for agriculture and subsequently reforested (spontaneous or planted; Flinn & Vellend 2005). In several regions, only a small fraction of the actual forest cover can be referred to as *ancient*. These ancient forests have no historical record (mainly cartographical) of agricultural land use and have generally been continuously wooded for at least *ca.* 150–400 years (reviews: Hermy *et al.* 1999; Flinn & Vellend 2005; Hermy & Verheyen 2007). Forest understorey plant species need to (re)colonise post-agricultural forest sites from relict populations in ancient forest or hedgerows, which are often scarce and isolated in contemporary landscapes.

Figure 1.2: The characteristic slow colonising forest understorey plant *Anemone nemorosa*

Especially the so-called *ancient forest species* (*sensu* Hermy *et al.* 1999) that only or mainly occur in ancient forests, display extremely low colonization rates (often $< 1\,\mathrm{m.year}^{-1}$; i.e. compared with the northward migration rate of the isotherms [$4\,\mathrm{km.year}^{-1}$] more than 4000 times slower), as documented both in Europe and in North America (Matlack 1994; Brunet & von Oheimb 1998a,b). Furthermore, it is also assumed that many understorey plants are still expanding northwards following deglaciation and that their distribution range is not yet in equilibrium with climatic factors (*cf.* **Box** on p. 6). Previous work has demonstrated that a large number of forest plants are mostly unsuccessful in (re)colonising isolated unoccupied forest patches so that, for example, the community composition bears the imprint of past land use for decades to centuries as post-agricultural forests regrow (Peterken & Game 1984; Whitney & Foster 1988; Dupouey *et al.* 2002; Plue *et al.* 2008). Today, there is a general consensus that two non-mutually exclusive hypotheses principally account for their low colonisation rates (*cf.* Baeten 2010). First, many forest plants show limited diaspore production and/or subsequent dispersal resulting in few potential diaspores arriving in a recently established forest patch (*dispersal limitation*; Verheyen *et al.* 2003c; Takahashi & Kamitani 2004; Matlack 2005). Second, also low germination and recruitment rates into the adult life stage and adult survival pose a bottleneck in the long-term colonisation of understorey species in post-agricultural forests (*establishment limitation*; Donohue *et al.* 2000; Jacquemyn & Brys 2008; Baeten *et al.* 2009). Hence, due to their low colonisation potential following land-use changes, it is assumed that forest understorey plants will also fail to track a shifting climate (Honnay *et al.* 2002b). However, because of the predicted temperature rise in the temperate zones, the actual observed colonisation capacity of these species can change (Graae *et al.* 2009b): it is likely, for instance, that intraspecific generation time and reproductive efficiency (e.g. flower and seed production, seed mass or germination) alters in response to rising temperatures (Baskin & Baskin 1998; Arft *et al.* 1999; Probert 2000; Hedhly *et al.* 2009).

Despite the ecological significance of understorey plants for temperate forest ecosystem biodiversity, competitive interactions, linkage with the overstorey and ecosystem functioning (Gilliam 2007), knowledge about the effects of climate warming on their growth, reproduction and colonisation capacity is largely lacking. Few *in situ* climate manipulation experiments have been performed in temperate deciduous forest ecosystems. One of the most famous records is the soil warming Long Term Ecological Research site at Harvard Forest, Massachusetts, USA that has been running since 1991 in a mixed deciduous forest (Farnsworth *et al.* 1995; Melillo *et al.* 2002; Bradford *et al.* 2008). Other research projects have mainly focused on the effect of warming on tree species in coniferous (Yin *et al.* 2008), broadleaved (Sager & Hutchinson 2005), mixed coniferous-broadleaved (Dabros *et al.* 2010) or boreal forest (Kilpeläinen *et al.* 2003) and on treeline dynamics (Danby & Hik 2007). Moreover, besides the Harvard Forest experiment, we are unaware of a single study that has experimentally investigated the *in situ* impact of climate warming on temperate herbaceous forest understorey plants. Hence, it remains largely unclear to what extent changing temperatures can affect the growth, reproduction and colonisation capacity of forest understorey plants. Skov & Svenning (2004) and Svenning & Skov (2006) already showed that the bioclimatic envelope of many herbaceous understorey plants might shift dramatically towards northern latitudes in Europe in the near future. Whether the future distribution of understorey plants will ever match their predicted bioclimatic envelope is very doubtful, with potentially large consequences for the long-term persistence of these species (Honnay *et al.* 2002b). Investigating the response of understorey plants to rising temperatures is thus extremely relevant.

Box: Climate changes in the past and forest plant dynamics

To assess likely future distributional shifts in response to climate change, it is of great importance to also understand the post-glacial migration patterns of species after the Pleistocene–Holocene transition. The temperature in Europe and North America fluctuated significantly during the Pleistocene glacials and interglacials (up to 7 °C increases within little more than a decade; Post 2003). It is commonly assumed that the redistributions of species started after the Last Glacial Maximum (± 20.000 year before present) from refugia south of 46 °N latitude or from microrefugia closer to the ice sheets (Hewitt 2000; Petit *et al.* 2003; Post 2003; McLachlan *et al.* 2005; Pearson 2006; Rull 2009). However, the northerly distribution range edge of many plant species is situated more towards the north than inferred from the present knowledge of life-history traits, demography and dispersal potential (Clark *et al.* 1998). The calculated migration rates necessary for plant populations responding to post-glacial warming to occupy their current distribution range ($100 - 1000\,\mathrm{m.year}^{-1}$) are several orders of magnitude larger than the present migration rates of many species (Clark *et al.* 1998; Malcolm *et al.* 2002). This discrepancy is termed ***Reid's paradox*** after Clement Reid who wondered more than 100 years ago how oak could migrate so rapidly to northern Britain after the ice ages given its slow spread at present (Clark *et al.* 1998; Graae *et al.* 2009b). Multiple explanations for this paradox have been put forward including the presence of occasional long-distance dispersal events (Cain *et al.* 1998; Pearson 2006), more local dispersal from microrefugia (*sensu* Rull 2009) containing populations that could survive closer to the ice caps (McLachlan *et al.* 2005) or even a change in the intrinsic colonisation capacity of plants (Graae *et al.* 2009b). However, it is also assumed that many trees and understorey plants are still expanding northwards following deglaciation and that their distribution range is not yet in equilibrium with climatic factors (Hermy 1985; Svenning & Skov 2004; Vanderveken *et al.* 2007; Svenning *et al.* 2008).

1.3 Latitudinal gradients and other warming methods

Potential plant responses to global warming can be examined using an array of approaches, including *in situ* investigations in natural communities along environmental (e.g. Alexander *et al.* 2009; Wilson & Nilsson 2009) or temporal gradients (Lenoir *et al.* 2008; Harrison *et al.* 2010; Verheyen *et al.* 2011), reciprocal transplanting (e.g. Kollmann & Bañuelos 2004; Reich & Oleksyn 2008; Haggerty & Galloway 2011), experiments with warming facilities (e.g. Walker *et al.* 2006; Shevtsova *et al.* 2009; Hoffmann *et al.* 2010), greenhouses or growth chambers (Beerling 1999; Jochum *et al.* 2007) or bioclimatic habitat models and process-based models (Svenning & Skov 2006; Fitzpatrick *et al.* 2008; reviewed in Pearson & Dawson 2003; Thuiller *et al.* 2008). There are specific advantages and shortcomings for each of these approaches (*cf.* Dunne *et al.* 2004; Rustad 2008; **Table 1.1**).

One way to study the influence of a variety of different temperatures on understorey forest species in natural plant communities is to make use of the temperature variation along latitudinal or altitudinal gradients (i.e. along a north–south or elevational gradient; Austin 1999; Fukami & Wardle 2005; Körner 2007). Gradients in species diversity and morphology between the tropics and the poles have fascinated ecologists ever since decades. Climatological (e.g. temperature, precipitation, relative humidity) and biogeochemical (e.g. soil characteristics, nitrogen (N) deposition) as well as physical variables (e.g. UV radiation, photoperiod) vary along such gradients, which in turn may affect patterns of land use. Disentangling the relative effects of these environmental factors is essential to be able to describe the effects of climate on biotic and abiotic processes in the past, present and future. The mean annual temperature generally decreases with 0.5–0.65 °C for every 100 m upward shift (Körner 2007; Jump *et al.* 2009) and with 1 °C for every 145 km northward shift between 23° and 67°N or S (Colwell *et al.* 2008; Jump *et al.* 2009). Hence, using such gradients can provide valuable insights into community and ecosystem responses to different climates (Berg *et al.* 1993; Gholz *et al.* 2000; Etterson 2004). Latitudinal and altitudinal gradients allow for assessing long-term ecosystem responses by using an indirect *space-for-time* substitution procedure by surveying different populations of the same target species along a latitudinal or altitudinal transect and using the existing differences to infer a potential response to climate change over time (Fukami & Wardle 2005). One major assumption here is that species and populations respond to temporal changes in the variable under study (say temperature) in the same way as they respond to spatial variability (Dunne *et al.* 2004; Fukami & Wardle 2005). However, the concurrent abiotic and biotic changes over space along altitudinal and latitudinal gradients such as precipitation, photoperiod, UV-B radiation, athmospheric pressure, or soil properties can be difficult to disentangle from temperature effects (Körner 2007). Moreover, directional selection may have favoured plant ecotypes adapted to the local environment (Leimu & Fischer 2008) making it difficult to assess whether the observed variation in response to temperature is due to phenotypic or genotypic causes (Magnani 2009).

To overcome these problems associated with spatial gradients, common garden transplant experiments offer the opportunity to transplant species or populations from a colder region into a warmer region with a longer growing season (e.g. Reich & Oleksyn 2008; Haggerty & Galloway 2011). A common garden experiment is an experiment in which genotypes or ecotypes (here defined as geographically distinct populations of the same species) are grown in a common environment (Moloney *et al.* 2009). Although transplant experiments always create some degree of disturbance and are generally only performed on the short-term, they can be used to assess phenotypic plasticity and genetically local adaptation across a species' range (Macel *et al.* 2007; Magnani 2009). Phenotypic plasticity is the ability of a genotype to change its phenotype in response to changes in the environment. Such plasticity may be considered a bet-hedging strategy of organisms to cope with environmental variation (recent review: Matesanz *et al.* 2010). Since phenotypic plasticity is also subject to evolution as other functional traits, it is only considered adaptive when plasticity in these life-history traits improves plant fitness. Plasticity is maladaptive in case it reduces fitness. Sometimes global change drivers may also select for reduced plasticity, such as in plants that maintain high photosynthetic efficiency both under dry and moist soil conditions (Matesanz *et al.* 2010).

Experimental warming devices such as open-top chambers, infrared heaters, soil heating cables or reflective curtains allow for the study of warming effects in a more controlled manner compared to gradient studies. However, they typically generate only short-term data obtained with a step-increase of temperatures, often disturb pollination or herbivory processes, and may alter other abiotic conditions such as CO_2 concentration or soil moisture content (Richardson *et al.* 2000; Kimball 2005; Rustad 2008; Aronson & McNulty 2009) all of which can have undesired effects on plant performance.

Bioclimatic habitat models and process-based studies, finally, mostly consider intraspecific genotypic and phenotypic variation in plant traits as static (Pearson & Dawson 2003; Thuiller *et al.* 2008) and may ignore competitive interactions, adaptive genetic variation and species-specific dispersal limitation (Hampe 2004). We further do not consider bioclimatic envelope models here, although they may serve as interesting first approximations of the potential impact of climate changes on plants and animal distributions alike (Pearson & Dawson 2003).

Combining experiments with investigations along environmental gradients should provide a better assessment of the likely response of plants to climate change across a range of environments (Dunne *et al.* 2004; Rustad 2008). For example, Gedan & Bertness (2009) experimentally warmed salt marshes along a latitudinal gradient in North America and found large warming-induced biodiversity declines in waterlogged pannes. Near the southern range limit, however, larger sensitivity to warming was detected compared to the north. Such combinations of gradient analyses and experiments, however, are extremely scarce in climate change research (Dunne *et al.* 2004; Rustad 2008). Here, we followed such an approach and chose to combine observational studies along a wide latitudinal gradient in Europe with open-top chambers, growth chambers programmed at different temperatures and common garden transplant experiments in the different chapters.

1.4 Objectives and outline of this thesis

The main objectives of this thesis were to:

- Quantify the response of the growth and reproduction of a set of selected herbaceous understorey plants to temperature variation along a latitudinal gradient and to experimentally elevated temperatures;
- Assess likely changes in forest understorey plant dynamics and colonisation capacity of these species along a latitudinal gradient in the face of climate change.

Therefore, we here study several phases of the life cycle of a set of characteristic herbaceous forest understorey plants along a latitudinal gradient. We specifically focused on the following successive stages of plant growth and recruitment (**Fig. 1.3**):

- Adult plant performance
- Seed production, seed quality and germination
- Seedling establishment

Although the different chapters may not always be clearly limited to one particular stage of the life cycle of understorey plants (e.g. seed mass is part of the leaf-height-seed strategy scheme [Chapter 2], and seedling establishment and seed production were also quantified in Chapters 5 and 7, respectively), the main findings of each chapter generally correspond to the life stage it was classified in (**Fig. 1.3**).

Besides growth and performance of adult plants, the main focus of this work is set on the recruitment from sexual reproduction of the study species, for mainly two reasons. Firstly, sexual plant reproduction is crucial for population persistence and spread of many plant species (review: Hedhly *et al.* 2009; Walck *et al.* 2011) and, more specifically, an important determinant of the colonisation capacity of understorey plants (Brunet & von Oheimb 1998a; Jacquemyn & Brys 2008). Secondly, few have explicitly studied the response of the seed and seedling life stage to climate change (Hovenden *et al.* 2008). However, temperature is a critical driver for seed dormancy, germination and seedling survival (Walck *et al.* 2011). Hence, changing temperatures are likely to alter recruitment from sexual reproduction of understorey plant species which could significantly affect community assembly.

Table 1.1: Non-exhaustive overview of the major advantages and drawbacks of (a) gradient, (b) experimental and (c) modelling methods to study the effects of climate change on plant individuals, species or community dynamics. Compiled from Dunne *et al.* (2004); Rustad (2008); D'hondt (2009); Willaert (2011).

	Method	Major advantages	Major drawbacks
(a)	Latitudinal and altitudinal gradient (×)	Long-term response to climate; no disturbance	Difficulty to unravel phenotypic from genetic effects
	Transplantation along gradient (×)	Allows to unravel phenotypic from genetic effects	Strong disturbance
	Temporal resurveys of (semi-)permanent plots	'Real' *in situ* ecosystem response to climate change	Difficulty to disentangle climate change from other temporal changes
(b)	Open-top chambers (×)	Simple, passive and economical	Little control of ΔT achieved
	Infrared radiation	Low degree of disturbance; control of ΔT	No direct warming of the air; soil drying
	Soil heating cables	Easily combined with other warming method; control of ΔT	High degree of soil disturbance; only soil warming; soil drying
	Nighttime reflective curtains	Mimicking of climate change due to GHGs	Little control of ΔT achieved
	Glasshouses and growth chambers (×)	Control of the treatment conditions achieved	Low resemblance with field conditions
(c)	Bioclimatic and process-based models	Large-scale projections are possible	Do not sufficiently include species interactions, microclimatic heterogeneity and migration limitations

GHGs: greenhouse gases; ΔT: temperature difference with the ambient control; ×: method applied in this PhD thesis.

We studied phenology, growth and recruitment of understorey plants both *in situ* in natural populations along a latitudinal gradient (Chapters 2–5) and with an open-top chamber validation experiment (Chapter 6). The *ex situ* part included experiments in three common garden transplant sites (Chapters 7 & 8) and in growth chambers programmed at different temperatures (Chapter 8).

More specifically, Chapters 2–5 follow an observational approach along a latitudinal gradient. In **Chapter 2**, we applied the leaf-height-seed (LHS) ecology strategy scheme (a combination of three ecologically important traits: specific leaf area (SLA), seed mass and plant height; Westoby 1998) intraspecifically to adult individuals of two widespread European understorey plants along a latitudinal gradient. The following three chapters describe seed production, seed quality and germination. **Chapter 3** assesses a potential latitudinal gradient in seed nutrient concentrations within the widespread European understorey forest herb *Anemone nemorosa*, a model species for slow colonising herbaceous forest plants. In **Chapter 4**, we unravel the effects of temperature, latitude and local environment, on reproductive traits of six widespread perennial forest plants, while **Chapter 5** focuses on the effects of temperature on sexual reproductive output (number of seeds, seed mass, germination percentage and seedling mass in forest sowing experiments) of one species (*A. nemorosa*) along the latitudinal gradient.

A more experimental approach is followed in Chapters 6–8. First, we validated the use of open-top chambers in forests to evaluate warming effects on understorey plants since knowledge on the performance of open-top chambers in deciduous forests was largely lacking (**Chapter 6**). Next, we combined common garden transplant experiments in three sites along a wide latitudinal gradient with an open-top chamber experiment in a single transplantation site to examine the effects of temperature on phenology, growth and reproductive performance of two species (**Chapter 7**). This set-up also allowed us to determine whether variation in the response of understorey plants to temperature is due to phenotypic plasticity or due to long-term adaptations to the local climate. Finally, **Chapter 8** describes phenotypic plasticity and adaptation in seedlings of two understorey plants resulting from seeds sampled along a latitudinal gradient and in the three common gardens to determine the acclimation potential of seedlings in response to temperature changes.

In **Chapter 9**, we integrated forest understorey plant performance and population spread by compiling data on 90 species and 812 species × study combinations from 18 published studies across Europe that determined species' distribution patterns in ancient (i.e. continuously forested since the first available land use maps) and post-agricultural forests. The recovery rate of species in post-agricultural forests in each landscape was then related to the species-specific life-history traits and local and regional factors (including climate).

The final chapter summarises and integrates the main findings of the observational approaches, experiments and meta-analysis (**Chapter 10**)

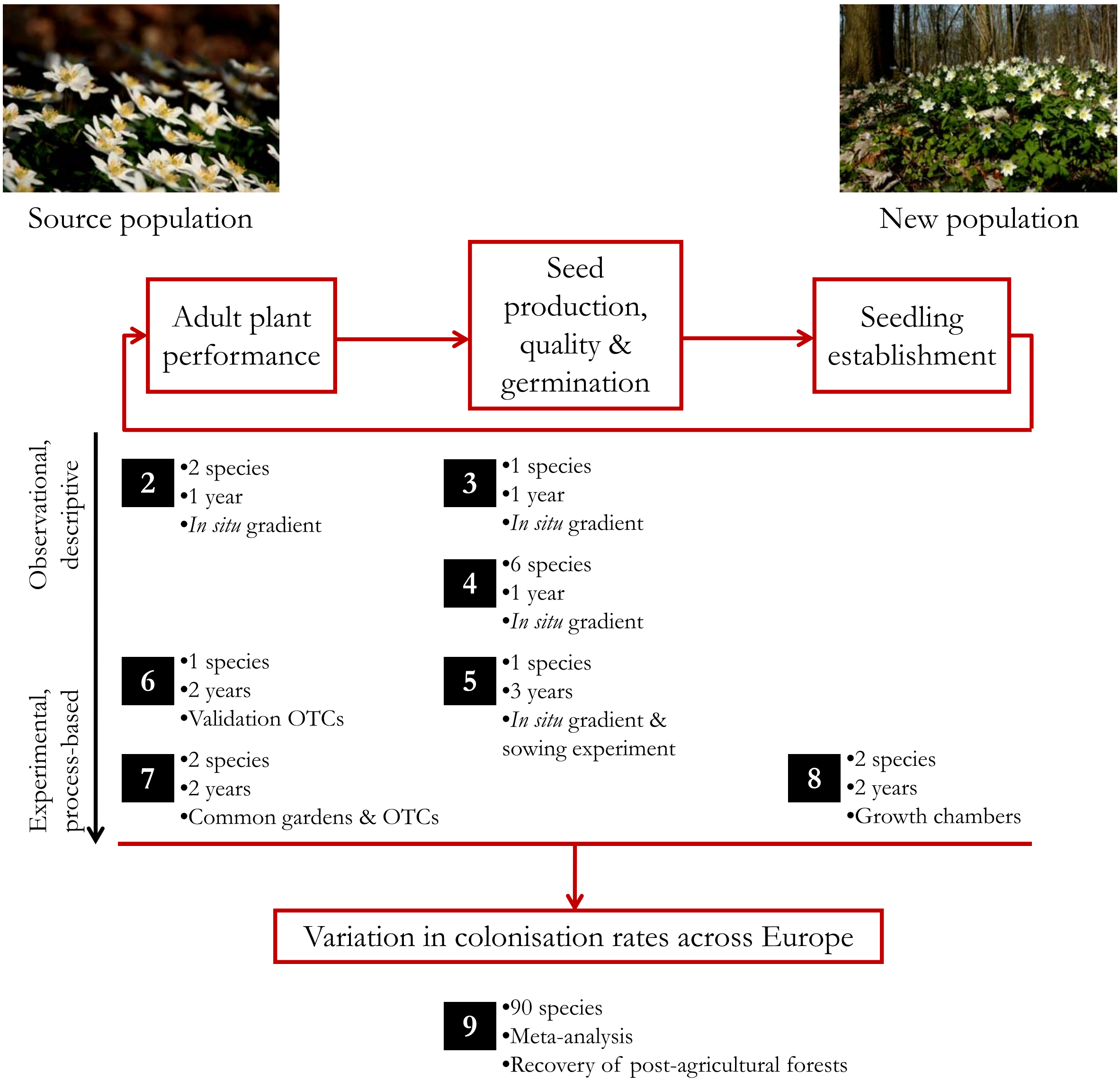


Figure 1.3: Outline of this PhD thesis. Chapter numbers in black frames. The number of species involved, duration and main methodology of each chapter is given. OTCs: open-top chambers.

2

An intraspecific application of the leaf-height-seed ecology strategy scheme

After: De Frenne P, Graae BJ, Kolb A, Shevtsova A, Baeten L, Brunet J, Chabrerie O, Cousins SAO, Decocq G, Dhondt R, Diekmann M, Gruwez R, Heinken T, Hermy M, Öster M, Saguez R, Stanton S, Tack W, Vanhellemont M, Verheyen K (2011) An intraspecific application of the leaf-height-seed ecology strategy scheme to forest herbs along a latitudinal gradient. *Ecography*, **34**, 132–140.

Abstract

We applied the leaf-height-seed (LHS) ecology strategy scheme (a combination of three ecologically important traits: specific leaf area (SLA), seed mass and plant height) intraspecifically to two widespread European forest herbs along a latitudinal gradient. The aims of this study were to quantify LHS trait variation, disentangle the environmental factors affecting these traits and compare the within-species LHS trait relationships with latitude to previously established cross-species comparisons. We measured LHS traits in 41 *Anemone nemorosa* and 44 *Milium effusum* populations along a 1900–2300 km latitudinal gradient from northern France to northern Sweden. We then applied mixed-effect models to identify the effects of regional (temperature, latitude) and local (soil fertility and acidity, overstorey canopy cover) environmental factors on LHS traits. Both species displayed a significant 4 % increase in plant height with every degree northward shift (almost a two-fold plant height difference between the southernmost and northernmost populations). Neither seed mass nor SLA showed a significant latitudinal cline. Temperature had a large effect on the three LHS traits of *A. nemorosa*. Latitude, canopy cover and soil nutrients were related to the SLA and plant height of *M. effusum*. None of the investigated variables appeared to be related to the seed mass of *M. effusum*. The variation in LHS traits indicates that the ecological strategy determined by the position of each population in this three-factor triangle is not constant along the latitudinal gradient. The significant increase in plant height suggests greater competitive abilities for both species in the northernmost populations. We also found that the studied environmental factors affected the LHS traits of the two species on various scales: spring-flowering *A. nemorosa* was affected more by temperature, whereas early-summer flowering *M. effusum* was affected more by local and other latitude-related factors. Finally, previously reported cross-species correlations between LHS traits and latitude were generally unsupported by our within-species approach.

2.1 Introduction

Functional life-history traits are increasingly used as the basis for a general ecological characterization and classification of a wide variety of organisms (Grime *et al.* 1988; Diaz *et al.* 2004; Freschet *et al.* 2010). Likewise, groupings of life-history traits that are consistent among different plant species have been widely used to predict changes in the abundance and distribution of individual species and species groups in response to processes such as disturbance (Lavorel *et al.* 1997; Graae & Sunde 2000; Verheyen *et al.* 2003c). The life-forms of Raunkiaer (1937) and the CSR-scheme of Grime (1974) have been applied and discussed for decades (see Craine 2005 for an overview). More recently, Westoby (1998) proposed the leaf-height-seed (LHS) ecology strategy scheme as an alternative explanation for plant responses to environmental variation. The LHS scheme differs from other classifications in that it is based on three easily measured 'soft' traits (sensu Diaz *et al.* 2004): (1) specific leaf area (SLA), (2) plant canopy height at maturity and (3) seed mass. SLA, for instance, contributes to the potential of a plant to respond to a change in stress exposure, whereas seed mass and plant height influence the ability of a plant to adjust to disturbance effects and other environmental changes (Westoby 1998; Poorter *et al.* 2009).

The LHS scheme has been used at the species level to compare species groups (Lavergne *et al.* 2003; Jardim & Batalha 2008) and at the community level to compare different forms of management or successional stages (Garnier *et al.* 2004; Moog *et al.* 2005; Golodets *et al.* 2009). To date, the LHS scheme has, to our knowledge, not been used to describe intraspecific trait variation among populations of a single species. Interspecific studies on how environmental factors influence a set of plant traits may be confounded by co-varying differences among taxa in, e.g. growth form, phenology, plant development time, predominant seed disperser assemblage or habitat type (Lavorel *et al.* 1997; Lord *et al.* 1997; Moles & Westoby 2003; Moles *et al.* 2007). For example, if seed mass or plant height across species increases towards the equator (Moles & Westoby 2003; Moles *et al.* 2007; 2009a,b), this pattern may be blurred by habitat variations, such as the decreasing proportion of trees towards the poles (Lord *et al.* 1997; Moles & Westoby 2003; Moles *et al.* 2007; Tautenhahn *et al.* 2008). These drawbacks are largely avoided by using a within-species approach. Moreover, comparisons between the intraspecific and interspecific relationships for traits such as plant height are ecologically important issues that require further investigation (Moles *et al.* 2009a; Albert *et al.* 2010; Fajardo & Piper 2010).

Quantitative knowledge about trait responses across various spatial scales is still largely lacking (Ordoñez *et al.* 2009). Such knowledge is urgently needed to better understand ecosystem functioning (McGill *et al.* 2006) and predict responses to future climate change. The macroclimatic variation along latitudinal gradients offers an opportunity to elucidate variation in plant traits and to unravel the relative importance of a set of environmental factors (temperature, soil fertility, etc.; Austin 1999; McGill *et al.* 2006; Graae *et al.* 2009b; see Fukami & Wardle 2005 for a recent review). Both observational studies and common-garden experiments along latitudinal transects at the inter- and intraspecific level show that seed mass and plant height generally decrease with latitude (Moles & Westoby 2003; Murray *et al.* 2004; Pakeman *et al.* 2008; Moles *et al.* 2009a,b), whereas SLA increases with latitude within species (Clevering *et al.* 2001; Etterson 2004; Miyazawa & Lechowicz 2004). Interspecific comparisons of SLA with latitude on a global scale are still lacking, and the three LHS traits have rarely been investigated intraspecifically from a combined perspective. Furthermore, little information is available about the scale on which the environmental factors affect the LHS traits, such as soil fertility on a local scale and climate on a regional scale (already stressed by Westoby 1998).

In the present study, we investigated intraspecific variation in the LHS traits for multiple populations of two common European herbaceous forest plant species along a latitudinal gradient. In an effort to make our results more widely applicable, the study species belong to two different phenological groups (Kudo *et al.* 2008) and life-forms: *Anemone nemorosa* L. is a spring flowering geophyte, and *Milium effusum* L. is an early-summer flowering hemicryptophytic grass. We specifically wanted to gain insight into (i) the extent of variation in the three LHS traits within these species along a latitudinal gradient, (ii) which environmental factors are most related to the observed variation in LHS traits and (iii) whether the within-species relationships differ from previously established cross-species comparisons.

2.2 Methods

2.2.1 Study species

Two species were specifically selected for this study due to their representativeness (they are characteristic for and abundant in broadleaved forests across Europe), reproductive traits (both clonal and sexual reproduction; see further in Chapter 7), distribution range (present in almost all regions along the latitudinal gradient), and, most importantly, based on their contrasting phenology and growth form: *Anemone nemorosa* L. (Ranunculaceae) and *Milium effusum* L. (Poaceae). Both species are widespread in the understorey of ancient (*sensu* Hermy *et al.* 1999; Hermy & Verheyen 2007) deciduous forests in northwestern and central Europe (Hultén & Fries 1986), but colonise secondary forests slowly (Verheyen *et al.* 2003c; Chapter 9). *A. nemorosa* typically has very low colonisation rates whereas *M. effusum* is somewhat faster (Verheyen *et al.* 2003c; Brunet 2007), probably due to zoochorous dispersal by large mammals (see below). Both species represent important phenological groups (*sensu* Kudo *et al.* 2008) and life-forms of forest understorey plants: *A. nemorosa* is a spring flowering geophytic forb while *M. effusum* is an early summer flowering hemicryptophytic grass. The northern distribution edge of *A. nemorosa* is situated around 67°N whereas *M. effusum* is distributed as far as 71°N. Close to their northern range edge, both species occur only in small deciduous forest patches within a largely boreal forest (*A. nemorosa*) or subarctic tundra (*M. effusum*) matrix. Their southern range limits are around 38–40°N (Hultén & Fries 1986). The flowers of *A. nemorosa* are hermaphroditic, pollinated by insects and mainly self-incompatible (Shirreffs 1985), although selfing sometimes occurs (Müller *et al.* 2000). The species usually produces 10–30 achenes (Chapter 5; hereafter referred to as *seeds*) per year and per flowering ramet (hereafter referred to as *individual*), and also propagates clonally with rhizomes (Shirreffs 1985). *M. effusum* is wind pollinated (Tyler 2002), usually produces 100–300 caryopses (hereafter referred to as *seeds*) per year and per shoot (hereafter referred to as *individual*), and also develops short stolons for re-sprouting. Seeds of the two species are mainly gravity-dispersed, but myrmecochory and slug-dispersal in *A. nemorosa* (Delatte & Chabrerie 2008; Türke *et al.* 2010) and epi- and endozoochory in *M. effusum* (Graae 2002; Heinken & Raudnitschka 2002; von Oheimb *et al.* 2005) can occur as well.

2.2.2 Study regions and populations

We studied the species in seven (*A. nemorosa*) and eight (*M. effusum*) regions located along an approximately 1900 km (*A. nemorosa*) to 2300 km (*M. effusum*) latitudinal gradient from northern France via Belgium and Germany to northern Sweden (**Fig. 2.1** excluding Estonia; **Appendix A**). The latitudinal gradient exhibits a mean annual temperature difference of 6.5 °C and 8.1 °C between the southernmost (Amiens) and northernmost (Umeå and Abisko; for *A. nemorosa* and *M. effusum*, respectively) populations (**Appendix A**; F.A.O. 2005). In 2008, we selected six populations of each species in each region (except five *A. nemorosa* populations in Umeå and two *M. effusum* populations in Stockholm), thus a total of 41 *A. nemorosa* and 44 *M. effusum* populations. Within each region, the populations were randomly selected within an area of *ca.* $20 \times 20\,\mathrm{km}^2$. All populations occurred in deciduous forests that were unaffected by major recent disturbances (management, grazing, etc.).

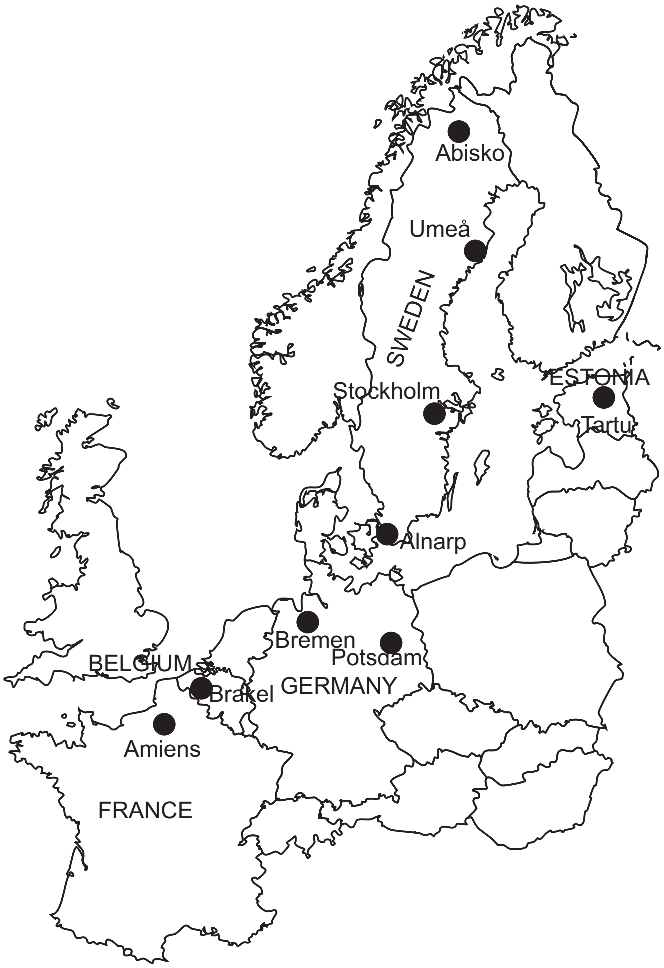


Figure 2.1: Study regions along the latitudinal gradient in northwestern Europe used for the *in situ* collections in Chapters 2–5. Note that not all regions were included in each chapter; this is specified in the text and in **Appendix A**.

2.2.3 LHS traits

At seed maturity, we measured plant height and collected all seeds and leaves of 15 randomly chosen individuals per population and species. Individuals that appeared heavily attacked by insects, pathogens or other organisms were avoided. Plants were considered mature when natural seed dispersal began, i.e. when seeds fell easily from the plant (Baskin & Baskin 1998). The collection dates are shown in **Table 2.1**. The following data collection protocol was adapted from Cornelissen *et al.* (2003). Plant canopy height at maturity was determined by measuring the total height from the ground level to the top for all 15 individuals per population. In *A. nemorosa*, we calculated mean air-dry seed mass as the total seed mass per individual (weighed to the nearest 0.1 mg) divided by the total number of seeds of each individual, whereas for *M. effusum*, we determined mean seed mass from a randomly selected 50-seed subsample per individual. Leaves of each individual were air-dried flat in dry paper for 3 days and shipped to Belgium. Subsequently, leaves were oven-dried (at 50 ℃) to constant mass for 3 days. Leaf area (LA; mm^2) was measured with a Li-Cor Portable Area Meter Li-3000 (Li-Cor Biosciences, Nebraska, USA). Hence, LA was measured on dry leaves and not on water-saturated leaves (Garnier *et al.* 2001) due to logistic constraints along the large geographic gradient, shipping restrictions and to standardize the LA measurements in a single lab with one LA meter. We believe that the introduced shrinkage error by drying leaves of these particular species is negligible in an intraspecific relative comparison. Leaf dry matter (LDM; mg) was weighed to the nearest 0.1 mg. Specific leaf area (SLA; $mm^2.mg^{-1}$) was calculated by dividing LA by LDM.

2.2.4 Environmental variables

We used the number of growing degree hours (GDH) above 5 ℃ from 1 January until the population- and species-specific seed collection dates as a measure of temperature (Lindsey & Newman 1956). The calculations of GDH followed those of Lindsey & Newman (1956) using daily minimum and maximum temperatures from weather stations as nearby as possible to the sampled populations (mostly $< 40\,km$). We included the latitude of each region in the analysis to account for regional environmental variables not measured directly along the gradient but expected to differ among sampling regions, such as precipitation, photoperiod and semi-darkness (Mills 2008), and land-use and climate history shaping distributions of species and populations (Jansson & Dynesius 2002; Moles *et al.* 2007).

Within each population, we visually estimated the percentage of overstorey canopy cover as a measure of light availability. In addition, we collected five 4-cm deep soil cores from below the litter layer. The soil samples from each population were combined, oven-dried (40 ℃) to constant mass and passed through a 2 mm-sieve. All samples were analysed for pH

Table 2.1: Collection dates (day of the year) of the leaf-height-seed samples along the latitudinal gradient (parentheses indicate the number of populations sampled in each region).

Lat. (°N)	Collection dates	
	A. nemorosa	*M. effusum*
49.8	140 (6)	157 (6)
50.9	130 & 131 (6)	157 (6)
52.6	136 & 137 (6)	162-164 & 167 (6)
53.3	142 (6)	165 (6)
55.6	141 & 143 (6)	170 & 171 (6)
59	157 (6)	198 (2)
63.8	176 & 177 (5)	187 & 196-198 (6)
68.4	- (0)	235, 239 & 242 (6)

(determined from a solution of 10 g of soil and 25 ml of 0.01 M $CaCl_2$ with a standard glass electrode), plant available phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K) (extraction with ammonium lactate and photometric determination by flow injection analysis for P and flame atomic absorption spectrophotometry, SpectrAA-220, Varian, for cations; all in mg per 100 g soil), carbon (C) and nitrogen (N) (elemental analyser; %).

2.2.5 Data analysis

We used population-level means for all analyses. Moreover, data were not transformed as assumptions of statistical tests in terms of normality and homogeneity of variances were fulfilled. To account for multicollinearity among soil variables, we performed a principal component analysis (PCA) with `VARIMAX`-rotation to reduce the set of soil variables to two components for both species. The first two PCA axes explained 67.4% and 71.2% of the variation in soil variables for *A. nemorosa* and *M. effusum*, respectively. For both species, the first PCA axis was a soil nutrient axis (Pearson correlations between axis 1 and soil N, Mg, C and K concentration: $r > 0.550$; $p < 0.001$) whereas the second PCA axis was a soil acidity axis (Pearson correlations between axis 2 and soil pH and Ca concentration: $r > 0.859$; $p < 0.001$). Sample scores for the first two principal components were used in the mixed-effect models (see below).

To explore whether and to what extent the three LHS traits varied along the latitudinal gradient, we calculated the variation in LHS trait values using *latitude* as a predictor variable in mixed-effect models (`SPSS Mixed` procedure with maximum likelihood estimation). A random effect term *region* was added to the mixed-effect models to account for the hierarchical nature of the data and the spatial autocorrelation between populations from the same region (Hox 2002). Secondly, the regional (temperature and latitude) and local (soil nutrients axis, soil acidity axis and canopy cover) predictor variables were related to the variation in LHS trait values using mixed-effect models. Again, the mixed-effect model acknowledges that regional environmental variables only account for regional level variance, omitting pseudoreplication of the predictors at the population level. To detect possible multicollinearity between the predictor variables, variance inflation factors (VIF) were calculated according to Quinn & Keough (2002). The calculated VIF were lower than 10 (< 4.85 for *A. nemorosa* and < 4.32 for *M. effusum*), indicating low collinearity (Quinn & Keough 2002). To avoid overfitting, the five predictors were first evaluated based on the -2 Log Likelihood information criterion (deviance; Hox 2002) as stand-alone predictors. Subsequently, extra predictors were added one-by-one to the first model with the lowest deviance. If the deviance decreased significantly (χ^2 tested; Hox 2002), this procedure was repeated. Only the final models are presented in the results. All analyses were performed with `SPSS 15.0`.

2.3 Results

There was clear variation in the three LHS traits along the latitudinal gradient for both species (**Fig. 2.2** and **Fig. 2.3**). *A. nemorosa* and *M. effusum* individuals growing in northern populations were significantly taller at maturity compared to individuals in more southerly distributed populations (**Table 2.2**). For instance, French *A. nemorosa* and *M. effusum* were on average (± S.E.) 14.2 (±0.3) cm and 82.9 (±2.4) cm tall, whereas the northernmost Swedish individuals measured 23.7 (±0.5) cm in Umeå and 148.7 (±1.8) cm in Abisko, respectively (**Fig. 2.4b**). This means that the plant height of *A. nemorosa* and *M. effusum* increased significantly by 4.8 % (0.43 cm) and 4.3 % (3.0 cm) respectively, per degree latitude poleward shift. In the case of *A. nemorosa*, seed mass and plant height were negatively correlated (**Fig. 2.2**), as were seed mass and SLA in *M. effusum* (**Fig. 2.3**). The remaining LHS traits of *A. nemorosa* and *M. effusum* were uncorrelated. Seed mass and SLA were unrelated to latitude in *M. effusum*, whereas in *A. nemorosa* the relation was only marginally significant (**Table 2.2**).

Table 2.2: Effects of latitude on leaf-height-seed traits of *Anemone nemorosa* and *Milium effusum* along the latitudinal gradient. Results from mixed-effect model analyses.

	LHS trait	P.E.	d.f.	t-value	p-value
A. nemorosa	Plant height	0.427	7.2	2.51	0.039 *
	SLA	0.526	7.2	1.93	0.094 (*)
	Seed mass	-0.037	7.3	-2.08	0.074 (*)
M. effusum	Plant height	3	3.9	6.84	0.003 **
	SLA	-0.163	7.4	-0.49	0.639 (ns)
	Seed mass	-0.003	7.8	-0.51	0.610 (ns)

The height of *A. nemorosa* and *M. effusum* increased significantly with increasing latitude (**Table 2.2**; **Fig. 2.4b**), but height differences in *A. nemorosa* were mainly related to accumulated temperature (**Table 2.3**). Hence, plant height of both species was determined by regional factors (temperature and latitude), although canopy cover also affected plant height of the summer-flowering *M. effusum* (**Table 2.3**), i.e. plants were taller under an open canopy. SLA of both species decreased with increasing soil nutrients and, for *A. nemorosa*, with increasing temperature (**Fig. 2.4a**). Finally, temperature (GDH) had a significant, positive effect on the seed mass of *A. nemorosa*, whereas none of the studied variables significantly affected the seed mass of *M. effusum* (**Table 2.3**).

2.4 Discussion

The LHS scheme (Westoby 1998) is used to classify species or communities according to three easily measured 'soft' (*sensu* Diaz *et al.* 2004) traits: SLA, canopy height and seed mass. This

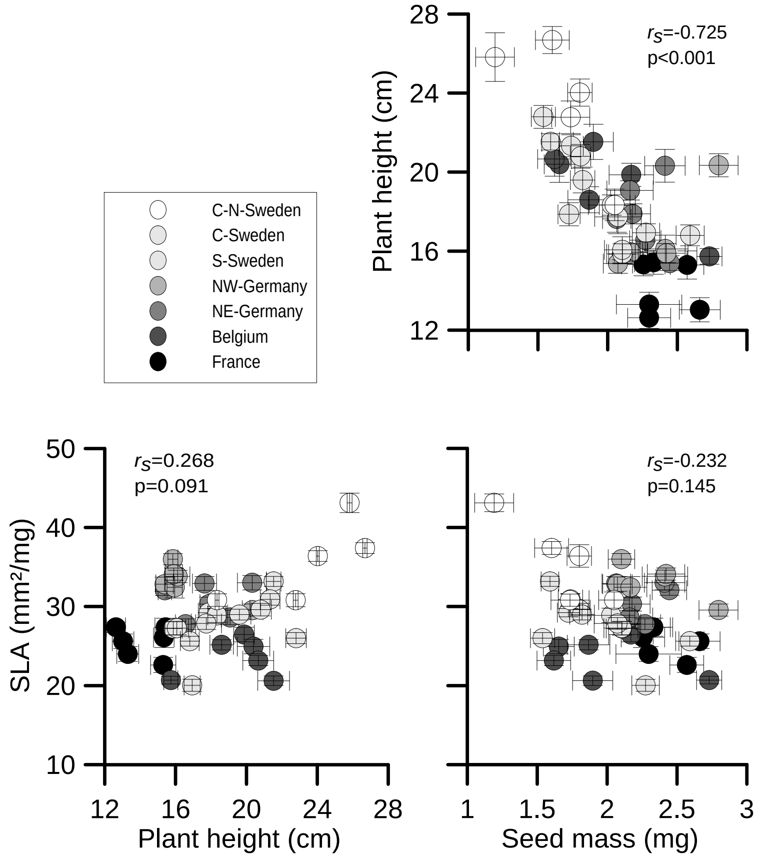


Figure 2.2: Leaf-height-seed relationships for *Anemone nemorosa* populations along the latitudinal gradient. Every dot represents a mean per population $\pm S.E.$ with the grey scaling indicating southerly (black) to northerly (white) regions. Spearman rank correlations between LHS traits (r_s) and p-value are also represented ($n = 41$).

study is the first to apply the LHS scheme intraspecifically in order to assess life-history trait variation of a single species along a (complex) environmental gradient. Using two forest herbs as a case study, the LHS scheme enabled us to clearly distinguish between different populations along the gradient. Our results demonstrate that LHS traits are useful for discriminating fundamental characteristics of growth and reproduction within a single species. Unlike cross-species observational quantifications, where a large part of the variability in plant traits occurs between coexisting species within a single site (Moles *et al.* 2007; 2009a), the within-species approach more clearly identifies the environmental variables shaping specific plant traits.

The variation in LHS traits for both species indicates that the ecological strategy determined by the position of each population in the LHS volume is not constant along the latitudinal gradient. Because taller plants possess greater competitive abilities than smaller individuals (Grime *et al.* 1988; Westoby 1998; Lavergne *et al.* 2003; Moog *et al.* 2005) and LHS traits

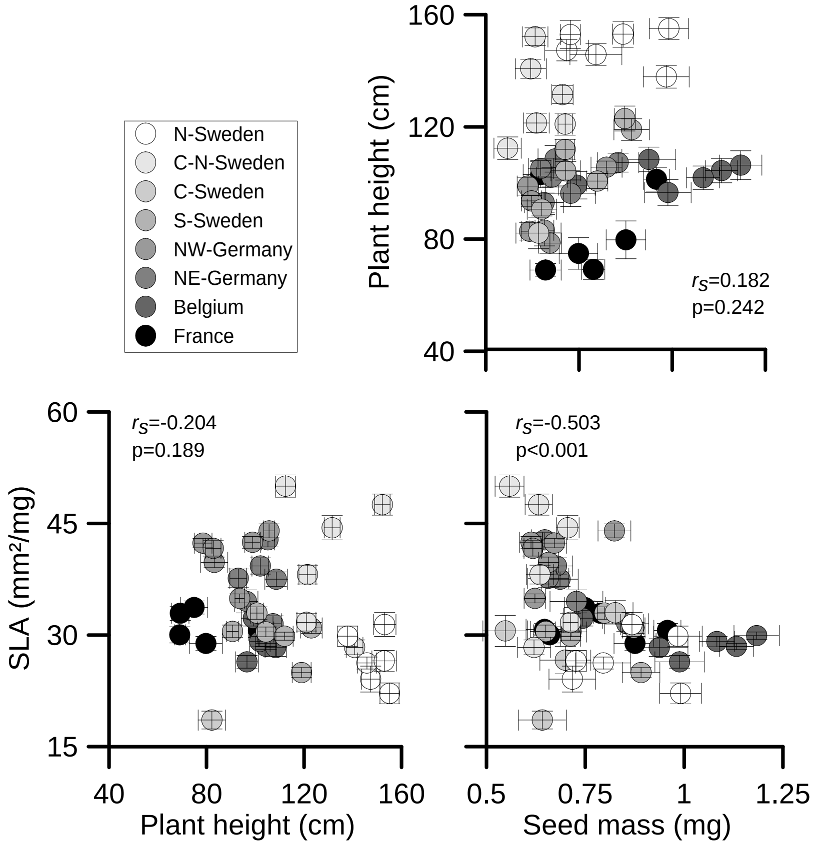


Figure 2.3: Leaf-height-seed relationships for *Milium effusum* populations along the latitudinal gradient. Every dot represents a mean per population $\pm S.E.$ with the grey scaling indicating southerly (black) to northerly (white) regions. Spearman rank correlations between LHS traits (r_s) and p-value are also represented ($n = 44$).

clearly correspond with CSR strategies (Moog *et al.* 2005), the significant latitudinal increase in plant height suggests that *A. nemorosa* and *M. effusum* individuals in the northern populations are more competitive. Of course, more research is needed to confirm this hypothesis in relation to coexisting species and to identify the factors driving this pattern experimentally. Furthermore, our analyses could be extended to a larger set of plant species to allow for generalisation among functional types or growth forms. Finally, the criterion of LHS trait independence (Westoby 1998; Golodets *et al.* 2009) was mostly upheld, as most correlations between LHS traits were not significant.

2.4.1 LHS trait variation and environmental factors

Firstly, plant height of both *A. nemorosa* and *M. effusum* increased by >4 % for every degree northward shift. Northern populations experience a longer photoperiod in the growing

Table 2.3: Effects of regional and local environmental factors on leaf-height-seed traits of *Anemone nemorosa* and *Milium effusum* along the latitudinal gradient. Results from multilevel models with predictor variables: (1) temperature [GDH], (2) latitude, (3) soil nutrients axis [first PCA axis], (4) soil acidity axis [second PCA axis] and (5) overstorey canopy cover.

	LHS trait	Env. factor	-2LL[a]	P.E.	d.f.	*t*-value	*p*-value
A. nemorosa	Plant height	Temperature	178.3	-1.67E-03	7.1	-4.33	0.003**
	SLA	Temperature	211.7	-2.15E-03	7.1	-3.04	0.019*
		Soil nutrients axis		-1.1	41	-1.8	0.079(*)
	Seed mass	Temperature	9.7	1.54E-04	7.2	3.61	0.008**
M. effusum	Plant height	Latitude	329	2.15	9.5	5.07	<0.001***
		Canopy cover		-0.4	34	-3.29	0.002**
	SLA	Soil nutrients axis	267.4	-1.98	44	-2.37	0.022*
	Seed mass	(ns)					

[a] -2 Log Likelihood (deviance) model fit information criterion

season when the temperature is above the threshold for plant development, and the studied forest type in the north is more open with lower canopy cover (from 90 % in France to 40 % in northern Sweden), which leads to a clear northward increase in potentially available radiation during the growing season. This increase in aboveground resource availability may result in a higher possibility of increased growth and a taller plant stature in northern populations, but may also influence interspecific competition and height growth of other species (see above). Secondly, increasing temperature (GDH) had a positive effect on the seed mass of *A. nemorosa*, but not on the seed mass of *M. effusum.* This relationship between GDH and reproductive output is explored in depth in Chapters 4 and 5. And thirdly, we found that both species displayed lower SLA values when they grew in a nutrient-rich environment. Two recent meta-analyses (Poorter *et al.* 2009; Ordoñez *et al.* 2009) demonstrated that irradiance, temperature, submergence and precipitation are the primary determinants of SLA, but that plants also tend to increase SLA with increasing soil fertility. The latter effect, however, is mostly observed in extremely nutrient-poor or nutrient-rich habitats, which differs from the growing situations of our study species. The comparison of the two species reveals that *A. nemorosa* (spring-flowering) is more temperature sensitive than the early-summer flowering *M. effusum*, which confirms previous divergent responses to temperature between species belonging to different phenological groups (Sherry *et al.* 2007; Kudo *et al.* 2008; see further Chapters 4, 7 and 10).

2.4.2 Intra- vs. interspecific relationships

Some of our results support previously reported interspecific relationships between seed mass, SLA or plant height (Westoby *et al.* 1996; Falster & Westoby 2003). For example, we found negative correlations between seed mass and height in *A. nemorosa* and between SLA and seed mass in *M. effusum.* These correlations may reflect a fundamental trade-off among

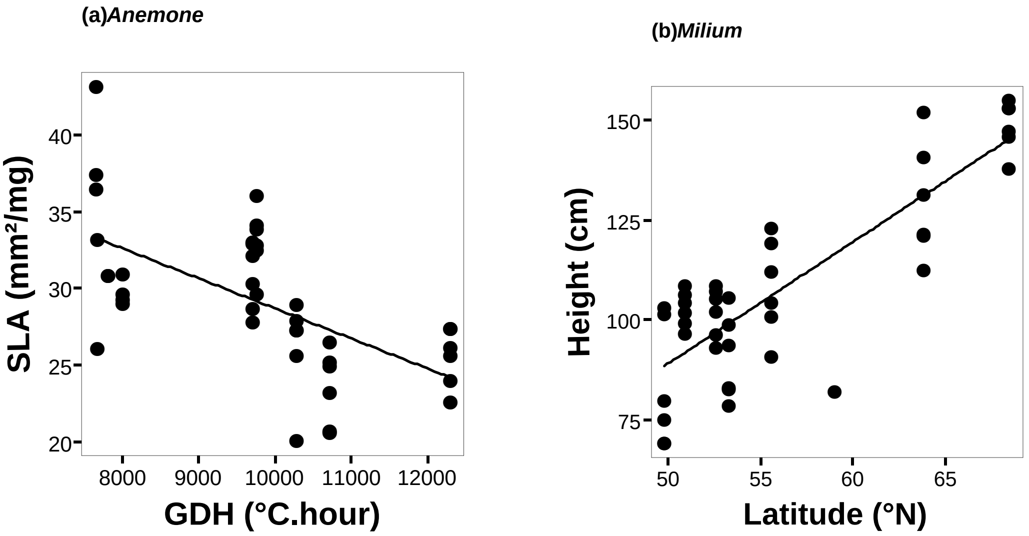


Figure 2.4: The effect of (a) temperature (growing degree hours, GDH) on specific leaf area (SLA) of *Anemone nemorosa* (n = 41) and (b) latitude on plant height of *Milium effusum* (n = 44) along the latitudinal gradient. See **Table 2.2** and **Table 2.3** for statistical significances.

plants, e.g. between investment in height growth (access to light) and reproduction (heavier seeds) (Falster & Westoby 2003; Diaz *et al.* 2004; Freschet *et al.* 2010). But, as most of the inter-trait correlations were not significant, the criterion of LHS trait independence (Westoby 1998; Golodets *et al.* 2009) was mostly (but not always) upheld. In contrast, the known interspecific trends with latitude are generally not confirmed by our intraspecific comparison. Both *A. nemorosa* and *M. effusum* individuals were significantly taller in the north, despite previous evidence that plant height across species and within herbs tends to decrease from the equator to the poles (Moles *et al.* 2009a). Moreover, species at high latitudes in general have a lower seed mass than species at low latitudes (Moles & Westoby 2003; Murray *et al.* 2004; Moles *et al.* 2007; Pakeman *et al.* 2008; Moles *et al.* 2009b), although it should be noted that this pattern is very weak within herbs (Moles *et al.* 2007). This trend is also absent in our data (see also Chapters 4 and 5). Finally, our study shows no relationship between SLA and latitude, but a global cross-species quantification of the relationship between latitude and SLA is still lacking. Hence, the existing cross-species LHS correlations with latitude were generally unsupported within our study species.

As our analyses are based on only two European temperate forest herbs, our findings do not cast doubt on the global cross-species patterns in plant height and seed mass. Yet, the results of our study do demonstrate that on a smaller scale (one continent) and within one functional plant type (forest herbs), the intraspecific variation of these important life-history traits with latitude may differ strongly from the overall interspecific patterns. This is probably due to

Figure 2.5: The plant height of *Milium effusum* almost doubled in natural populations from northern France to northern Sweden, while its seed mass was relatively constant across latitude

a combination of several factors, including the exclusion of confounding cross-species effects through growth form, seed disperser assemblage (e.g. tropical species that are more adapted to dispersal by vertebrates) or habitat type (Lord *et al.* 1997; Lavorel *et al.* 1997; Moles & Westoby 2003; Moles *et al.* 2007; Tautenhahn *et al.* 2008; Moles *et al.* 2009a), as well as the latitudinal range studied.

All of our study populations were situated within deciduous forest, whereas large-scale cross-species patterns in plant height were derived by combining different ecosystems (tropical forest, desert, tundra, etc.; Moles *et al.* 2009a) along a much larger latitudinal range. The 29–fold decrease in mean height between plant species growing within 15° of the equator compared to those growing between 60 and 75 °N is mostly driven by differences in growth form and precipitation. For instance, there is a lack of small species in sites with high temperature, precipitation and/or productivity and there are large treeless areas in the north (Moles *et al.* 2009a). In contrast, growth form and precipitation are of minor importance in our study because we investigated intraspecific patterns and there are no extreme differences in precipitation among the study regions (**Appendix A**). Our approach probably increases the significance of the effect of light availability (see above) on LHS traits, as compared to growth form and precipitation.

Moles & Westoby (2003) found a significant within-species relationship between seed mass and latitude for 78 species, but the intraspecific slope was only two-sevenths of the interspecific slope. They also found that species with a wider latitudinal range were more likely to show a decline in seed mass with latitude. However, the sampled latitudinal ranges for *A. nemorosa* and *M. effusum* in our study were 14 ° and 18.6 °, respectively, which is a wide range when compared to the other studies compiled in Moles & Westoby (2003). Hence, the within-species decline in seed mass towards the poles is rather idiosyncratic (see also Murray *et al.* 2004) compared to the decline found across species.

2.4.3 Conclusions

The LHS traits of multiple populations of a single species varied clearly along the latitudinal gradient. We found an almost two-fold increase in plant height between the southernmost and northernmost *A. nemorosa* (14° latitudinal range) and *M. effusum* (18.6° latitudinal range) populations, which suggests increased competitive abilities in the north for both species. Furthermore, we found evidence that environmental factors on different scales (local vs. regional) influence the two study species differently: *A. nemorosa*, a spring-flowering herb, is influenced most by temperature, whereas *M. effusum*, an early-summer flowering grass, is affected more by factors operating on the local scale (canopy cover, soil nutrients) and by other latitude-related variables. Finally, most previously established cross-species correlations are unsupported within our study species, which suggests that the global patterns in LHS traits found across species are not as widespread within species as previously thought. Further research into the causes (pheno- vs. genotypic) and relativity (in relationship to coexisting species) of the within-species variation in LHS traits and ecological strategy of these forest herbs and other plant species with latitude is required.

3

A latitudinal gradient in seed nutrients of the forest herb *Anemone nemorosa*

After: De Frenne P, Kolb A, Graae BJ, Decocq G, Baltora S, De Schrijver A, Brunet J, Chabrerie O, Cousins SAO, Dhondt R, Diekmann M, Gruwez R, Heinken T, Hermy M, Liira J, Saguez R, Shevtsova A, Baskin CC, Verheyen K (2011) A latitudinal gradient in seed nutrients of the forest herb *Anemone nemorosa*. *Plant Biology*, **13**, 493–501.

Abstract

The nutrient concentration in seeds determines many aspects of the potential success of the sexual reproductive phase of plants including the seed predation probability, efficiency of seed dispersal and seedling performance. Despite considerable research interest in latitudinal gradients of foliar nutrients, a similar gradient for seeds remains unexplored. We investigated a potential latitudinal gradient in seed nutrient concentrations within the widespread European understorey forest herb *Anemone nemorosa* L. We sampled seeds of *A. nemorosa* in 15 populations along a 1900 km-long latitudinal gradient at three to seven seed collection dates post-anthesis and investigated the relative effects of growing degree hours >5 °C, soil characteristics and latitude on seed nutrient concentrations. Seed nitrogen, nitrogen:phosphorus ratio and calcium concentration decreased towards northern latitudes, while carbon:nitrogen ratio increased. When taking differences of the growing degree hours and measured soil characteristics into account and only considering the most mature seeds, the latitudinal decline remained particularly significant for the seed nitrogen concentration. We argue that the decline in seed nitrogen concentration can be attributed to northward decreasing seed provisioning due to lower soil nitrogen availability or greater investment in clonal reproduction. This pattern may have large implications for the reproductive performance of this forest herb as the degree of seed provisioning ultimately co-determines seedling survival and reproductive success.

3.1 Introduction

The sexual reproductive phase from gamete development to seedling emergence is one of the most critical stages in the life-cycle of plants (Harper 1977). The success of an individual in passing through this phase is greatly dependent on the maternal environment (Roach & Wulff 1987; Donohue 2009). Effects of the maternal environment on seed set, maturation, predation, germination and seedling survival are all well documented (e.g. Meunier *et al.* 2007). With respect to seed traits, however, seed nutrient concentration is often overlooked, although for multiple reasons it deserves as much attention as e.g. seed size (Kitajima 2002): the seed nutrient concentration has important implications for several processes within the sexual reproductive phase including interactions with nitrogen-limited seed predators (Mattson 1980; Jolivet & Bernasconi 2004; De Menezes *et al.* 2010), efficiency of seed dispersal (Boulay *et al.* 2006; Delatte & Chabrerie 2008) and seedling performance (Parrish & Bazzaz 1985; Stock *et al.* 1990; Naegle *et al.* 2005).

One approach to assess the impact of the maternal environment on plant performance in general and on seed nutrient concentrations in particular, is to take advantage of the variation in climate and soil characteristics induced by large latitudinal gradients (Fukami & Wardle 2005). Investigating latitudinal gradients of seed nutrient concentrations would not only make it possible to predict how seed nutrients might be affected by global warming using a space-for-time substitution procedure (*sensu* Fukami & Wardle 2005), but would also advance our understanding of quantitative trait responses, variation in reproductive performance and ecosystem functioning across large environmental gradients (McGill *et al.* 2006) as knowledge about latitudinal variation in seed nutrient concentrations is largely lacking. One meta-analysis compiled data on the nitrogen (N) concentration of fleshy, vertebrate-dispersed fruits in four European sites along a north-south transect but found conflicting trends and did not investigate seed nutrients (Hampe 2003).

To our knowledge, latitudinal gradients of seed nutrient concentrations (e.g. seed N, carbon [C] and phosphorus [P] but also potassium [K], calcium [Ca] or magnesium [Mg]) have not yet been reported. This observation is somewhat surprising as the latitudinal variation in the C, N and P status of both soils and leaves has received considerable attention. On a global scale, foliar N and P generally increase from the tropics to the mid-latitudes (ca. 40-50 °N) and then level off or decrease towards the poles whereas leaf N:P ratios tend to increase towards the tropics (Oleksyn *et al.* 2003; Güsewell 2004; McGroddy *et al.* 2004; Reich & Oleksyn 2004; Han *et al.* 2005; Kerkhoff *et al.* 2006; Lovelock *et al.* 2007; Townsend *et al.* 2007). These patterns can be explained by, for instance, direct temperature effects on plant biochemistry and physiology (growth rate hypothesis) and by limitation of soil P and N near the equator and at higher latitudes, respectively (geochemical hypothesis) (Oleksyn *et al.* 2003; McGroddy *et al.* 2004; Reich & Oleksyn 2004; Lovelock *et al.* 2007). Furthermore, a proxy for cation content (pH of foliar extracts) and leaf K has also been suggested as part of the leaf and plant economics spectrum (Wright *et al.* 2005; Cornelissen *et al.* 2006; Freschet *et al.* 2010). We are aware of only one study that has investigated a latitudinal gradient in leaf K, Ca or Mg, and this found no correlation between these leaf traits and latitude in Scandinavia (Johansson 1995). Nevertheless, the seed K concentration is also important since it has a large influence on seed germination (Zerche & Ewald 2005), as K primarily functions as an osmoregulator and activator of many enzymes (Larcher 2003). Generally, due to the strong relocation of minerals and carbohydrates between mother plant and embryo and endosperm during seed maturation (Patrick & Offler 2001), however, seed nutrient concentration and stoichiometry is not necessarily comparable to the patterns observed in leaves (Kerkhoff *et al.* 2006).

Here, we investigate latitudinal variation in seed nutrient concentrations of a widespread understorey forest herb in Europe. *Anemone nemorosa* L. (Ranunculaceae) may be considered as a model species for slow colonising perennial understorey forest herbs. Given its wide distribution (Hultén & Fries 1986) and high degree of phenotypic plasticity (Shirreffs 1985), *A. nemorosa* is potentially an excellent species to study variation in seed nutrients. Given the presence of a gradient in seed nutrient concentration, it also offers the possibility to examine effects of growing degree hours >5 °C (*cf.* growth rate hypothesis) and soil characteristics (*cf.* geochemical hypothesis), relative to latitude itself. Therefore, two main research questions were formulated: (i) Is there a latitudinal gradient in seed nutrient concentration and stoichiometry of *A. nemorosa*? (ii) What is the relative importance of growing degree hours, soil characteristics and latitude on seed nutrient concentrations and stoichiometry? We further consider possible causes, other than temperature and soil characteristics, and discuss consequences of the possible presence/absence of a latitudinal gradient in seed nutrients.

3.2 Methods

3.2.1 Study species

Anemone nemorosa L. (wood anemone) is a widespread perennial herb mainly occurring in the understorey of temperate deciduous forests (Shirreffs 1985). Although some information on *A. nemorosa* was already given in section 2.2.1, this section here briefly provides more specific information. *A. nemorosa* occurs from northern Spain to northern Scandinavia and from Ireland to western Russia (Hultén & Fries 1986). It grows on rich, usually not too acidic, relatively dry to wet soils and can sustain in open areas (Shirreffs, 1985). Shoots emerge in early spring and flowering starts a few weeks later. Flowers (one per shoot) are typically white, hermaphrodite, mostly self-incompatible and pollinated by insects, although selfing occurs (Müller *et al.* 2000). Each ramet produces on average 18.1 (0.6 S.E.) achenes that contain a single seed (achenes are referred to as *seeds* hereafter) with a mature seed mass of 1.96 (0.04) mg (Chapter 5). Seeds are gravity-, ant- and slug-dispersed (Delatte & Chabrerie 2008; Türke *et al.* 2010), show epicotyl morphophysiological dormancy (Mondoni *et al.* 2008) and germination rates ranging from 5 % to 100 % across its distribution range (Eriksson 1995; Mondoni *et al.* 2008; Chapter 5). Seeds do not form a persistent seed bank (Eriksson 1995). Vegetative growth through a branching horizontal rhizome system is common but very slow (*ca.* 30 mm.year^{-1} in Denmark; Philipp & Petersen 2007) and it is assumed that seeds are the most important dispersal organ accounting for population persistence and spread (Brunet & von Oheimb 1998; Müller *et al.* 2000; Stehlik & Holderegger 2000). *A. nemorosa* may be considered one of the most intensively studied forest herbs in Europe (Baeten *et al.* 2010a).

3.2.2 Seed collection

In 2009, two *A. nemorosa* populations were sampled in each of eight regions along a 1900 km latitudinal gradient, spanning 14° (we sampled only one population in northern Sweden due to its rarity in that region) (**Fig. 2.1** on p. 19 excluding Abisko; **Appendix A**). All populations were large (> 10,000 ramets), situated in deciduous forest (mixed forests in northern Sweden and Estonia) with 40-95 % canopy cover and were > 1 km apart in each region.

Sampling occurred on three to seven seed collection dates: because of the nutrient relocation between mother plant and embryo and endosperm, the variation in growing season length and large phenological differences with latitude, the correct estimation of seed maturity is extremely important. It is likely that the seed collection date strongly affects seed nutrient concentrations (*cf.* Zerche & Ewald 2005) and was therefore incorporated into the sampling design. Usually, seed ecologists harvest seeds at only one point in time, although it is known that the collection date can have clear influence on, for example, germination and dormancy (Hay & Probert 1995; Baskin & Baskin 1998; Handley & Davy 2005). Seeds were therefore collected every fifth day, from the date when the first seeds formed (27–39 days post-anthesis) to when most seeds had already been shed (53–60 days post-anthesis; **Appendix B**). This resulted in 79 samples from 15 population × 3–7 collection date combinations (**Fig. 3.1**). At every collection date, we sampled and pooled the seeds of 15–20 randomly chosen ramets per population.

3.2.3 Environmental variables

We collected five 4 cm deep soil cores from below the litter layer in each population in 2008 (France, Belgium, northeastern Germany, southern Sweden, northern Sweden) or 2009 (north-

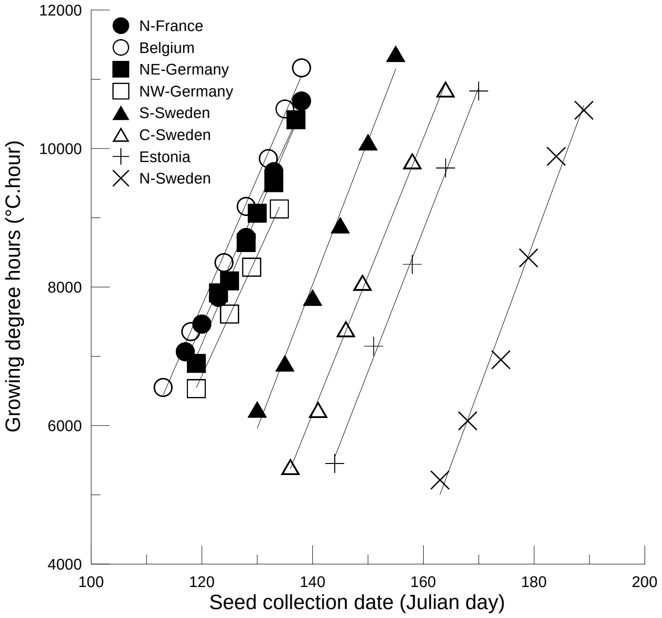


Figure 3.1: Relationship between the seed collection date of *Anemone nemorosa* seeds and growing degree hours >5 °C between January 1 and the collection date for the eight regions along the latitudinal gradient. The subdivision of the regions into the three regional zones for **Fig. 3.3** is based on this graph: the southern zone encompasses northern France, Belgium and both German regions (earliest mature *A. nemorosa* seeds); the central zone contains southern Sweden, central Sweden and Estonia; northern Sweden forms the northern zone with the last *A. nemorosa* seed maturation.

western Germany, central Sweden, Estonia). Soil samples from each population were pooled, air-dried to constant mass and passed through a 2 mm-sieve. All samples were analysed for pH (determined from a solution of 10 g of soil and 25 ml of 0.01 M $CaCl_2$ using a standard glass electrode). Furthermore, 5 g dry soil was extracted in 100 ml ammonium lactate solution (9.01 ml lactic acid (88 %) + 18.75 ml acetic acid (99 %) + 7.75 ml NH_4^+-acetate diluted to 1 L) according to the modified method of Egner *et al.* (1960) and analysed for Ca, Mg and K using Atomic Absorption Spectrometry (Varian, SpecrAA-220) and for P in a spectrophotometer (Varian, Cary 50) according to the colorimetric method of Scheel (1936) with molybdenum vanadate as colour reagent. Finally, the percentages of carbon (C) and nitrogen (N) were analysed with an element analyser. Growing degree hours >5 °C were used for the accumulated temperature sum until the seed collection date (Meunier *et al.* 2007). Growing degree hours between 1 January and the collection date were calculated according to Lindsey & Newman (1956) using data obtained from weather stations close to the sampled populations (in all cases < 30 km). Since the growing degree hours and seed collection dates were closely correlated in each region (**Fig. 3.1**) and since the amount of degree hours is the ecologically more relevant variable, we only included this variable in the data analyses. Latitude and growing degree hours were not correlated when considering all seed collections ($r = -0.157$, $n = 79$, $p = 0.168$) or only the last seed collection ($r = 0.171$, $n = 15$, $p = 0.543$).

3.2.4 Seed traits

The seeds were air-dried to constant mass for *ca.* one week. We determined mean seed mass by weighing 50 seeds (to the nearest 0.1 mg) per seed sample and dividing the obtained mass by 50. For each seed sample, three Petri dishes were lined with moist filter paper, and 20–50 randomly chosen seeds were distributed in each dish (the number of seeds within each dish depended on seed availability) within 15 days of collection for all seed samples. Based on earlier studies (Mondoni *et al.* 2008), seeds were given eight weeks of warm stratification (simulating summer; 23 ℃) before they were placed in cool stratification (simulating autumn; 10 ℃) in temperature-controlled growth chambers. The number of germinated seeds (seeds with emerged radicle) was recorded weekly for at least 8 weeks (from the start of the cool stratification) until germination had ceased in all dishes. The sets of dishes were randomly rotated after each weekly recording and distilled water was supplied as necessary.

A subsample of seeds not used for the germination trials was oven-dried to constant mass (50 ℃ for 72 hours), analysed for C and N (element analyser; %) and, subsequent to wet acid digestion with HNO_3 and $HClO_4$, analysed for P (colorimetrically as above), K, Ca and Mg (Atomic Absorption Spectrometry as above; P and cations in ppm or μg.g^{-1} seed dry mass). C:N and N:P ratios were calculated from these results as C/N and $N/P \times 10,000$. Since we found a clear latitudinal gradient in seed N (see §3.3), we also did a selective extraction of alkaloids on the two seed lots with extreme total N concentration (i.e. the northernmost and southernmost seed provenances) in a second step. Plants may resist herbivory using physical and/or chemical defence mechanisms (e.g. spines, hairs, physical toughness or secondary compounds), most of which are said to increase towards the equator (Schemske *et al.* 2009). Also, increasing concentrations of secondary compounds are related to better defence mechanisms (Bennet & Walsgrove 1994; Chen 2008). Therefore, we aimed to check whether the higher N concentrations in seeds of our southernmost populations are related to increased chemical seed defence. The most widespread secondary compounds involved in chemical plant defence, containing N and occurring in the Ranunculaceae are alkaloids (Larcher 2003). Therefore, we used a microwave technique on the southernmost and northernmost *A. nemorosa* seeds, and subsequently screened for alkaloids combining thin layer chromatography (TLC) with the Dragendorff's reagent for visualisation.

3.2.5 Data analysis

To explore the relative importance of growing degree hours (which includes the seed collection date effect due to the strong correlation within regions; see **Fig. 3.1**), soil variables and latitude on seed traits, mixed-effect model analyses were performed both considering all collection dates or only the last collection date per population (i.e. the most mature seeds). *Region* was included in the models as a random-effect term to account for possible spatial autocorrelation of the populations within a region. *Population* was included as random-effect term in models in which repeated measurements within one population were analysed (i.e. the different collection dates). To examine the proportion of variance explained by the grouping structure in the mixed-effect models (i.e. by the random-effect terms *region* and *population*), the intraclass correlation was calculated according to Hox (2002). The results showed that between 0 % and 69 % (when considering all seed collections) or between 1 % and 93 % (only final seed collection) of the variance in seed traits of *A. nemorosa* was caused by the grouping structure.

Since some soil variables were highly correlated, we first performed a principal components analysis (PCA) with `VARIMAX`-rotation on the soil variables (pH, C, N, P, K, Ca, Mg), extracted the first two PCA axes (comprising 49.1 and 31.4 % of the variability in soil variables, respectively) and used these axes for analysis. The first PCA axis was positively correlated with plant available soil nutrients (P, K, Ca, Mg; $r > 0.607$) and pH ($r = 0.852$), whereas the second PCA axis was positively correlated with total soil C and N ($r > 0.980$; i.e. organic matter axis). The variance inflation factors of the growing degree hours, the two soil PCA axes and latitude were lower than ten (< 1.57) indicating low multicollinearity among the predictor variables (Quinn & Keough 2002). To avoid overfitting, maximal models were simplified by subsequently excluding predictors with $p > 0.1$; only the final reduced models are presented in the results. To fulfil normality and homoscedasticity assumptions, germination percentage and seed P concentration were first arcsine square-root and $\log_{10}$-transformed, respectively. All other data were untransformed. All data were on the population × collection date level and analysed in `SPSS (VERSION 15.0)`.

3.3 Results

Seed N and N:P ratio were significantly negatively related to latitude, whereas the seed C:N ratio increased with increasing latitude (**Table 3.1**; **Fig. 3.2**). Seed Ca was negatively related to latitude only when all seed collection dates were considered (**Table 3.1**). Across all seed collection dates, mean seed N and seed N:P ratio (with S.E. in parentheses) decreased from 3.33 % (0.22) and 8.4 (0.5) in northern France to 2.52 % (0.07) and 6.5 (0.2) in northern Sweden, respectively (**Fig. 3.2a,e**). Seed C:N ratio increased from 15.4 (0.8) in the southernmost populations to 20.1 (0.6) in the northernmost population (**Fig. 3.2**c). When considering only the last collection date in every population (i.e. the most mature seeds), the strength of the relationships with latitude increased (**Table 3.1** and **Fig. 3.2**; comparison of parameter estimates and *t*-values). Across all collection dates, seed Ca decreased from 5245 ppm (450) in northern France to 3502 ppm (607) in northern Sweden (**Table 3.1** and **Appendix B**).

The other seed nutrient concentrations showed no significant latitudinal cline. Soil conditions consistently affected seed C (positive effect of soil PCA 2) and seed N:P ratio (negative effect of soil PCA 1), while positive effects on germination and negative effects on seed Ca were apparent only when all or only the last collection date was considered, respectively (effects of both soil PCA 1 and 2; **Table 3.1**). In the TLC analysis, no alkaloids were found in any of the seed lots with extreme total N concentration (i.e. the northernmost and the southernmost seed provenances).

Furthermore, all seed traits of *A. nemorosa* were affected by the seed collection date (effects of growing degree hours in **Table 3.1**). For example, the earliest collected seeds with the lowest degree hours failed to germinate (0 % for all regions except the two German regions; data not shown) whereas later collected seeds showed germination rates up to 94 %. Overall, **Table 3.2** shows that germination percentages were positively affected by seed mass, seed C and N as well as seed N:P ratio but negatively affected by seed K, Ca and Mg concentrations. Seed mass and seed C showed a threshold-effect and a clear correlation with germination percentage. This pattern was independent of the climatic zone where the seeds were collected (**Fig. 3.3**).

3.4 Discussion

Seeds of *A. nemorosa* collected along a 1900 km-long latitudinal gradient from northern France to northern Sweden showed a significant latitudinal cline in seed N and Ca concentrations and in seed C:N and N:P ratios. Seed nutrients displayed variations in concentration depending on the seed collection date, but the latitudinal clines were still significant when considering only the most mature seeds (final collection date; except for seed Ca).

Our results clearly show that seed N concentrations and N:P ratios decrease and C:N ratios increase with increasing latitude in *A. nemorosa.* For example, seed N:P decreased on average by two units between northern France and northern Sweden. This agrees with findings of Reich & Oleksyn (2004) who showed that leaf N:P across 244 herb species and within the *Vaccinium* genus dropped from *ca.* 10 to 7.5 between sites with a mean annual temperature of 9.7 ℃ (i.e. corresponding to temperatures of our southernmost region) and 3.4 ℃ (our northernmost region), respectively. The leaf C:N:P stoichiometry appears to be primarily driven by both soil characteristics and temperature (Reich & Oleksyn, 2004; Han *et al.* 2005; Lovelock *et al.* 2007). In our study, the N:P stochiometry of seeds, for example, was affected by a combined effect of pH, plant available soil P and cations (soil PCA 1) and temperature (growing degree hours). However, the effect of latitude on seed nutrients of A. nemorosa was still present after the measured soil characteristics and temperature effects had been accounted for, indicating that other factors should also be considered. Moreover, the latitudinal pattern in seed C:N and N:P in *A. nemorosa* is largely driven by the latitudinal decline in seed N.

Table 3.1: Effects of temperature (growing degree hours, GDH), soil variables (two soil-PCA axes) and latitude on seed traits of *Anemone nemorosa* along a latitudinal gradient. In (a) all seed collection dates are considered, in (b) only the last seed collection (i.e. most mature seeds) of each population. Soil PCA1 was positively correlated with plant available soil nutrients (P, K, Ca, Mg; $r > 0.607$) and pH ($r = 0.852$), whereas soil PCA2 was positively correlated with total soil C and N ($r > 0.980$; i.e. organic matter axis).

	(a) All seed collection dates				**(b) Final seed collection date**			
Seed trait	Predictor	P.E.	d.f.	*t*-value	Predictor	P.E.	d.f.	*t*-value
Seed mass (mg)	GDH	4.20E-04	69	9.06***	(ns)[a]			
Germination (%)	GDH	1.90E-04	62	12.65***	(ns)[a]			
	Soil PCA2	1.34E-01	13	2.60*				
	Soil PCA1	1.40E-01	12	2.36*				
	Latitude	-2.95E-02	11	-2.05(*)				
Seed C (%)	GDH	1.83E-03	64	17.63***	GDH	1.27E-03	14.2	2.60*
	Soil PCA2	8.88E-01	15	2.47*	Soil PCA2	8.61E-01	14.1	2.55*
Seed N (%)	Latitude	-7.93E-02	13	-3.52**	Latitude	-1.50E-01	8.2	-5.10***
	GDH	8.10E-05	63	2.97**				
Seed P (ppm)	(ns)[a]				GDH	-4.04E-05	9.3	-1.88(*)
Seed K (ppm)	GDH	-1.17	65	-6.90***	GDH	-3.9	12.6	-3.29**
Seed Ca (ppm)	GDH	-5.11E-01	66	-7.06***	Soil PCA1	-5.48E+02	14.6	-2.02(*)
	Latitude	-1.40E+02	15	-2.43*	Soil PCA2	-5.64E+02	11	-3.47*
Seed Mg (ppm)	GDH	-1.11E-01	67	-4.70***	(ns)[a]			
Seed C:N	Latitude	4.36E-01	13	4.62***	Latitude	7.93E-01	7.8	4.99**
	GDH	3.47E-04	65	2.47*				
Seed N:P	GDH	2.14E-04	64	5.48***				
	Soil PCA1	2.01E-01	15	-2.26*	Soil PCA1	-7.00E-01	15	-2.37*
	Latitude	-8.96E-02	15	-1.87(*)	Latitude	-1.38E-01	15	-1.95(*)

[a] None of the studied factors was significant

Table 3.2: Correlations among seed traits of *Anemone nemorosa* along a latitudinal gradient ($n = 79$ population × seed collection date combinations). Significant Pearson correlation coefficients (r) are shown ($p < 0.05$). ns: $p > 0.05$.

	Seed mass	**Germ.**	**C**	**N**	**P**	**K**	**Ca**	**Mg**	**C:N**	**N:P**
Germ.	0.656									
C	0.558	0.834								
N	0.4	0.331	ns							
P	ns	ns	ns	0.618						
K	ns	-0.251	-0.536	0.281	0.349					
Ca	-0.328	-0.434	-0.631	ns	ns	0.615				
Mg	-0.227	-0.263	-0.504	0.315	0.474	0.767	0.514			
C:N	ns	ns	0.292	-0.894	-0.654	-0.416	ns	-0.478		
N:P	0.334	0.268	0.344	0.576	-0.273	ns	ns	ns	-0.417	
C:P	ns	ns	0.557	-0.453	-0.906	-0.512	-0.299	-0.611	0.659	0.389

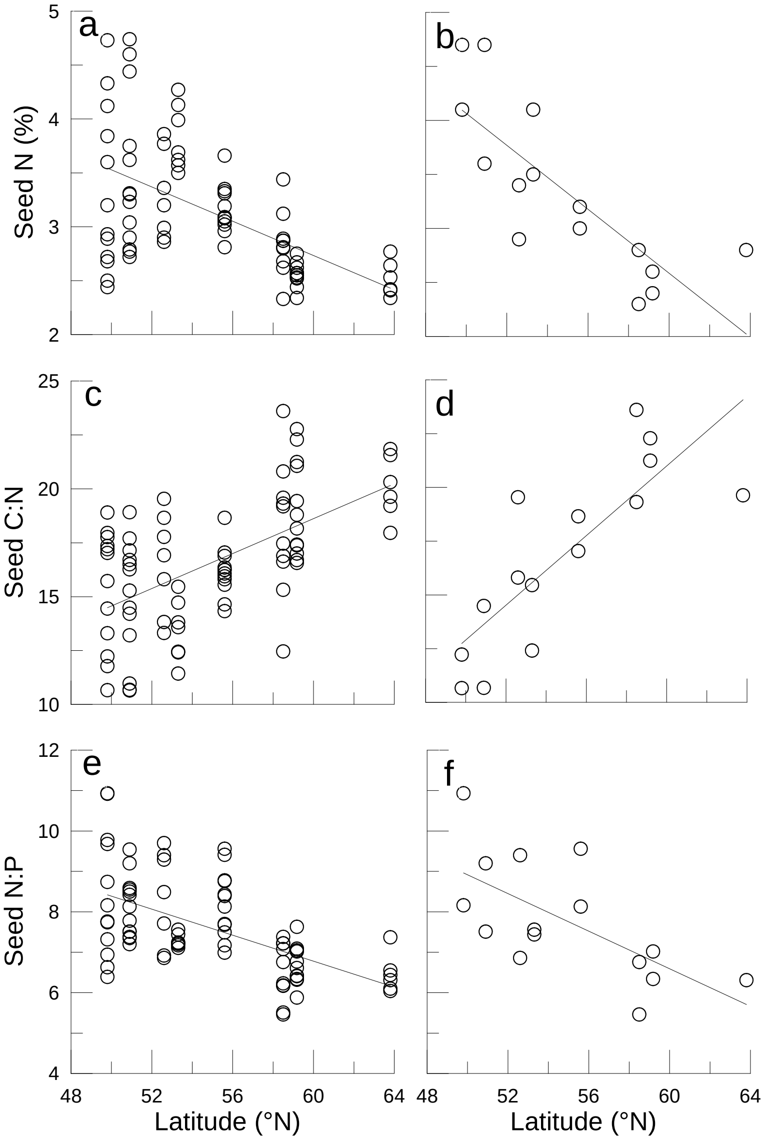


Figure 3.2: Nitrogen concentration (N), carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) ratio of seeds of *Anemone nemorosa* along a latitudinal gradient from northern France to northern Sweden. In (a), (c) and (e) all seed collections are considered. In (b), (d) and (f) only the last seed collection date (i.e. the most mature seeds) of each population is included. Significance values are given in **Table 3.1**.

Deducing possible causes other than temperature and soil characteristics for the observed latitudinal pattern in seed nutrients is difficult because of the observational nature of this study. However, differences in (i) seed defence mechanisms and (ii) seed provisioning represent potential mechanisms for the increase in N concentration (resulting in wider C:N and more narrow N:P ratios) of *A. nemorosa* seeds along the studied latitudinal gradient. First, physical

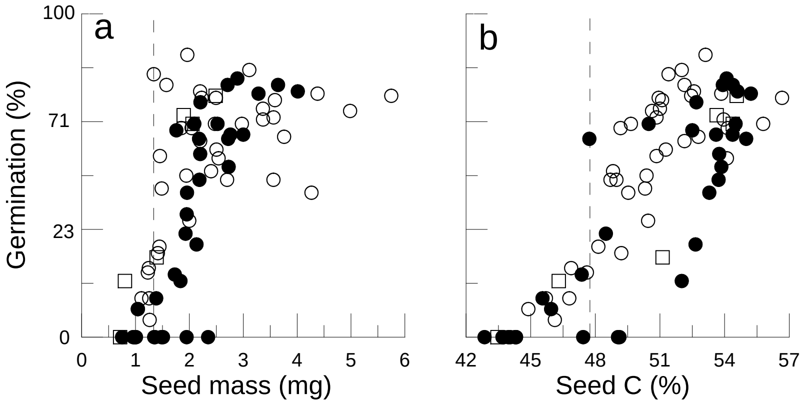


Figure 3.3: Effect of (a) seed mass and (b) seed carbon concentration on germination percentage (arcsine square root-transformed Y-axis) of *Anemone nemorosa* seeds collected at different collection dates along a latitudinal gradient in southern (open circles), central (filled circles) and northern (open squares) regions (see **Fig. 3.1**). The vertical dashed lines in (a) and (b) represent the threshold seed mass (1.34 mg) and seed carbon concentration (47.7 %), respectively (defined as the situation where for the first time at least 50 % germination occurs).

and chemical plant defence mechanisms are generally said to increase towards the equator (Levin & York 1978; Siska *et al.* 2002; Pennings *et al.* 2007; review: Schemske *et al.* 2009), although other studies have shown an increasing or stable latitudinal pattern in defence mechanisms (Andrew & Hughes 2005; Adams *et al.* 2009; Moles *et al.* 2011). However, no alkaloids were found in any of the seed lots with extreme total N concentration. From this analysis, we thus conclude that no latitudinal gradient in alkaloid concentration occurs in *A. nemorosa* seeds, and that seed defence is unlikely to explain the higher N concentrations in the south.

Alternatively, the higher seed N concentration in southern plants may result from increased seed provisioning due to increased soil N availability or a potential shift in reproductive allocation. Indeed, although we found no effect of total soil N (soil PCA 2) on seed N, total soil N is not a very good measure of plant available N. Mineralisation rates in northern boreal forests are much slower than in temperate forests mainly due to low temperatures and recalcitrant organic matter (Coûteaux *et al.* 2001; Robinson 2002). Further, Holland *et al.* (2005) clearly show a northward decline in N deposition input across Europe (from ca. 20 kg N.ha^{-1}.yr^{-1} in northern France to nearly zero in northern Sweden). This potentially increased N availability for growth and reproduction of *A. nemorosa* in the south may result in more available resources for seed provisioning with N-rich compounds (i.e. mostly proteins). Second, northern peripheral populations of clonal plants tend to increase the ratio of investment in asexual reproduction over investment in sexual reproduction (Mooney & Billings

1961; Houle & Babeux 1994; Dorken & Eckert 2001). This suggests that lower provisioning of seeds but greater investment in clonal reproduction may be an adaptive strategy in northern *A. nemorosa* populations. To conclude, two possible mechanisms may explain why the seed N concentration decreases to the north: potentially lower soil N availability and greater investment in clonal reproduction. Hence, further (experimental) research seems worthwhile to explicitly identify the environmental factors driving the latitudinal clines in seed nutrient concentrations and to investigate how global change (e.g. increasing temperatures may alter soil N availability through changes in decomposition; Rustad *et al.* 2001) affects the seed nutrient concentrations of this forest understorey plant. Finally, also differences in the inter-population genetic ability to store N in seeds may also account for the observed differences, but this can only be investigated using genetic analyses or common-garden experiments.

Whatever the cause, the latitudinal gradient in seed N has important ecological implications. Southern forest herb seedlings may have to cope with more environmental hazards, e.g. due to higher canopy cover and drought risk in summer compared to the north. Seedlings resulting from seeds with higher N concentrations are known to perform better under poor environmental conditions than those with lower concentrations due to the extra provisioning (Parrish & Bazzaz 1985; Stock *et al.* 1990; Naegle *et al.* 2005). This also suggests that sexual recruitment in northern populations may be hampered in e.g. nutrient-poor soils, with potentially large implications for distribution patterns of this species. Finally, as diaspores of numerous forest herbs, including *A. nemorosa*, are dispersed by ants (Delatte & Chabrerie 2008) or slugs (Türke *et al.* 2010), geographic variations in their chemical composition may affect plant-animal dispersal relationships (i.e. the probability of diaspore removal) which potentially determine plant demography (Boulay *et al.* 2006).

Our results also highlight the importance of the seed collection date for ecologists studying plant sexual reproduction. Harvesting *A. nemorosa* seeds at an earlier date biased seed mass and germination towards lower values (Hay & Probert 1995; Handley & Davy 2005) and affected seed nutrient concentrations. Also the absolute value of the regression slope of the C:N:P stoichiometry with latitude increased when only considering the final collection date. Thus, prediction of the optimal (i.e. with the highest germination) collection date is crucial for ecological studies. Seed K concentration decline has already been suggested to be a key-determinant of the optimal collection date in *Primula vulgaris* (Zerche & Ewald 2005). The effect of decreasing seed K in later seed collections is confirmed here for *A. nemorosa*. This highlights the synergistic role of abscisic acid and K in osmoregulation, cell extension, assimilate translocation and enzyme system activation in cell growth (Zerche & Ewald 2005). Also seed mass, seed C and the other cations (Ca, Mg) are valuable predictors of optimal seed maturity, of which seed mass and C are the most easy to determine (see e.g. Handley & Davy 2005; Bedane *et al.* 2006). The relationship between seed mass (when exceeding 1.34 mg [critical range 1.3–2 mg]) and seed C (when exceeding 47.7 % [critical range 47–50 %])

on the one hand and consequently of seed mass and germination on the other hand offers a basis for relatively rapid estimation of maturity of *A. nemorosa* seeds.

In summary, we found a significant latitudinal gradient in nutrient concentrations of *A. nemorosa* seeds. Seed N, Ca and N:P ratios decreased towards northern latitudes, while C:N ratios increased. This previously unreported latitudinal cline in seed nutrients resembles observed general latitudinal patterns in leaf nutrient concentrations. We hypothesize that the northward decline in seed N concentration can be attributed to lower seed provisioning, but not to differences in seed defence mechanisms. This pattern may have large implications for the reproductive performance of this forest herb across its distribution range in the face of, e.g. climate warming, as the degree of seed provisioning ultimately co-determines seedling survival and reproductive success.

4

Unravelling the effects of temperature, latitude and local environment on the reproduction of understorey plants

After: De Frenne P, Kolb A, Verheyen K, Brunet J, Chabrerie O, Decocq G, Diekmann M, Eriksson O, Heinken T, Hermy M, Jõgar Ü, Stanton S, Quataert P, Zindel R, Zobel M, Graae BJ (2009) Unravelling the effects of temperature, latitude and local environment on the reproduction of forest herbs. *Global Ecology and Biogeography*, **18**, 641–651.

Abstract

The aim of this study was to investigate the effect of temperature, latitude and local environment, on reproductive traits of widespread perennial understorey plants to better understand the potential impacts of rising temperatures on their population dynamics and colonisation capacities. Within six regions along a latitudinal gradient from France to Sweden, we collected data from three to five populations of up to six species. For each species, several variables were recorded in each region (temperature, latitude) and population (local abiotic and biotic environmental variables). Seed production and germination were determined. Seed production was quantified as resource investment in reproduction (RIR) (seed number × seed mass), while germination was expressed as germinable seed output (GSO) (seed number × germination percentage). We performed linear regression and mixed-effect models to investigate the effects of temperature (growing degree hours), latitude and local abiotic and biotic environment on RIR and GSO. Temperature and latitude explained most of the variation in RIR and GSO for early flowering species with a northerly distribution edge range (*Anemone nemorosa*, *Paris quadrifolia* and *Oxalis acetosella*). Reproduction of the more southerly distributed species (*Brachypodium sylvaticum*, *Circaea lutetiana* and *Primula elatior*), in contrast, was independent of temperature/latitude. In the late summer species, *B. sylvaticum* and *C. lutetiana*, variation in RIR and GSO was best explained by local environmental variables, while none of the investigated variables appeared to be related to reproduction in *P. elatior*. Hence, reproduction of two early flowering, northerly distributed species was related to temperature. This suggests that the potential reproductive response of understorey plants to climate warming partly depends on their phenology and distribution, but also that the response is to some extent species dependent. These findings should be taken into account when predictions about future distribution range shifts are made.

4.1 Introduction

Evidence of the influence of climate change on natural ecosystems worldwide is increasing (Root *et al.* 2003; Thomas *et al.* 2004; IPCC 2007). The predicted rise in temperature is expected to induce large-scale latitudinal and altitudinal plant migration (Pitelka *et al.* 1997; Malcolm *et al.* 2002; Parolo & Rossi 2008), requiring high colonisation capacity for plant species. However, it has been shown that many plant species, and understorey plants in particular, are slow colonisers (Verheyen *et al.* 2003c; reviewed in Chapter 1), and how the reproduction and distribution of these species will be altered under a changing climate remains uncertain.

Forecasting plant distributions following climate change is often performed using bio-climatic envelope models (Thuiller *et al.* 2005; Svenning & Skov 2006), sometimes combined with soil or landscape data (Iverson *et al.* 2008; Pompe *et al.* 2008), but mostly considering intraspecific genotypic and phenotypic variation in plant traits as static (Pearson & Dawson 2003; Thuiller *et al.* 2008). It is likely, however, that, for instance, intraspecific generation time and reproductive efficiency (e.g. flower and seed production, seed mass or germination) will alter in response to rising temperatures (Baskin & Baskin 1998; Arft *et al.* 1999; Probert 2000; Hedhly *et al.* 2009).

One way to study the influence of a variety of different temperatures on the reproduction of perennial understorey plants in natural plant communities is to make use of the temperature variation along large latitudinal gradients (Austin 1999; Fukami & Wardle 2005). This approach can provide valuable insights into community and ecosystem responses (Berg *et al.* 1993; Gholz *et al.* 2000; Etterson 2004). Latitudinal gradients induce intraspecific phenotypic and genotypic variation of plant traits (Potvin 1986; Clevering *et al.* 2001; Etterson 2004) and avoid the drawbacks associated with experimental warming studies, such as the use of infrared heaters (Hovenden *et al.* 2008) or open-top chambers (Arft *et al.* 1999) which can disturb pollination, increase CO_2 concentration or dry the soil surface unnaturally (Richardson *et al.* 2000; Kimball 2005; *cf.* Chapter 1). Latitudinal gradients provide the opportunity to disentangle the effect of temperature while taking other confounding variables, such as photoperiod and soil properties, into account.

Unraveling the importance of the direct effect of temperature on possible future vegetation range shifts compared with other local abiotic and biotic environmental variables is highly relevant, since there is an urgent need for knowledge about the potential effect of warming on seed production and germination (Hovenden *et al.* 2008). The main purpose of this study was therefore to clarify if we can expect an immediate response of reproduction of understorey plants to changes in temperature in the *resource investment in reproduction* (RIR), i.e. the product of seed number and seed mass, which is an estimate of how many resources are used for sexual reproduction, and *germinable seed output* (GSO), i.e. the product of seed number and germination percentage, which is a very important estimate for population dynamics and persistence. We also test for the influences of latitude and the local abiotic and biotic environment and thus for the indirect effects of temperature (e.g. soil parameters) or non-temperature dependent effects (e.g. photoperiod) on reproductive traits of perennial understorey plants. We used an indirect space-for-time substitution procedure (*sensu* Fukami & Wardle 2005) by surveying different populations of the same target species along a latitudinal transect and using the existing differences to infer a potential response to climate change over time.

Using six herbaceous understorey plants growing in natural communities along a 1400 km latitudinal gradient, we specifically addressed the following question: what is the importance of temperature, latitude and local abiotic and biotic factors on RIR and GSO?

4.2 Methods

4.2.1 Study species and area

We selected six perennial herbaceous forest understorey plants for this study: *Anemone nemorosa* L. (Ranunculaceae), *Brachypodium sylvaticum* (L.) P. Beauv. (Poaceae), *Circaea lutetiana* L. (Onagraceae), *Oxalis acetosella* L. (Oxalidaceae), *Paris quadrifolia* L. (Liliaceae) and *Primula elatior* (L.) Hill (Primulaceae) (nomenclature follows Hultén & Fries 1986). The species belong to three phenological groups (following the same subdivision system as Kudo *et al.* 2008): *A. nemorosa* and *O. acetosella* (spring flowering species), *P. quadrifolia* and *P. elatior* (early summer flowering species) and *B. sylvaticum* and *C. lutetiana* (late summer flowering species). Furthermore, *A. nemorosa*, *O. acetosella* and *P. quadrifolia* [referred to as *northerly (distributed) species*] have a very northerly distribution range edge (range up to

67 °N, 69 °N and 71 °N, respectively) while the northerly distribution edge of *P. elatior*, *B. sylvaticum* and *C. lutetiana* [referred to as *southerly (distributed) species*] is situated more to the south (range up to 53 °N, 55 °N and 55 °N, respectively; Hultén & Fries, 1986).

Within each of six regions located along an approximately 1400 km latitudinal gradient from northern France via Belgium, northeastern and northwestern Germany to southern and central Sweden (see **Fig. 2.1** on p. 19 excluding Tartu, Umeå and Abisko; **Appendix A**), five populations (except in Belgium, where $n = 3$ due to resource constraints) of three to six species were selected for data collection. There are no natural occurrences of *P. elatior* in northeastern Germany and of *B. sylvaticum*, *C. lutetiana* and *P. elatior* in central Sweden (Hultén & Fries, 1986). *Paris quadrifolia* could not be sampled in Belgium due to its rarity in that study region, hence a total of 145 populations of all the species were sampled in the study. Within each region, the populations were randomly selected within an area of *ca.* $20 \times 20\,\mathrm{km}^2$ and were at least 1 km apart. All populations occurred in deciduous hardwood forest (with *Quercus robur* L., *Fagus sylvatica* L., *Fraxinus excelsior* L. and/or *Betula pubescens* L.) with 50-95 % canopy cover.

4.2.2 Temperature, latitude and abiotic and biotic environmental factors

To test for the influence of temperature relative to latitude and other abiotic and biotic factors on reproduction of the six understorey plants, we collected environmental data for each region (temperature and latitude) and population (all other variables).

We used the number of growing degree hours (GDH) above a base temperature (5 °C) from 1 January until the species- and population-specific seed collection dates as a measure of temperature (Lindsey & Newman 1956). The GDH-concept is often used as a substitute for temperature because of the demonstrated relationship between plant development and GDH (Diekmann 1996; Meunier *et al.* 2007). Calculations of GDH followed those of Lindsey & Newman (1956) using daily minimum and maximum temperatures from weather stations in Amiens (northern France, < 15 km), Bevekom (Belgium, < 15 km), Potsdam (northeastern Germany, < 20 km), Bremen (northwestern Germany, < 60 km), Lund (southern Sweden, < 25 km) and Stockholm (central Sweden, < 70 km). The mentioned distances are the maximum distances between the respective weather stations and the farthest population for every region and species. Across all species and populations, this GDH measure was strongly positively correlated to the number of growing degree hours 30 and 60 days before seed collection ($r = 0.719$, $p < 0.001$, $n = 31$ and $r = 0.970$, $p < 0.001$, $n = 31$, respectively), to the mean temperature 30 and 60 days before seed collection ($r = 0.617$, $p < 0.001$, $n = 31$ and $r = 0.958$, $p < 0.001$, $n = 31$, respectively) and also to the number of growing degree hours from 1 February and 1 March until seed collection (in both cases $r > 0.999$, $p < 0.001$, $n = 31$).

Within each population we visually estimated the percentage of canopy cover, estimated soil moisture according to four different classes (1: dry soil conditions, water table always deep below ground level; 2: fresh soil conditions, water table always below ground level; 3: moist soil conditions, moist indicating species present, water table often on ground level during winter; or 4: wet soil conditions, sometimes inundated, water table almost permanently on ground level) and collected five 4 cm deep soil cores from below the litter layer. The soil samples from each population were combined, air-dried to constant mass and passed through a 2 mm-sieve. All samples were analysed for pH (determined from a solution of 10 g of soil and 25 ml of 0.01 M $CaCl_2$ with a standard glass electrode), plant available phosphorus (P), calcium (Ca), magnesium (Mg) and potassium (K) (extraction with ammonium lactate and photometric determination by flow injection analysis for P and Atomic Absorption Spectroscopy (AAS) for cations; all in mg per 100 g soil), carbon (C) and nitrogen (N) (elemental analyser; %). We calculated the C:N ratio by dividing the carbon by the nitrogen percentage. The total size of each population (hereafter referred to as *population size*) was estimated according to six classes, namely < 50, $50 - 100$, $100 - 500$, $1000 - 5000$, > 5000 flowering individuals (*P. elatior*), ramets (*A. nemorosa, C. lutetiana, O. acetosella, P. quadrifolia*) or tussocks (*B. sylvaticum*) (hereafter, ramets and tussocks will be referred to as *individuals*). The density of each population was estimated as the number of flowering individuals m^{-2} (hereafter referred to as *population density*), either by counting all individuals within a circle (20 m radius) around the center of each area or by counting the number of individuals in ten randomly placed quadrats within this circle.

Finally, the latitude of each region was included in the analysis to account for environmental variables not measured directly along the gradient but expected to differ among sampling regions, such as photoperiod, precipitation, microevolutionary adaptations and genotypic plasticity or past climate variations shaping species and population distributions (Moles *et al.* 2007). It is important to note that GDH and latitude are not correlated (**Table 4.1**).

4.2.3 Seed production and germination

At seed maturity, we collected the diaspores of 20 (unless fewer were present) randomly chosen individuals located within the area in which we had measured the environmental variables. Seeds were considered mature when natural dispersal began (Baskin & Baskin 1998), i.e. when seeds fell easily from the plant. Whole diaspores (fruits) were sampled, but are herein referred to as seeds. Seeds were extracted from the *P. quadrifolia* berries (necessary for the germination trials). The seeds were air-dried at room temperature and sent to the Climate Impacts Research Centre (CIRC) in Abisko, Sweden for further processing. For each sampled plant in each population, we determined the total number of seeds and mean seed mass. In *A. nemorosa*, *C. lutetiana*, *O. acetosella* and *P. quadrifolia* we counted and weighted all seeds of each individual and calculated mean seed mass as the total seed mass divided by the

Table 4.1: Relationships between the predictor variables temperature (GDH), latitude (Lat) and local abiotic and biotic environment with PCA1.soil (Soil), PCA1.population (Pop) and Canopy Cover (CC) in forest plant populations along a latitudinal gradient. Data were standardized by dividing by the mean per species (GDH and Lat)[a] or species and region (Soil, Pop, CC)[b]. Pearson correlation coefficients (r), significance levels and sample sizes (n) are shown.

		GDH[a]	**Lat**[a]	**Soil**[b]	**Pop**[b]
Lat[a]	r	-0.077 (ns)			
	n	31			
Soil[b]	r	-0.058 (ns)	-0.427 *		
	n	31	31		
Pop[b]	r	0.323 (ns)	0.179 (ns)	-0.050 (ns)	
	n	31	31	145	
CC[b]	r	-0.228 (ns)	-0.367 *	-0.081 (ns)	-0.167 *
	n	31	31	145	145

[a] Data available for each region and species ($n = 31$)

[b] Data available for each population and species ($n = 145$)

total number of seeds. In *B. sylvaticum* and *P. elatior*, which usually produce at least 200 seeds per individual, we first weighed all seeds from each individual to determine total seed mass. Afterwards, we randomly selected 50 seeds from all seeds per individual and weighed this 50-seed subsample. We determined the mean seed mass by dividing the mass of the 50 seeds subsample by 50. Finally, we calculated the total number of seeds per individual as the total seed mass per individual divided by the mean seed mass. For each species and population (i.e. for each seed sample), nine Petri dishes were lined with moist filter paper (Munktell 00A), and 50 (40 in *B. sylvaticum*) randomly chosen seeds were distributed in each dish (most samples within one month after sampling). In some populations of *O. acetosella* fewer seeds were available, ranging from 20–50 seeds per dish.

Based on earlier studies (Baskin & Baskin 1998; Endels *et al.* 2004), we subjected the seeds to the dormancy break treatments that we expected to be most efficient. *B. sylvaticum* seeds were cold stratified (2 ℃ and darkness) directly after collection whereas the remaining species were given six weeks of warm stratification (diurnal cycles of 20 ℃ and full light (240 μmol.m^{-2}.s^{-1}) for 14 hours and 10 ℃ and darkness for 10 hours) before they were also placed in cold stratification for 20 weeks. After the 20 weeks of cold stratification, three Petri dishes from each seed sample were placed in each of three incubators set on diurnal cycles of 14 h day/10 h night temperatures of 20/10 ℃, 15/5 ℃ and 10/5 ℃. Daytime always had full light as described above and night always complete darkness. The dishes were stored in ziplock bags to avoid desiccation during stratification and incubation, and additional water was supplied as needed.

The number of germinated seeds (seeds with an emerged radicle) was recorded weekly for 6 weeks. The sets of dishes were randomly rotated in the incubator after each weekly recording.

In *P. quadrifolia* no seeds germinated during the incubation. These seeds were given another 20 weeks of cold stratification and were then subjected to the same incubation treatments as above. After eight weeks some seeds germinated. The dishes were kept for an additional 11 weeks for a total of 71 weeks until germination had ceased in all dishes.

For some species, a large proportion of seeds germinated during cold stratification: in *A. nemorosa*: 85.3 %, *B. sylvaticum*: 71.8 % and *P. elatior*: 72.5 % vs. *C. lutetiana*: 0.3 %, *O. acetosella*: 11.4 % and *P. quadrifolia*: 0.0 % (calculated across all regions and populations as mean germination percentage after stratification / mean total germination percentage × 100). We use maximum germination percentage (MaxGerm%) per population across treatments in all calculations because it represents the highest potential incubator germination percentage among our treatments and comparing all species no consistent differences could be found between incubation temperatures.

4.2.4 Data analysis

We used seed number, seed mass and MaxGerm% (all means at the population level; except for (a) in **Table 4.2**) to calculate two compound measures of reproductive success: *resource investment in reproduction* (RIR) as the product of mean seed number and mean seed mass per population, and *germinable seed output* (GSO) as the product of mean seed number and mean MaxGerm% per population divided by 100 (one value per variable for each population). To meet assumptions of statistical tests in terms of normality (tested with histograms, QQ-plots and Kolmogorov-Smirnov tests) and homogeneity of variances (Quinn & Keough 2002), RIR and GSO data were log-transformed prior to analysis.

Since we measured a large number of environmental variables that were partly correlated, we performed a separate principal component analysis (PCA) with `VARIMAX`-rotation (Kaiser 1960) per species across regions to reduce the set of environmental variables to two components: a soil PCA (pH, K, Ca, Mg, P, C, N, C:N, soil moisture) and a population PCA (population size and density). Sample scores for the first principal component (PCA1.soil and PCA1.population) were used for the linear and mixed-effect models (see below) as a weighted average of the soil and population variables, explaining the highest proportion of variance (here 30.9–52.9 % for soil and 51.5–80.5 % for population variables).

We used linear regression models (LM) and mixed-effect models (MM) to explore the relative importance of the five different predictor variables or variable groups (1) temperature (expressed as GDH), (2) latitude, (3) soil variables (as PCA1.soil), (4) population variables (as PCA1.population) and (5) canopy cover (CC) on the log-transformed RIR and GSO of the six understorey plants. In comparison to the linear model, a random-effect term *region* was added to the mixed-effect models to address the likelihood that populations from the same region share spatially autocorrelated characteristics. However, modelling the similarity of the populations with a random-effect term with a random normal distribution is also a strong assumption which cannot easily be verified because of the low number of replicates. Hence, we preferred to keep both the simple linear model and the mixed-effect model and to discuss the results in relation to each other. In this way we could assess the sampling design effect on the significance of the terms of the model. Also we assert that the structure imposed by the mixed-effect model can eliminate interesting effects. To avoid overfitting and for model simplification, only variables with $p < 0.05$ from the full models were considered for the final simplified models. Correlation coefficients between the different predictor variables were calculated to check for multicollinearity in linear regression models (Graham 2003). In general, correlations were absent or weak (**Table 4.1**) indicating low collinearity (Quinn & Keough 2002). This confirms that latitude, soil and population variables and canopy cover can be statistically unraveled from temperature. All analyses were conducted with `SPSS 15.0` and `S-Plus 8.0`.

4.3 Results

Among regions, seeds from the spring flowering species (*A. nemorosa* and *O. acetosella*) ripened after receiving (mean with coefficient of variation in brackets) 10200 GDH (17.8 %) and 13600 GDH (14.5 %), respectively. Seeds of the early summer species (*P. quadrifolia* and *P. elatior*) were mature after 27900 GDH (15.9 %) and 25600 GDH (16.3 %), respectively, and seeds of the late summer species (*B. sylvaticum* and *C. lutetiana*) after 41400 GDH (10.2 %) and 40900 GDH (8.6 %), respectively. The number of seeds per plant and mean seed mass (multiplied to calculate the RIR) were negatively correlated in *A. nemorosa* and *P. elatior*, while in *O. acetosella*, *C. lutetiana* and *B. sylvaticum* mean seed mass increased with seed number (**Table 4.2**). Seed number and MaxGerm% (multiplied to calculate the GSO) were positively correlated in *A. nemorosa*, *O. acetosella* and *B. sylvaticum* and not related in the other species. In *O. acetosella*, *C. lutetiana* and *B. sylvaticum* mean seed mass and MaxGerm% were positively correlated, whereas for *P. quadrifolia* the opposite was true. Seed mass showed no relationship with MaxGerm% in *A. nemorosa* and *P. elatior* (**Table 4.2**).

Among species, RIR and GSO varied between a minimum of 2.4 mg and 0.09, respectively, for Swedish *O. acetosella* and a maximum 620.7 mg and 187.9 for Belgian *B. sylvaticum* (**Table 4.3**). The GDH was the most important variable to explain variation in RIR and GSO of *P. quadrifolia* and GSO of *A. nemorosa* (**Fig. 4.1** and **Fig. 4.2**). Hence, temperature had a positive effect and explained the largest part of the variability in the GSO of the spring flowering species *A. nemorosa* (**Fig. 4.3a**), while it had a negative effect on the GSO of early summer flowering *P. quadrifolia* (**Fig. 4.3b**). Thus, *A. nemorosa* produces more seeds with higher germination when the mother plant received more GDH, whereas for *P. quadri-*

Table 4.2: Relationships between (a) the number of seeds per individual and the mean seed mass of that individual, (b) the mean number of seeds per population and the maximum germination percentage across treatments of that population (MaxGerm%) and (c) the mean seed mass of a population and MaxGerm% for populations of six forest herbs along a latitudinal gradient. Spearman's rank correlation coefficients (r_s), significance levels and sample sizes (n; individuals in (a) and populations in (b) and (c)) are shown. [a]One lost population in the germination trials.

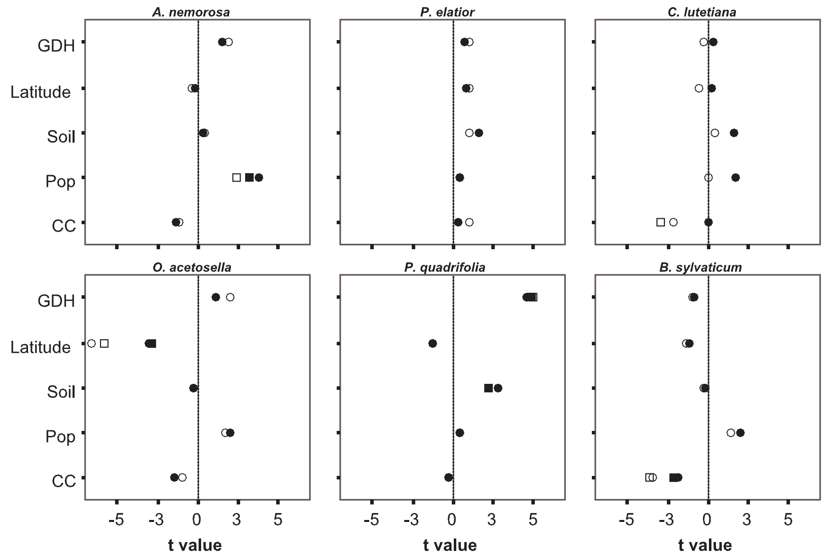
	(a) Seed number vs. mass	n	(b) Seed number vs. MaxGerm%	n	(c) Seed mass vs. MaxGerm%	n
A. nemorosa	-0.171 ***	556	0.321 (*)	28	0.099 (ns)	28
O. acetosella [a]	0.290 ***	540	0.368 (*)	27	0.395 *	27
P. quadrifolia	0.011 (ns)	496	-0.066 (ns)	25	-0.356 (*)	25
P. elatior [a]	-0.221 ***	355	-0.010 (ns)	17	0.000 (ns)	17
B. sylvaticum	0.348 ***	412	0.662 ***	23	0.775 ***	23
C. lutetiana	0.171 ***	456	0.025 (ns)	23	0.409 (*)	23

folia the opposite is true. In general, across species, is the RIR less temperature sensitive than the GSO. Latitude influenced RIR and GSO of *O. acetosella* negatively (**Fig. 4.1** and **Fig. 4.4a**). Latitude also made a significant positive contribution to GSO of *A. nemorosa* and *P. quadrifolia* . Hence, northern populations of *O. acetosella* had lower RIR and GSO. Northern *A. nemorosa* and *P. quadrifolia* populations showed higher GSO. The soil variables explained a significant part of the variation in RIR of *P. quadrifolia* (both LM and MM). The most important rotated component loadings in this PCA1.soil (absolute value higher than 0.7) for *P. quadrifolia* were C, N and soil moisture and for *C. lutetiana* C, N, and Mg. The population variables made a significant contribution to variation in RIR for *A. nemorosa* (both models). Canopy cover was the most important variable for RIR and GSO of *B. sylvaticum* (both models; **Fig. 4.4b**) and for RIR of *C. lutetiana* (LM).

4.4 Discussion

Sampling herbaceous forest understorey plants in natural communities along a latitudinal gradient allowed us to unravel the influence of accumulated temperature (growing degree hours), latitude and environment on reproductive characteristics, and revealed interesting patterns and insights into the potential impact of climate warming on a variety of understorey plants. Combining linear regression (LM) and mixed-effect models (MM) provided both the direction of the influence of a variable under study and its relative importance compared to other explanatory variables. Comparing the LM and MM, the trends in the results are that most of the absolute values of the t-values decrease and p-values increase from the LM to the MM (mostly because of low sample sizes inherent in this type of study) but the relative importance of a variable compared with other variables stays similar in both models. Hence this justifies our approach to retain the simple LM as an alternative to the MM.

(a) log(RIR)



(b) log(GSO)

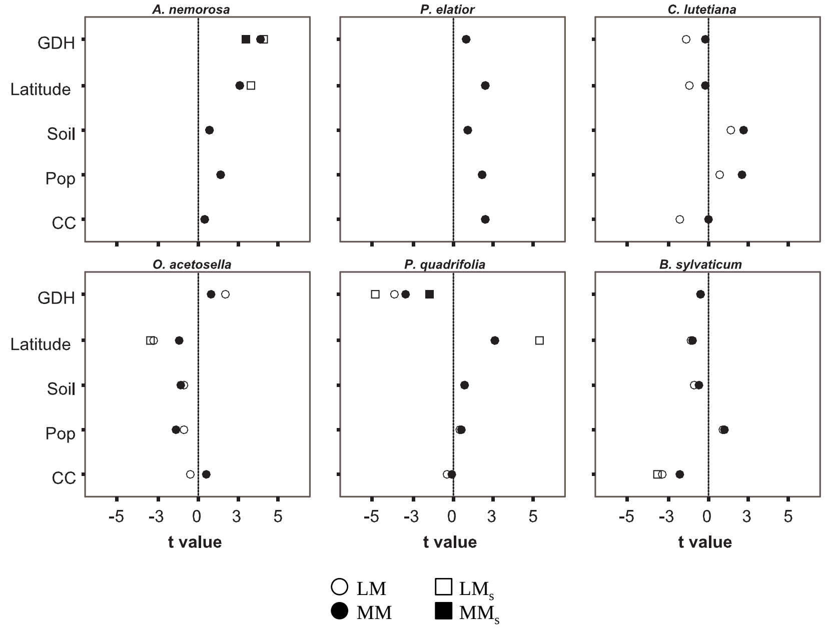
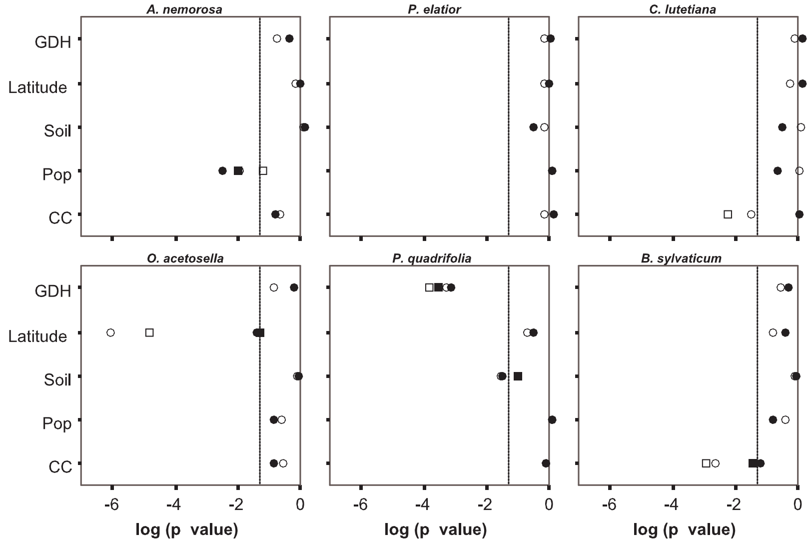


Figure 4.1: Relative importance (*t*-value) of temperature (GDH), latitude and local abiotic and biotic environment with PCA1.soil (Soil), PCA1.population (Pop) and Canopy Cover (CC) on the log-transformed resource investment in reproduction (RIR; above) and germinable seed output (GSO; below) for six understorey plants. Results from full linear regression models (LM, open circles), full mixed-effect models (MM, closed circles), simplified (only variables with $p < 0.05$ included) linear regression models (LM_S, open squares) and simplified mixed effect models (MM_S, closed squares) with plus and minus signs of the *t*-value representing signs of the coefficients. The vertical dashed line represents t-value= 0.

(a) log(RIR)



(b) log(GSO)

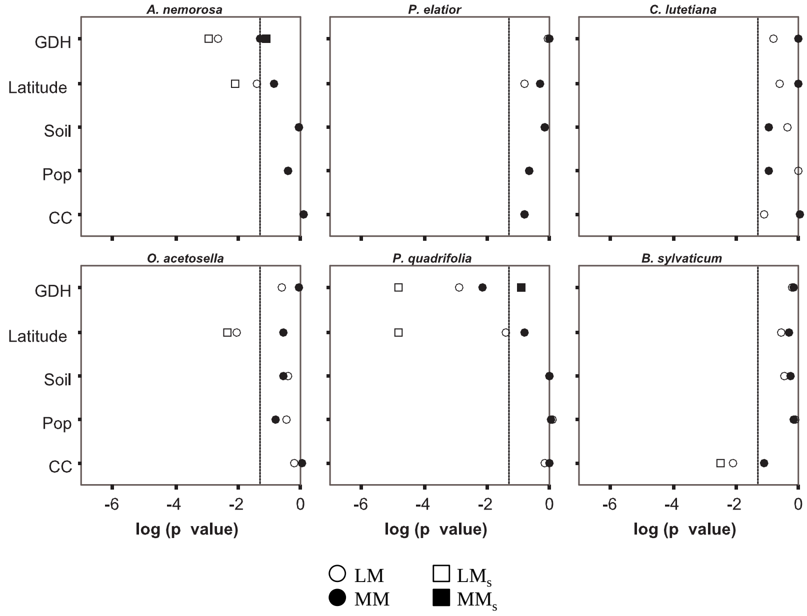
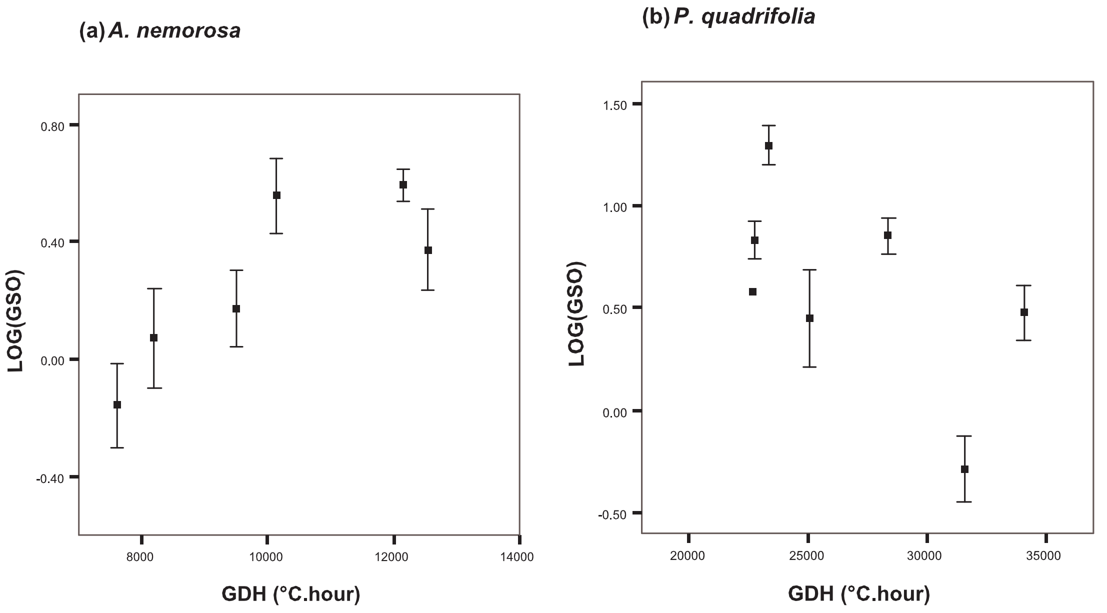


Figure 4.2: Significance (logarithm of p-value) of temperature (GDH), latitude and local abiotic and biotic environment with PCA1.soil (Soil), PCA1.population (Pop) and Canopy Cover (CC) on the log-transformed resource investment in reproduction (RIR; above) and germinable seed output (GSO; below) for six understorey plants. Results from full linear regression models (LM, open circles), full mixed effect models (MM, closed circles), simplified (only variables with $p < 0.05$ included) linear regression models (LM$_S$, open squares) and simplified mixed effect models (MM$_S$, closed squares). The vertical dashed line represents $p = 0.05$. Values left of this line ($p < 0.05$) are considered significant.

Table 4.3: Resource investment in reproduction and germinable seed output for populations of six understorey plants along a latitudinal gradient. Values are means (with S.D.).

Resource investment in reproduction (mg)						
Species	N-France	Belgium	NE-Germ.	NW-Germ.	S-Sweden	C-Sweden
A. nemorosa	36.6 (6.4)	31.8 (9.3)	38 (4.6)	30.5 (5.8)	38.2 (6.3)	31.7 (7.0)
O. acetosella	16.1 (5.0)	7.9 (2.3)	7.0 (0.4)	3.5 (1.3)	6.2 (2.2)	2.4 (0.5)
P. quadrifolia	118.4 (5.1)	-	85 (14.6)	83.8 (12.8)	98.0 (40.0)	128.3 (16.6)
P. elatior	175.1 (73.7)	98.1 (18.1)	-	138.8 (25.8)	115.5 (33.4)	-
B. sylvaticum	576.8 (129.0)	620.7 (259.7)	184.9 (77.9)	165.3 (75.2)	455.6 (220.0)	-
C. lutetiana	120.3 (54.2)	56.6 (20.6)	43.1 (21.0)	66.4 (26.0)	76.1 (26.4)	-
Germinable seed output (-)						
Species	N-France	Belgium	NE-Germ.	NW-Germ.	S-Sweden	C-Sweden
A. nemorosa	2.9 (2.2)	0.78 (0.48)	1.8 (1.2)	1.6 (1.4)	4.1 (1.2)	4.3 (2.9)
O. acetosella	7.6 (3.5)	0.40 (0.53)	0.67 (0.46)	0.27 (0.16)	0.61 (0.41)	0.09 (0.11)
P. quadrifolia	0.65 (0.42)	-	4.4 (3.4)	6.5 (3.0	12.5 (7.8)	3.8 (3.3)
P. elatior	234.3 (97.0)	122.1 (29.4)	-	181.6 (61.5)	150.7 (26.0)	-
B. sylvaticum	127.4 (32.3)	187.9 (94.0)	40.0 (24.0)	20.4 (15.2)	79.6 (26.9)	-
C. lutetiana	18.4 (5.4)	16.5 (8.5)	6.9 (5.8)	11.7 (8.1)	19.4 (3.0)	-


(a) ***A. nemorosa***

(b) ***P. quadrifolia***

Figure 4.3: The influence of temperature (GDH) on the log-transformed output of germinable seeds (log GSO) for *A. nemorosa* (a) and *P. quadrifolia* (b) along the latitudinal gradient. Means ± S.E. are shown.

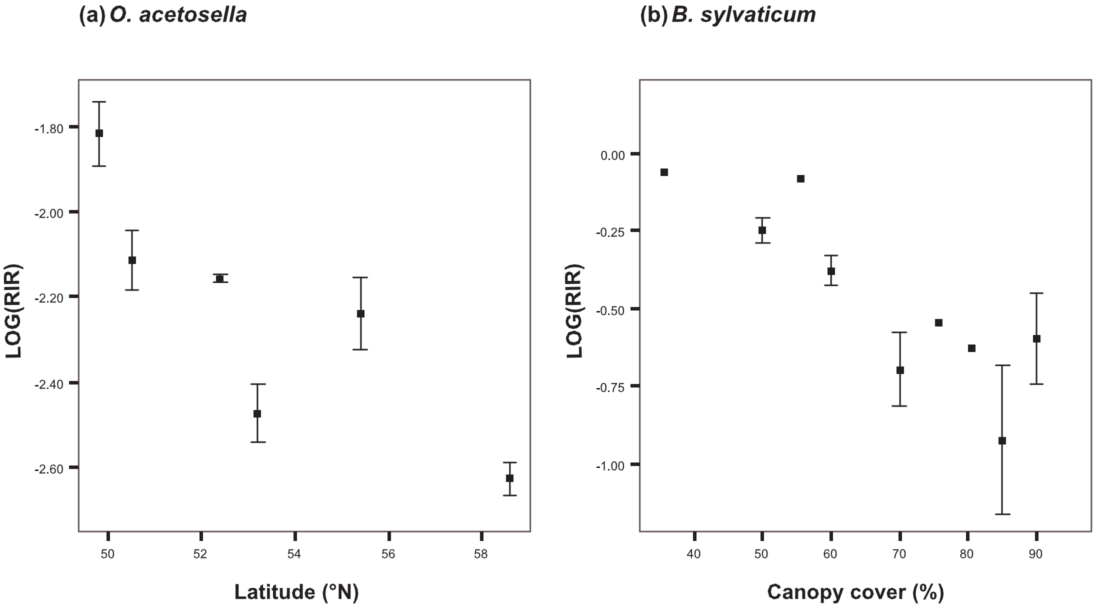


Figure 4.4: The influence of latitude (°N) on the log-transformed resource investment in reproduction (log RIR) for *O. acetosella* (a) and the influence of canopy cover (%) on the log-transformed resource investment in reproduction (log RIR) for *B. sylvaticum* (b) along the latitudinal gradient. Means ± S.E. are shown.

We show that the impact of accumulated temperature, latitude and local environment on RIR and GSO of understorey plants primarily depends on both flowering time and/or distribution range. For the northerly distributed and spring or early summer flowering species *A. nemorosa* and *P. quadrifolia*, accumulated temperature was the most important variable increasing and decreasing GSO, respectively. Rising temperatures may therefore have pronounced effects on the population dynamics of these species. Similar patterns as for *P. quadrifolia* were found experimentally for *Austrodanthonia caespitosa*, an Australian perennial grassland species: seed number and seed mass showed no response to a warming treatment, whereas the germinability of the seeds decreased by 12 % (Hovenden *et al.* 2008).

Seed traits of *O. acetosella* , a northerly distributed spring flowering species, showed a latitudinal trend but no response to accumulated temperature. Also *A. nemorosa* and *P. quadrifolia* showed a latitudinal response. Two opposite latitudinal mechanisms are at work: (1) northern populations experience a longer photoperiod in the growing season, when temperature is mostly above the threshold for plant development, and, (2) on the other hand, the shorter growing season experienced by northern populations limits the plant development time (Moles & Westoby, 2003). Previous studies have confirmed the latitudinal hypothesis that seed mass decreases within and between species towards higher latitudes (e.g. Moles & Westoby 2003), but little is known about the relationship between latitude and seed number or germination percentage (but see Hernandez-Verdugo *et al.* 2001). However, Berg & Redbo-Torstensson (1998) consider the cleistogamous reproductive biology in *O. acetosella* as a bet-hedging strategy optimizing reproductive output in changing environments (comparable to reproductive assurance in Baker's law; Baker, 1955). This suggests that *O. acetosella*'s northernmost populations may reproduce more in the cleistogamous way. Since we only collected chasmogamous seeds in spring, this can be a reason for the unimportance of accumulated temperature for the chasmogamous GSO of *O. acetosella* along the gradient in the present study. Additionally, *O. acetosella* may simply be less temperature sensitive than *A. nemorosa* and *P. quadrifolia.*

Canopy cover, but not accumulated temperature or latitude, was the variable most influencing RIR and GSO of the southern species. Since the seeds of late summer flowering species (*C. lutetiana* and *B. sylvaticum*) mature after canopy closure, the negative effect of a higher canopy cover and decreasing light availability and photosynthesis on resources available for reproduction is easily understood (see e.g. Kudo *et al.* 2008). Seeds of spring species mature before or shortly after canopy closure, and thus canopy cover is of no importance.

Sherry *et al.* (2007) also reported contrasting phenological responses to experimental warming of plant species in a North American prairie. Early flowering species advanced their flowering and fruiting phenology, whereas late flowering species flowered and fruited later. Kudo *et al.* (2008) suggest that the influence of climate warming on plant species phenology and reproduction will be most pronounced in the group of spring bloomers. The expected divergent response of plant species with different phenology to temperature is only partly confirmed. The response based on phenology and distribution established here, can be caused by the following: (1) Ambient temperatures that are higher in summer than in spring. An increase in spring GDH is marginally more important than an increase in summer GDH (Sherry *et al.* 2007; confirmed by the lower coefficient of variation of the GDH values of late summer species [*ca.* 10 %] than early species [*ca.* 15 %]). (2) This also results in a smaller temperature range in our analyses for the southern distributed species. (3) Forest plants with a more southerly distribution range edge (*in casu C. lutetiana* , *B. sylvaticum* and *P. elatior*) may show a distributional time-lag as a legacy of the last Ice Age. Supporting this last point, many forest plant species have not yet reached their equilibrium distribution after the Last Glacial Maximum due to poor colonisation rather than climate or other environmental factors (Van Der Veken *et al.* 2007; Svenning *et al.* 2008). This also may contribute to the fact that, at present, these southern species are not phenotypically responsive to temperature as such along the latitudinal gradient, despite the fact that they may show microevolutionary adaptations to rising temperatures in the future.

It is also generally accepted that interspecific seed number decreases and germinability increases with seed mass (Leishman *et al.* 2000; Fenner & Thompson 2005). However, in the present study, some of these relationships were intraspecifically confirmed, others were absent or inverse. First, this may be due to the clustering of these traits with region: data points of seed traits from the same region tend to cluster together (results not shown), possibly caused by differences in resources or genetics among populations and regions along the gradient, since this influences the intraspecific relationship. Second, seed number, seed mass and germination percentage may be influenced by other environmental variables or to another extent by the same environmental variables along the gradient (as shown), which also influences the intraspecific relationship. Hence, when populations of the same species are compared along a latitudinal gradient, the intraspecific seed number, seed mass and germinability relationships are not clarified by nor easily grasped in one standard formula. Using seed mass, for example, as a surrogate for germinability seems therefore not always warranted.

We conclude that responses of the reproduction and population dynamics of understorey plants to temperature are primarily dependent upon phenology and distribution, but are still difficult to predict. There is a need for studies that compare species responses to temperature since results cannot just be transferred from model species. We also show that the GSO is more temperature sensitive than the RIR across species. Hence, caution should be exercised when estimating the impact of climate warming on colonisation capacity based on seed number and seed mass changes alone, as the GSO is especially important in the light of population dynamics and distributional changes caused by rising temperatures compared with the RIR. The GSO trait is considered as static in, for instance, bio-climatic envelope models and here we clearly show that when climate changes this trait can alter. Since both *A. nemorosa* and *P. quadrifolia* are slow-colonising understorey plants (Verheyen *et al.* 2003c), changes in their GSO may have pronounced effects on their future population dynamics when temperature rises. Hence, bio-climatic envelope models would benefit from including the temperature dependence of this trait when forecasting future distributions of plant species.

However, to fully assess the effects of climate change and temperature on the colonisation capacity of understorey plants, further research is needed: (i) production, maturation and germination of seeds is only the first stage in the recruitment process from mother to adult daughter plant; (ii) our analyses could be extended to a larger set of herbaceous forest plant species to allow us to generalise among functional types, as we are far from understanding how different functional species groups and species occurring close or far from their distribution edge, react to temperature changes; and (iii) the mechanisms explaining the observed variation in sexual reproduction and especially the divergent response of different species, remain unclear. Common-garden experiments (e.g. Macel *et al.* 2007) in particular can generate valuable information about reproduction of herbaceous forest species along a geographical gradient and whether variations have genotypic and/or phenotypic causes.

5

Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L.

After: De Frenne P, Graae BJ, Kolb A, Brunet J, Chabrerie O, Cousins, SAO, Decocq G, Dhondt R, Diekmann M, Eriksson O, Heinken T, Hermy M, Jõgar Ü, Saguez R, Shevtsova A, Stanton S, Zindel R, Zobel M, Verheyen K (2010) Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. *Forest Ecology and Management*, **259**, 809–817.

Abstract

Climate warming is already influencing plant migration in different parts of the world. Numerous models have been developed to forecast future plant distributions. Few studies, however, have investigated the potential effect of warming on the reproductive output of plants. Understorey forest plants in particular, have received little attention in the debate on climate change impacts. This study focuses on the effect of temperature on sexual reproductive output (number of seeds, seed mass, germination percentage and seedling mass) of *Anemone nemorosa* L., a model species for slow colonising herbaceous forest plants. We sampled seeds of *A. nemorosa* in populations along a 2400 km latitudinal gradient from northern France to northern Sweden during three growing seasons (2005, 2006 and 2008). This study design allowed us to isolate the effects of accumulated temperature (growing degree hours; GDH) from latitude and the local abiotic and biotic environment. Germination and seed sowing trials were performed in incubators, a greenhouse and under field conditions in a forest. Finally, we disentangled correlations between the different reproductive traits of *A. nemorosa* along the latitudinal gradient. We found a clear positive relationship between accumulated temperature and seed and seedling traits: reproductive output of *A. nemorosa* improved with increasing GDH along the latitudinal gradient. Seed mass and seedling mass, for instance, increased by 9.7 % and 10.4 %, respectively, for every 1000 °C.hour increase in GDH. We also derived strong correlations between several seed and seedling traits both under field conditions and in incubators. Our results indicate that seed mass, incubator-based germination percentage (Germ$\%_{Inc}$) and the output of germinable seeds (product of number of seeds and Germ$\%_{Inc}$ divided by 100) from plants grown along a latitudinal gradient (i.e. at different temperature regimes) provide valuable proxies to parameterize key population processes in models. We conclude that (i) climate warming may have a pronounced positive impact on sexual reproduction of *A. nemorosa* and (ii) climate models forecasting plant distributions would benefit from including the temperature sensitivity of key seed traits and population processes.

5.1 Introduction

Climate warming is already influencing plants in different parts of the world. It is predicted that the enhanced greenhouse effect and associated temperature rises (IPCC 2007) will induce migration and/or require adaptation of plant species worldwide, because temperature is one of the most significant factors controlling plant distributions at broad spatial scales (e.g. Woodward 1987). Many plant species, however, are dispersal limited, and only fast migrating species are expected to be able to track the changing climate (*cf.* Chapter 1). In particular, many herbaceous understorey forest plants are known to be very slow colonisers (Verheyen *et al.* 2003c) and some have not yet reached their equilibrium distribution after the last glacial maximum (Van der Veken *et al.* 2007; Svenning *et al.* 2008; **Box** on p. 6). Our understanding of the effect of climate warming on plant reproductive output such as number of seeds, seed mass, germination capacity or seedling survival, is especially limited (Hovenden *et al.* 2008). Since the sexual reproductive stage is considered amongst the most sensitive phases of the life cycle of plants, global warming is likely to influence gamete development, the progamic phase from pollination to fertilization and the postzygotic early embryo development as well as subsequent stages such as fruit and seed set, germination rate and seedling emergence (Hedhly *et al.* 2009).

One way to study the influence of variable temperatures on responses in reproductive output of plants, is to take advantage of the temperature variation along large latitudinal gradients (reviewed in Austin 1999; Fukami & Wardle 2005; Chapter 1). This temperature variation results in inter- and intraspecific variation of plant traits (e.g. Etterson 2004; Chapter 2–4). An advantage of a latitudinal gradient approach is that it offers a geographic gradient along which temperatures vary, which allows for separation of the direct effects of temperature from confounding effects related to site-specific characteristics (e.g. photoperiod, precipitation, canopy cover, soil and population properties) in the absence of multicollinearity (Chapter 4).

For example, one can imagine a colder than average spring in northern France in the same year as a warmer than average spring in northern Sweden. Thus, growing season (*in casu* spring) temperature does not necessarily decline in a predictable manner with latitude, when compared to mean annual temperature (Graae *et al.* 2011).

In this chapter, we examined sexual reproductive output (number of seeds, seed mass, germinability and seedling mass) of wood anemone, *Anemone nemorosa* L. (Ranunculaceae), a characteristic species representing the group of slow colonising understorey plants, growing in natural populations along a 2400 km latitudinal gradient in three different growing seasons (2005, 2006 & 2008). While many studies have focused on genetics (Stehlik & Holderegger 2000; Rusterholz *et al.* 2009), phenology (Ahas 1999; Tyler 2001), ecological interactions (Garcia-Guzman & Wennström 2001), reproductive strategies and colonisation (Meyer & Hellwig 1997; Brunet & von Oheimb 1998a,b; Holderegger *et al.* 1998; Müller *et al.* 2000; Philipp & Petersen 2007; Wittig 2008) as well as germination behaviour and embryo growth (Ali *et al.* 2007; Mondoni *et al.* 2008) of *A. nemorosa*, very little is known about the impact of climate warming on this species (but see Graae *et al.* 2009b).

The main purpose of this chapter was to extend the findings on the RIR and GSO of *A. nemorosa* from Chapter 4 and to test whether temperature variations along a latitudinal gradient has a direct effect on a wider range of reproductive traits of *A. nemorosa* from seed set to seedling establishment in field conditions. Our results can contribute to parameterization of key population processes (such as germination and establishment) in climate change impact models by providing easily measurable quantitative traits from plants growing at different temperatures (Weiher *et al.* 1999; Diaz *et al.* 2004; Jeltsch *et al.* 2008). We specifically addressed the following questions: (i) what is the importance of accumulated temperature for reproductive output (number of seeds, seed mass, germination percentage in incubators, a greenhouse or in the forest, and seedling mass) of *A. nemorosa* compared to latitude and local abiotic and biotic environmental factors along the gradient and (ii) which correlations exist between these reproductive traits and which traits can be used in climate warming models?

We hypothesize that populations of *A. nemorosa* from warmer sites show more efficient reproductive output because the flowering and fruiting phenology of early flowering species tend to react positively to increased temperatures (Sherry *et al.* 2007). Furthermore, the high degree of phenotypic plasticity ascribed to *A. nemorosa* (Shirrefs 1985) suggests that it has the capacity to cope with climate changes. In addition, we expect to find correlations among reproductive traits within *A. nemorosa* to be similar to those reported between different species, such as a negative relationship between number of seeds and seed mass (Leishman 2001; Fenner & Thompson 2005) and a positive one between seed mass and germination percentage and between seed mass and seedling mass (Moles & Westoby 2004a).

5.2 Methods

5.2.1 Study species

Anemone nemorosa L. (wood anemone) is a perennial and vernal forest understorey geophyte common in ancient deciduous European woodland. More information on the species is given in sections 2.2.1 and 3.2.1.

5.2.2 Study region and populations

We used an indirect space-for-time substitution procedure (*sensu* Fukami & Wardle 2005) by surveying different populations of the same target species along a latitudinal transect and using the existing climatic differences to infer a potential response to climate change over time. Within each of seven regions located along an approximately 2400 km (13.7°) latitudinal gradient from northern France (49.8°N) via Belgium, northwestern and northeastern Germany to southern, central and northern Sweden (63.5°N) (**Fig. 2.1** on p. 19 excluding Estonia; **Appendix A**), one, up to five and up to six lowland populations per region were selected for data collection in 2005, 2006 and 2008, respectively (**Table 5.1**). Hence, a total of 7, 28 and 41 populations were sampled in 2005, 2006 and 2008, respectively. Within each region, populations were randomly selected within an area of *ca.* $20 \times 20\,\text{km}^2$ and were usually at least 1 km apart. All populations occurred in deciduous hardwood forest (with *Quercus robur* L., *Fagus sylvatica* L., *Fraxinus excelsior* L. and/or *Betula pubescens* L. as dominant tree species) with 50–95 % canopy cover.

5.2.3 Temperature, abiotic and biotic environmental factors and latitude

To test for the influence of temperature relative to latitude and other abiotic and biotic factors on reproduction of *A. nemorosa*, we collected environmental data for each region

Table 5.1: Seed collection dates (Julian day) by region and year for *A. nemorosa* populations along a 2400 km latitudinal gradient from northern France to northern Sweden. Parentheses indicate the number of populations sampled in each region and year.

	2005	**2006**	**2008**[a]
northern France	143 (1)	143 (5)	140 (6)
Belgium[b]	131 (1)	131 (3)	130 & 131 (6)
northeastern Germany	143 (1)	143 (5)	136 & 137 (6)
northwestern Germany	125 (1)	143 (5)	142 (6)
southern Sweden	150 (1)	170 (5)	141 & 143 (6)
central Sweden	164 (1)	164 (5)	157 (6)
northern Sweden	173 (1)	(0)	176 & 177 (5)

[a] 2008 was a leap year, Julian days thus present a one day difference compared to 2005 and 2006.

[b] Sampling in Belgium was performed near Leuven in 2005 and 2006, and near Brakel in 2008.

(temperature and latitude; in 2005, 2006 and 2008) and population (all other variables; only in 2006 and 2008; **Table 5.2**). We used the number of growing degree hours (GDH) above a base temperature (5 ℃) from 1 January until the population- and year-specific seed collection dates as a measure of accumulated temperature (Lindsey & Newman 1956; Lischke *et al.* 1997). The GDH-concept is often used as a proxy for accumulated temperature because of the demonstrated relationship between plant development and GDH (e.g. Diekmann 1996; Meunier *et al.* 2007). Calculations of GDH followed those of Lindsey & Newman (1956) using daily minimum and maximum temperatures from weather stations near Amiens (northern France, < 15 km), Leuven (2005 and 2006) and Gontrode (2008) (Belgium, < 20 km), Potsdam (northeastern Germany, < 20 km), Bremen (northwestern Germany, < 60 km), Alnarp (southern Sweden, < 25 km), Stockholm (central Sweden, < 70 km) and Umeå (northern Sweden, < 50 km). The mentioned distances are the maximum distances between the respective weather stations and the farthest sampled population for every region and year.

Within each population, in 2006 and 2008, we visually estimated the percentage of canopy cover, estimated soil moisture according to four different classes (1: dry, 2: fresh, 3: moist or 4: wet) and collected five 4 cm deep soil cores from below the litter layer. The soil samples from each population were combined, air-dried to constant mass and passed through a 2 mm-sieve. All samples were analysed for pH (determined from a solution of 10 g of soil and 25 ml of 0.01 M $CaCl_2$ with a standard glass electrode), plant available phosphorus (P), calcium (Ca), magnesium (Mg) and potassium (K) (extraction with ammonium lactate and photometric determination by flow injection analysis for P and Atomic Absorption Spectroscopy (AAS) for cations; all in mg per 100 g soil). The total size of each population (hereafter referred to as *population size*) was estimated according to six classes, namely < 50, $50 - 100$, $100 - 500$, $1000 - 5000$, > 5000 flowering ramets (hereafter, ramets will be referred to as *individuals*). The density of each population was estimated as the number of flowering individuals.m^{-2} (hereafter referred to as *population density*), by counting the number of flowering individuals in ten randomly placed $0.5 \times 0.5\,m^2$ quadrats within a 20 m radius circle.

The latitude of each region was included in the analysis to account for environmental variables not measured directly along the gradient but expected to differ among sampling regions, such as photoperiod (Jackson 2009), precipitation or past climate variations shaping species (microevolutionary adaptations and plasticity) and population distributions (Moles *et al.* 2007). It is important to note that GDH and latitude were not correlated within any sampling year ($r = -0.674$, $n = 7$, $p = 0.097$ for 2005; $r = 0.103$, $n = 6$, $p = 0.846$ for 2006; $r = -0.642$, $n = 7$, $p = 0.120$ for 2008), confirming that the variables temperature and latitude can be statistically separated.

Table 5.2: Abiotic and biotic variables measured in *A. nemorosa* populations along a 2400 km latitudinal gradient from northern France to northern Sweden

Variable group	Variable	Unit	Sampling years
Temperature	GDH	°C.hours	2005; 2006; 2008
Latitude	-	°N	2005; 2006; 2008
Overstorey canopy cover	-	%	2006; 2008
Population characteristics	Population density	Flowering individuals.m^{-2}	2006; 2008
	Population size	Total number of individuals	2006; 2008
Soil characteristics	pH	-	2006; 2008
	P	mg.100 g^{-1} dry soil	2006; 2008
	K	mg.100 g^{-1} dry soil	2006; 2008
	Ca	mg.100 g^{-1} dry soil	2006; 2008
	Mg	mg.100 g^{-1} dry soil	2006; 2008
	Soil moisture class	-	2006; 2008

5.2.4 Seed production and germination

At seed maturity, we collected the diaspores (hereafter referred to as *seeds*) of 15–20 randomly chosen individuals located within the area in which we had measured the environmental variables. Seeds were considered mature when natural dispersal began (Baskin & Baskin 1998), indicated by seeds falling easily off the plant (region-specific seed collection dates are listed per year in **Table 5.1**). The seeds were air-dried at room temperature. For each sample plant in all populations, we determined the total number of seeds (only in 2006 and 2008) and mean seed mass (all years). For each population, in 2005 and 2006, three Petri dishes were lined with moist filter paper (Munktell 00A), and 50 randomly chosen seeds were distributed in each dish (most samples within 1 month after sampling).

Based on earlier studies (Baskin & Baskin 1998; Endels *et al.* 2004) seeds were given six weeks of warm stratification (diurnal cycles of 20 °C and full light (240 μmol.m^{-2}.s^{-1}) for 14 hours and 10 °C and darkness for 10 hours) before they were placed in cold stratification (2 °C and darkness) for 20 weeks. After the 20 weeks of cold stratification, the dishes were placed in warm stratification as above. The dishes were stored in ziplock bags to avoid desiccation during stratification and incubation, and additional distilled water was supplied as needed. The number of germinated seeds (seeds with an emerged radicle) was recorded weekly for six weeks. The sets of dishes were randomly rotated in the incubator after each weekly recording. Mean germination percentage in the incubator was calculated per population (Germ%$_{Inc}$). We also calculated the output of germinable seeds (GSO$_{Inc}$) based on the Germ%$_{Inc}$ from 2006, i.e. the product of number of seeds and Germ%$_{Inc}$ per population divided by 100 as an important measure of reproductive success.

5.2.5 Seed sowing experiments

We conducted two seed sowing experiments, one in 2006 and one in 2008, in order to compare incubator germination percentages with germination percentages under field conditions in the forest, to evaluate correlations between traits (seed mass, incubator germination percentage, seedling mass, etc.) under field conditions and to determine seedling performance of the different provenances of *A. nemorosa* in two common gardens. In a Danish beech forest (in early June 2006: Hvalsø Forest, 55.5 °N and 11.9 °E) and Belgian oak forest (in June 2008: Aelmoeseneie forest, Gontrode, 50.9 °N, 3.8 °E), 3 × 50 seeds from each population ($n = 28$ in 2006 and $n = 41$ in 2008) were sown into three different plots, where *A. nemorosa* dominates the ground vegetation. To homogenize the soil substrate, we removed the litter layer and placed $50 \times 30\,\mathrm{cm}^2$ plastic bags (Denmark) or $55 \times 35\,\mathrm{cm}^2$ pots (Belgium) with standard potting soil (5 cm deep) on the soil surface. The bottoms of the bags and pots were perforated so that water could move between the potting soil and the underlying soil. We sowed the seed samples at both sites randomly in a grid system where each subplot was about $10 \times 5\,\mathrm{cm}^2$. Two control plots were free of sowings per bag or pot to account for germination from naturally dispersed seeds (no *A. nemorosa* seedlings emerged from these plots). The plots were covered with the litter that had been removed from each plot before placing the bags and pots.

At the Danish site, we counted and harvested all seedlings on 1 April 2007. Mean germination percentage in the forest per population ($\mathrm{Germ\%}_{For}$) was calculated as the number of seedlings divided by 50 seeds. Seedlings were dried to constant mass at 40 °C. Due to difficulties in keeping the seedlings (mainly their roots) intact during harvest, we decided to only use those seedlings where the aboveground part (i.e. the shoots) were intact, cut away the root part and estimated the mean dry weight of these aboveground seedlings from each sample (hereafter referred to as *seedling mass*). We calculated the output of germinable seeds in the Danish forest (GSO_{For}) as the product of the number of seeds and forest germination percentage per population divided by 100.

At the Belgian site, the pots were transferred to a greenhouse (20 ± 1 °C) on 4 December 2008 after the seeds had received sufficient cold stratification in the forest to determine germination percentage. The pots were randomly rotated in the greenhouse every week and supplied with water as necessary. The number of germinated seeds (seeds with emerged radicle) was recorded after seven weeks (on 20 January 2009) per replicate of 50 seeds. Mean germination percentage in the greenhouse ($\mathrm{Germ\%}_{GH}$) was calculated per population. We also calculated the output of germinable seeds based on the greenhouse germination percentage (GSO_{GH}) as the product of the number of seeds and greenhouse germination percentage per population divided by 100.

5.2.6 Data analysis

We used population-level means for all analyses and variables (except for the correlations between seed number and mass in which individual-level data were used). Number of seeds, seed mass, seedling mass, GSO_{Inc}, GSO_{For} and GSO_{GH} data were log-transformed and germination percentage data were arcsine square root-transformed to meet assumptions of statistical tests in terms of normality and homogeneity of variances (Quinn & Keough 2002).

To account for correlations among environmental and population variables measured in 2006 and 2008, we performed a separate principal component analysis (PCA) with `VARIMAX`-rotation (Kaiser 1960) to reduce the set of environmental variables to two components: a soil PCA (pH, K, Ca, Mg, P, soil moisture) and a population PCA (population size and density). Sample scores for the first principal components (PCA1.soil and PCA1.population) were used for the mixed-effect models (see below) as a weighted average of the soil and population variables, explaining the highest proportion of variance (here 35.6 % and 37.0 % for soil and 62.0 % and 56.6 % for population variables for 2006 and 2008, respectively).

The effects of accumulated temperature, latitude and local environment along the latitudinal gradient were separated using linear regression or mixed effect models. For the 2005 data (when only one population per region was sampled), we used linear regression models to explore the relative importance of two different predictor variables: accumulated temperature (expressed as GDH) and latitude. To avoid overfitting and for model simplification, only variables with $p < 0.2$ (high p-value because of limited sample size in 2005) from the full model were considered for the final linear model. For the 2006 and 2008 data, we used mixed-effect models to explore the relative importance of the five different predictor variables or variable groups: (1) accumulated temperature (expressed as GDH), (2) latitude, (3) soil variables (as PCA1.soil), (4) population variables (as PCA1.population) and (5) canopy cover (CC) on reproductive traits of *A. nemorosa*. A random effect term *region* was added to the mixed effect models to address the likelihood that populations from the same region share spatially autocorrelated characteristics. To avoid overfitting and for model simplification, only variables with $p < 0.1$ from the full mixed-effect models were considered for the final models. These final reduced models are presented in the results section. To detect possible multicollinearity (Graham, 2003) between the different predictor variables, variance inflation factors (VIF) were calculated according to Quinn & Keough (2002). VIF were low (< 3.13 for 2006 and < 3.19 for 2008) indicating low collinearity (Quinn & Keough 2002). This confirms that the effects of latitude, soil and population variables and canopy cover can be statistically unraveled from temperature effects. Correlations between reproductive traits were established by calculating Pearson correlation coefficients (r) on the transformed data. All analyses were conducted with `SPSS 15.0` and `S-Plus 8.0`.

5.3 Results

Averaged across years and regions, *A. nemorosa* produced 18.1 seeds per individual of 1.96 mg each. The absolute value of the mean germination percentages was lower in the incubators (mean for 2005 and 2006: 11.7 %) and in the greenhouse (2008: 7.5 %) than in the forest (2006: 28.9 %; **Table 5.3**).

Accumulated temperature (GDH) had a mostly significant positive effect on most reproductive traits of *A. nemorosa* along the latitudinal gradient (**Table 5.4**). For instance, seed mass consistently increased with accumulated temperature during all study years (**Fig. 5.1**). The parameter estimate of the predictor GDH in the equation of log-transformed seed mass was 4.0×10^{-5} for 2005 and 2008 (**Table 5.4**), which means that seed mass increased roughly with a factor $10^{0.04} = 1.097$ for every 1000 °C.hour increase in GDH. Similar calculations yield a factor of 1.104 for seedling mass. The arcsine sqrt-transformation of germination percentages makes it difficult to interpret the parameter estimate for these variables. The GDH effect on the parent plant is also reflected in the sowing experiment in the forest. More seeds of warmer grown parent plants germinated (**Fig. 5.2a**) and seedlings were heavier (**Fig. 5.2b**). The soil PCA gradient was only important for the number of seeds in 2006 and the population PCA for the number of seeds in 2006 and seed mass in 2008. Latitude and canopy cover were never related to the reproductive traits under study (**Table 5.4**).

The number of seeds per individual was significantly negatively correlated with seed mass, but not with other reproductive traits in 2006 (**Table 5.5**). The number of seeds was negatively correlated with the seed mass ($r = -0.121$, $p = 0.003$, $n = 620$ individuals) and Germ$\%_{GH}$ ($r = -0.301$, $p = 0.059$, $n = 40$) in 2008. Seed mass was positively correlated with seedling mass and Germ$\%_{For}$ in 2006 (**Table 5.5**), Germ$\%_{Inc}$ in 2005 ($r = 0.671$, $p = 0.099$, $n = 7$) and Germ$\%_{GH}$ in 2008 ($r = 0.384$, $p = 0.015$, $n = 40$), but not with Germ$\%_{Inc}$ in 2006 (Table 5). Additionally, we found significant positive relationships between Germ$\%_{Inc}$, Germ$\%_{For}$ and seedling mass (**Table 5.5**). Significant positive relationships per region between the germination trials from 2005 and 2006 were detected, but not between 2005 and 2006 on the one hand and 2008 on the other hand (**Table 5.6**). Forest germination percentage and incubator germination percentage (both 2006) from populations along the gradient were highly correlated (**Table 5.5** and **Table 5.6**).

5.4 Discussion

There is an urgent need for knowledge about the potential effect of warming on seed production and germination for plants in general (Hovenden *et al.* 2008) and for understorey plants in particular. In this chapter, we showed that increasing accumulated temperatures may have a pronounced positive impact on a wide variety of reproductive traits of *A. nemorosa*, inde-

Table 5.3: Reproductive output (mean and S.E.) of *A. nemorosa* populations along the latitudinal gradient

	Seed number	Seed mass (mg)	Germ$\%_{Inc}$ (%)	Germ$\%_{For}$ (%)	Seedling mass (mg)	Germ$\%_{GH}$ (%)	GSO$\%_{Inc}$	GSO$\%_{For}$	GSO$\%_{GH}$
Sampling years	2006; 2008	2005; 2006; 2008	2005; 2006	2006	2006	2008	2006	2006	2008
Mean	18.1 (0.6)	1.96 (0.04)	11.7 (1.7)	28.9 (2.2)	0.52 (0.04)	7.5 (1.3)	2.19 (0.34)	5.60 (0.47)	1.20 (0.15)
France	14.2 (1.7)	2.24 (0.09)	18.2 (5.4)	35.9 (3.1)	0.54 (0.04)	15.5 (7.3)	2.82 (1.01)	6.93 (1.13)	1.59 (0.75)
Belgium	21.5 (1.3)	1.85 (0.14)	2.3 (1.3)	7.1 (2.4)	0.33 (0.05)	8.6 (1.4)	0.56 (0.40)	1.49 (0.47)	1.85 (0.42)
NE-Germ	19.9 (0.6)	2.09 (0.07)	7.0 (2.5)	32.5 (3.9)	0.42 (0.04)	6.0 (1.6)	1.44 (0.65)	6.26 (0.88)	1.26 (0.36)
NW-Germ	16.0 (0.6)	2.06 (0.10)	8.9 (3.1)	22.9 (4.2)	0.44 (0.03)	7.0 (1.3)	1.51 (0.67)	3.90 (0.82)	1.10 (0.23)
S-Sweden	19.0 (0.8)	1.83 (0.06)	16.9 (2.5)	38.3 (3.1)	0.66 (0.03)	4.1 (0.9)	2.80 (0.30)	7.20 (0.24)	0.76 (0.15)
C-Sweden	17.4 (1.7)	1.87 (0.12)	15.7 (4.0)	27.7 (3.8)	0.44 (0.04)	7.9 (1.6)	3.35 (1.03)	6.15 (1.03)	1.10 (0.23)
N-Sweden	21.1 (2.9)	1.58 (0.15)	0.0 (0.0)	-	-	2.8 (0.7)	-	-	0.59 (0.16)

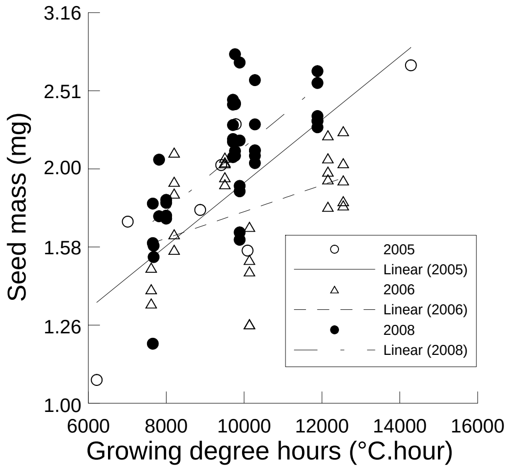


Figure 5.1: Relationship between accumulated temperature (growing degree hours) and seed mass of *A. nemorosa* seeds collected in 2005, 2006 and 2008 along the latitudinal gradient. See **Table 5.4** for significance values. Note the log-transformed Y axis.

Table 5.4: Effects of (1) accumulated temperature (GDH), (2) latitude, (3) canopy cover and (4) soil (PCA1.soil) and (5) population (PCA1.population) variables on the reproductive output of *A. nemorosa* populations along the latitudinal gradient in 2005, 2006 and 2008

Year	Response variable	Transformation	Predictor	P.E.	d.f.	*t*-value
2005	Seed mass	Log	GDH	4.04E-05	1, 5	3.17*
	Germ%$_{Inc}$	Arcsine sqrt	GDH	6.28E-05	1, 5	2.09(*)
2006	Seed number	Log	Soil	2.87E-02	1, 20	2.04(*)
			Population	2.71E-02	1, 20	2.04(*)
	Seed mass	Log	-	-	-	(ns)
	Germ%$_{Inc}$	Arcsine sqrt	GDH	4.06E-05	1, 21	2.85**
	GSO$_{Inc}$	Log	GDH	1.88E-04	1, 21	2.83**
	Germ%$_{For}$	Arcsine sqrt	GDH	5.95E-05	1, 21	3.05**
	Seedling mass	Log	GDH	4.29E-05	1, 19	3.27**
	GSO$_{For}$	Log	GDH	1.07E-04	1, 21	2.71*
2008	Seed number	Log	GDH	-6.80E-05	1, 33	-2.83**
	Seed mass	Log	GDH	4.00E-05	1, 32	4.07***
			Population	-1.65E-02	1, 32	-1.96 (*)
	Germ%$_{GH}$	Arcsine sqrt	GDH	4.49E-05	1, 32	3.76***
	GSO$_{GH}$	Log	GDH	7.03E-05	1, 32	1.74(*)

pendent of other latitudinal and regional differences. We successfully derived clear positive relationships between accumulated temperature and most seed and seedling traits. Seed and seedling mass, for instance, increased by 9.7 % and 10.4 %, respectively, for every 1000 °C.hour increase in GDH (1000 °C.hour increase = an increase of 1 °C during 42 days).

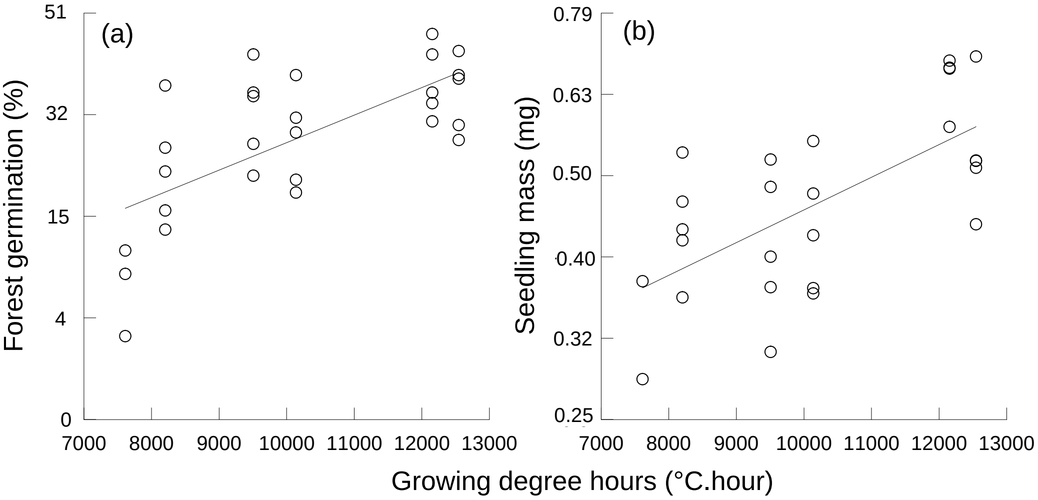


Figure 5.2: Relationship between accumulated temperature (growing degree hours) the parent plant experienced and (a) germination (%) and (b) the mass of *A. nemorosa* seedlings (mg) grown in Hvalsø forest (Denmark) between June 2006 and April 2007. Source seeds were collected along the latitudinal gradient. See **Table 5.4** for significance values. Note the arcsine square root (a) and log-transformed (b) Y axes.

5.4.1 Significance of accumulated temperature

It is known that the timing of embryo growth and germination of seeds is finely tuned to the local climate, as shown in a comparison of lowland and mountain populations of *A. nemorosa* (Mondoni *et al.* 2008). In the present study, we clearly show that accumulated temperature is amongst the most important factors controlling reproductive traits of *A. nemorosa*. Seed mass, germination percentage (Germ$\%_{Inc}$, Germ$\%_{For}$, Germ$\%_{GH}$), germinable seed output (GSO$_{Inc}$, GSO$_{For}$, and GSO$_{GH}$) and seedling mass all show a positive response to increased GDH experienced by the parent plant. These results suggest that if climate warms, sexual reproduction of the slow colonising *A. nemorosa* may improve. One of the few studies that also investigated the effect of warming on seed production and germination of plant species, found that seed mass of an Australian perennial grassland species (*Austrodanthonia caespitosa*) showed no response to a warming treatment with infra red heaters, but seed germinability and aboveground seedling biomass decreased by 12 % and 26 %, respectively (Hovenden *et al.* 2008). These results contrast with our findings that *A. nemorosa*'s seed and seedling mass and germination percentages tended to increase under higher accumulated temperatures. Sherry *et al.* (2007) detected that experimental warming in a North-American tallgrass prairie advanced flowering and fruiting phenology for early flowering species whereas later flowering species flowered and fruited later. Since *A. nemorosa* is a vernal species, positive effects of temperature on its reproduction could be expected. The number of seeds per *A. nemorosa*

Table 5.5: Correlations (r) between the log-transformed number of seeds and seed mass and arcsine square root-transformed germination percentages in incubators (Germ%$_{Inc}$) and a forest (%$_{For}$) of *A. nemorosa* seeds sampled along the latitudinal gradient in 2006.

		Seed number	Seed mass	Seedling mass	Germ%$_{Inc}$
Seed mass	r	-0.157***			
	n	556			
Seedling mass	r	-0.025 (ns)	0.391*		
	n	26[a]	26[a]		
Germ%$_{Inc}$	r	0.159 (ns)	0.072 (ns)	0.457*	
	n	28	28	26	
Germ%$_{For}$	r	-0.084 (ns)	0.454*	0.653***	0.744***
	n	28	28	26[a]	28

[a] Two lost populations in the seedling mass determination

Table 5.6: Correlations (r) between the arcsine square root-transformed germination percentages in incubators (Germ%$_{Inc}$), a forest (Germ%$_{For}$) and a greenhouse (Germ%$_{GH}$) of *A. nemorosa* seeds sampled along the latitudinal gradient (Umeå, northern Sweden, was not included in this analysis) in 2005, 2006 or 2008.

		Germ%$_{Inc}$ 2005	Germ%$_{Inc}$ 2006	Germ%$_{For}$ 2006
Germ%$_{Inc}$	r	0.880*		
2006	n	6		
Germ%$_{For}$	r	0.784(*)	0.744***	
2006	n	6	28[a]	
Germ%$_{GH}$	r	0.373 (ns)	0.046 (ns)	-0.063 (ns)
2008	n	6	6	6

[a] Comparison within the same year and populations, therefore $n = 28$ (**Table 5.5**)

individual is the reproductive trait least influenced by accumulated temperature, probably because of preformation during the previous growing season (comparable to bud preformation in arctic plants; e.g. Diggle 1997). In 2008, this trait was negatively affected by increasing accumulated temperature, suggesting that the effect is idiosyncratic. Canopy cover is of minimal importance because *A. nemorosa* has the major part of its photosynthetic active period before canopy flush (Shirreffs 1985).

5.4.2 Seed size, incubator studies and reproductive output in the forest

Many studies have investigated interspecific relationships between seed size and environmental conditions (Aizen & Woodcock 1992; Li *et al.* 1998, Moles & Westoby 2003; Meunier *et al.* 2007; Tautenhahn *et al.* 2008) and correlations between seed size and field germinability or seedling growth (Saverimuttu & Westoby 1996; Leishman 2001; Moles & Westoby 2004a; Lehtilä & Ehrlén 2005). Fewer studies, however, have established such correlations for multiple populations of a single species (but see e.g. Meyer & Carlson 2001; Brys *et al.* 2004).

In the present study, seed mass and especially Germ$\%_{Inc}$ appear to be valuable traits for predicting forest germination percentage and seedling mass in the forest. This finding is supported by recent research of *A. nemorosa* in northern Italy which found a link between forest and incubator germination in terms of germination timing and behaviour and embryo growth (Mondoni *et al.* 2008). However, Mondoni *et al.* (2008) did not investigate the number of seeds and mass, nor did they link germination percentages in the laboratory to percentages in the wild.

The output of germinable seeds is an important measure in population dynamics and persistence, but can be difficult to quantify under natural conditions. Our results demonstrate that germination percentage obtained in an incubator can be extrapolated to germination in the field, suggesting that GSO_{Inc} calculated from incubator tests can be a useful variable for modelling population dynamics (see Weiher *et al.* 1999; Diaz *et al.* 2004; Jeltsch *et al.* 2008) of *A. nemorosa*. This GSO measure will determine the output of seedlings and their fitness (e.g. seedling mass), but to use climate models to predict species responses and distributional changes, clear relationships between GSO and temperature are needed (*cf.* Jeltsch *et al.* 2008). These relationships could then, for instance, be integrated in coupled matrix population models with integrodifference equations of the type suggested by Neubert & Caswell (2000).

5.4.3 Potential impact of climate warming

This study demonstrates that climate models forecasting plant distributions would benefit from including the relationships between temperature and reproductive output. However, the limitations of our study must be considered. For example, when comparing germinability from the different years, taking into account that laboratory and field results are highly correlated within the same year (2006), regions do not always display similar levels of germination in all sampling years. This suggests that the temperature response of *A. nemorosa* is due to phenotypic flexibility rather than genotypic determination of the reproductive output within regions along the gradient (see further Chapter 6, 7 and 8). A lack of interannual variation in germination percentage within a specific region would be a strong suggestion for genotypic control over germination. Of course, phenotypic flexibility may be genotype-specific (Pigliucci 2005). The reasons for the observed variations in reproductive output remain uncertain in the absence of reciprocal transplant experiments, and this will be treated in detail in Chapters 7 and 8.

It remains unclear whether reproduction of *A. nemorosa* will also benefit in warm and dry habitats (e.g. northern Italy; Mondoni *et al.* 2008) within its distribution range. Such habitats were not included in the present study and post-dispersal seed drying negatively affects the life span of *A. nemorosa* seeds (Ali *et al.* 2007). This risk may be higher in warm and dry habitats. Also, the significance of an increased output of germinable seeds on dispersal and colonisation rates remains to be quantified. This should therefore be a focus of future research.

Nevertheless, our results suggest that if climate warms, this will have a pronounced positive impact on the reproduction of *A. nemorosa*, especially in terms of seed mass, germination percentage and seedling mass. If more seeds germinate and resulting seedlings show higher fitness, more individuals may be recruited to the adult stage. As rhizome growth also is likely to benefit from higher winter temperatures (Philipp & Petersen 2007), it can be hypothesized that the migration potential of *A. nemorosa* may increase as the climate in northwestern Europe becomes warmer in the coming decades.

6

The use of open-top chambers for evaluating warming effects on forest understorey plants

After: De Frenne P, De Schrijver A, Graae BJ, Gruwez R, Tack W, Vandelook F, Hermy M, Verheyen K (2010) The use of open-top chambers in forests for evaluating warming effects on herbaceous understorey plants. *Ecological Research*, **25**, 163–171.

Abstract

Open-top chambers (OTCs) are widely used experimental warming devices in open-field ecosystems such as tundra and alpine heath. However, knowledge on their performance in temperate deciduous forest ecosystems is largely lacking. The application of OTCs in forests might become important in the future since the effects of climate warming on growth, reproduction and future distribution of understorey forest plants have rarely been investigated. Therefore, polycarbonate OTCs covered with (OTCs+GF) and without permeable polypropylene GardenFleece (OTCs–GF) were installed in a temperate deciduous forest to create an experimental warming gradient. Short-term responses in phenology, growth and reproduction of a model understorey forest plant (*Anemone nemorosa* L.) to OTC installation were determined. In a second growing season, an in-depth study of multiple abiotic conditions inside OTCs–GF was performed. Both OTCs+GF and OTCs–GF raised air and soil temperature in a realistic manner (*ca.* +0.4 °C to +1.15 °C), but OTCs–GF only in the leafless period (up to +1.5 °C monthly average soil temperature). The early flowering forest plant *A. nemorosa* also showed a clear phenotypic response to OTC installation. Based on these facts and the large ecological drawbacks associated with OTCs+GF (mostly in connection with a higher relative air humidity and a lower light quantity) and very modest abiotic changes in OTCs–GF, we encourage the use of OTCs–GF in deciduous forest ecosystems for evaluating climate warming effects on early flowering understorey plants. There is also a potential to use this warming method on later flowering species, but this needs further research.

6.1 Introduction

It has been demonstrated that global warming already induces plant responses in different parts of the world (e.g. Parmesan & Yohe 2003). Despite the ecological significance of herbaceous forest understorey plants for temperate forest ecosystem biodiversity (Gilliam 2007), knowledge about the effects of climate change on their phenology, growth, reproduction and future distribution is largely lacking (*cf.* Chapter 1).

Various temperature manipulation systems have been applied worldwide to study the influence of climate warming on ecosystems (e.g. Marion *et al.* 1997; Kimball 2005; Netten *et al.* 2008). The use of open-top chambers (OTCs) is the most common, simplest and cheapest approach. OTCs are small greenhouses made of translucent plexiglass with inclined walls and an open top. They passively heat a small vegetation plot by capturing solar radiation and protecting against wind. Several studies report on the use of OTCs in open-field ecosystems such as tundra (Arft *et al.* 1999; Hollister & Webber 2000; Danby & Hik 2007), alpine meadows (Totland & Nylehn 1998; Totland 1999; Kudernatsch *et al.* 2008) and South African quartz-fields (Musil *et al.* 2005). OTCs have less frequently been used in deciduous forests; we are aware of only one single study using OTCs to create a rise in temperature in a deciduous forest ecosystem in Ontario, Canada (Sager & Hutchinson 2005).

In this chapter, we aimed to find out whether OTCs can be used in a deciduous temperate forest ecosystem to assess the impact of a rise in temperature on herbaceous understorey plants. We examined *Anemone nemorosa* L. (Ranunculaceae) which may be considered a model species for (European) slow colonising understorey plants. The present study is, according to our knowledge, the first to use open-top chambers for warming purposes examining herbaceous understorey plants in deciduous forest. Since it can be expected that the efficiency of OTCs is lower in forests than in open-field ecosystems due to its specific properties (shading, moist soils, lower wind speed, etc.), half of the OTCs were additionally covered with a permeable polypropylene fleece allowing both air and moisture exchange, but minimizing turbulence and potentially increasing the temperature difference with the ambient controls.

We specifically addressed the following question from a purely methodological point of view: are OTCs useful devices to gain insight in the impact of a warming climate on herbaceous understorey plants in a temperate deciduous forest ecosystem? Hence, the OTCs must fulfill the following criteria: (i) temperature is increased adequately and in a realistic manner compared to the IPCC predictions (IPCC 2007) and (ii) ecological abiotic and biotic drawbacks of the OTCs are limited. Furthermore, given the high degree of phenotypic plasticity of *A. nemorosa* (Shirreffs 1985), a certain response in a trait of this forest plant to OTC installation, increases the potential usefulness of this experimental warming method.

6.2 Methods

6.2.1 Study species

Anemone nemorosa L. (wood anemone) is a perennial and vernal forest understorey geophyte common in ancient deciduous European woodland. More information on the species is given in sections 2.2.1 and 3.2.1.

6.2.2 Study area

The Aelmoeseneie forest (50°58'N, 3°49'E, 16 m a.s.l.) is a temperate mixed deciduous forest near Ghent in central Belgium, *ca.* 60 km from the North Sea. The forest has a total area of 28 ha and the dominant trees are about 85 years old. Mean (1961–1990) annual precipitation amounts to 821 mm and is evenly distributed throughout the year. Mean annual temperature is 9.7 °C, mean temperature of the coldest and warmest month is 2.5 °C and 17.2 °C, respectively (F.A.O. 2005). The fenced study area is located in a forest stand dominated by ash (*Fraxinus excelsior* L.) and pedunculate oak (*Quercus robur* L.). The approximate time of canopy flush for *Q. robur* (expressed as the date when the first completely unfolded leaves are visible) was around 5 to 10 April in 2008 and 2009. The forest soil has developed from a quaternary layer of sand loam (0.5–1 m) on a shallow impermeable clay and sand complex of tertiary origin and is classified as Gleyic Cambisol in the World Reference Base (ISS-ISRIC-FAO 1998). *Anemone nemorosa* is widely distributed, carpeting the forest soil and dominating the ground vegetation until June. Understorey species such as *Lamiastrum galeobdolon* (L.) Ehrend. & Polatschek, *Oxalis acetosella* L., *Polygonatum multiflorum* (L.) All. and *Hedera helix* L. co-occur.

6.2.3 Experimental design

To simulate climate warming, six hexagonal open-top chambers (OTCs) (*cf.* Marion *et al.* 1997; Hollister & Webber 2000) were installed to passively warm small vegetation plots within the natural carpeting *A. nemorosa* community with >90 % cover. The OTCs were 60 cm high; one side was 66.4 cm at the base and 34.6 cm at the top, covered a surface area of 1.15 m^2 and had 60° inclined walls. The OTCs were made of extruded polycarbonate (visible & UV light transmission 86 %, IR light transmission < 5 %, DIN 5036) (Quinn Plastics, Derrylin, Northern Ireland). The open-top design allows free air exchange and is said to minimize undesirable chamber effects as changed radiation input, precipitation, gas and humidity concentrations and exclusion of pollinators or herbivores (Marion *et al.* 1997; Kanerva *et al.* 2005; Netten *et al.* 2008). To create a warming gradient, half of the open-top chambers ($n = 3$) were additionally covered with permeable 17 g.m^{-2} polypropylene GardenFleece (GF; light transmission is 90.3 % between 250–800 nm wavelength; Freudenberg, Vliesstoffe KG, Kaiserslautern, Germany) allowing both air and moisture exchange with the environment, but minimizing turbulence. The GF was attached on the top edges of the OTCs, into a small, light and hexagonal wooden frame which could be lifted to carry out the field work inside the plots.

In the near vicinity of each OTC, a $80 \times 80\,\text{cm}^2$ control plot ($0.64\,\text{m}^2$; $n = 6$) was established. The OTCs and control plots were randomly installed in areas with expected equal cover percentages of *A. nemorosa* on 1 February 2008 before emergence of the shoots. Hence, three treatments were applied: control plots (C), open-top chambers (OTC–GF) and open-top chambers covered with GF (OTC+GF). Measurements of the first growing season were finished on 8 May 2008.

6.2.4 Abiotic variables

To characterise the abiotic conditions in the OTCs in comparison with the control plots, soil surface temperature (in the litter layer; 0 cm depth) and air temperature (10 cm above ground level) were measured at 15 minutes intervals inside two OTCs–GF, two OTCs+GF and two control plots using Decagon ECT Temperature probes connected to Decagon Em50 dataloggers (Decagon Devices Inc., Pullman, WA, USA). Furthermore, daytime relative air humidity (10 cm above ground level; RH) was measured in all plots every five days with an Eijkelkamp Portable P4.01 logger (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) in April 2008 ($n = 6$). To evaluate the effect of the permeable GF on precipitation amount, six manual rain gauges were installed in an open-field grassland area adjacent to the forest stand, of which half were covered with GF ($n = 3$). Cumulative precipitation was measured weekly between 20 March and 16 May 2008 (see **Table 6.1** for an overview).

6.2.5 Biotic variables

To have an estimate of phenological development, the number of open flowers per plot was counted twice a week (every 3–5 days) from the date the first individual flowered (25 February 2008) until all flowers had disappeared (1 May 2008). Flowers were considered open when the anthers were visible and thus exposed to pollinators. To have an estimate of maximal plant growth, total plant height of the eight highest wood anemones per plot (evenly spread per quadrant) was measured weekly. Furthermore, at the stage of seed maturity (see further), we harvested and determined the total oven-dried (40 °C) aboveground dry matter biomass per plot (TADM; kg.ha^{-1}).

Table 6.1: Response variable groups and variables, units, abbreviations and the level on which variables are analysed

Type	Variable group	Response variable	Level[a]	Year[b]
Abiotic	Temperature	Soil temperature (T_{Soil}, -5 cm)	-	2
		Surface temperature ($T_{Surface}$, 0 cm)	-	1, 2
		Air temperature (T_{Air}, 10 cm)	-	1, 2
	Relative humidity	Relative humidity	-	1, 2
	Precipitation	Precipitation interception by GardenFleece	-	1
	Gas concentration	CO_2 concentration	-	2
	Moisture	Soil moisture	-	2
	Light	Photosynthetically active radiation	-	2
Biotic	Phenology	Number of flowering ramets	Plot	1
	Growth	Plant height	Ramet[c]	1
		Total aboveground biomass	Plot	1
		Specific leaf area	Ramet[d]	1
		Chemical composition leaves	Plot	1
	Reproduction	Seed number per ramet	Ramet[d]	1
		Seed mass	Ramet[d]	1
		Germination percentage	Plot	1

[a]Level of biotic variables: plot or ramet level; [b]Year: 1: first growing season (2008); 2: second growing season (2009); [c]Eight highest ramets per plot; [d]Ten randomly selected ramets per plot.

Ten random individuals (aboveground ramets) per plot were selected for further analysis from the harvested material. These ramets were flat oven-dried to constant mass (50 ℃). Leaf area (LA; mm^2) was measured with a Li-Cor Portable Area Meter Li-3000 (Li-Cor Biosciences, Nebraska, USA). Leaf dry matter (LDM; mg) was weighed to the nearest 0.1 mg. Specific leaf area (SLA; $mm^2.mg^{-1}$) for the ten ramets per plot was calculated by dividing LA by LDM. Finally, leaf samples were pooled per plot and analysed for total N (modified method of Kjeldahl) and total estimated C (loss on ignition, four hours at increasing temperature until 450 ℃ [%C=(100−% of ashes residue)/2]).

At the stage of seed maturity (8 May 2008), we collected the achenes (referred to as *seeds*) of 10 randomly chosen ramets per plot. Seeds were considered mature when natural dispersal began (Baskin & Baskin 1998), i.e. when seeds fell easily from the plant. The seeds were air-dried at room temperature for two to three weeks. For each ramet, all seeds were counted, weighted (to the nearest 0.1 mg) and total number of seeds and mean seed mass (total seed mass/total number of seeds) were calculated. Per plot, 20 to 50 seeds (number was dependent on the availability) were randomly distributed in three Petri dishes lined with moist filter paper and placed in temperature-controlled incubators. Since *A. nemorosa* seeds germinate in autumn (Mondoni *et al.* 2008), seeds were given 8 weeks warm stratification (23 ℃) and subsequent cold stratification (10 ℃). Filter papers were kept moist with distilled water during the experiment. The number of germinated seeds (seeds with emerged radicle) was recorded weekly for 16 weeks after transfer to 10 ℃ until germination had ceased in all dishes.

6.2.6 In-depth study OTCs–GF

To give a well documented judgment on the OTCs–GF, an in-depth study of these open-top chambers was performed to gain insight in other possibly co-varying abiotic conditions. Therefore, eleven OTCs–GF and five control plots (all characteristics of plots and OTCs were identical to the 2008 design) were installed in a similar site *ca.* 100 m from the site where the first growing season measurements were performed (measurements from 17 September 2008 to 8 May 2009; see Chapter 7 for more information on this experiment).

Soil temperature (5 cm depth), soil surface temperature (in the litter layer; 0 cm depth), air temperature (10 cm above ground level), relative air humidity (10 cm above ground level; %) and soil moisture (0–5 cm depth) were measured at 40 minutes intervals between 17 September 2008 and 8 May 2009 inside two randomly selected OTCs–GF and two control plots using Decagon EHT RH/Temperature, ECT Temperature and EC-5 Soil Moisture probes connected to Decagon Em50 dataloggers. Furthermore, changes in light quantity and CO_2 concentration were quantified. Photosynthetically active radiation (PAR) was measured with a Spectrum Technologies (Illinois, USA) PAR 3668 sensor connected to a Watchdog 425 datalogger at a 1 min interval for five days in February 2009 (before canopy flush). The sensors were placed randomly within the OTCs and control plots. A Bruël & Kjaer (Veenendaal, The Netherlands) Multigasmonitor Type 1302 was used to measure CO_2 concentration in the five OTCs–GF and control plots on 7 April 2009 (before canopy flush) at 10 cm above ground level (**Table 6.1**).

6.2.7 Data analysis

Phenological advancement of height growth was analysed by a Repeated Measures General Linear Model (GLM) with time as within-subjects factor and treatment as between-subjects factor. Flowering phenology from OTCs and controls within one time period was compared using a Kruskal-Wallis test.

To compare characteristics of *A. nemorosa* (see **Table 6.1**) between the OTCs and control plots, their effect size was calculated as the natural logarithm of the ratio of the experimental mean to the control mean (according to Arft *et al.* 1999; Hedges *et al.* 1999):

$$E = \frac{\overline{x}_{OTC}}{\overline{x}_{control}}$$

with E = effect size and $\overline{x}$ = means of response variables for the OTCs and control plots. Use of the natural logarithm linearizes the metric ratio (it treats deviations in the numerator the same as deviations in the denominator) and provides a more normal sampling distribution in small samples (Hedges *et al.* 1999).

In the case of germination percentage, an adapted effect size E' was calculated as

$$E' = \frac{\overline{x}_{OTC} + 1}{\overline{x}_{control} + 1}$$

since some OTC plots had zero germination percentage.

Biotic data available on the ramet level (**Table 6.1**) were first tested on assumptions of normality and homogeneity of variances and then analysed with a GLM with *plot* as block factor nested within treatment (three levels: OTC+GF, OTC–GF and control) and a Bonferroni post-hoc test. Because of limited sample size, effect sizes of data available on plot level (**Table 6.1**) were analysed with a Wilcoxon signed rank test. All analyses were performed with `SPSS 15.0` and `S-Plus 8.0`.

6.3 Results

6.3.1 Abiotic effects first growing season

Between 1 February and 8 May 2008, average air and soil surface temperature were higher in the OTCs compared to the control plots: in the OTCs–GF, average air and soil temperature were +0.06 °C and +0.28 °C higher, while in the OTCs+GF, air and soil temperature increased with +0.44 °C and +1.19 °C (**Table 6.2**). Daytime relative humidity was +10.6 % [95 % confidence interval: 7.2–14.1 %] and +31.0 % [27.9–34.2 %] higher in the OTC–GF and the OTC+GF plots, respectively, compared to the control plots in April 2008 (for all time periods: Kruskal-Wallis $\chi^2 > 9.57$, $d.f. = 2$, $p < 0.01$). Cumulative precipitation decreased with 13.4 % in the rain gauges covered with GF compared to the open rain gauges (Wilcoxon rank-sum statistic $Z = 1.964$, $n = 3$, $p = 0.050$). Considering the fact that the variability of the precipitation flux in our study plot ranges between 40 and 80 % of the open-field precipitation (Staelens *et al.* 2006), this 13 % decreased precipitation flux in the OTC+GF-plots falls within the natural internal variation of our study plot.

6.3.2 Biotic effects

Both open-top chamber types altered some aspects of the growth and reproduction of *A. nemorosa*. While flower emergence was not affected, flower senescence was accelerated in the OTC+GF treatment compared to OTC–GF and the control plots (**Fig. 6.1**). Plant height was significantly increased ($F = 5.88$, $d.f. = 14, 208$, $p < 0.001$) in both OTC treatments compared to the controls (**Fig. 6.2**). Total aboveground dry matter (TADM; signed-rank statistic $Z = -0.53$, $p = 0.593$ and $Z = 0$, $p = 1$, respectively for OTCs+GF and OTCs–GF), specific leaf area (SLA; $F = 0.784$, $d.f. = 2, 9$, $p = 0.485$), leaf N, leaf C and leaf C:N ratio (all $Z < 1.34$; $p > 0.180$) did not change significantly in both OTCs in comparison to the controls (**Fig. 6.3**). A negative effect on number of seeds per individual in the OTCs+GF

Table 6.2: Mean and monthly air temperature (T_{Air}; ℃) at 10 cm above ground level, soil surface temperature ($T_{Surface}$; ℃) in the litter layer and soil temperature (T_{Soil}; ℃) at 5 cm depth below the soil surface in the control plots between 1 February and 8 May 2008 and 17 September 2008 and 8 May 2009 in the Aelmoeseneie forest (left). Mean and monthly differences between open-top chambers without GardenFleece (OTC–GF) vs. the control plots and between open-top chambers covered with GardenFleece (OTC+GF) vs. the control plots are shown in the other columns. Positive numbers there denote a temperature increase in the OTCs.

	Control (℃)			OTC–GF (℃)			OTC+GF (℃)	
	T_{Air}	$T_{Surface}$	T_{Soil}	T_{Air}	$T_{Surface}$	T_{Soil}	T_{Air}	$T_{Surface}$
Feb-08	7.69	7.28	-	1.16	1.05	-	0.97	1.08
Mar-08	6.37	6.55	-	0.04	0.26	-	0.60	1.32
Apr-08	9.01	8.68	-	-0.52	-0.04	-	0.19	1.14
May-08	12.41	11.81	-	-0.20	-0.22	-	0.26	0.89
Average	8.06	7.92	-	0.06	0.28	-	0.44	1.19
Sep-08	11.3	10.50	11.69	0.31	0.68	0.77	-	-
Oct-08	8.20	7.75	9.74	0.38	0.68	0.98	-	-
Nov-08	4.95	4.89	7.03	0.45	0.77	1.15	-	-
Dec-08	1.39	1.71	4.01	0.39	1.00	1.37	-	-
Jan-09	0.37	0.72	2.06	0.24	0.51	1.50	-	-
Feb-09	3.55	3.58	3.48	0.19	0.02	1.29	-	-
Mar-09	6.79	6.84	6.03	0.72	0.05	1.31	-	-
Apr-09	10.38	10.25	10.07	0.38	0.11	0.68	-	-
May-09	12.06	11.03	10.88	0.12	0.07	0.40	-	-
Average	5.48	5.44	6.20	0.38	0.41	1.15	-	-

($F = 9.95$, $d.f. = 2, 9.15$, $p = 0.005$) was detected, while seed mass ($F = 0.64$, $d.f. = 2, 9.05$, $p = 0.550$) and germination percentage ($Z = -1.604$, $p = 0.109$) were not significantly influenced (**Fig. 6.4**).

6.3.3 In-depth study OTCs–GF

Temperature differences between the OTCs–GF and control plots in the second study period were comparable to the first growing season and increased from the air (+0.38 ℃) over the surface (+0.41 ℃) to the soil temperature (+1.15 ℃). As in the first growing season, (i) temperature differences between the OTCs–GF and controls decreased after canopy flush, and (ii) temperature differences increase from the air temperature, over the soil surface temperature to the soil temperature at 10 cm depth (**Table 6.2**; see also **Table C.1** in **Appendix C** for measurements of a third growing season for the experiments described in Chapter 7).

Continuous RH measurements in the OTCs–GF gave a slight increase of +1.2 % RH in the OTCs–GF compared to the control plots in the second growing season. Photosynthetically active radiation (PAR) decreased by −7.5 % (from an average of 67.4 μmol.m^{-2}.s^{-1} to 62.4 μmol.m^{-2}.s^{-1}, respectively) in the OTCs–GF compared to the control plots before

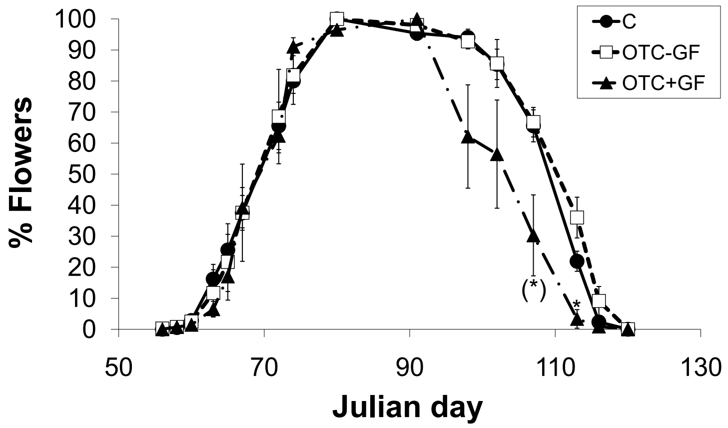


Figure 6.1: Influence of open-top chamber installation on phenological advancement of *A. nemorosa*'s flowers expressed as the percentage of flowering ramets at a particular date to the maximum number of flowering ramets per plot (mean ± S.E.) in the control (C) and open-top chambers covered with (OTC+GF) and without (OTC–GF) GardenFleece. Significance levels from Kruskal-Wallis test.

canopy flush. The concentration of CO_2 gas in the OTCs–GF was not significantly different from the CO_2 concentration in the control plots (466.4 ppm in the control plots vs. 470.0 ppm in the OTCs–GF; Wilcoxon's $Z = -1.273$, $n = 5$, $p = 0.203$). Furthermore, volumetric soil moisture was +1.6 % higher in the OTCs–GF compared to the control plots (18.1 vol % vs. 16.5 vol %, respectively).

6.4 Discussion

6.4.1 Temperature elevation in OTCs

Mean air, soil surface and soil temperature were found to increase with 0.06 °C to 1.2 °C in OTCs. The temperature increase in the OTCs–GF compared to the control plots is highest in the leafless period (February and March 2008 and October 2008 to March 2009), whereas the OTCs+GF maintained a similar temperature elevation after canopy flush. Although the IPCC (2007) predicts a median increase in annual temperature of +3.2 °C by 2080-2099 in northern Europe, this increase will only be reached over a century. Hence, an abrupt increase of 3 °C is more unrealistic than an increase of 1 °C. Comparable temperature elevations were reported by Sager & Hutchinson (2005) for OTCs placed in a deciduous forest dominated by sugar maple (*Acer saccharum* Marsh.) in Oregon, Canada, wherein air temperature (5 cm height) rose with 0.4–0.9 °C and soil temperature (5 cm depth) with 0.2–0.7 °C between May and September. Several studies, however, report on significantly higher temperature elevations when OTCs are placed in open-field ecosystems. Musil *et al.* (2005) installed OTCs in arid African quartz-fields and measured +5.5 °C average warming. Also Marion *et al.* (1997) report

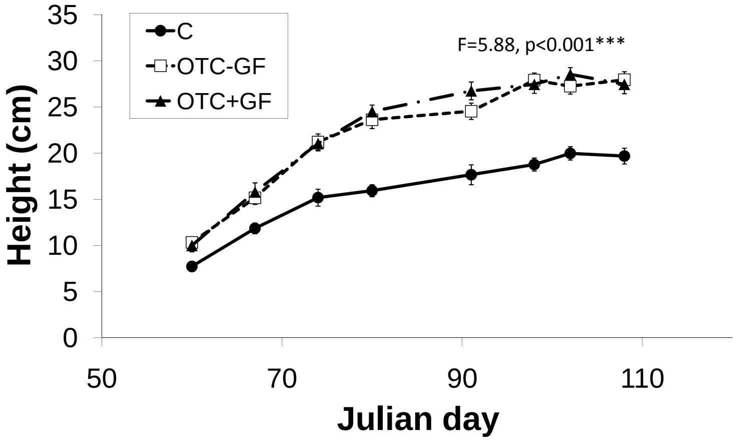


Figure 6.2: Influence of open-top chamber installation on height growth (mean ± S.E.) with time (Julian day) of *A. nemorosa* per plot in the control (C) and open-top chambers covered with (OTC+GF) and without (OTC–GF) GardenFleece. Significance level from Repeated Measures GLM.

an average temperature elevation of 1.2-1.8 ℃ in OTCs placed in tundra ecosystems around the world. In German alpine meadows, Kudernatsch *et al.* (2008) measured an increase of 0.7-1.4 ℃ for air temperature and 0.2-0.8 ℃ for soil temperature. The apparently lower temperature enhancements in forest ecosystems after canopy flush compared to open-field ecosystems are probably due to specific characteristics of forests: (i) the overstorey produces shade, (ii) causes spectral shifting of penetrating solar radiation (Daws *et al.* 2002) which lowers the radiation-capturing capacity of the polycarbonate OTCs, (iii) tempers average wind speed making the OTCs less effective and (iv) moist litter layers furthermore tend to heat slower than for example dry African quartz-fields or mineral arctic soils (see also Sager & Hutchinson 2005; Yin *et al.* 2008).

6.4.2 Response of *A. nemorosa* to OTC installation

Several global meta-analyses and warming experiments document significant advancements of spring events such as flowering and fruiting (Fitter *et al.* 1995; Dunne *et al.* 2003; Parmesan & Yohe 2003; Sherry *et al.* 2007). In the present study, however, we did not observe a phenological acceleration of *A. nemorosa* flowers in the first spring after experimental warming was started. However, this is likely due to the fact that OTCs were installed in February, only a few weeks before the first *A. nemorosa* shoots emerged (*vs.* Chapter 7).

The accelerated flower senescence in the OTCs+GF plots cannot be attributed to the absence of pollinators by the GF barrier (see further), since it is known that pollination accelerates senescence and cell death in the petals of flowers (Hadfield & Bennett 1997). The earlier senescence may be caused by two other (co-)occuring factors: (i) temperature elevation leads

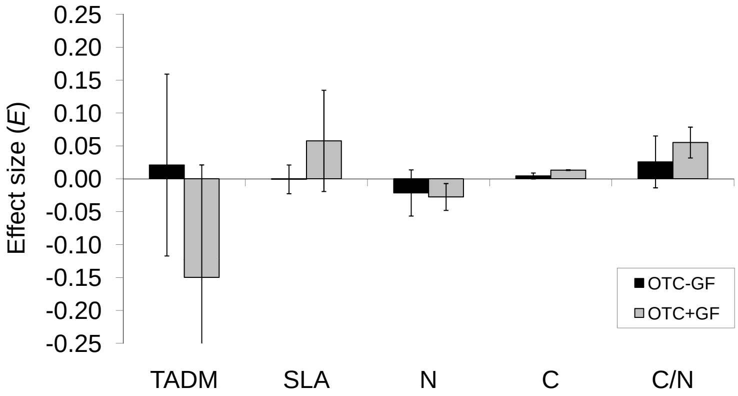


Figure 6.3: Influence of open-top chamber installation on growth and chemical composition of the leaves of *A. nemorosa* (mean effect size ± S.E.). Total aboveground biomass (TADM), specific leaf area (SLA), leaf nitrogen (N), leaf carbon (C) and C:N ratio of *A. nemorosa* in the open-top chambers covered with (OTC+GF) and without (OTC–GF) GardenFleece compared to the control. No significant differences.

to faster development and senescence of flowers (Rubinstein 2000) and (ii) relative air humidity elevation in the OTCs+GF may have caused growth of fungi and petal senescence is often hastened by wounding of the flower parts (Rubinstein 2000).

Both temperature treatments induced similar height growth of *A. nemorosa*, which might be caused by the fact that soil temperature at −5 cm increased more than soil surface temperature in open OTCs. This is likely important for geophytes such as *A. nemorosa*. These results are consistent with other warming experiments that showed plant species to grow better or higher when there are no other limiting factors (nutrients, water, light, etc.) (e.g. Parsons *et al.* 1994; Totland & Nylehn 1998; Arft *et al.* 1999; Kudo & Suzuki 2003; Danby & Hik 2007; Yin *et al.* 2008; Lin *et al.* 2010).

Specific leaf area nor leaf C, N or C:N ratio were affected in both treatments. Earlier studies found both increased (Loveys *et al.* 2002; Yin *et al.* 2008) and stable SLA (Suzuki & Kudo 1997; Lemmens *et al.* 2006) for different plant species following warming manipulations (for an overview, see Poorter *et al.* 2009). Despite the fact that individuals grew taller (see above), TADM (plot level) was not affected in both treatments as was the case in subarctic tundra vegetation warmed with OTCs in Abisko (Sweden) (Parsons *et al.* 1994). This might be caused by size-density compensation (Totland & Nylehn 1998).

To conclude: the vernal forest geophyte *A. nemorosa* showed a phenotypic response in certain plant traits to the rise in temperature before canopy flush. However, in this study we did not investigate a later flowering species. There is also a potential to use this experimental warm-

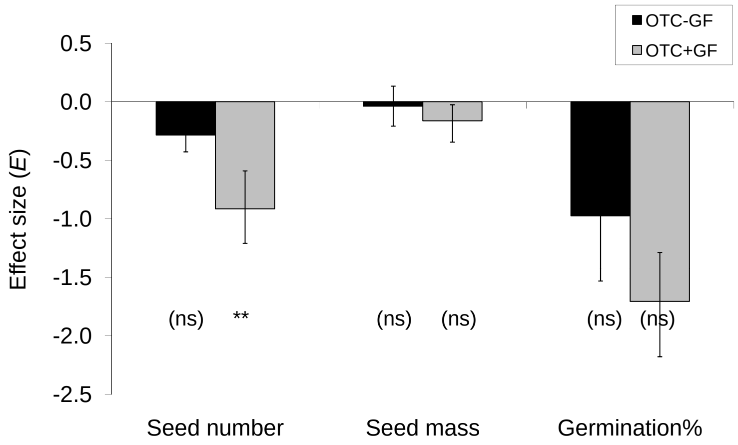


Figure 6.4: Influence of open-top chamber installation on reproduction of *A. nemorosa*. Seed number per individual, seed mass and germination percentage (%) (mean effect size ± S.E.) of *A. nemorosa* in the open-top chambers covered with (OTC+GF) and without (OTC–GF) GardenFleece compared to the control plots. Significance levels from Wilcoxon test for germination percentage (plot level) and Bonferroni post-hoc test for seed number and seed mass (ramet level).

ing method to study later flowering species, but this needs further research as temperature differences decreased after canopy flush and hence OTCs–GF comprehend only a *pre-canopy flush* warming treatment.

6.4.3 Biotic drawbacks of OTCs

Since *A. nemorosa* flowers are mostly self-incompatible and pollinated by hive bees (*Apis mellifera* L.) and other small insects (Shirreffs 1985), the GF imposed a near-impossibility of pollination of flowers. The only possible ways of pollination in the OTCs+GF were occasional (i) selfing, (ii) wind cross-pollination or (iii) insect cross-pollination. Probably because of this phenomenon, plants grown in the OTCs+GF had a lower germination percentage.

The design of the present study makes it difficult to distinguish the effect of selfing from warming on regeneration processes. Richardson *et al.* (2000) studied the presence of Hemiptera inside and outside OTCs in a subarctic tundra ecosystem in Abisko (Sweden) and reported a significant barrier effect through which the density of Hemiptera was reduced. They also suggest that insects present on plants in the chamber may prolong their stay relative to insects on plants outside chambers. If this would also be the case in our study, and given the fact that genetic variation within *A. nemorosa* is small (Stehlik & Holderegger 2000) on the spatial scale of our plots (*ca.* $1\,\mathrm{m}^2$), pollination by insects in our OTCs is presumably within genetically related individuals. It is generally accepted that self-fertilization in (mostly) self-incompatible plant populations causes an inbreeding depression (in e.g. germination percentage) in comparison to outcrossing (Brys *et al.* 2003; Busch 2005). Open-top chambers with GF totally exclude pollinators and herbivores. Hence, the lower seed number and germination percentage in the OTC+GF plots (mostly selfed individuals) is an indication that this might have happened. The lower germination percentage in the OTCs–GF may therefore also be, at least partly, caused by self-fertilization effects, which hampers distinguishing of direct warming effects. Since *A. nemorosa*'s seed production and germination growing along a 2400 km latitudinal gradient from northern France to northern Sweden showed a strong positive response to increased temperature (Chapters 4 and 5), the results obtained from the present OTC study will probably be biased by this selfing problem. Hence, quantifying this selfing effect inside open-top chambers — not only in forests, but also in other ecosystems (Richardson *et al.* 2000) — should be the scope of future research.

6.4.4 Abiotic drawbacks of OTCs

Open-top chambers seem very promising and useful in studying warming effects on early flowering herbaceous understorey plants in temperate deciduous forests. Although the OTCs covered with GF appeared to be more effective in manipulating temperature than the open OTCs, we prefer the latter for two main reasons: (i) temperature increase was significant and realistic and (ii) abiotic and biotic drawbacks are smaller in OTCs–GF than in OTCs+GF.

1. The open OTCs increased mean monthly temperature up to +1.5 ℃ in the leafless period, which is during shoot emergence and vegetative growth of early flowering understorey plants. The IPCC (2007) also predicts a higher median temperature rise in December, January and February (+4.3 ℃) than in June, July and August (+2.7 ℃) for northern Europe by 2080-2099 compared to 1980-1999.

2. The open OTCs changed other abiotic conditions (RH, CO_2 concentration, PAR, soil moisture, precipitation, etc.) only to a small extent. Relative air humidity was increased with only 1.2 % (measurements 2009; this chapter) or 0.4 % (measurements 2008–2010; **Table C.1**) in the OTCs–GF plots. RH changes have a significant influence on plant growth and development (Leuschner 2002; Lendzion & Leuschner 2007, 2008). In the case of the OTCs–GF, however, RH elevations are limited, what makes us suppose that forest understorey plants are not or only marginally influenced, also since these plant species are already adapted to humid environments and small fluctuations in humidity under natural conditions (Leuschner 2002; Lendzion & Leuschner 2007). In the OTC+GF, however, RH was increased with 31.0 % (discrete daytime values; 2008) adding a major drawback to this type of OTCs. Other abiotic variables (PAR, CO_2 concentration and soil moisture) showed no or only small differences between the OTCs–GF and controls.

6.4.5 Are OTCs useful warming devices in forest ecosystems?

A certain warming device may be considered *useful* when it fulfills the following criteria: (i) it increases temperatures adequately and in a realistic manner and (ii) ecological abiotic and biotic drawbacks of the warming device are limited. According to our opinion, both criteria are fulfilled in case of the OTCs–GF. Furthermore, *A. nemorosa* responded clearly to installation of both OTC types. Hence, we recommend the use of OTCs–GF to gain insight into the response of early flowering forest understorey plants to global warming — if done so with caution and taking the ecological drawbacks into consideration, especially in connection with reproduction. In this way open-top chambers can become a valuable tool for predicting the potential response of particularly early flowering forest plants to climate warming in temperate forest ecosystems. There is also a potential to use this method for later flowering species, but this needs further research.

7

Temperature effects on understorey plants assessed by warming and transplant experiments along a latitudinal gradient

After: De Frenne P, Brunet J, Shevtsova A, Kolb A, Graae BJ, Chabrerie O, Cousins SAO, Decocq G, De Schrijver A, Diekmann M, Gruwez R, Heinken T, Hermy M, Nilsson C, Stanton S, Tack W, Willaert J, Verheyen K (2011) Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Global Change Biology*, in press.

Abstract

Slow colonising forest understorey plants are probably not able to rapidly adjust their distribution range following large-scale climate change. Therefore, the acclimation potential to climate change within their actual occupied habitats will likely be key for their short- and long-term persistence. Here, we assessed the effects of temperature on phenology, growth and reproductive performance of multiple populations of slow colonising understorey plants, using the spring flowering geophytic forb *Anemone nemorosa* and the early summer flowering grass *Milium effusum* as study species. In both species, emergence time and start of flowering clearly advanced with increasing temperatures. Vegetative growth (plant height, aboveground biomass) and reproductive success (seed mass, seed germination and germinable seed output) of *A. nemorosa* benefited from higher temperatures. Climate change may thus increase future competitive ability and colonisation rates of this species. Apart from the effects on phenology, growth and reproductive performance of

M. effusum generally decreased when transplanted southwards (e.g. plant size and number of individuals decreased towards the south) and was probably more limited by light availability in the south. Specific leaf area of both species increased when transplanted southwards, but decreased with open-top chamber installation in *A. nemorosa*. In general, individuals of both species transplanted at the home site performed best, suggesting local adaptation. We conclude that contrasting understorey plants may display divergent plasticity in response to changing temperatures which may alter future understorey community dynamics.

7.1 Introduction

Climate change is considered to be one of the major threats to biological diversity (Butchart *et al.* 2010). The global average earth surface temperature has increased by 0.8 °C between 1956 and 2005 (NASA 2010) and is expected to increase by an additional 1.8–4.0 °C until 2090–2099 relative to 1980–1999 (IPCC 2007). Such temperature increases induce a latitudinal and altitudinal advance of the isotherms (Hansen *et al.* 2006), thereby moving the bioclimatic envelope of many species (recent review: Walther 2010).

Range shifts have been documented primarily for habitat generalists, pioneers or species with a short life cycle that disperse relatively easily (Warren *et al.* 2001; Lenoir *et al.* 2008; le Roux & McGeoch 2008). Slow colonising species, on the other hand, may not be able to rapidly extend their distribution range (Parolo & Rossi 2008; Engler *et al.* 2009; Vittoz *et al.* 2009), an effect which is further aggravated by large-scale habitat loss and fragmentation (Honnay *et al.* 2002b; Leimu *et al.* 2010; Chapter 9). Many herbaceous forest understorey plants are known to have extremely low colonisation rates, as documented both in Europe and in North America (Brunet & von Oheimb 1998a,b; Cain *et al.* 1998; Verheyen *et al.* 2003c). They are generally unable to colonise isolated habitat patches even in relatively densely forested landscapes (Chapter 9) and thus acclimation or adaptation (*sensu* Magnani 2009) to a warmer climate within their current distribution range will likely be important for their short- and long-term persistence (Honnay *et al.* 2002b). Due to the significance of forest understorey plant species for forest biodiversity (Gilliam 2007), it is important to determine to what extent they will be able to respond to climate change in the near future.

Plant responses to rising temperatures vary between species with different phenologies (Sherry *et al.* 2007; Kudo *et al.* 2008) and life forms (Dormann & Woodin 2002; Walker *et al.* 2006). Sherry *et al.* (2007), for instance, found that tallgrass prairie plant species that flower before the summer heat peak, tended to advance their phenology in response to warming, whereas later flowering species delayed their phenology. Also Kudo *et al.* (2008) suggested that climate change will have the largest impact on bee-pollinated spring flowering understorey plants. On the other hand, graminoids generally show more plasticity to resource alterations such as nitrogen enrichment and temperature increases than forbs (Dormann & Woodin 2002; Walker *et al.* 2006; Hollister & Flaherty 2010; De Schrijver *et al.* 2011).

Potential plant responses to global warming can be examined using multiple approaches, including observational investigations along environmental gradients (e.g. Alexander *et al.* 2009; Wilson & Nilsson 2009), reciprocal transplantations (e.g. Kollmann & Bañuelos 2004; Haggerty & Galloway 2011), or experiments with warming facilities (e.g. Walker *et al.* 2006; Shevtsova *et al.* 2009). The specific advantages and shortcomings that are associated with each of these approaches have been discussed in section 1.3 and are shown in **Table 1.1**. For instance, environmental gradients allow the assessment of long-term ecosystem responses by using a space-for-time substitution, although the concurrent abiotic and biotic changes over space can be difficult to disentangle (e.g. correlated latitudinal temperature patterns with other latitudinal clines such as photoperiod). Moreover, directional selection may have favoured plant ecotypes adapted to local environmental conditions (Leimu & Fischer 2008). To overcome these problems associated with spatial gradients, common garden transplant experiments (e.g. see the pioneering work of Clausen *et al.* 1940) offer the opportunity to relocate ecotypes from a colder region into a warmer region with a longer growing season (or vice versa; e.g. Haggerty & Galloway 2011) and can be used to separate phenotypic plasticity and local adaptation across a species' range (Macel *et al.* 2007; Magnani 2009). However, transplant experiments always create some degree of disturbance (e.g. due to soil mixing or transplantation stress). Experimental warming devices such as open-top chambers or infrared heaters allow for the study of warming effects in a more controlled manner compared to gradient studies, but typically generate relatively short-term data, often disturb biotic interactions, and may alter abiotic conditions such as CO_2 concentration or soil moisture content (Richardson *et al.* 2000; Aronson & McNulty 2009). Therefore, combining multiple approaches should provide a better assessment of the likely response of plants to global warming across a range of environments.

Here, we assessed the effects of temperature on phenology, growth and reproductive performance of multiple populations of slow colonising understorey plants. We selected the spring flowering geophytic forb *Anemone nemorosa* and the early summer flowering grass *Milium effusum* as study species. We combined common garden transplant experiments in three sites along a 2300 km latitudinal gradient with an open-top chamber experiment in a single site which allowed us to determine variation in the plastic response of understorey plants to temperature and long-term adaptation to the local environment (Magnani 2009). Divergent responses of the study species are to be expected due to their contrasting phenology (early vs. later flowering) and life forms (forb vs. grass). We specifically addressed (i) how the contrasting study species responded to temperature variations, (ii) whether southward transplantation and experimental warming resulted in a similar response and (iii) whether home transplants performed better than away transplants.

7.2 Methods

7.2.1 Study species

Two species were specifically selected for this study: *Anemone nemorosa* L. (Ranunculaceae) is a spring flowering geophytic forb, and *Milium effusum* L. (Poaceae) is an early summer flowering hemicryptophytic grass. More information on the species is given in sections 2.2.1 and 3.2.1.

7.2.2 Experimental design

We conducted a common garden transplant experiment in three sites along a latitudinal gradient and applied an experimental warming treatment in one of the common gardens. Both seeds and adult individuals were used as source material to be able to study temperature effects on recruitment through sexual (germination and seedling performance) and vegetative reproduction (adult plant performance) and due to the slow recruitment of *A. nemorosa* into the adult life stage. Source material was collected in 2008 in two populations in each of seven (*A. nemorosa*) or eight (*M. effusum*) regions located along an approximately 1900–2300 km latitudinal gradient from northern France to northern Sweden, and transplanted into three common gardens near Gontrode (Belgium), Alnarp (southern Sweden), and Umeå (northern Sweden; **Fig. 7.1**). This resulted in a total of 14 and 16 source populations for *A. nemorosa* and *M. effusum*, respectively. All source populations were located in deciduous forests that were unaffected by recent major disturbances and that were at least 1 km apart in each region. Within each population of each species, the seeds of 15 randomly chosen individuals were collected at the time of seed maturity and pooled. The seeds of each population were then sown into 40 ml pots (20 *A. nemorosa* seeds and 40 *M. effusum* seeds per pot) containing standard potting soil (see further for specifications) to avoid seed desiccation. Simultaneously, rhizome fragments of adult individuals of *A. nemorosa* and whole root lumps of adult individuals of *M. effusum* were collected and stored in shade in containers with soil from the collection sites. In September 2008, when all populations of both species were sampled along the gradient, the seed-soil mixtures and adult individuals (two 7.5 cm long *A. nemorosa* rhizome fragments or one *M. effusum* root lump per pot) were transferred into 1.5 l-pots containing standard potting soil and transplanted into the three common gardens in a randomized block design ($n = 4$ replicates per species, population and establishment method [seeds or adult material]). The total sample size was thus 112 pots for *A. nemorosa* (4 replicates × 2 populations × 7 regions × 2 establishment methods) and 128 pots for *M. effusum* (4 × 2 × 8 × 2) in each of the three transplant sites. The *A. nemorosa* pots in southern and northern Sweden and the *M. effusum* pots at all sites were randomly placed in one large block per site. The *A. nemorosa* pots in Belgium were randomly distributed across five control blocks to have a similar spatial distribution of the pots as in the experimental warming treatment (see below). The pots were dug into the soil so that the top edge of the pots was level with the soil surface. The potting soil (Peltracom NV, Overpelt, Belgium) consisted of a 2:1 volume mixture of peat and coconut fibers that resembled litter-rich forest soil and had the following characteristics: pH-KCl of 5.5, ammonium acetate-EDTA extractable P of 0.35 mg.kg^{-1} dry soil, and KCl extractable NO_3^- of 2.68 mg.kg^{-1} dry soil. A patterned release 15:8:11:2.5 N:P:K:Mg fertilizer containing trace elements (Osmocote Exact Lo.Start 16–18M, Scotts International BV, Geldermalsen, the Netherlands) was added at an application rate of 6 g fertilizer per liter potting soil at the beginning of the experiment. The common gardens were located in sites similar to deciduous forest ecosystems characteristic of that specific latitude in terms of tree species composition and overstorey canopy cover (Chapter 2): (i) in Belgium, the pots were placed in a mixed ash (*Fraxinus excelsior*), pedunculate oak (*Quercus robur*) and beech (*Fagus sylvatica*) forest with 89.9 % (± 0.4 S.E.) canopy cover; (ii) in southern Sweden, the common garden was situated in a mixed pedunculate oak and beech forest with 85.0 % (± 1.4) canopy cover; (iii) in northern Sweden, the common garden was installed in the experimental garden at the campus of Umeå University with 28.5 % (± 17.1) canopy cover with an overstorey consisting of *Salix* sp., *Betula pubescens* and *Sorbus* sp. The percentage canopy cover was determined from hemispherical fish-eye photographs taken with a Nikon D70S with a Sigma 8 mm fisheye lens after canopy flush in May–June 2009 and subsequent calculations in Gap Light Analyser Version 2.0 (`www.ecostudies.org/gla/`; $n = 3$ per common garden).

Second, an experimental warming treatment was used to examine the direct effects of increased temperature on *A. nemorosa*. In the southernmost common garden (Belgium, **Fig. 7.1**), the above-described experimental design of *A. nemorosa* (for both seeds and adult individuals, again: $n = 4$ replicates, or 112 pots in total) was replicated inside eleven hexagonal open-top chambers (OTCs). OTCs are small plexiglas greenhouses with inclined walls and an open top that passively heat a small vegetation plot. Passive OTCs have frequently been used in Arctic or (sub)alpine open-field ecosystems (e.g. Walker *et al.* 2006) but less often in deciduous forests (e.g. Sager & Hutchinson 2005; Chapter 6). The OTCs resembled those from the International Tundra Experiment (Walker *et al.* 2006), were 60 cm high and covered a surface area of 1.15 m^2 (Chapter 6). Commonly observed abiotic side effects (in terms of altered precipitation, soil moisture, relative humidity, photosynthetically active radiation, and CO_2 concentration inside the OTCs) have been shown to be negligible in deciduous forests (Dabros *et al.* 2010; Chapter 6). Due to the large size of adult *M. effusum* individuals (plant height of up to 160–180 cm; Chapter 2), OTCs could not be applied to this species. Detailed information on the design, installation and performance of the OTCs is provided in Chapter 6. The OTCs remained in place throughout the experiment.

Finally, to monitor the air temperature and relative humidity at 10 cm height above the soil surface, the temperature of the litter layer at the soil surface and the soil temperature at 5 cm below the soil surface, two microclimate Em50 dataloggers (Decagon Inc., Pullman, WA,

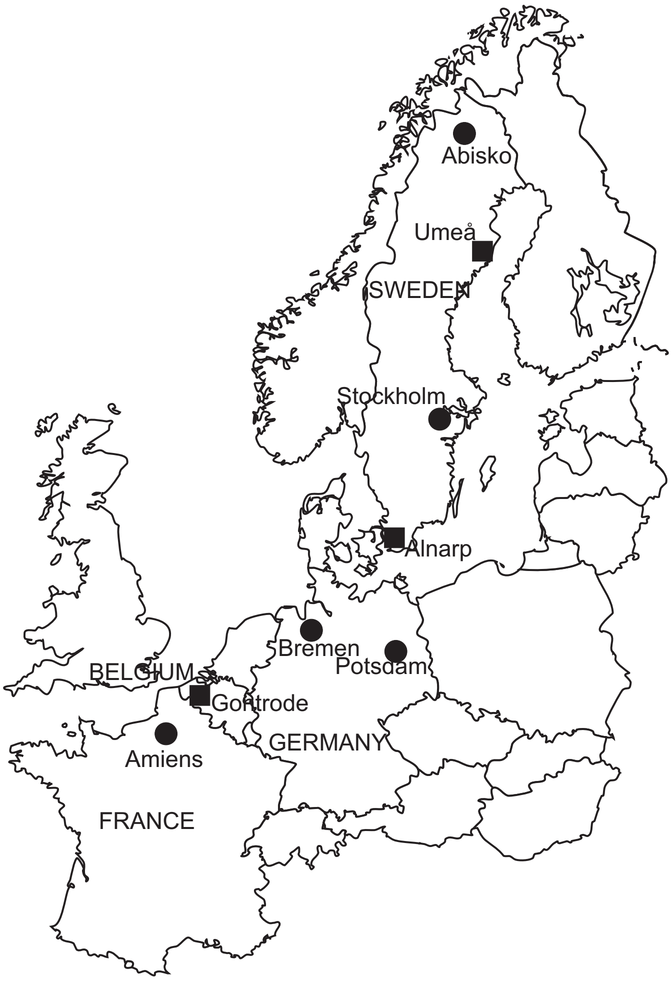


Figure 7.1: Location of the source populations of *Anemone nemorosa* and *Milium effusum* in seven or eight regions, respectively, along a 1900–2300 km latitudinal gradient from northern France to northern Sweden (circles and squares; excluding Abisko for *A. nemorosa*) as well as of the three common gardens (squares).

USA) each consisting of three temperature sensors and one relative humidity sensor were installed in each of the three common gardens and logged at two-hourly intervals. In the Belgian common garden, two additional dataloggers were installed in two randomly selected OTCs. The mean air temperature during the course of the experiment (Sept. 2008 – July 2010) was 8.6 ℃ in Belgium, 8.1 ℃ in southern Sweden and 3.5 ℃ in northern Sweden (see detailed microclimatic data in **Table C.1** in **Appendix C**). The average (Sept. 2008 – July 2010) temperature inside the OTCs was passively increased by 0.2 ℃ at 10 cm height, by 1.0 ℃ at the soil surface and by 1.6 ℃ at 5 cm depth in the soil compared to the ambient controls in the same common garden (**Table C.1**). The largest warming occurred in the soil (probably due to the buffering effect and dark colour of the moist litter layer in forests)

Figure 7.2: *A. nemorosa* in an open-top chamber in the Belgian common garden

and during winter and spring before tree canopy flush (see also Sager & Hutchinson 2005; Chapter 6). The effect of experimental warming was especially evident in the accumulated heat sum: growing degree-hours above 5 ℃ between 1 January and 15 June at 10 cm height, for instance, were 16,850 ℃.hour and 16,250 ℃.hour in the ambient controls and 19,625 ℃.hour and 17,885 ℃.hour in the OTCs in 2009 and 2010, respectively in Belgium (**Table C.1**).

7.2.3 Measurements of phenology and fitness-related life-history traits

Several life-history traits were measured during two consecutive growing seasons (2009 and 2010) in both transplant treatments (further referred to as *adult (individuals)* versus *seeds*). They include a wide range of traits that each can have an impact on plant performance and competitive ability (e.g. plant height, biomass, number of individuals) and colonisation rates (reproductive traits such as number of seeds, seed mass and germination) of these species. For each adult individual (as emerged from the transplanted rhizomes or root lumps), the day of emergence (the date when the first leaf was completely unfolded; only in *A. nemorosa*) and the start of flowering (the date when the anthers were visible) were assessed *ca.* every three days at the beginning of each growing season. At the time of seed maturity (monitored *ca.* every three days), we counted the total numbers of adult and fruiting individuals, measured the height of each adult individual and collected its seeds to determine the total number of seeds as well as mean seed mass. Plant height was measured as the maximal height of the foliage (following Cornelissen *et al.* 2003). We also measured the height of the inflorescences of *M. effusum*. In *A. nemorosa*, we counted the total number of seeds per individual and determined the mean air-dry seed mass as the total seed mass per individual (weighed to the nearest 0.1 mg) divided by the total number of seeds. In *M. effusum* (which produces up to 200–300 seeds per individual), all seeds and a random subsample of 50 seeds were weighted per individual. The number of seeds per individual was then calculated as the total seed mass divided by the 50-seed mass and multiplied by 50. The mean seed mass was determined as the 50-seed mass divided by 50. In 2010, we also determined the aboveground biomass and specific leaf area (SLA) of each adult individual at the time of seed maturity, and assessed the germination percentage of the seeds produced by each adult individual. Accordingly, all aboveground parts were harvested, oven-dried (50 ℃) for three days and weighed. The total biomass of each individual was estimated as the mass of all aboveground vegetative and reproductive tissue. SLA was determined on one randomly selected leaf per pot (flat dried between dry sheets of paper for three days at 50 ℃) by dividing the leaf area measured with a Li-Cor Portable Area Meter Li-3000 (Li-Cor Biosciences, Nebraska, USA) by the leaf mass.

Finally, germination trials were conducted to determine the germinable seed output of each individual. For each seed sample, one Petri dish was lined with moist filter paper, and 10–50 randomly chosen seeds that were collected in the common gardens in 2010 were distributed in each dish (the number of seeds within each dish depended on seed availability) within one month after collection. Based on earlier studies (Thompson 1980; Mondoni *et al.* 2008; Graae *et al.* 2009b), seeds were given eight weeks of warm stratification (simulating summer; 23 ℃) in temperature-controlled growth chambers before they were placed in cool stratification for six weeks (simulating autumn; 10 ℃), cold stratification for four weeks (simulating winter; 2 ℃) and cool stratification (simulating spring; 10 ℃). The number of germinated seeds (seeds with an emerged radicle) was recorded weekly until germination had ceased in all dishes. The sets of dishes were randomly rotated after each weekly recording and distilled water was supplied as necessary. The germinable seed output was calculated as the number of seeds per individual × germination fraction.

Due to their different generation times, different variables were recorded for *A. nemorosa* and *M. effusum* in the pots into which seeds had been sown. In *A. nemorosa*, the number of seedlings was counted in each pot (once in early spring and once in late spring) in each common garden and year. Germination rates were then calculated by dividing the number of seedlings by the number of seeds originally sown into each pot. In *M. effusum*, seeds germinate and usually grow into juvenile plants in the first year and reproduce in the second year. We therefore counted the number of individuals (as a proxy for the germination percentage) and measured plant height in the first year (2009), and performed exactly the same measurements as for the transplanted adult individuals in 2010.

7.2.4 Data analysis

To examine the effects of (i) the common garden transplant site, (ii) the latitude of origin of each population and (iii) the year of sampling on the phenology and fitness-related traits of both species in the three common gardens (excluding the OTCs), linear mixed-effect models were performed using the *lmer*-function of the *lme4*-library in `R 2.11.1` (R Development Core Team 2010) with maximum likelihood estimation. A similar model for the southernmost common garden was used to assess the effects of (i) warming (i.e. OTC installation), (ii) the latitude of origin of each population and (iii) the year of sampling on phenology and plant performance. *Region* and *population* nested within *region* were added to the models as random-effect terms to address the autocorrelation and hierarchy of the two populations within one region and the replicates per population, respectively. For the analyses of the *A. nemorosa* data, a random-effect term *block* was also added to the model to account for the block (ambient) and OTC effects (see §7.2.2). Model assessment followed Zuur *et al.* (2009) by starting with a full model (all predictors included), using a likelihood ratio test (χ^2 test statistic) of nested models, and dropping the least significant terms. In other words, we started with a model including the three explanatory variables (and all two-way interactions) and compared this model with a model that dropped the least significant term to test the significance of that particular variable. This procedure was repeated until all remaining terms were significant. Only the final models are presented in the results section.

In a second step of the analyses, we scrutinized the effects of the common garden transplant site and the home-away distance (quantified as the absolute value of the latitudinal difference between the latitude of origin and the latitude of the common garden transplant site [ΔLatitude]) on the phenology and fitness-related traits of both species in the three common gardens (excluding the OTCs), by using similar linear mixed-effect model assessment as above. Since the year of sampling was already analysed in the first step of the analyses, we here used year as random-effects term instead of fixed-effects term.

To meet the assumptions of the statistical tests, the data were $\log_{10}$ and arcsine square-root transformed, respectively, prior to the analyses if necessary (Quinn & Keough 2002; Zuur *et al.* 2009). For count data (e.g. number of ramets per pot), we used the Poisson family in the *lmer*-function instead of the Gaussian family (Zuur *et al.* 2009). The effect of the year of sampling could not be analysed for response variables that were only measured in the second growing season (SLA, biomass, etc.).

7.3 Results

Among all variables, the transplant site generally best explained differences in phenology, growth and reproduction of both species in the common garden experiment (**Table 7.1** and **Table 7.2**). Both emergence time and flowering phenology were delayed with increasing latitude of the transplant site (**Table 7.1**; **Fig. 7.3**). For example, compared to the southern common garden, the flowering start of adult *A. nemorosa* individuals was delayed by 15.9 and 55.8 days in 2009 and by 22.4 and 48.7 days in 2010 in the central and northernmost site, respectively. In *M. effusum*, differences in flowering phenology amounted to 25.8 and 49.5 days in 2009 and to 27.2 and 38.0 days in 2010, respectively. Plant height, number of individuals and aboveground biomass decreased from the southern to the northern common garden across all populations in *A. nemorosa*, while they increased in *M. effusum*. SLA increased from the northern to the southern common garden in both species (**Table 7.1**). Also the year of sampling and latitude of origin had significant impacts (**Table 7.1**). For example, emergence and flowering of both species initiated earlier in populations of southern origin compared to the northern populations in the southernmost common garden ('ambient' in **Fig. 7.4**), while this difference disappeared in the northern common gardens (**Fig. 7.3**); the aboveground biomass of *A. nemorosa* adults of Belgian origin amounted to 1038 mg in the Belgian common garden, but to 384 mg and 159 mg in southern and northern Sweden, respectively. Especially when the home-away distance was taken into account (i.e. the latitudinal distance between the transplant site and the site of origin), significant adaptation to the local site conditions was apparent: e.g. plant height, number of individuals, fruiting probability and aboveground biomass decreased significantly with increasing home-away distance in adult individuals of both species (**Table 7.2** and **Fig. 7.3**; see also 'ambient' in **Fig. 7.4**). Thus, individuals that were transplanted closer to the home site performed better than at the other sites or than the other populations at that site.

Experimental warming in the OTCs significantly advanced phenology and had important effects on growth and reproduction of the transplanted *A. nemorosa* adults (**Table 7.3a**). Across all populations, experimental warming advanced emergence and flowering phenology by 3.3 and 3.1 days in 2009 and by 2.2 and 4.5 days in 2010 (**Fig. 7.4a,b**). SLA and the number of seeds per individual decreased, while plant height, the percentage of fruiting individuals, seed mass and seed germination increased with experimental warming (**Table 7.3a**, **Fig. 7.4c–f**). In general, the different populations responded similarly to experimental warming; only in a few cases we found the warming × latitude of origin interaction to be significant (**Table 7.3**). For instance, warming had a larger positive effect on the height of individuals from the southern populations than on the height of individuals from more northern populations (**Fig. 7.4c**; *cf.* interaction term between common garden and warming in **Table 7.3**). Germination rates of the wild-collected seeds originally sown into the pots were not affected by experimental warming (**Table 7.3b**).

Finally, the day of leaf emergence, flowering start, plant height and germination percentage of seeds of *A. nemorosa* adults showed a similar response to increasing temperatures with experimental warming and southward transplantation, while the percentage of fruiting individuals, SLA and number of seeds displayed a different response to temperature variations when results of the experimental warming and transplant approach are compared (**Table 7.1** and **Table 7.3**). The remainder of the traits of *A. nemorosa* only responded significantly to one of the temperature manipulation methods: while the number of individuals, aboveground biomass and germinable seed output increased with southward transplantation, experimental

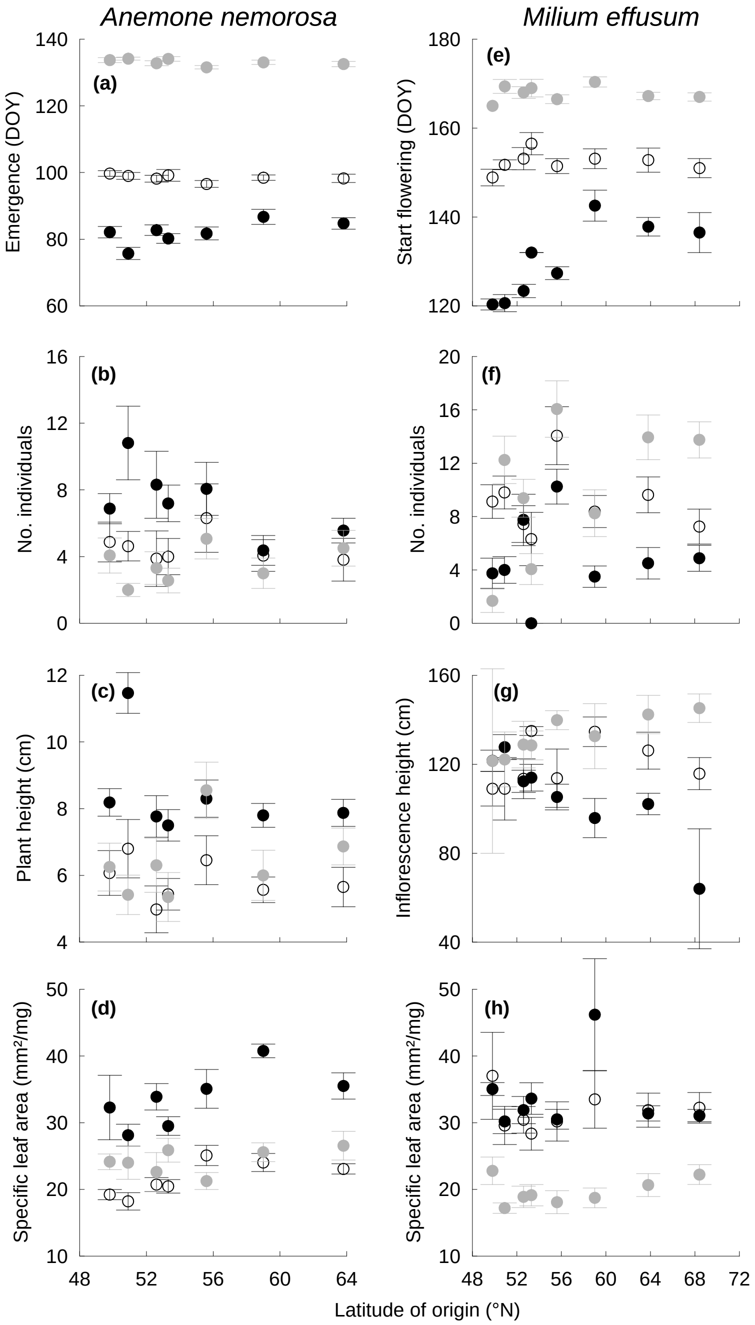


Figure 7.3: The effects of the latitude of population origin on phenology (day of emergence or start of flowering) and fitness-related traits of the transplanted adult individuals of *Anemone nemorosa* (a–d) and *Milium effusum* (e–h) collected along a latitudinal gradient and transplanted in the common garden in Belgium (black circles), southern Sweden (open circles) and northern Sweden (grey circles). The averages (± S.E.) of both years and populations per region are given for visualisation. DOY: day of the year. Significance values: see **Table 7.1**.

warming had no significant effect on these traits. Conversely, experimental warming increased seed mass of *A. nemorosa*, but this trait was unaffected by southward transplantation (**Table 7.1** and **Table 7.3**).

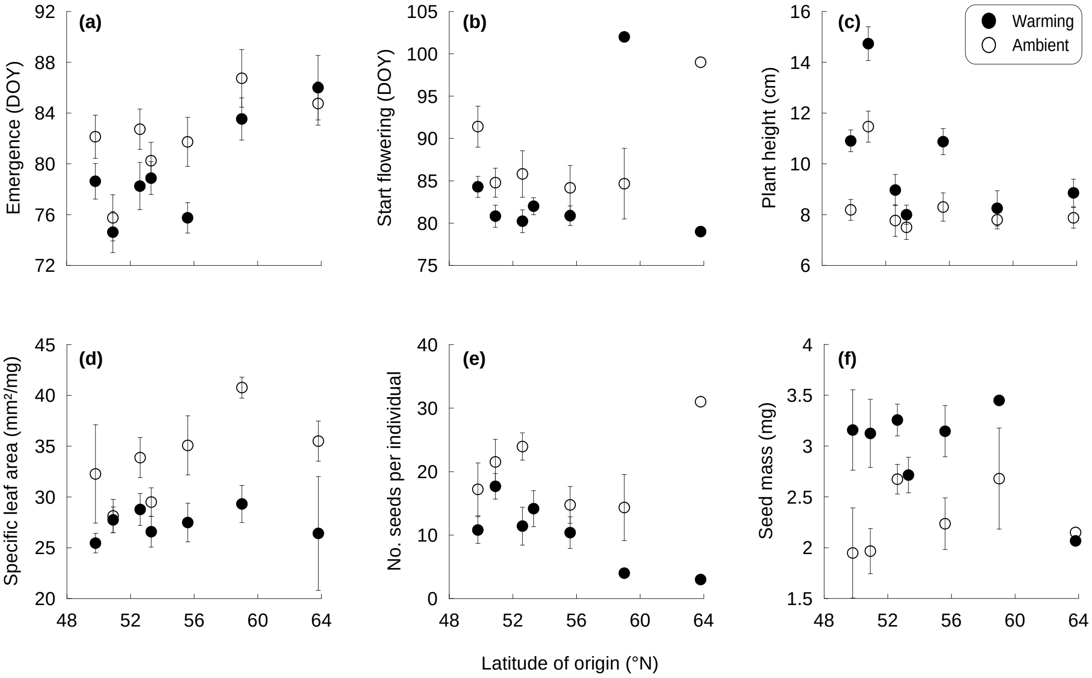


Figure 7.4: The effects of experimental warming on phenology (day of emergence and start of flowering) and fitness-related traits of the transplanted adult individuals of *Anemone nemorosa* collected along a latitudinal gradient and transplanted into the southernmost common garden (at 50.9 °N latitude). DOY: day of the year. The means (±S.E.) of both years and populations per region are shown. See **Table 7.3** for significance values using mixed-effect models that account for nesting.

7.4 Discussion

7.4.1 Response of phenology and fitness-related life-history traits to increasing temperatures

Increasing temperatures advanced emergence time and start of flowering across the investigated populations of the understorey plants *A. nemorosa* and *M. effusum*, confirming evidence from a variety of species across the globe (Parmesan 2007; Ibáñez *et al.* 2010). An earlier flowering date of *A. nemorosa* with increasing mean spring temperature was already revealed by time-series analyses (reviewed in Baeten *et al.* 2010a), but an experimental approach to corroborate this for multiple populations across its distribution was still lacking. Photoperiodic ecotypes have been described for plant species with large north–south distribution

Table 7.1: The effects of the common garden transplant site (CG), latitude of origin (Lat) and year of sampling (Yr) on phenology and fitness-related traits of (a) the transplanted adult individuals and (b) the sown seeds of *Anemone nemorosa* and *Milium effusum*. Seeds and adult individuals were sampled along a latitudinal gradient and transplanted into each of three common gardens (see Methods for details).

Anemone nemorosa		CG	Yr	Lat	CG×Yr	CG×Lat	Yr×Lat
(a)	Emergence^{a}	↓32.1***	↑66.7***	ns	30.7***	23.7***	ns
	Flowering start^{a}	↓20.3***	↑19.5***	ns	25.3***	ns	ns
	Plant height	↑12.6**	↑31.8***	ns	27.3***	ns	ns
	No. individuals^{b}	↑13.8***	↑234.1***	ns	ns	24.4***	ns
	% Fruiting individuals^{c}	↓10.5**	↑11.3***	ns	9.6**	ns	ns
	Specific leaf area	↑10.5**	-	↓6.8**	-	ns	-
	Biomass^{a}	↑6.9*	-	ns	-	ns	-
	No. seeds per individual^{a}	↑18.8***	ns	ns	ns	ns	ns
	Seed mass	ns	ns	ns	ns	ns	ns
	Germination%^{c,d}	↑6.3*	-	ns	-	ns	-
	Germinable seed output^{a}	↑14.9***	-	ns	-	ns	-
(b)	Germ.^{c} (early spring 1)	↕12.7**	-	ns	-	ns	-
	Germ.^{c} (late spring 1)	↑9.5**	-	ns	-	ns	-
	Germ.^{c} (early spring 2)	ns	-	ns	-	ns	-
	Germ.^{c} (late spring 2)	↑3.7(*)	-	ns	-	9.6**	-
Milium effusum							
(a)	Flowering start^{a}	↓342.4***	↑25.7***	↓3.9*	28.2***	71.0***	ns
	Plant height^{a}	↓24.2***	↑189.5***	ns	13.8***	12.5**	17.7***
	Inflorescence height	↓22.8***	↑10.3**	ns	15.7***	21.3***	ns
	No. individuals^{b}	↓97.2***	↑17.2***	ns	ns	58.1***	8.5**
	% Fruiting individuals^{c}	ns	↑60.0***	ns	ns	ns	ns
	Specific leaf area^{a}	↑123.2***	-	ns	-	ns	-
	Biomass^{a}	↓16.8**	-	ns	-	15.5***	-
	No. seeds per individual	↑4.8(*)	ns	↑3.4(*)	ns	31.3***	ns
	Seed mass	↓17.8***	↓14.0***	↑4.8*	9.3**	14.3***	ns
	Germination%^{c,d}	↕26.4***	-	ns	-	14.2***	-
	Germinable seed output	↕9.4**	-	ns	-	20.8***	-
(b)	Flowering start^{a}	↓327.9***	-	ns	-	26.0***	-
	Plant height^{a}	↓45.0***	↑424.4***	↑10.1**	28.4***	18.3***	ns
	Inflorescence height	↓39.3***	-	↓3.7(*)	-	11.4**	-
	No. individuals^{b,e}	↓449.4***	↑139.1***	↑11.8***	ns	41.4***	ns
	% Fruiting individuals^{c}	↓25.2***	-	↑9.9**	-	12.4**	-
	Specific leaf area^{a}	↑47.6***	-	↓3.8(*)	-	ns	-
	Biomass^{a}	↓6.9*	-	↑14.5***	-	ns	-
	No. seeds per individual^{a}	↕18.1***	-	↑5.0*	-	ns	-
	Seed mass^{a}	↕18.4***	-	ns	-	14.6***	-
	Germination%^{c,d}	↕68.5***	-	ns	-	11.4**	-
	Germinable seed output^{a}	↕40.2***	-	↑4.9*	-	10.8**	-

Legend: see p. 107

Table 7.2: Effects of the common garden transplant site and the home-away distance (i.e., the absolute value of the latitudinal difference between the latitude of origin and the latitude of the common garden transplant site; ΔLatitude) on phenology and fitness-related traits of (a) the transplanted adult individuals and (b) the sown seeds of *Anemone nemorosa* and *Milium effusum.* Seeds and adult individuals were sampled along a latitudinal gradient and transplanted into each of three common gardens (see Methods for details).

Anemone nemorosa		Common garden	ΔLatitude	Common garden × ΔLatitude
(a)	Emergence^a	↓27.8***	↑17.1***	11.1**
	Flowering start^a	↓18.7***	ns	ns
	Plant height	↑11.5**	↓7.8**	ns
	No. individuals^b	↑10.8**	↓30.1***	ns
	% Fruiting individuals^c	↓11.3**	↓5.9*	ns
	Specific leaf area	↑10.1**	ns	ns
	Biomass^a	↑5.9(*)	↓4.3*	ns
	No. seeds per individual^a	↑21.8***	ns	ns
	Seed mass	ns	ns	ns
	Germination%^{c,d}	↑6.6*	ns	ns
	Germinable seed output^a	↑15.5***	ns	ns
(b)	Germ.^{c} (early spring 1)	↕11.8**	ns	ns
	Germ.^{c} (late spring 1)	↑8.1*	↓4.7*	ns
	Germ.^{c} (early spring 2)	ns	↓5.6*	ns
	Germ.^{c} (late spring 2)	ns	↓7.5**	ns
Milium effusum				
(a)	Flowering start^c	↓365.5***	↑39.3*	26.7***
	Plant height^c	↓28.6***	↓8.9**	ns
	Inflorescence height	↓31.5***	↓12.6***	ns
	No. individuals^b	↓108.9***	↓37.8***	9.4**
	% Fruiting individuals^c	ns	↓4.1*	ns
	Specific leaf area^a	↑119.9***	ns	ns
	Biomass^a	↓23.4***	↓15.4***	ns
	No. seeds per individua^a	ns	↓18.1***	ns
	Seed mass	↓21.3***	↓7.8**	9.6**
	Germination%^{c,d}	↕32.6***	↓7.1**	ns
	Germinable seed output	↕23.5***	↓20.0***	10.1**
(b)	Flowering start^a	↓301.8***	↑12.7***	ns
	Plant height^a	↓53.9***	↓14.8***	ns
	Inflorescence height	↓45.9***	↓7.5**	ns
	No. individuals^{b,e}	↓428.6***	ns	34.0***
	% Fruiting individuals^c	↓25.0***	ns	ns
	Specific leaf area^a	↑43.3***	ns	ns
	Biomass^a	↓7.3*	↓3.6(*)	ns
	No. seeds per individual^a	↕19.0***	↓3.0(*)	ns
	Seed mass^a	↕19.5***	ns	12.1**
	Germination%^{c,d}	↕68.7***	↓3.1(*)	ns
	Germinable seed output^a	↕38.4***	ns	10.4**

Legend: see p. 107

←Legend to Table 7.1 and Table 7.2

Values and symbols are χ^2-values and significances, respectively, from likelihood ratio tests of mixed-effect models. The open-top chamber treatment was excluded from these analyses.

The direction of the effect is given: ↑ corresponds to a delay in phenology (i.e. an increase of the day of the year) or an increase in the trait value to the south (*common garden*, *latitude*), with increasing home-away distance (Δ*Latitude*) or over time (*year*), whereas ↓ corresponds to the opposite pattern. ↕ specifies mixed effects in the common gardens.

-: year is not applicable since this trait was only measured in the final growing season.

^{a}Log10 transformed. ^{b}A Poisson error distribution was applied for count data. ^{c}Arcsine sqrt-transformed. ^{d}Germination of the seeds produced in the common gardens. ^{e}Proxy for the germination of the originally sown seeds.

Table 7.3: Effects of warming with open-top chambers (OTC), latitude of origin (Lat) and year of sampling (Yr) on (a) phenology and fitness-related traits of the transplanted adult individuals and (b) on the germination of the sown seeds of *Anemone nemorosa*. Seeds and adult individuals were sampled along a latitudinal gradient, transplanted into the southernmost common garden in Belgium and subject to ambient temperatures or to experimental warming (see Methods for details).

	Trait	OTC	Yr	Lat	OTC × Yr	OTC × Lat	Yr × Lat
(a)	Emergence^a	↓6.9**	↑100.7***	↓6.2*	ns	ns	ns
	Flowering start^a	↓6.0*	↑27.8***	ns	ns	ns	ns
	Plant height^a	↑6.9**	ns	ns	ns	4.8*	ns
	No. individuals^b	ns	↑296.0***	↑5.9	ns	13.6***	6.1*
	% Fruiting individuals^c	↑4.9*	↑13.8***	↑3.5(*)	ns	ns	12.2***
	Specific leaf area	↓6.7**	-	↑3.4(*)	-	ns	-
	Biomass^a	ns	-	↑5.7*	-	ns	-
	No. seeds per individual^a	↓7.5**	↑5.9*	ns	ns	ns	ns
	Seed mass	↑13.5***	ns	ns	ns	ns	ns
	Germination%^{c}	↑5.2*	-	ns	-	ns	-
	Germinable seed output^a	ns	-	ns	-	5.4*	-
(b)	Germ.^{c} (early spring 1)	ns	-	ns	-	ns	-
	Germ.^{c} (late spring 1)	ns	-	ns	-	ns	-
	Germ.^{c} (early spring 2)	ns	-	ns	-	ns	-
	Germ.^{c} (late spring 2)	ns	-	ns	-	ns	-

Values and symbols are χ^2-values and significances, respectively, from likelihood ratio tests of mixed-effect models. The direction of the effect is given: ↑ corresponds to a delay in phenology (i.e. an increase of the day of the year) or an increase in the trait value with warming, to the south (*latitude*) or over time (*year*), whereas ↓ corresponds to the opposite (i.e. advance of the phenology or decrease of a trait value). -: year is not applicable since that trait was only measured in the final growing season. ^{a}Log10 transformed. ^{b}A Poisson error distribution was applied for count data. ^{c}Arcsine sqrt-transformed.

ranges, but generally northern ecotypes flower before southern ecotypes when transplanted in a common environment (Winn & Gross 1993; Raven *et al.* 1999; Kollmann & Bañuelos 2004). Our results suggest that flowering initiation of the two observed species is mainly plastically responsive to temperature: we detected earlier flowering of southern than northern populations in the southern common garden but no latitudinal cline in flowering initiation in the common gardens at mid and northern latitudes. We also found (i) interannual differences for both species and (ii) *A. nemorosa* to flower earlier in open-top chambers (OTCs). First, the winter and spring of 2010 were colder and longer than the winter and spring of 2009 in much of Europe, which is confirmed by the observed differences in the growing degree hours calculated from the microclimatic measurements at the transplantation sites. Because of these lower temperatures in the spring, *A. nemorosa* and *M. effusum* were forced to start flowering later in the season. Second, the phenology of *A. nemorosa* was advanced in the experimental warming treatment (OTCs). This observation allows us to separate photoperiodic from temperature effects, since day length is also known to be a major cue for growth or flowering initiation in many plant species (Raven *et al.* 1999). Moreover, the emergence time of *A. nemorosa* in forests cannot be attributed to differences in day length as the emerging shoots (and thus the photoreceptors; Raven *et al.* 1999) are situated beneath the soil surface or below a thick litter layer during winter. Together, our findings stress the temperature-sensitivity of the phenology of the study species across their distribution range.

Warming also increased the size and reproductive success of *A. nemorosa*. However, warming had a larger positive effect on the height growth of individuals from the southern populations than on individuals from more northern populations, suggesting that increasing temperatures can have a differential impact within the distribution range of this species. The size of *M. effusum*, on the other hand, decreased towards the south. While *A. nemorosa* is a spring flowering geophyte and completes its life cycle almost entirely before canopy flush, the early summer flowering *M. effusum* grows and flowers below a dense tree canopy. The common gardens in the present study displayed a decreasing canopy cover from 89.9 % in Belgium, over 85.0 % in southern Sweden to 28.5 % in northern Sweden (comparable to the latitudinal decrease of the canopy cover in natural populations of these species; Chapter 2). Hence, we argue that northerly increasing light availability related to lower canopy cover and longer photoperiod in the growing season probably drives the latitudinal cline in plant size of *M. effusum* (*cf.* Jonasson & Widerberg 1988), suggesting that *M. effusum* is limited more by light availability than by temperature. Further research is needed to elucidate whether this differential response of species with contrasting phenology and life form to variation in temperatures is a widespread phenomenon. Based on the results of an earlier study in which adult plants of *A. nemorosa* were sampled *in situ* along a latitudinal gradient, significant positive effects of increasing temperature on reproductive output were determined, but it remained unclear whether these patterns were due to phenotypic plasticity or local adaptation along the latitudinal gradient (Chapters 4 & 5). Here we stressed the plastic increase of *A. nemorosa*'s seed mass, germination percentage and germinable seed output with increasing temperatures (these traits showed a significant positive response with at least one warming method).

7.4.2 Local adaptation

Even though transplantation or experimental warming induced plastic phenotypic responses, local adaptation was apparent. Local adaptation enables populations to perform better when transplanted within their home site (Joshi *et al.* 2001; Leimu & Fischer 2008). Since plants were transplanted into comparable pots, differences in life-history traits among common gardens cannot be attributed to soil characteristics or competition effects. The adaptation is thus probably to the local climate and light regime (see also Macel *et al.* 2007). Given that differentiation between low- and high-latitude populations is likely adaptive (i.e. improvement of plant fitness, for instance by growing taller), we may also expect evolutionary responses to climate change in the future (e.g. Haggerty & Galloway 2011). However, the existence of local adaptation during the past does not necessarily suggest that adaptation to rapid climate change will take place: the rate of adaptive evolution of many species (especially perennials with a long life span) is likely slower than the predicted rate of climate change (reviewed in Jump & Peñuelas 2005; but see Jump *et al.* 2008). The plasticity of these species to rising temperatures (established by experimental warming or southward transplantation) is also subject to evolution, and is considered adaptive when plasticity in these life-history traits improves plant fitness (Matesanz *et al.* 2010).

7.4.3 Southward transplantation vs. experimental warming

While the plant height and reproductive success of *A. nemorosa* generally increased both with increasing temperatures at more southern latitudes and under experimental warming, e.g. SLA and the number of seeds of *A. nemorosa* showed contrasting results when compar-

Figure 7.5: *A. nemorosa* from Germany in the common garden in Umeå (left) and Belgian *A. nemorosa* rhizomes in the Belgian common garden (right)

ing their responses to temperature in the two approaches. At first we observed SLA, a plant trait central to growth and ecological strategy (Poorter *et al.* 2009), to decrease under experimental warming in the open-top chambers but to increase when plants were transplanted southwards. The concurrent changes with southwards transplantation (especially related to light availability) will likely have inverted the response of SLA to temperature, as irradiance is one of the primary determinants of SLA (Poorter *et al.* 2009). Second, we observed a lower number of seeds per individual in the OTCs, while the seed number increased with southward transplantation. Previous research has shown that the density of insects is generally lower inside OTCs, while the duration of the insects' visit may increase (Richardson *et al.* 2000). A disturbed pollinator abundance and behaviour might be the cause for the observed lower seed number in the OTCs. In natural populations of clonal plants, pollination on the scale of a chamber then presumably occurs within genetically related individuals (Stehlik & Holderegger 2000; Chapter 6). In this experiment, however, genotypes from different populations across Europe were randomly mixed within a single chamber (and in the control blocks), reducing possible inbreeding depression effects on seed mass and germination. There was also no trade-off between the number of seeds and seed mass (data not shown). The higher seed mass and germination of *A. nemorosa* in the OTCs can thus likely be attributed to warming effects.

7.4.4 Possible impacts of climate warming

The combination of possible future changes in phenology, growth and reproduction of understorey forest plants may have important implications for co-adaptations between species, competitive interactions, linkages with the overstorey, colonisation rates as well as forest ecosystem biodiversity and functioning in general (Gilliam 2007; Baeten *et al.* 2010a). First, we clearly showed that both study species will most likely advance their phenology in response to climate warming (*cf.* Parmesan 2007; Ibáñez *et al.* 2010). Hence, we speculate that specifically in forest ecosystems, these phenological advances may alter the *phenological escape*, i.e. the discrepancy between overstorey and understorey phenology, with potential feedbacks on understorey productivity (Routhier & Lapointe 2002; Neufeld & Young 2003) if leaf unfolding of trees responds at a slower or faster rate (Menzel 2003). Secondly, we showed that not all understorey plants respond equally to temperature variation, which may alter understorey community composition and dynamics in the future. *A. nemorosa* is considered to be a relatively stress tolerant species that dominates the understorey of many ancient European deciduous forests during several weeks in the spring (Shirreffs 1985). Hence, if its height and thus competitive ability for light would increase (Neufeld & Young 2003) in the future and if smaller associates such as *Adoxa moschatellina*, *Viola riviniana*, *V. reichenbachiana*, *Hepatica nobilis* and *Ranunculus ficaria* or even tree seedlings would less benefit from climate warming, these species might increasingly be unable to establish below the dense *A. nemorosa* carpet due to competition for light (Pigott 1982; Neufeld & Young 2003). Furthermore, the accumulating evidence of the significant positive effects of temperature on the reproductive output of *A. nemorosa* established here extends the findings from the observational Chapter 5. This makes us hypothesize that the persistence of *A. nemorosa* populations and the colonisation potential into unoccupied habitats might increase in the future. Besides the observed direct effect on plant reproduction and growth, climate warming might also indirectly influence plant growth through its effect on nitrogen (N) availability. Atmospheric deposition has resulted in altered ecosystem N cycling (Lu *et al.* 2011) and large amounts of stored soil organic N in forests (Dörr *et al.* 2010). A larger fraction of this organic N can become available for plant growth if the predicted climate warming increases soil N mineralization rates (Rustad *et al.* 2001). Elevated N bioavailability can then result in altered growth depending on the deposition legacy of each respective region. This hypothesis certainly deserves further research.

7.4.5 Conclusions

Nesting experimental manipulations within a larger biogeographic framework can be useful to understand future ecosystem functioning in response to environmental change (Dunne *et al.* 2004; Rustad 2008). Using an innovative approach by combining transplant experiments along a latitudinal gradient with experimental warming, we have shown that integrating these techniques allows for a better understanding of the potential effects of global warming on plant species. Although our results clearly indicate that the phenology of multiple populations across the distribution range of the investigated understorey species will probably plastically advance with increasing temperatures in the future, the effects on growth and reproductive performance were species-dependent. Generally, individuals transplanted at the home site performed best and were thus locally adapted. While climate warming may increase the vegetative and reproductive performance of the spring flowering forb *A. nemorosa*, the early summer flowering grass *M. effusum* was probably more limited by light availability along the gradient. Hence, if these divergent responses of understorey plants differing in phenology and life form are a widespread phenomenon, this could alter future forest understorey dynamics.

8

Latitudinal variation in transgenerational plasticity in seedlings of temperate understorey perennials

After: De Frenne P, Graae BJ, Brunet J, Shevtsova A, De Schrijver A, Chabrerie O, Cousins SAO, Decocq G, Diekmann M, Hermy M, Heinken T, Kolb A, Nilsson C, Stanton S, Verheyen K. Latitudinal variation in transgenerational plasticity in seedlings of temperate understorey perennials. *Oecologia*, submitted.

Abstract

Determining the acclimation potential of species is necessary for forecasting their likely response to environmental change. Little is known, however, about variation in phenotypic plasticity to different temperatures of plant seedlings across species' ranges despite the importance of sexual recruitment and seedling establishment for population persistence and community assembly. We quantified phenotypic plasticity and examined adaptation in seedlings of two slow colonising understorey perennials (*Anemone nemorosa* and *Milium effusum*) along a latitudinal gradient in Europe. First, we calculated the plasticity of seedlings grown from seeds from natural populations subjected to three spring warming scenarios. Next, we compared the functional traits of seedlings grown from seeds from common gardens with those from natural populations. Performance of the seedlings (in terms of biomass) decreased with the latitude of origin irrespective of whether seeds were wild-collected or in the common gardens. The emergence percentage, on the other hand, decreased with increasing distance between the latitude of origin and the transplant site, which suggests that the maternal plants were best adapted to their home-environment resulting in higher seed quality. There was a latitudinal cline of increasing plasticity in

M. effusum seedling biomass but not in *A. nemorosa* seedling biomass nor in specific leaf area of both species. Higher plasticity in the north in *M. effusum* seedling growth is likely adaptive and may thus offer an important way to cope with climatic changes. Together, our findings stress the importance of intraspecific variation in transgenerational plasticity and local adaptation on seedling performance across the distribution range of species.

8.1 Introduction

Phenotypic plasticity is the ability of a genotype to change its phenotype in response to changes in the environment. Such plasticity may be considered a bet-hedging strategy of organisms to cope with environmental variation (recent review: Matesanz *et al.* 2010). Phenotypic plasticity is believed to be particularly important for perennial plants experiencing climate change as (i) species with high phenotypic plasticity are better suited to withstand the changing climate (Bradley *et al.* 1999; Bret-Harte *et al.* 2001; Valladares 2008) and (ii) the specific life-history traits of perennial plants together with large-scale habitat fragmentation in contemporary landscapes strongly limit their potential for fast migration (Öster *et al.* 2009; Chapter 9) and genetic evolution (Davis & Shaw 2001; Jump & Peñuelas 2005; Svenning *et al.* 2008; but see Maron *et al.* 2004; Kramer *et al.* 2010). Therefore, slow colonising plants can be expected to require a certain amount of phenotypic plasticity to survive in a changing climate. Since phenotypic plasticity is also subject to evolution as other functional traits, it is considered adaptive when plasticity in these life-history traits improves plant fitness (Matesanz *et al.* 2010).

Since recruitment from seeds is one of the most sensitive phases of the life cycle of plants, climate change will likely affect gamete and embryo development, seed set, germination rates and seedling survival (Hedhly *et al.* 2009; Walck *et al.* 2011). Hence, determining the phenotypic plasticity of seedling growth is critical for forecasting the likely response of populations and communities to climate change. However, little is known about intraspecific variability in the degree of plasticity to temperature across a species' range (but see Black-Samuelsson & Andersson 1997). For instance, alpine or northern (sub)arctic populations have to cope with more climatic and microclimatic variability and a more pronounced small-scale mosaics of environments than populations of the same species in a temperate climate or lowland region (Armbruster *et al.* 2007; Scherrer & Körner 2010; Graae *et al.* 2011). Thus, it can be hypothesized that seedlings in northern or alpine populations possess more plasticity to respond to such microclimatic environmental heterogeneity as it is known that plants growing in heterogeneous environments generally possess greater plasticity (reviewed in Matesanz *et al.* 2010). Black-Samuelsson & Andersson (1997), however, found no relationship between the biogeographical location (core vs. northern periphery of the range) of populations of two *Vicia* species and the degree of phenotypic plasticity to temperature.

Phenotypic plasticity of seedlings across a species' range can be examined by raising offspring from natural populations in different environments, but also by comparing responses of offspring from mother plants grown in the wild and in common gardens. From the former, the phenotypic plasticity of seedlings to temperature can be examined (following Valladares *et al.* 2006) across the range of a species. The latter approach allows examining the role of plasticity and adaptation: Magnani (2009) recently summarised the possible responses of plant functional traits to environmental variation along geographic transects (*in situ*) and at the site of seed origin in common garden experiments (*ex situ*) in a synthetic scheme (see also Kawecki & Ebert 2004). A parallel response of the plant trait under study *in* and *ex situ* demonstrates long-term adaptations to the local conditions. In other words, when the functional trait value increases or decreases with an environmental driver both in the wild and in the common garden, this indicates directional selection. A contrasting response, on the other hand, suggests genetic control over phenotypic plasticity (genetic × environment interaction). Finally, a constant pattern (slope = 0) of the functional trait values of plants grown in the common garden demonstrates pure phenotypic plasticity (i.e. acclimation to the local site conditions). Unravelling the relative effect of plasticity vs. adaptation and, subsequently forecasting the likely effects of future climate change is only possible by combining the *in situ* and *ex situ* records, and of high ecological and evolutionary significance (Magnani 2009).

Given that we considered the seedling stage, we actually studied maternal environmental effects (reviews: Roach & Wulff 1987; Donohue 2009). Since the environment where a mother plant grows, flowers, and sets seeds co-determines the phenotype of the offspring, the developmental contingency of later life stages upon earlier stages is a special kind of phenotypic plasticity of the offspring (Donohue 2009). For instance, growth responses to elevated concentrations of carbon dioxide (CO_2) in the air diverge between the progeny of mother plants grown in elevated versus ambient CO_2 conditions (Bezemer *et al.* 1998; Lau *et al.* 2008; but see Teng *et al.* 2009). Also, the growth response to drought differs between progeny of mother plants grown in dry versus moist soils (Sultan *et al.* 2009). Maternal environmental effects can result in altered plasticity to the offspring conditions (e.g. Latzel *et al.* 2010) and might be adaptive if this plasticity improves fitness of the progeny (Rohde & Junttila 2007; Bischoff & Müller-Schärer 2010; Matesanz *et al.* 2010).

Here, we investigated phenotypic plasticity of two perennial slow colonising forest understorey plants. We followed a transgenerational approach and determined the emergence percentage, biomass and specific leaf area of seedlings grown in growth chambers from *in situ* seeds (collected from mother plants growing in natural populations along a latitudinal gradient) and from *ex situ* seeds (collected from mother plants growing in three common gardens along the same gradient). We chose specifically these functional life-history traits since emergence percentage and seedling biomass are determinants of plant fitness, while specific leaf area is a plant trait central to growth and ecological strategy (Westoby *et al.* 2002; Poorter *et al.* 2009). Hence, these traits reflect both maternal and seedling plant fitness. Additionally, to assess the degree of phenotypic plasticity to temperature, we combined this gradient with a warming experiment by growing the *in situ* seeds in three temperature scenarios and calculating the amount of phenotypic plasticity to temperature (Valladares *et al.* 2006). We explicitly addressed the hypothesis that the magnitude of phenotypic plasticity to temperature of seedlings increases with latitude of origin, which would enable these seedlings to cope with a more pronounced small-scale mosaics of environments in the north. Finally, we integrated the *in situ* and *ex situ* data to disentangle the relative effects of acclimation vs. adaptation, following the synthetic scheme of Magnani (2009).

8.2 Methods

8.2.1 Study species

Two species were specifically selected for this study: *Anemone nemorosa* L. (Ranunculaceae) is a spring flowering geophytic forb, and *Milium effusum* L. (Poaceae) is an early summer flowering hemicryptophytic grass. More information on the species is given in sections 2.2.1 and 3.2.1.

8.2.2 Latitudinal gradient and *in situ* seed collection

In situ seeds were collected in 2008 in seven (*A. nemorosa*) and eight (*M. effusum*) regions located along an approximately 1900 km (*A. nemorosa*) to 2300 km (*M. effusum*) latitudinal gradient from northern France via Belgium and Germany to northern Sweden (**Fig. 7.1**). We selected two populations of each species in each region, resulting in a total of 14 *A. nemorosa* and 16 *M. effusum* populations. Within each region, the populations were selected within an area of *ca.* $20 \times 20\,\mathrm{km}^2$. All populations occurred in deciduous forests with no major recent disturbances in terms of management, grazing, etc. Within each population, the seeds of 15 randomly chosen individuals were collected and pooled. The region- and species-specific seed collection dates are shown in **Appendix D**.

8.2.3 *Ex situ* seed collection

Adult individuals (i.e. rhizome fragments in the case of *A. nemorosa* and whole root lumps in the case of *M. effusum*) were collected in each of the two populations in the seven or eight regions along the latitudinal gradient and transplanted in three common gardens near Gontrode (Belgium), Alnarp (southern Sweden) and Umeå (northern Sweden) (**Fig. 7.1**). The maternal plants for the *ex situ* study (as well as for Chapter 7) originated from the same populations as for the *in situ* study. The adult individuals were planted in pots filled with standard potting soil. Similar pots, potting soil, fertilizer mixture and experimental design were used in all the three common gardens in Belgium, southern and northern Sweden. The pots with both species were placed in a randomized design in forest ecosystems characteristic of the region where the common garden was established. More information on the design of the common gardens is provided in section 7.2.2. Only the transplanted adult individuals were used in this chapter. The *ex situ* seeds were collected in 2009 in each of three common garden sites; again, the exact collection dates were site-dependent (**Appendix D**) and seeds were pooled per population × common garden combination. *A. nemorosa* had to be omitted from the *ex situ* analyses because individuals produced no or only few seeds in the common gardens in 2009 (of 56 individuals in each common garden, only eight produced seeds in Belgium, none in southern Sweden and four in northern Sweden).

8.2.4 Growth-chamber experiment: seed sowing

The *in situ* and *ex situ* seeds were subsequently handled in exactly the same way in the growth-chamber experiments. Seeds of *A. nemorosa* were warm stratified (21 ℃) in moistened white sand to avoid desiccation and viability loss (Ali *et al.* 2007) between seed collection and the start of the experiment (*ca.* 12 weeks). Distilled water was added when needed to the sand-seed mixture. Seeds of *M. effusum* were stored in dry air until sowing as short-term desiccation poses no threat to viability of *M. effusum* seeds (Thompson 1980). On 1 September 2008 (*in situ*) and 1 September 2009 (*ex situ*), the seeds of both species (in *A. nemorosa* only *in situ* seeds) were sown in containers filled with potting soil (same soil-fertilizer mixture as above in the common gardens). The containers were subdivided into 28 cells of 17 cm^3 each. In each cell, ten seeds were sown. Each population (2009) and population × common garden combination (2010) was randomly assigned to a cell (seven replicates per population and per treatment). The containers were then placed in temperature- and light-controlled growth chambers (Sanyo MLR-351 Versatile Environmental Test Chamber; Osaka, Japan) and underwent the same seasonal cycle in both years (starting on 1 September): (1) autumn simulation at 10 ℃ for eight weeks (continuous light), (2) winter simulation at 2 ℃ for 11 weeks (8 h light, 16 h darkness photoperiod) and (3) early spring simulation at 10 ℃ for two weeks (8 h light, 16 h darkness). During the following six weeks in the *in situ* experiment, the containers were randomly assigned to three different growth chambers programmed to represent three possible spring scenarios across the species' range: cool (10 ℃), intermediate (15 ℃) or warm (20 ℃; always 12 h light, 12 h darkness). In the *ex situ* experiment, the containers were placed in a growth chamber at an intermediate 15 ℃ spring scenario (12 h light, 12 h darkness). The number of growing degree hours >5 ℃ between 1 January and the end of the experiment (GDH, following Lindsey & Newman 1956) calculated for the three scenarios amounted to 6960, 12240 and 17520 ℃.hours, respectively. These values correspond to values obtained in the species' natural habitats between northern France and northern Sweden (seed maturity of *A. nemorosa* and *M. effusum* occurs at *ca.* 8,000 to 12,000 ℃.hours and *ca.* 12,000 to 18,000 °C.hours, respectively; Chapter 2). Thus, provenances were grown in environments similar to their natural habitats. Photosynthesis photon flux density in the centre of the growth chambers was always set to 112 μmol.m^{-2}.s^{-1} when light; darkness was 0 μmol.m^{-2}.s^{-1}. Throughout the experiment, the containers were rotated weekly in the growth chambers and watered as necessary.

8.2.5 Measurements

All seedlings were harvested on 10–12 March 2009 (*in situ* seeds) and 11–12 March 2010 (*ex situ* seeds). Newly emerged seedlings (defined as shoot emergence) were recorded weekly from the start of the experiment and the final emergence percentage was calculated as the number of seedlings at the end of the experiment divided by the seed input. One whole leaf per cell was chosen randomly for both species and used for the determination of the specific leaf area (SLA). Fresh leaf area was immediately measured using a JVC TK-5310 camera with Cosmicar 12.5 mm lens connected to a computer and a Digital Image Analysis System (Decagon Devices Inc., Pullman, USA) in 2009 and with a Li-Cor Portable Area Meter Li-3000 (Li-Cor Biosciences, Nebraska, USA) in 2010 following procedures described in Cornelissen *et al.* (2003). The rest of the aboveground biomass of that cell was harvested together. All biomass was dried for 48h at 60 °C and weighted; subsequently, the aboveground biomass per cell was divided by the number of seedlings to determine the biomass per seedling. Finally, SLA was calculated by dividing the leaf area by the leaf dry matter.

8.2.6 Data analysis

The effects of latitude of origin and temperature treatment (*in situ*) or of latitude of origin and common garden site (*ex situ*) on the functional seedling traits were analysed with linear mixed-effect models, using the *lme*-function of the *nlme*-library in `R 2.11.1` (R Development Core Team 2010). *Region* and *population* nested within *region* were added to the models as random-effect terms to account for potential autocorrelation of populations from the same region and replicates within populations. The effects of the latitudinal home-away distance (i.e. the absolute value of the latitudinal difference between the latitude of origin and the latitude of the common garden transplant site; ΔLatitude) was analysed with a similar mixed-effect model. In a second step of the analyses, we compared the latitudinal clines in functional seedling traits of *M. effusum* in the *in situ* experiment (only the 15 °C treatment for comparability with the *ex situ* experiment) and in each common garden with a mixed-effect model with *latitude* as predictor variable and *region* and *population* nested within *region* as random-effect terms. To improve normality and homoscedasticity, emergence data were arcsine square-root transformed and biomass and SLA data were log$_{10}$-transformed (except SLA data for *A. nemorosa*) prior to the analyses.

Next, to quantify the amount of phenotypic plasticity in biomass and SLA response to temperature of the *in situ* seeds, the simplified relative distance plasticity index ($\mathrm{RDPI}_{\mathrm{s,k}}$) for population k was adapted from Valladares *et al.* (2006):

$$RDPI_{s,k} = \sum \frac{\mid x_j - x_{j'} \mid}{x_j + x_{j'}} / n_1$$

with x_j and $x_{j'}$ representing the value of the functional trait in temperature treatment j and temperature treatment j', respectively; and n_1 representing the total number of tested environments (i.e. three temperature scenarios). The $\mathrm{RDPI}_{\mathrm{s}}$ varies between zero and one, representing a population possessing no or an infinitely high amount of phenotypic plasticity. The same linear mixed-effect model analyses were also performed for the $\mathrm{RDPI}_{\mathrm{s}}$ with *latitude* as a predictor and *region* and *population* nested within *region* as random effects. The plasticity to spring temperature was not calculated for the emergence percentage as germination generally occurs already in autumn or early spring (Thompson 1980; Graae *et al.* 2009) and was expected to depend little on the spring temperature treatment.

8.3 Results

Aboveground seedling biomass across temperature treatments decreased in the seedlings from the southern to the northern populations for both species in the *in situ* experiment (**Table 8.1**; **Fig. 8.1e**). Unsurprisingly, seedling biomass consistently increased with the spring temperature (**Table 8.1**; **Fig. D.1** and **Fig. D.2** in **Appendix D**). Emergence and SLA decreased and increased, respectively, with latitude of origin in *M. effusum* (**Fig. 8.1a,i**), but not in *A. nemorosa* (**Fig. D.1**). Moreover, SLA decreased with increasing temperatures in *A. nemorosa*, and increased in *M. effusum.* No interactions between latitude and temperature were significant for *A. nemorosa*, but significant interaction terms were present for *M. effusum* which indicates a differential response to temperature depending on the latitude of seed origin (**Table 8.1**). The phenotypic plasticity ($\mathrm{RDPI}_{\mathrm{s}}$) to temperature of biomass and SLA of *A. nemorosa* seedlings showed no significant linear latitudinal pattern (**Fig. 8.2a,c**). However, the *in situ M. effusum* seedlings of northern origin displayed a significant 4.9-fold higher plasticity in biomass growth than the southern populations (difference between average plasticity of the southernmost and northernmost region, respectively; **Fig. 8.2b**). Finally, also no latitudinal cline in plasticity in SLA was present in *M. effusum* (**Fig. 8.2d**).

In the second experiment, *ex situ M. effusum* seedlings showed a significant response to common garden transplant site of the mother plant (**Table 8.2a**). Likewise to the *in situ* experiment, aboveground seedling biomass decreased significantly from the southern, French populations to the northernmost Swedish populations (**Table 8.2a**; **Fig. 8.1e–h**). The emergence percentage was significantly higher for the seeds produced by the mother plants that were transplanted closer to the home-site (i.e. with lower ΔLatitude), suggesting local

Table 8.1: The effects of latitude of seed origin, spring temperature treatment and their interaction on seedling traits of *in situ* collected seeds of (a) *Anemone nemorosa* and (b) *Milium effusum* along a latitudinal gradient

	Latitude		Temperature		Latitude × Temperature	
(a) *A. nemorosa*	D.f.	F-value	D.f.	F-value	D.f.	F-value
Emergence	5	0.58 ns	267	↕7.28**	267	0.55 ns
Biomass	5	↓15.52*	200	↑3.96*	200	0.13 ns
Specific leaf area	5	0.09 ns	200	↓10.07***	200	0.05 ns
(b) *M. effusum*						
Emergence	6	↓16.13**	334	1.05 ns	334	3.47*
Biomass	6	↓6.93*	324	↑35.05***	324	28.13***
Specific leaf area	6	↑27.76**	326	↑60.13***	326	8.84***

Directions of the effect are only reported for the main effects and if $p < 0.1$: ↓ and ↑ indicate a negative and positive effect, respectively. In the case of the temperature scenarios, ↕ specifies mixed effects (i.e., a negative or positive effect for the 15 °C or 20 °C scenario compared to the 10 °C scenario).

Table 8.2: Effects of (a) latitude of origin of the mother plant, common garden transplant site and their interaction and (b) the latitudinal home-away distance (i.e. the absolute value of the latitudinal difference between the latitude of origin and the latitude of the common garden transplant site; ΔLatitude), common garden transplant site and their interaction on seedling traits of *ex situ* seeds of *Milium effusum* collected in three common garden transplant sites along a latitudinal gradient

(a)	Latitude		Common garden		Latitude × Common garden	
	D.f.	F-value	D.f.	F-value	D.f.	F-value
Emergence	5	0.03 ns	152	14.03***	152	20.04***
Biomass	5	↓19.31**	145	8.33***	145	1.78 ns
Specific leaf area	5	3.89 ns	145	3.51*	145	0.76 ns
(b)	ΔLatitude		Common garden		ΔLatitude × Common garden	
	D.f.	F-value	D.f.	F-value	D.f.	F-value
Emergence	151	↓32.62***	151	13.95***	151	1.80ns
Biomass	144	0.09ns	144	8.83***	144	0.44ns
Specific leaf area	144	1.97ns	144	3.44*	144	0.23ns

Direction of the effects of Latitude and ΔLatitude if $p < 0.1$: ↓ and ↑ indicate a negative and positive effect, respectively.

adaptation (**Table 8.2b**; **Fig. 8.1b–d**). This effect was, however, not present in the seedling biomass and SLA. The slope of the relationship between the functional traits and latitude was significantly different from zero in the seedlings resulting from *in situ* seeds (15 °C only) and in the seedling biomass from seeds sampled in the central common garden (**Fig. 8.1**).

8.4 Discussion

For many adult plants, common garden experiments have shown that individuals from northern populations grow smaller or produce less aboveground biomass than individuals from

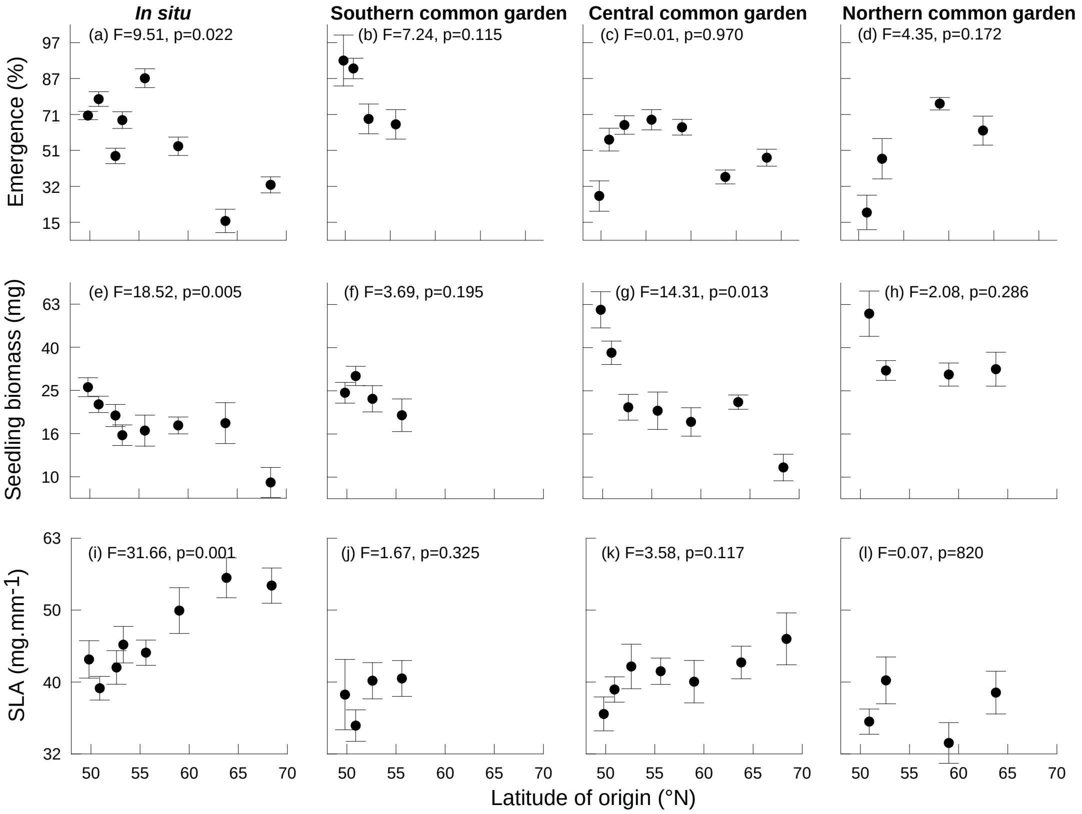


Figure 8.1: Emergence percentage, biomass and specific leaf area (SLA) of seedlings of the understorey forest plant *Milium effusum* along a latitudinal gradient: (a, e, i) *in situ* collected seeds, (b, f, j) *ex situ* collected seeds from the southernmost common garden (Belgium), (c, g, k) *ex situ* collected seeds from the central common garden (southern Sweden) and (d, h, l) *ex situ* collected seeds from the northernmost common garden (northern Sweden). The data were averaged across the two populations within a region (only for this figure; not for statistical analyses). Note the arcsine square-root (emergence) or $\log_{10}$-transformed (biomass and SLA) Y axis. Error bars represent standard errors. F and p-values from mixed-effect models with *latitude* as predictor variable and *region* and *population* nested within *region* as random-effect terms. Only the 15 °C treatment from the *in situ* part was included for comparability with the second experiment; *ex situ* data were here analysed per common garden.

southern populations (e.g. Santamaria *et al.* 2003; Kollmann & Bañuelos 2004). Here, we demonstrated that this effect is also present in a transgenerational approach: the seedling biomass of *in situ* collected seeds of *A. nemorosa* and *M. effusum* decreased with latitude of origin of the mother plant. Additionally, intraspecific SLA generally increases with latitude of origin in common gardens (Clevering *et al.* 2001; Etterson 2004), which was confirmed here for *M. effusum* seedlings. We basically used two approaches to address phenotypic plasticity and local adaptation: (1) comparing the functional traits of seedlings resulting from *in situ* collected seeds exposed to different temperatures and (2) comparing the functional traits of

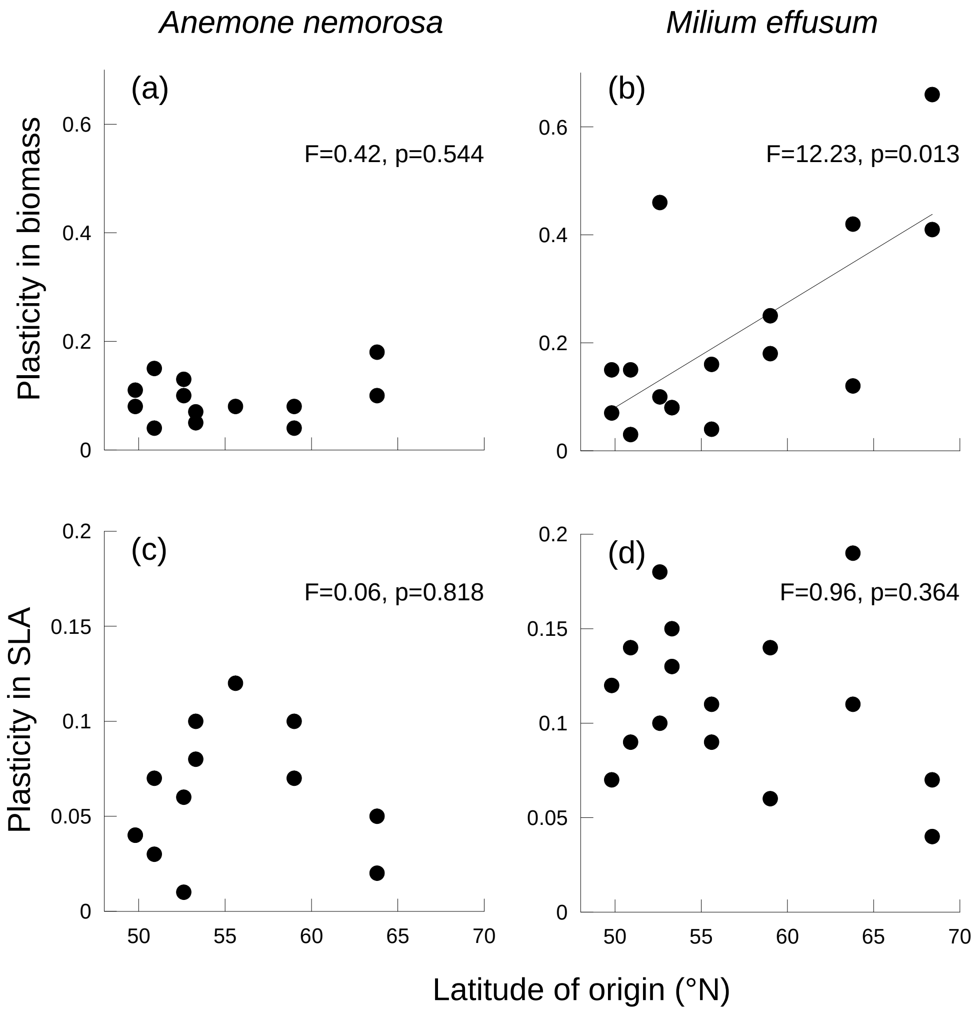


Figure 8.2: Phenotypic plasticity to temperature for seedling biomass and specific leaf area (SLA) of the understorey forest species *Anemone nemorosa* (a, c) and *Milium effusum* (b, d) resulting from seeds collected *in situ* in natural populations along a latitudinal gradient. Phenotypic plasticity was quantified by means of the average simplified relative distance plasticity index. F and p-values from mixed-effect models with *latitude* as predictor variable and *region* and *population* nested within *region* as random effects.

progeny of adult mother plants grown *in situ* and in common gardens. When the seedlings of both species were grown under different spring temperature scenarios, northern populations of *M. effusum* showed more than fourfold the amount of phenotypic plasticity in biomass growth compared to the southern populations, confirming our hypothesis (i.e. that plasticity increases with latitude) for *M. effusum* biomass, but not for *A. nemorosa* nor for SLA of *M. effusum.* This increased amount of plasticity in biomass production (i.e. a proxy for growth and fitness) for *M. effusum* seedlings might enable these populations to cope with more extreme microclimatic heterogeneity in the northern subarctic, than in temperate regions (e.g. Armbruster *et al.* 2007) and possibly with climatic changes in the coming decades. However, the plasticity in seedling SLA was relatively constant across latitude for both species. Still, the local environment seems more unfavourable in the north than in the south taking the latitudinal decrease in *in situ* seedling trait values (e.g. related to seedling biomass or emergence) for both species into account. Hence, seedlings of *M. effusum* of southern origin appear to be less plastic to variations in spring temperature, but they still can, e.g. grow taller. Also, high plasticity in biomass accumulation might be adaptive at northern latitudes when plasticity in this trait improves plant fitness (Matesanz *et al.* 2010).

Many plants display an *in situ* decrease in vegetative and reproductive fitness and resulting population density decline from the core populations to the periphery of their range (e.g. Jump & Woodward 2003), although this is not always true (Sexton *et al.* 2009). Populations at the range edges are also suggested to possess fewer possibilities for fast evolutionary adaptations compared to core populations (Davis & Shaw 2001; Bridle & Vines 2007; but see van der Merwe *et al.* 2010) and, subsequently, this can affect the amount of phenotypic plasticity (Mägi *et al.* 2011). Furthermore, increased plasticity is expected in expanding populations (e.g. in invasive species; Lavergne & Molofsky 2007). Our latitudinal gradient coincides with a gradient from the core of the distribution range of both species to the periphery (Hultén & Fries 1986) and many understorey plants are still expanding northwards following deglaciation (Svenning *et al.* 2008). However, our results contrast with those of Mägi *et al.* (2011) who found a reduction in the degree of phenotypic plasticity to light availability in marginal areas and with Stewart & Nilsen (1995) and Black-Samuelsson & Andersson (1997) who found no biogeographical pattern in plasticity to nutrients or temperature, respectively. It remains unclear why *M. effusum* shows northwards increasing phenotypic plasticity in biomass growth and *A. nemorosa* does not. A speculative explanation is that *A. nemorosa* is a relatively small forb whereas *M. effusum* is a tall grass. It has been shown previously that graminoids generally show more plasticity to resource alterations such as nitrogen enrichment and temperature increases than forbs (Dormann & Woodin 2002; Walker *et al.* 2006; Hollister & Flaherty 2010; De Schrijver *et al.* 2011). An alternative account could be that *M. effusum* is distributed farther to the north than *A. nemorosa* where environmental heterogeneity is also higher.

Since seeds from the *in situ* experiment were wild-collected, variation in seedling trait expressions and plasticity can both result from a plastic response to the maternal environment, or have a genetic basis. To address this issue, we designed the second experiment in which seeds were harvested in three common gardens (i.e. similar maternal environment) along a latitudinal gradient and seedlings grown in the intermediate 15°C spring temperature scenario. There, common garden transplant site had a significant effect on the investigated seedling traits of *M. effusum* which indicates a plastic maternal environmental (i.e. transgenerational) effect on

Figure 8.3: *A. nemorosa* and *M. effusum* in the growth chambers

seedling traits. However, also a genetic component was present. Firstly, the emergence percentage decreased with increasing distance between the latitude of origin and the transplant site, which suggests that the maternal plants were best adapted to their home-environment resulting in higher seed quality: emergence of the seedlings originating from the northern populations grown in the northernmost common garden was larger than the emergence of the seedlings of southern provenance, and vice versa. Populations of widespread plant species generally display adaptation to the local site conditions due to divergent natural selection (Leimu & Fischer 2008; Magnani 2009; *cf.* Chapter 7). The transgenerational local adaptation observed here cannot be attributed to soil properties or competitive interactions because all mother plants were grown principally without competition in exactly the same soil in the three common gardens. Rather, this pattern must be due to adaptation to other local (but often co-varying) factors, including climate, photoperiod or canopy cover (see also Macel *et al.* 2007 and Chapter 7). It is important to note that variation in emergence percentage could also be due to, e.g. seed dormancy so that it could conceivably be adaptive to have lower emergence. However, given the germination strategy of these forest understorey species (Thompson 1980; Graae *et al.* 2009b) and the temperature treatments, this is unlikely. Additionally, the transplanted mother plants that were collected north of 57°N even failed to flower and set seed in the southernmost garden.

Secondly, the functional traits of *M. effusum* seedlings did not always show a similar latitudinal cline in seeds from *in situ* and *ex situ* collections. The synthetic scheme of Magnani (2009) presents a summary for the possible responses of plant functional traits to the prevailing environmental conditions along climatic transects and at the site of seed origin in common gardens. In our specific case, using three common gardens instead of one and investigating transgenerational relationships, a complex picture develops, namely a parallel response (slope significantly different from zero) of the seedling biomass to the latitude of origin *in situ* and in the central common garden and a constant pattern (slope not significantly different from zero) of the seedling biomass in the two other common gardens. Hence, these results indicate a mixture of adaptation in the central common garden and phenotypic plasticity in the northern and southern common garden (following Magnani 2009). Together, our findings indicate the importance of both plastic transgenerational environmental, and genetic effects on seedling performance. Thus, based on the complex responses to environmental variation *in situ* and *ex situ*, we argue that the intraspecific variation in plasticity needs to be taken into account when forecasting the influence of global change factors on plant communities because even populations of the same species from different origin may display differential plastic responses to a changing environment.

A drawback of the methodology in this chapter is that we were unable to separate responses of maternal plants and bulked seeds on the population level. Theoretically, phenotypic plasticity should be studied using genetically identical individuals. This is, however, difficult to achieve in ecological studies, therefore plasticity is often measured in a broader sense at the population level (see Valladares *et al.* 2006 and references therein; Baeten *et al.* 2010b). We attempted to have similar levels of genetic variability among populations by randomly collecting the adult individuals of each species in large populations of ancient forest sites along the latitudinal gradient. Furthermore, the seedlings were completely randomized over the treatments, levelling out the within-population genetic differences. However, by neglecting the genetic structure present within populations of clonal plants (in turn depending on e.g. environmental variables or anthropogenic pressures; e.g. Stehlik & Holderegger 2000; Rusterholz *et al.* 2009) we did not take the sampled intrapopulation genetic variation into account that can affect the level of phenotypic plasticity and natural directional selection in the future (Jump & Peñuelas 2005; Jump *et al.* 2008). For instance, Jump *et al.* (2008) showed that the Mediterranean shrub *Fumana thymifolia* has the ability to rapidly adapt to warming and drought manipulations due to existing genetic variation already present within the population.

In summary, using a combination of *in situ* and *ex situ* experiments, we were able to show that the performance of seedlings (in terms of biomass growth) decreased with latitude of origin when seeds were wild-collected and across the common gardens for both species. The emergence percentage decreased with increasing home-away distance, suggesting that the maternal plants were best adapted to their home-environment resulting in higher seed quality. However, *M. effusum* seedlings originating from northern populations displayed more plasticity in biomass growth in response to spring temperature than seedlings from southern populations. This was not the case for *A. nemorosa.* Higher plasticity in seedling growth in the north is likely adaptive and may thus offer one way to cope with climatic changes to secure seedling survival of this species in the future.

9

Interregional variation in the floristic recovery of post-agricultural forests

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Abstract

Worldwide, the floristic composition of temperate forests bears the imprint of past land use for decades to centuries as forests regrow on agricultural land. Many species, however, display significant interregional variation in their ability to (re)colonise post-agricultural forests. This variation in colonisation across regions and the underlying factors remain largely unexplored. We compiled data on 90 species and 812 species × study combinations from 18 studies across Europe that determined species' distribution patterns in ancient (i.e. continuously forested since the first available land use maps) and post-agricultural forests. The recovery rate of species in each landscape was quantified as the log-response ratio of the percentage occurrence in post-agricultural over ancient forest and related to the species-specific life-history traits and local (soil characteristics and light availability) and regional factors (landscape properties as habitat availability, time available for colonisation, and climate). For the herb species, we demonstrate a strong (interactive) effect of species' life-history traits and forest habitat availability on the recovery rate of post-agricultural forest. In graminoids, however, none of the investigated variables were significantly related to the recovery rate. The better colonising species that mainly belonged to the short-lived herbs group showed the largest interregional variability. Their recovery significantly increased with the amount of forest habitat within the landscape, whereas, surprisingly, climate, the time available for colonisation, soil characteristics and light availability had no effect. We conclude that by analysing 18 independent studies across Europe, we clearly showed for the first time on a continental scale that the recovery of short-lived forest herbs increased with the forest habitat availability in the landscape. Small perennial forest herbs, however, were generally unsuccessful in colonising post-agricultural forest — even in relatively densely forested landscapes. Hence, our results stress the need to avoid ancient forest clearance to preserve the typical woodland flora.

9.1 Introduction

Large parts of the present-day forest area in Europe and eastern North America have been cleared for agriculture and subsequently reforested (spontaneous or planted) during the past centuries (Flinn & Vellend 2005). In several regions, only a small fraction of the actual forest cover can be referred to as *ancient*. These ancient forests have no historical record (mainly cartographical) of agricultural land use and have generally been continuously wooded for at least *c.* 150–400 years (recent reviews: Hermy *et al.* 1999; Flinn & Vellend 2005; Hermy & Verheyen 2007). Forest understorey plant species need to (re)colonise post-agricultural forest sites from relict populations in ancient forest or hedgerows, which are often scarce and isolated in contemporary landscapes. Previous work has demonstrated that a large number of forest plants are mostly unsuccessful in (re)colonising isolated post-agricultural forests so that the community composition bears the imprint of past land use for decades to centuries (Peterken & Game 1984; Whitney & Foster 1988; Dupouey *et al.* 2002). Today, there is a general consensus that the low dispersal capacity of many forest plants may principally account for their low colonisation rates (Verheyen *et al.* 2003c; Takahashi & Kamitani 2004; Matlack 2005). Also the low recruitment rates into the adult life stage and adult survival pose a bottleneck in the long-term colonisation of understorey species (Donohue *et al.* 2000; Jacquemyn & Brys 2008; Baeten 2010).

Many studies have compared the actual floristic composition of ancient and post-agricultural forest in a specific region or local landscape and classified species into fast vs. slow colonising subclasses (Peterken & Game 1984; Wulf 1997; Honnay *et al.* 1998; Graae 2000; Verheyen *et al.* 2003c, 2006) or calculated species-specific colonisation rates (Brunet & von Oheimb 1998a,b; Bossuyt *et al.* 1999; Dzwonko 2001b; Orczewska 2009b). Other studies compared the life-history traits (e.g. seed production, dispersal type, capacities for vegetative spread, etc.) of slow- vs. faster colonising forest plants (Honnay *et al.* 1998; Hermy *et al.* 1999; Graae & Sunde 2000; Verheyen *et al.* 2003c). However, a comparison of the colonisation rates within species and across regions suggests that forest plants may differ considerably in their capacity to colonise post-agricultural sites (Hermy & Stieperaere 1981; Hermy *et al.* 1999; Orczewska 2009b). Peterken (1974) has stressed the need to develop region-specific lists of fast vs. slow colonising species.

Yet, a broader biogeographical approach in which the (interactive) effects of local and regional factors such as climate, soils and habitat availability are assessed is still lacking. Hence, little is known about the factors controlling the interregional differences in recovery rates of understorey forest plant populations into post-agricultural forests on a continental scale. It can be expected that several factors are of influence (*cf.* Wright & Fridley 2010). First, slow-colonising forest plants tend to produce heavier seeds, which show higher germinability and better performance of seedlings in warmer regions (Graae *et al.* 2009b; Chapters 4, 5, 7). Temperature also has a positive effect on the potential growth rates of some understorey plants (Farnsworth *et al.* 1995; Chapter 6 and 7). Together, this suggests that variables related to climate may explain differences in colonisation behaviour of forest plants among regions (Peterken 1974; Wright & Fridley 2010). Second, it can be expected that local site conditions affect colonisation rates since variation in soil characteristics (e.g. pH, nutrients) and light availability across sites have been shown to affect the abundance and performance of understorey plants (Dupré & Ehrlen 2002; Graae *et al.* 2004; Kolb & Diekmann 2004; Verheyen *et al.* 2003a, 2006; Baeten *et al.* 2010b). Moreover, Wright & Fridley (2010) recently showed that the succession rate of woody species (expressed as the years to 10 % and 50 % woody cover) in old fields across eastern North America is significantly related to latitudinal gradients in temperature (expressed as growing degree-days) and soil fertility (indirectly measured through cation exchange capacity and pre-agroindustrial maize yields). Finally, regional differences in landscape properties such as the habitat availability (proportion of forest cover within a landscape) and the time since agricultural abandonment (age of the post-agricultural forests) can also affect the colonisation chances of forest herbs (Grashof-Bokdam & Geertsema 1998; Jacquemyn *et al.* 2001; Graae *et al.* 2004; Brunet 2007). By combining a meta-analysis of ten studies in Europe and eastern North America with a mainland-island metapopulation model, Vellend (2003), for instance, showed that the ratio of the species richness of post-agricultural over ancient forests decreased significantly when the proportion of ancient forest in the landscape dropped below 10 %.

To get insight into the broad-scale variation in recovery rates of forest plant populations, we carried out a meta-analysis of 18 studies that determined distribution patterns of in total 90 individual understorey species within ancient and post-agricultural forest patches in forested landscapes across Europe. We also included the functional traits related to colonisation capacity (Verheyen *et al.* 2003c) in the analyses to compare the effects of each predictor variable among species with contrasting life-histories. More specifically, we aimed (i) to quantify the interregional variation in recovery rates of understorey plants in post-agricultural forest in Europe, (ii) to examine the effects of species' life-history traits on this variation and (iii) to investigate the importance of both local (soil characteristics and light availability) and regional factors (habitat availability, time available for colonisation, and climate) potentially altering this recovery rate.

9.2 Methods

9.2.1 Data collection

We searched the literature (peer-reviewed papers but also reports and unpublished theses) for field studies investigating the floristic composition in both ancient and post-agricultural deciduous forests in landscapes across Europe. Studies were only included if they met the following criteria (adapted from Verheyen *et al.* 2003c): (i) the inventoried forests were situated in lowland or lower mountainous ($< 600\,\mathrm{m}$ a.s.l.) Europe, (ii) frequency data of the forest plants were available for all (or a representative subsample of) ancient and post-agricultural forest patches in the landscape, (iii) the land-use history and sufficient information on the other predictor variables (see below) of both forest types or the region was given and (iv) canopy closure had already occurred in post-agricultural forests (thus, the analyses were restricted to closed-forest ecosystems). Eighteen geographically non-overlapping studies from nine countries (from central Italy in the south to central Sweden in the north and from the United Kingdom in the west to Poland in the east; see **Fig. 9.1**) complied with these four criteria and yielded 2231 species × study combinations (693 species) from 1098 ancient and 1370 post-agricultural data collection units (patches or plots; **Table 9.1** and **Table E.1** in **Appendix E**). However, to avoid a biased estimation of the parameters in the statistical models for species with low frequency, we only focused on those species that were cited in more than five studies. This reduced the species × study combinations to 1332 (155 species).

To explore the effects of life-history traits related to plant colonisation on the recovery rate of post-agricultural forest, we used the emergent groups of plant species (*sensu* Lavorel *et al.* 1997) identified by Verheyen *et al.* (2003c) according to 13 reproductive, vegetative and phenological traits: seed mass, seed size, seed shape, seed production, dispersal type, seed bank, germination requirements, age of first reproduction, growth form, life cycle, vegetative spread, maximum height and flowering phenology. Based on these traits, each species was classified into one of four (herbs) or three (graminoids) emergent groups as delineated by Verheyen *et al.* (2003c) by combining Gower's Similarity Coefficients, non-metric multidimensional scaling (NMDS) and clustering with Ward's method (see Verheyen *et al.* 2003c for more information). For the herbs the four groups were (i) short-lived herbs, (ii) tall perennials with heavy seeds, (iii) tall perennials with light seeds and (iv) small perennials with heavy seeds; for the graminoids the three groups were (i) large, summer flowering graminoids, (ii) small, summer flowering vegetatively spreading graminoids, and (iii) early flowering graminoids (the char-

Table 9.1: Summary of the 18 studies included in the meta-analysis (from north to south). The location of each study area (ID) is depicted in **Fig. 9.1**. A more extensive overview of the characteristics of each study area is given in **Table E.1** in **Appendix E**.

ID	Publication	Country[a]	Lat. (°N)	Long. (°E)	Collection unit[b]	Ancient units	PA[c] units	No. species
1	Cousins & Eriksson (2008)	S	59.4	17.2	5 plots ($4\,\mathrm{m}^2$) patch^{-1}	54	35	51
2	Graae (2000)	DK	56.8	9.6	Plots ($625\,\mathrm{m}^2$)	38	10	45
3	Petersen (1994)	DK	55.7	10.9	Plots (100–400 m^2)	9	9	16
4	Brunet (2007)	S	55.5	13.2	Patch	137	126	30
5	Kolb & Diekmann (2004)	GE	53.4	9.4	Patch	71	40	73
6	Peterken & Game (1984)	UK	53.3	-0.3	Patch	89	273	64
7	Wulf (2003)	GE	53.2	12.0	Plots (200–400 m^2)	304	174	42
8	Grashof-Bokdam & Geertsema (1998)	NL	52.3	7.0	Patch	54	110	10
9	Zacharias (1994)	GE	52.1	10.6	Patch	11	11	68
10	Jakubowska-Gabara & Mitka (2007)	PO	52.0	20.2	Plots ($100\,\mathrm{m}^2$)	16	18	31
11	Honnay *et al.* (1998)	B & F	51.0	3.5	Patch	58	46	66
12	Orczewska (2009a)	PO	51.0	17.8	Patch	23	22	65
13	Jacquemyn *et al.* (2001)	B	50.9	4.9	Patch	35	204	60
14	Verheyen *et al.* (2003b)	B	50.8	4.6	Patch	15	182	11
15	Dzwonko & Loster (1989)	PO	49.9	19.7	Patch	63	6	32
16	Jamoneau (2010)	F	49.8	3.6	Patch	14	15	70
17	Sciama (1999); Sciama *et al.* (2009)	F	46.7	5.6	Patch	62	65	55
18	De Sanctis *et al.* (2010)	I	41.6	12.2	Patch	45	24	23
					Sum	1098	1370	812

[a] Countries: S: Sweden; DK: Denmark; GE: Germany; UK: United Kingdom; NL: The Netherlands; PO: Poland; B: Belgium; F: France; I: Italy. [b] Data collection units: if the data collection units were randomly distributed plots, plot size is given. Otherwise whole forest patches were surveyed. [c] Post-agricultural.

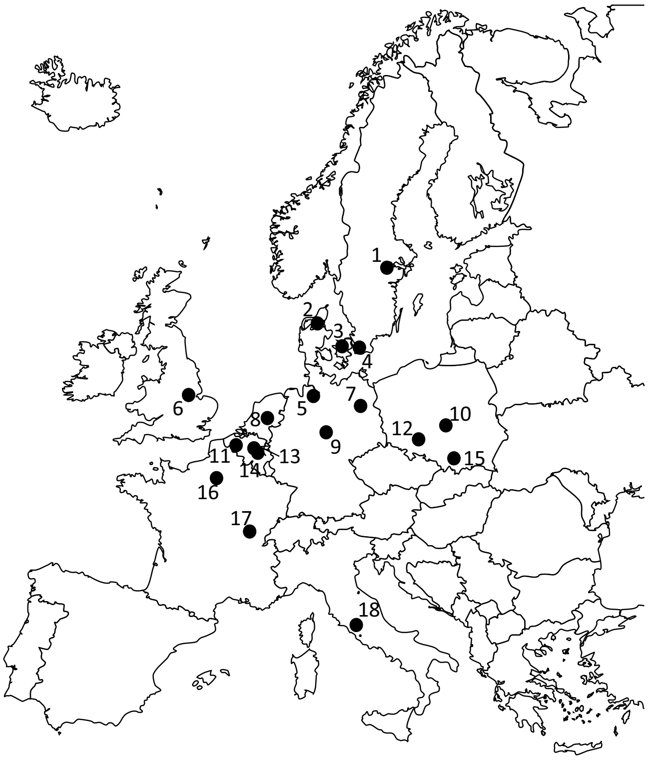


Figure 9.1: Map of the study sites included in this meta-analysis. The numbers refer to the studies in **Table 9.1** and **Appendix E**.

acteristic life-history traits per emergent group are shown in **Table E.2** and the emergent group per species in **Table E.3** in **Appendix E**). Only those species for which $> 50\,\%$ of the life-history traits were available were used in the analyses. The 18 studies then yielded a final set of 90 species (71 herbs and 19 graminoids) and 812 species × study combinations (**Table 9.1** and **Table E.3**). The emergent groups were calculated across all species and individual species can thus display a deviating value for some life-history traits. The species nomenclature in this chapter follows Wisskirchen & Haeupler (1998).

To conduct the interregional analyses, four groups of predictor variables were gathered for each study. These were related to habitat availability, time available for colonisation, climate and soil and light characteristics.

Similar to Vellend (2003), we focus on habitat loss and not habitat fragmentation *sensu stricto* (*cf.* Fahrig 1997, 2003) as information to calculate the absolute patch sizes or patch isolation distances was unavailable for most studies. Therefore, to account for habitat availability and time available for colonisation, data on the total proportion of forest in the landscape (ancient plus post-agricultural; TF) and of ancient and post-agricultural forest separately (AF and PAF, respectively) as well as the mean and maximum post-agricultural forest age were collected instead. It should be kept in mind, however, that the amount of forest cover is not necessarily correlated with spatial isolation. Forest cover data were provided by the authors, compiled from the original publications, taken from Vellend (2003) or, in the case of Zacharias (1994) and Jakubowska-Gabara & Mitka (2007), obtained by digitising maps provided in the original publication using `Image J` (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, `http://rsb.info.nih.gov/ij/`). The total forest and ancient forest cover in the landscape encompasses a broad range: 5.4–50.7 % and 0.9–27 %, respectively. The total forest cover was significantly correlated with the ancient forest cover ($r = 0.489$, $p = 0.039$) and the post-agricultural forest cover ($r = 0.859$, $p < 0.001$) across our studies ($n = 18$).

The mean and maximum age of the post-agricultural forests (further referred to as the mean and maximum colonisation time, respectively) was mostly available in the original publications or provided by the authors. We calculated the mean colonisation time as the average of the minimum and maximum colonisation time within the landscape when the mean was not explicitly mentioned. It should be noted that forest age *sensu stricto* is not the main focus here. Due to data availability in the different regions, an ancient forest in one region can be younger than a post-agricultural forest in another region (**Table E.1**). Although an old post-agricultural forest is probably more likely to be already colonised, we consider forest continuity here (i.e. whether a forest has always been forest or cleared for agriculture at some point). Hence, we take both the mean and maximum colonisation time of the post-agricultural forests in a region into account. The mean and maximum colonisation time of the post-agricultural forests varied between 24–135 and 54–240 years (excluding Peterken & Game 1984). The British study by Peterken & Game (1984) forms a notable exception because much older detailed maps are available in Britain (Goldberg *et al.* 2007; mean and maximum colonisation time of the post-agricultural forests up to 201 and 370 years in Peterken & Game 1984). Exclusion of this study, however, yielded similar statistical results (data not shown).

Next, climate data were obtained from the `NewLocClim 1.10` software (F.A.O. 2005) using nearest-neighbour interpolation with ten weather stations. We gathered latitude, longitude and altitude data of the centre of each study region and used these values to deduce mean annual temperature (MAT; 1961-1990), mean annual precipitation (MAP) and potential evapotranspiration (PET). Also the effects of latitude and longitude themselves were tested for. Subsequently, an aridity index was calculated according to F.A.O. (2005) as the ratio of MAP and PET. The MAT and aridity index varied between 6.6-15.0 °C and 0.74-2.58.

Finally, as detailed soil data or light measurements were not available for most studies so that we could rigorously test for their effects, we used the mean frequency-weighted Ellenberg indicator values (Ellenberg *et al.* 1992) for nutrients (mN_j), reaction (mR_j), moisture (mF_j) and light (mL_j) of the ancient forest as rough proxies for the local soil nutrient availability, soil acidity, soil moisture and light availability, respectively. Ellenberg values are known to be very good correlates of in situ measured environmental characteristics in ancient forests but not in post-agricultural forests (Dzwonko 2001a). Therefore we only used the Ellenberg values of the ancient forests as proxy for these environmental variables to allow for a general interregional ranking in soil and light characteristics. For mN_j, for example, this was calculated as:

$$mN_j = \frac{Freq_{sp1,j}.N_1 + Freq_{sp2,j}.N_2 + \cdots + Freq_{spi,j}.N_i}{Freq_{sp1,j} + Freq_{sp2,j} + \cdots + Freq_{spi,j}}$$

with $\mathrm{Freq}_{spi,j}$ the frequency of species i in the ancient forests in study j and N_i the Ellenberg N value for species i. The calculated mN values ranged between 2.5 (nutrient-poor soils) and 5.8 (nutrient-rich), the mR between 2.7 (acid soils) and 4.9 (more neutral), the mF between 2.6 (dry soils) and 6.0 (moister) and the mL between 3.1 (forests with low light availability in the understorey) and 4.9 (higher light availability) (**Table E.1**). Hereafter, the mN, mR, mF and mL values are referred to as soil nutrient availability, soil acidity, soil moisture and light availability, respectively.

9.2.2 Data analysis

The recovery rate (RR) of post-agricultural forests was calculated for each species × study combination as the risk ratio with binary data (2 × 2 tables) in standard meta-analytical procedures (Borenstein *et al.* 2009):

$$RR_{ij} = ln\left[\frac{PAF_{ij} + 0.01}{AF_{ij} + 0.01}\right]$$

with RR_{ij} being the recovery rate for species i in study j and PAF_{ij} and AF_{ij} the percentages of data collection units (patches or plots; **Table 9.1**) occupied by species i in post-agricultural and ancient forest in study j, respectively. This equation includes a correction (+0.01) in both the numerator and denominator to account for zero-percentages in both forest types. Zero values of RR thus correspond to equal percentages of the species in ancient and post-agricultural forest, whereas positive and negative values correspond to a lower and higher affinity to ancient than to post-agricultural forest, respectively. A species with an $RR = -1$, for instance, showed a percentage in ancient forest that was approximately 2.7 times the percentage in post-agricultural forest.

Subsequently, the effect of the life-history traits of each species (emergent groups) on RR was tested with mixed-effect models in `R 2.11.0`, using the *lmer*-function of the *lme4*-library (R Development Core Team 2010). According to Zuur *et al.* (2009), we first selected the optimal random-effects structure based on a likelihood ratio test between models with a similar fixed component (no predictor variables included), but a different random component. The optimal model included both *study* and *species* as non-nested random effects. Modelling the hierarchical nature of the data using two non-nested random effect terms in a mixed-effect model then leads to partial pooling across the different levels (Qian *et al.* 2010), and hence, this takes the possible autocorrelated characteristics of (i) species from the same study region and (ii) similar species in different regions into account. Next, we compared the null model (only including the two non-nested random effects) with a model that included the emergent groups (species level) (χ^2-test statistic with likelihood ratio test; Zuur *et al.* 2009); these analyses were conducted for all species together and afterwards also separately for herbs and graminoids.

Next, to quantify the interregional variation in the RR for each species, we calculated the coefficient of interregional variation (CIV$_{RR}$) of species i as $CIV_{RR} = SD_i/(\overline{x}_i+1)$, with SD_i the standard deviation and $\overline{x}_i$ the mean RR for species i (hence, one CIV$_{RR}$ value per species). A correction factor of 1 was added to the denominator to prevent CIV values from skyrocketing for species with an RR close to zero. We then performed a one-way ANOVA using a General Linear Model (GLM) with Bonferroni post hoc test (using `SPSS 15.0`) to investigate whether there were differences in the CIV$_{RR}$ between the different emergent groups; again, herbs and graminoids were analysed separately.

To explore the effects of all the environmental predictor variables on the RR, namely (i) the proportion of total, ancient and post-agricultural forest in the landscape, (ii) the mean and maximum colonisation time, (iii) latitude, (iv) longitude, (v) temperature, (vi) aridity index, (vii) soil nutrient availability, (viii) soil acidity, (ix) soil moisture and (x) light availability (all study-level), we again applied mixed-effect modelling in `R` following a similar approach as above. The model again included *study* and *species* as non-nested random effects and the null model (only including random effects) was compared with a model that included one of the predictor variables (on a one-by-one basis mainly to avoid multicollinearity problems) to test the significance of that particular variable (χ^2-test statistic with likelihood ratio test; Zuur *et al.* 2009). We also estimated the percentage of variation explained by adding the predictor variables to the null model through calculations of the ratio of the difference in residuals between the null model and the final model over the residuals of the null model (Hox 2002). Finally, we tested for additive models and interactions among all significant predictor variables with the life-histories of the species (emergent groups) by comparing (i) a model that included the emergent groups plus that particular predictor variable with a model that included only the emergent groups as main effect (i.e. test of the additive effects) and (ii) a full factorial model with a model that included the emergent groups plus that particular predictor variable (i.e. test of the interaction term). Again, the χ^2-test statistic with likelihood ratio test was used for this purpose.

9.3 Results

Across Europe, many species displayed large interregional variability in their recovery rate (RR) in post-agricultural forest. The RR across species ranged between 0.46 (*Galium aparine*) and -2.29 (*Luzula pilosa*) and amounted to -0.60 (± 0.04 S.E.) on average indicating that the 90 investigated understorey species showed an overall *c.* 1.8 (=$e^{0.60}$) times higher affinity to ancient than to post-agricultural forest. Looking at individual species, the within-species variation in RR was high: for example, RR varied strongly in the herbs *Anemone nemorosa* [0.03 to -2.72, $n = 17$], *Lamium galeobdolon* [0.15 to -2.60; $n = 14$] and *Paris quadrifolia* [0.61 to -2.40; $n = 12$], and the graminoids *Festuca gigantea* [3.05 to -1.67; $n = 11$], *Holcus mollis* [0.10 to -2.89; $n = 8$] and *Melica uniflora* [-0.07 to -3.53; $n = 13$] (see **Table E.3** for the mean RR per species). In general, however, large differences were apparent among the different emergent groups (**Fig. 9.2a**; **Table 9.2**). Species that were rather indifferent to forest continuity (RR close to zero) were members of the *short-lived herbs*-emergent group (e.g. *Moehringia trinervia* and *Geranium robertianum*) and the *large summer flowering graminoids*-emergent group (e.g. *Brachypodium sylvaticum* and *Festuca gigantea*). The poorer colonisers (i.e. species with a negative RR), on the other hand, were small perennials with heavy seeds (e.g. *Anemone nemorosa* and *Convallaria majalis*) and early flowering graminoids (e.g. *Melica uniflora* and *Luzula pilosa*). The emergent groups (species-level predictor) explained a large proportion of the variability in the RR for herbs (percentage explained from the species and study level variance: 57.0 % and 2.8 %, respectively), while the RR of the graminoids was not significantly different between the emergent groups (percentage explained from the species and study level variance: 33.8 % and 1.1 %, respectively; **Table 9.2**). Finally, we found significant differences in the coefficient of interregional variation (CIV_{RR}) between the herbs, but not between the graminoids (**Fig. 9.2b**; **Table E.3**).

The habitat availability in a given region appeared to be an important landscape property to explain differences in the RR between regions; the species' recovery rate increased with the proportion of forest (ancient plus post-agricultural; study level predictor) in the landscape (percentage explained from the species and study level variance: 0.9 % and 24.9 % across species, respectively; **Table 9.2** and **Fig. 9.3a**). There was also a significant interaction between the total forest cover and the emergent groups across all species and within the group of the herb species; all emergent groups displayed a positive slope between the total forest cover and the RR (**Table 9.2**; **Fig. 9.3b,c**). When analysed for each emergent group separately, the effect of forest cover was not significant ($\chi^2 < 2.21$, $p > 0.137$) except within the short-lived herbs ($\chi^2 = 5.20$, $p = 0.022$). However, the positive trend between the average RR of each emergent group and the slope of the relationship between the RR and total forest cover (slopes from **Fig. 9.3b** and **c**) suggests that emergent groups with a higher average RR tend to profit more from increased habitat availability. The post-agricultural forest cover within a landscape also had a significant positive impact on the RR for all species and for

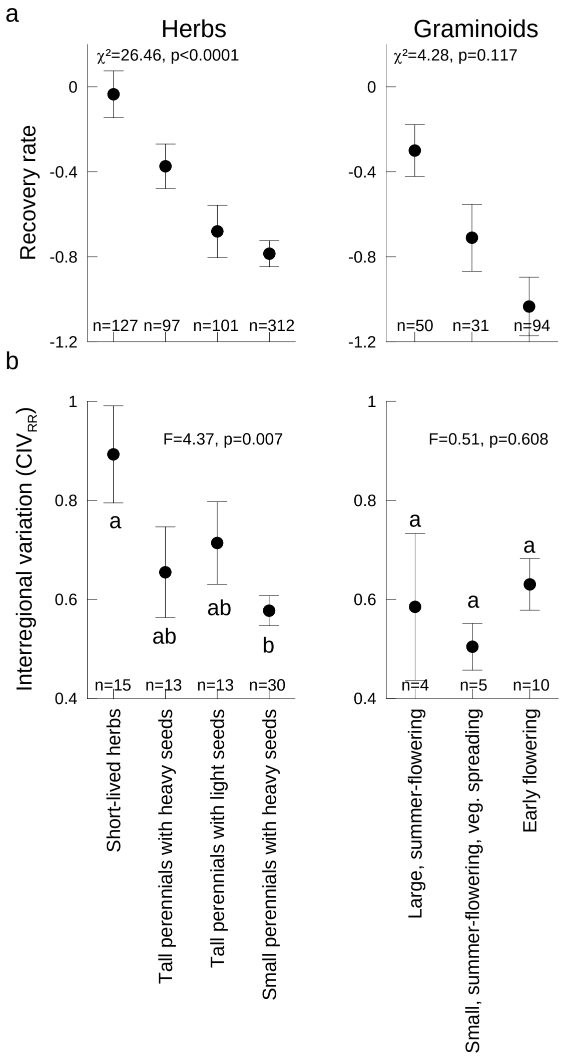
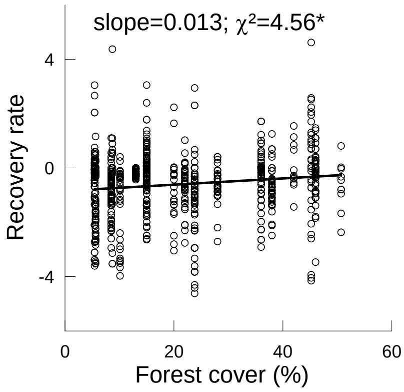


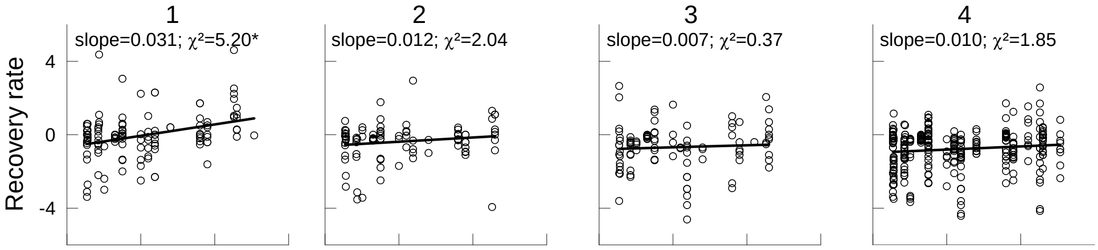
Figure 9.2: The recovery rate of forest herbs (left) and graminoids (right) (RR; a) and the coefficient of interregional variation (CIV_{RR}; b) for the different emergent groups across Europe. Significance values of the RR were obtained from a mixed-effect model with *study* and *species* as non-nested random effect terms; significance values of the CIV_{RR} were obtained from a one-way ANOVA using a GLM with Bonferroni post hoc test. Error bars depict standard errors; numbers of replicates (n) indicate species × study combinations in (a) and species in (b).

herbs and graminoids separately (positive slope for all emergent groups; percentage explained from the species and study level variance: 0.6 % and 31.0 % across all species, respectively; **Table 9.2**). In contrast, neither the ancient forest cover nor the climatic variables, latitude and longitude, soil nutrient and light availability, soil acidity, soil moisture, and mean and maximum colonisation time had a significant effect on the RR (**Table 9.2**).

(a) All species



(b) Herbs



(c) Graminoids

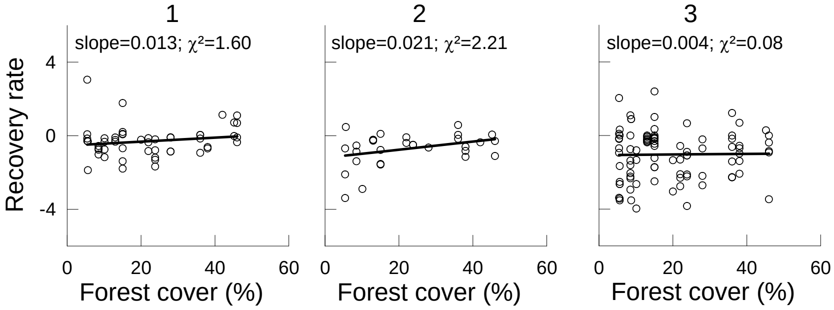


Figure 9.3: The effect of total forest habitat availability (forest cover within the landscape) on the recovery rate of understorey species in post-agricultural forest (a) across all species and within the different emergent groups of the (b) herbs and (c) graminoids. The emergent groups for the herbs were: 1. short-lived herbs, 2. tall perennials with heavy seeds, 3. tall perennials with light seeds and 4. small perennials with heavy seeds. For the graminoids the three groups were 1. large, summer flowering graminoids, 2. small, summer flowering vegetatively spreading graminoids, and 3. early flowering graminoids (ranked according to the mean recovery rate). Slope and significance values from mixed-effect models with *study* and *species* as non-nested random effect terms which takes the possible autocorrelation of species and studies into account.

Table 9.2: Effects of the predictor variables on the recovery rate of forest understorey plants in post-agricultural forests in Europe for all species and for the herbs and graminoids separately. Results from mixed-effect models with *study* and *species* as non-nested random effect terms. χ^2-test statistic from likelihood ratio test.

Predictor(s)	All species ($n = 90$)		Herbs ($n = 71$)		Graminoids ($n = 19$)	
	χ^2	p-value	χ^2	p-value	χ^2	p-value
Emergent group (EG)	32.56	<0.001 ***	26.46	<0.001 ***	4.28	0.117
Total forest cover (TF)	4.56	0.033 *	4.54	0.033 *	1.92	0.165
Ancient forest cover (AF)	0.04	0.841	0.14	0.709	0.03	0.858
PA[a] forest cover (PAF)	5.89	0.015 *	5.29	0.022 *	3.64	0.057(*)
Mean colonization time	0.13	0.723	0.33	0.566	0.00	0.973
Maximum colonization time	0.02	0.883	0.05	0.818	0.02	0.902
Latitude	0.07	0.789	0.00	0.959	0.58	0.447
Longitude	0.01	0.939	0.03	0.872	0.05	0.83
Temperature	0.21	0.648	0.12	0.724	0.52	0.469
Aridity index	0.51	0.475	0.82	0.364	0.02	0.892
Soil nutrient availability	0.19	0.667	0.17	0.677	0.02	0.878
Soil acidity	0.04	0.845	0.08	0.772	0.10	0.758
Soil moisture	1.23	0.267	1.29	0.256	0.32	0.572
Light availability	0.37	0.542	0.55	0.458	0.04	0.841
Additive EG + TF[b]	4.62	0.032*	4.95	0.026*		
Interaction EG*TF[c]	12.62	0.049*	10.39	0.016*		
Additive EG + PAF[b]	6.43	0.011*	6.14	0.013*	3.55	0.059(*)
Interaction EG*PAF[c]	7.46	0.281	7.11	0.069(*)	0.05	0.975

[a] Post agricultural. [b] Additive model with the factor variable emergent group was tested among the significant environmental variables from the first step and compared to a model that included the emergent groups as main effect. [c] Interaction with the factor variable emergent group was tested among the significant variables from the first step (full factorial) and compared to an additive model that included the emergent groups and that particular environmental variable.

9.4 Discussion

By applying a quantitative meta-analytical approach across Europe, we provide evidence for large among-region variability in the recovery rate of understorey species into post-agricultural forest. The slope of the effect of the forest habitat availability within the landscape on the recovery rate varied for the (life-history trait-based) emergent groups. The short-lived herbs showed the highest interregional variability in their recovery rate (CIV$_{RR}$), and this variability was significantly related to the total amount of forest cover within the landscape. The small perennial herbs with heavy seeds and early flowering graminoids were generally confined to ancient forest patches throughout Europe. The effect of increased habitat availability appeared to be favourable across all species, but when this effect was analysed per emergent group, it seems only beneficial for the short-lived herbs. This finding suggests that even a high proportion of forest within the landscape (in the present study up to 51 %; Grashof-Bokdam &

Geertsema 1998) has no positive effect on the colonisation rates into post-agricultural forests by the species with the lowest recovery rate, confining these mostly to ancient forest patches. This also implies that these species strongly depend on temporal habitat continuity, occurring mainly as relict populations in ancient forests or hedgerows, whereas the short-lived herbs can show a metapopulation behaviour between the different forest patches within a largely agricultural and urban matrix (Verheyen *et al.* 2004). The latter species may colonise new forest patches soon after canopy closure.

Various studies have shown the beneficial effect of forest cover on species migration into post-agricultural forest on a local scale (Honnay *et al.* 2002b; Graae *et al.* 2004; Verheyen *et al.* 2006) or for species richness (Vellend 2003). Here, we quantified this effect for the first time on a continental scale by combining 18 independent studies across Europe and also show contrasting effects depending on the life-history traits of the understorey species. Contrary to Vellend (2003), who highlighted the importance of ancient forest cover in the landscape for species richness in post-agricultural forest in Europe and eastern North America, not ancient forest cover *per se* but the availability of total and post-agricultural forest habitat was found to have a significant positive effect on the recovery rate. Since the total and post-agricultural forest habitat availabilities were strongly correlated in the present study, densely forested landscapes generally have a high proportion of post-agricultural forests. This could be caused by historical afforestation practices in lowland Europe such as the tendency to afforest (mostly little-productive) regions that had lost most of their (ancient) forests until the 1800s. In (often more productive) regions where few ancient forests remained, on the other hand, hardly any afforestation effort was made. Consequently, the low total forest cover there is associated with low post-agricultural forest cover (e.g. noble estates, state-owned land; Rackham 2003). Most species displayed a high affinity to ancient forest in forest-poor regions whereas only the short-lived herb species appeared to be able to benefit from increased possibilities for migration in densely forested landscapes. Finally, in spite of a different methodology, the present study clearly confirms the findings of Verheyen *et al.* (2003c) that plant traits associated with a low dispersal capacity result in a high affinity to ancient forest. Our results corroborate that plant height (e.g. tall herbs have greater chances for epizoochorous dispersal), possibilities for long-distance dispersal (e.g. light seeds) and abilities to reproduce easily (e.g. vegetative spread) together account for the interspecific differences (Graae 2002; Verheyen *et al.* 2003c).

Surprisingly, climate, soil and light characteristics, and colonisation time were of no influence to the recovery. Although an effect of, for instance, climate, soil nutrient availability and colonisation time has been shown for the performance and abundance of individual species (Kolb & Diekmann 2004; Baeten *et al.* 2010b; Chapters 4–8), this effect was not apparent in these analyses. This may be due to multiple factors including long-term adaptations to the local environment (genetic control), a changing species pool across soil types and a complex mosaic of different soil types and age classes in post-agricultural forests within a landscape.

First, long-term adaptations to the local environment (both to climate and to soil characteristics) result in a bias in observational studies along geographic transects. For instance, some ecotypes of the same species may be adapted to growing in warmer climates whereas others perform equally well in colder conditions (Joshi *et al.* 2001) leading to a *homogenization* of colonisation rates across climates. Second, the species pool on inherently richer soils (in the present study, high Ellenberg indicator value for nutrients) may differ drastically from the species pool on poorer soils (Zobel & Pärtel 2008), again making an effect of soil nutrient availability on the floristic recovery rate difficult to extract. The heterogeneity in soil conditions within large study areas (e.g. in Honnay *et al.* 1998) reduces the chance of finding an interregional effect. Also, mean Ellenberg indicator values for ancient forests alone may not represent the whole range of regional environmental variation in post-agricultural forests. Besides, also the position of ancient forest within a landscape may a priori be determined by the local soil conditions due to historical deforestation and farming practices (Flinn *et al.* 2005; Cousins 2009). Finally, there is also large variation in mean and maximum colonisation time available since agricultural abandonment between the post-agricultural patches within a landscape, thereby reducing the effect of colonisation time (see also *Methods* or Vellend 2003).

Still, our results clearly suggest that, even within the maximum time horizon studied here (up to 370 years), the species with life-history traits that limit dispersal are most likely to be missing from the floristic community of post-agricultural forests across Europe. In summary, we clearly showed that the degree to which a species manages to colonise post-agricultural forests depends mainly on (i) life-history traits related to dispersal and colonisation and (ii) the forest habitat availability in the landscape. Short-lived herbs showed the highest interregional variability. Of this variability, a significant part could be explained by the total and post-agricultural forest habitat availability in the landscape. Hence, short-lived herbs are most likely to be the first colonisers into a newly established forest patch and also show the greatest variability in recovery rates across Europe. Forest-rich landscapes thereby provide the best setting for high permeability from remnant ancient forest or hedgerows into post-agricultural patches for these species. Furthermore, we stress the need to avoid ancient forest clearance to preserve the typical woodland flora with an intrinsically low recovery rate.

10

General discussion and conclusions

In spite of the ecological significance of understorey plants for temperate forest ecosystem biodiversity, competitive interactions, linkage with overstorey and ecosystem functioning in general (Gilliam 2007), knowledge about the effects of climate change on their phenology, growth, reproduction and colonisation capacity is relatively scarce (*cf.* Chapter 1). Hence, it remains largely unclear to what extent changing temperatures can affect herbaceous understorey plant dynamics.

Here, we assessed the response of understorey plants to variation in temperature using a variety of observational and experimental approaches along a latitudinal gradient. By sampling natural populations, it was impossible to determine the reasons for the observed variations in functional traits (i.e. whether latitudinal plant trait variation has genotypic and/or phenotypic causes; Chapters 2–5). Experimental warming and transplant experiments enabled to disentangle phenotypic plasticity (*acclimation*) to variation in temperatures from genetic local adaptation across a species' range (Chapters 6–8). Finally, we quantified the recovery rate of post-agricultural forests across Europe in Chapter 9 and related this variation in recovery rate to local and regional factors (including climate). In this closing chapter, we summarise the main findings for each of the successive phases of plant growth and recruitment presented in **Fig. 1.3**, suggest implications for ecological conservation and discuss limitations of this study and options for further research.

10.1 Climate-change effects on understorey plants

10.1.1 Phenology and adult plant performance

Multiple lines of evidence (Chapter 2, 6, 7) suggest future advancing emergence and flowering phenology, and increased growth for the characteristic understorey herb *Anemone nemorosa* L. in a future, warmer climate. For instance, plant height and aboveground biomass of *A. nemorosa* is expected to increase with rising temperatures in the future (Chapters 6 and 7). On the other hand, apart from the effects on phenology, the impact of rising temperatures on adult plant performance of summer-flowering species such as *Milium effusum* (Chapters 2 and 7) are expected to be less pronounced.

We showed that our study species will most likely advance their emergence and flowering phenology in response to experimental warming (Parmesan 2007; Ibáñez *et al.* 2010; Chapter 7). This confirms the negative relationship between the flowering date of *A. nemorosa* and the mean spring temperature using a 78-year and 154-year time series from Estonia and England, respectively (Ahas 1999; Sparks & Menzel 2002; but see Tyler 2001). In forest ecosystems specifically, these phenological advances may increase the phenological escape (i.e. the discrepancy between overstorey and understorey phenology) when understorey plants would advance their phenology and if trees respond slower or to a lesser extent (Menzel 2003). This could positively feedback to the understorey productivity if plants can acquire more resources in spring (Routhier & Lapointe 2002), especially at northern latitudes where the favorable period for growth of understorey plants is shorter than in the south (Neufeld & Young 2003). Conversely, phenological advances of flowering plants may generate temporal plant-animal mismatches related to pollination, dispersal, or herbivory (Memmott *et al.* 2007; Tylianakis *et al.* 2008; Berg *et al.* 2010). Kudo *et al.* (2008) stressed already that the group of bee-pollinated early flowering understorey plants will probably be negatively affected by climate warming due to possible mismatches between trophic levels.

The investigations of the leaf-height-seed ecology strategy scheme revealed that plant height showed a clear intraspecific latitudinal increase for *A. nemorosa* and *M. effusum* plants that could be attributed to northward decreasing canopy cover and increasing light availability in the growing season (Chapter 2). This increase in understorey plant height suggests increased competitive abilities in the north for both species. Since light is a unidirectional resource (only coming from above), taller plants that are able to display their leaves above other plants have an assymmetric competitive advantage (Grime *et al.* 1988; Westoby 1998; Craine 2009). Hence, it can be hypothesized that *A. nemorosa* and *M. effusum* individuals in the northern populations are more competitive to maximize light interception since they are taller than in the south. These intraspecific findings, however, contrast with the global decreasing patterns in plant height across plant species towards the poles (Moles *et al.* 2009a). Changing intraspecific body size with latitude does also occur within animals (Bergmann's rule and converse Bergmann's rule) due to latitudinal clines in temperature, growing season length or prey size (McNab 1971; Blanckenhorn & Demont 2004; De Block *et al.* 2008). Hence, one might speculate that also latitudinal co-evolution between the size of plants and animals due to plant-animal interactions is not impossible (e.g. pollinating or herbivorous animals can be adapted to the size of the flower or the foliage morphology, and vice versa; Herrera 1996; Mopper 1996). Clearly, further research is needed to confirm this intraspecifc hypothesis in relation to coexisting plant (and animal) species and a larger set of plant species to allow for generalisations among ecosystems, functional groups of species and/or growth forms (see further §10.3).

Figure 10.1: *Anemone nemorosa*: one of the winners in a warmer climate?

If the plant height and aboveground biomass of a particular species would increase following climate change this would support that particular species in it's race for the top and its possibilities to suppress other species (Craine 2009). Other experimental warming studies, mainly from arctic, antarctic and alpine ecosystems, have indicated changed competitive and facilitative interactions due to divergent responses in height or biomass growth among coexisting species (Harte & Shaw 1995; Klanderud 2005; Walker *et al.* 2006; Kardol *et al.* 2010; Yang *et al.* 2011). For instance, graminoids may respond differently to rising temperatures than shrubs or forbs (Dormann & Woodin 2002; Walker *et al.* 2006; Hollister & Flaherty 2010). Likewise, Farnsworth *et al.* (1995) showed already that herbaceous understorey plants respond more strongly to warming using soil heating cables than shrubs and trees. Consequently, changing community compositions can, in turn, affect ecosystem functioning (Tilman *et al.* 1997; Van Peer *et al.* 2001; Wookey *et al.* 2009; Paquette & Messier 2011) and even feedback to the climate system (Sturm *et al.* 2001; Chapin *et al.* 2005). *Anemone nemorosa* is considered to be a relatively stress tolerant species that dominates the understorey of many ancient European deciduous forests during the spring (Shirreffs 1985). Hence, we speculate that if its competitive ability would increase and if associates such as *Adoxa moschatellina*, *Viola riviniana*, *V. reichenbachiana*, *Hepatica nobilis*, *Hyacinthoides non-scripta* and *Ranunculus ficaria* would less benefit from climate warming, these species might increasingly be unable to establish below the dense *A. nemorosa* carpet. For instance, Pigott (1982) determined competitive exclusion of *H. non-scripta* by *A. nemorosa*: *A. nemorosa* attained local dominance when it was able to raise its leaves above the *H. non-scripta* canopy. Similarly, the significant increase in the abundance of *A. nemorosa* since the 1970s in Dalby Söderskog forest, southern Sweden, may have contributed to the decline of co-occurring species such as *A. moschatellina* (von Oheimb & Brunet 2007). Obviously, it remains unclear whether *A. nemorosa* is more favoured by warming than these associates, but as competition for light is asymmetric, small changes among co-existing species could have significant effects on community assembly. Also competition for light with recruiting tree seedlings might be enforced since competition between tree seedlings and herbaceous understorey plants depends on the maximal plant height of the latter group (Neufeld & Young 2003). We emphasise that many questions remain to be addressed in this field of forest understorey ecology (*cf.* §10.3) as few *in situ* climate manipulation research has been performed in temperate deciduous forests.

10.1.2 Seed production, quality, germination and seedling establishment

Our understanding of the effects of global change on plant recruitment is much more limited than the effects on vegetative responses (Hovenden *et al.* 2008; HilleRisLambers *et al.* 2009; Walck *et al.* 2011). Since recruitment from seeds is considered one of the most important phases of the life cycle of plants for population persistence and spread (e.g. Jacquemyn & Brys 2008; Dalgleish *et al.* 2010) but also one of the most temperature-sensitive phases, global warming is likely to influence gamete development, the progamic phase from pollination to fertilization and the postzygotic early embryo development as well as subsequent stages such as fruit and seed maturation, germination rate, and seedling establishment (reviewed in Hedhly *et al.* 2009 and Walck *et al.* 2011).

Here, we clearly showed that increasing temperatures have a pronounced positive impact on a wide variety of reproductive traits of *A. nemorosa*. Seed mass, for instance, increased by 9.7 % for every 1000 °C.hour increase in GDH along the latitudinal gradient (Chapter 5). The average experimental increase in GDH due to OTC installation in 2010 (+3200 °C.hour averaged over air, surface and soil temperatures; **Appendix C**), on the other hand, resulted in a significant increase of the mass of *A. nemorosa* seeds from 2.17 mg to 3.20 mg (i.e. an increase of 47 % or 14.7 % per 1000 °C.hour; Chapter 7). Hence, temperature variation brought about by natural variability along a climatic gradient and by experimental manipulation, induced a remarkable comparable response in mass of *A. nemorosa* seeds.

These results suggest that if climate warms, this will have a pronounced positive impact on the reproduction of *A. nemorosa*, especially in terms of seed mass, germination percentage and seedling mass. If more seeds germinate and resulting seedlings show higher fitness, more individuals might be recruited to the adult stage. As rhizome growth also is likely to benefit from higher winter temperatures (Philipp & Petersen 2007), it can be hypothesized that the colonisation potential of *A. nemorosa* populations (e.g. into post-agricultural forests; Chapter 9) may increase as the climate in northwestern Europe becomes warmer in the coming decades (see also §10.1.4).

The impact of changing temperatures on reproduction was lower on summer flowering species such as *B. sylvaticum* and *C. lutetiana* (Chapter 4). However, *M. effusum* seedlings originating from seeds collected in northern populations displayed more plasticity in biomass growth in response to variation in spring temperature than seedlings of southern origin. This was not the case for *A. nemorosa* (Chapter 8). Higher plasticity in seedling growth in the north is likely adaptive and may thus offer one way to cope with spatial and temporal environmental heterogeneity. Plasticity in seedling growth also offers a way to secure recruitment in a future, warmer climate. Other studies investigating seed production, germination and/or seedling establishment of plants showed both positive (Wookey *et al.* 1995; Hobbie & Chapin 1998; Milbau *et al.* 2009; Landhäusser *et al.* 2010; Klady *et al.* 2011) and negative (Hovenden *et al.* 2008; Graae *et al.* 2009a; Shevtsova *et al.* 2009) effects of rising temperatures on sexual reproduction (see Walck *et al.* 2011 for an overview). Of course is the impact of changing temperatures on germination and seedling establishment of some species also moisture-dependent (Shevtsova *et al.* 2009; Walck *et al.* 2011), but this was not investigated here. Hence, whether rising temperatures will confer or constrain plant recruitment and migration in the future will be largely species-dependent and thus future understorey community dynamics may be altered if the divergent responses of understorey plants are a widespread phenomenon.

The investigations of the seed nutrient concentrations (Chapter 3) revealed a previously unreported phenomenon: the seed nitrogen (N) concentration declined to the north in natural *A. nemorosa* populations. This can likely be attributed to lower seed provisioning due to lower N availability in the north. This relationship can have large implications for future changes in the seedling survival and reproductive success as seedlings resulting from seeds with higher N concentrations are known to be more competitive or perform better under poor environmental conditions (Parrish & Bazzaz 1985; Stock *et al.* 1990; Naegle *et al.* 2005; Chapter 3). Worldwide atmospheric deposition of biologically reactive N more than tripled between 1860 and the early 1990s (Galloway *et al.* 2004) and shows a northward decline across Europe (Holland *et al.* 2004; Bobbink *et al.* 2010). Chronic N enrichment has thus resulted in a changed N cycle (Lu *et al.* 2011) and large amounts of stored soil organic N in forests (Dörr *et al.* 2010). This organic N can decompose and become readily available for plant growth if the predicted climate warming would increase N mineralization rates (Rustad *et al.* 2001;

Turner & Henry 2010). Enrichment of bioavailable N could then result in a higher seed N concentration and seedling survival, but also increase herbivory and fungal pathogen attack (Gilliam 2006; Nordin *et al.* 2009).

10.1.3 Phenological syndromes

A classical phenological subdivision system of understorey plants in temperate deciduous forests consists of three groups (Dierschke 1982; Hermy 1985; Kudo *et al.* 2008):

1. spring flowering species that flower and fruit before canopy flush,
2. early summer flowering species that flower during the progress of canopy closure and fruit after canopy flush,
3. late summer flowering species that flower and fruit after canopy flush.

Kudo *et al.* (2008) recently showed for 18 perennial herbaceous understorey plants occurring in deciduous forest in Japan that species within each group possess a set of co-occurring physiological and reproductive characteristics (*syndromes*). They argued that this was mainly due to the canopy flush and related dramatic light availability decline in deciduous forests in spring (see also Neufeld & Young 2003). Sherry *et al.* (2007) found that plant species in a tallgrass prairie that flower before the summer heat peak tended to advance their phenology in response to experimental warming, whereas later flowering plants delayed their phenology.

We here found that spring flowering species seem to be more temperature-responsive than later flowering species. For instance, the leaf-height-seed traits of spring-flowering *A. nemorosa* were affected more by temperature, whereas early summer flowering *M. effusum* was affected more by local and other latitude-related factors. We therefore argue that the degree to which understorey plants respond to variation in temperatures is partly phenology-dependent. This extends the set of characteristics that co-occur within phenological groups (*syndromes*). Since our analyses are only based on a limited set of species, however, far-stretching generalisations could not be made.

Although this has already been discussed in detail in Chapters 1 and 4, we also highlight the importance of the current distribution range of understorey species here. Forest understorey species with a more southerly northern distribution range edge may show a distributional time lag as a legacy of the last Ice Age (*cf.* **Box** on page 6): many forest plant species have not yet reached their equilibrium distribution after the last glacial maximum due to poor colonisation (Van der Veken *et al.* 2007; Svenning *et al.* 2008). This can contribute to the fact that these more southerly distributed species (such as *B. sylvaticum* and *C. lutetiana*) are less responsive to temperature as they are not yet limited by climatic constraints. It is important to note that the species investigated here do not only contrast with regards to their phenology and distribution range, but also in connection to their growth form (grasses vs. forbs; see Chapter 7), colonisation rates, clonality, CSR-signature, etc.

10.1.4 Colonisation rates across Europe

By compiling data on 90 species from 18 studies across Europe, we demonstrated interactive effects of species' life-history traits and forest habitat availability on the recovery rate of post-agricultural forests. The recovery of short-lived herbs increased with the habitat availability in the landscape. Small perennial herbs, however, were generally unsuccessful in colonising post-agricultural forests — even in relatively densely forested landscapes.

Surprisingly, variation in mean annual temperature across Europe (6.6–15.0 °C; Chapter 9) had no effect on the floristic recovery of post-agricultural forests, although Chapters 2–8 stressed the impacts of rising temperatures on the growth and recruitment of individual species as *A. nemorosa*. This may be due to multiple factors which have already been glanced at in Chapter 9, but of which some are underlined here.

First, long-term adaptations to the local environment result in a bias in observational studies along geographic transects. For instance, some ecotypes of the same species may be adapted to growing in warmer climates whereas others perform equally well in colder conditions (Joshi *et al.* 2001; Leimu & Fischer 2008) leading to a *homogenisation* of colonisation rates across climates. As shown in Chapters 7 and 8, understorey species as *A. nemorosa* and *M. effusum* show local adaptation together with plastic responses to temperature variation. Hence, these species do not necessarily perform better when transplanted southwards in a region with a longer growing season, even though experimental warming with OTCs may have increased their performance. Thus, although we found no effects of temperature variation on the recovery of post-agricultural forests in the meta-analysis across Europe at present, climatic changes in the future may still have a plastic effect on colonisation and recovery rates.

Secondly, the impacts of more seed production and better seedling establishment on the population dynamics and migration rates of understorey species remain to be quantified. Production, maturation and germination of seeds and seedling establishment are only the first steps in the recruitment process from mother to adult daughter plants. Especially in highly fragmented contemporary landscapes, habitat availability appears to be the primary determinant of the recovery rate of post-agricultural forests (Chapter 9). Hence, dispersal limitation of diaspores between forest patches within a largely agricultural and urban matrix appears to be simply more important than variation in temperatures for the recovery rate of post-agricultural forests. Nevertheless, rising temperatures can still have a significant impact on competitive interactions within the current distribution range of species or positively affect migration of understorey plants within adjacent forest patches in the future (i.e. where no dispersal barrier occurs; see also §10.3).

Thirdly, the variable *mean annual temperature* calculated from weather station data (as applied in Chapter 9) alone may not represent the whole range of microclimatic heterogeneity within forests in each study region. Growing degree hours (GDH) based on data from temperature loggers (e.g. Chapter 7) are better suited to reflect plastic effects of plants to temperature variation during the growing season (*cf.* Graae *et al.* 2011). Thus, even though regions display different long-term mean annual temperatures (e.g. continental vs. maritime climates), plants might experience similar microclimatic temperatures or GDH during the growing season. However, detailed microclimatic temperature data from each forest patch are required to address this issue. Related to this subject is the use of GDH as a proxy for temperature in Chapters 2–5. Also this method displays specific advantages and drawbacks. The rather arbitrary choice of the threshold value as well as the possibility that plants respond to warming by advancing their phenology (and thus would experience the same GDH at maturity) deserves further consideration (Graae *et al.* 2011). The degree-hours between 1 January and seed maturity might as such be an evolved plant trait in response to environmental variation. For instance, the threshold value (5 °C) might change across the distribution range of species. The concept of accumulation of daily air temperatures (degree-days or degree-hours) above a certain threshold, on the other hand, generally performs better as approximation to predict, for instance, flowering phenology of perennial forest herbs than e.g. daily mean temperature (Diekmann 1996). Both the temperature as well as the duration the temperature is above the threshold for plant development are important for seed development and maturation of plant species (Meunier *et al.* 2007). The embryo in the seed still attached to the mother plant (sporophytic generation), for instance, needs a certain time to develop (Baskin & Baskin 1998). The GDH-concept is therefore often used as a proxy for temperature because of the demonstrated relationship between plant development and GDH (see e.g. Diekmann 1996; Meunier *et al.* 2007). Moreover, the non-significant correlation between latitude and GDH is essential to be able to statistically disentangle these factors (e.g. sections 3.2.3, 4.2.2 and 5.2.3). Finally, the mean temperature during the four to eight weeks before seed maturity and GDH were highly correlated in the present study (e.g. section 4.2.2). Hence, increasing temperatures in the month preceding seed maturity resulted in higher GDH values confirming the use of GDH as sound temperature proxy in climate warming studies (see also Prentice *et al.* 1992; Thuiller *et al.* 2005; Meunier *et al.* 2007).

10.2 Implications for conservation

Based on the results of this PhD thesis, we here formulate some implications of climate change for the conservation of plant species diversity in temperate deciduous forests.

Given the slow colonising character of perennial herbaceous understorey plants together with the low habitat availability in much of western Europe (Chapter 9), we believe that these species will be unable to fill their potential distribution ranges as predicted from shifts in the suitable bioclimatic envelope at the end of the 21st century (Svenning & Skov 2006). However, we here clearly showed that the performance and reproductive efficiency of understorey plants may alter in response to rising temperatures. This might affect the actual observed intrinsic colonisation capacity of these species. Hence, the effectiveness of dispersal corridors and stepping stones such as hedgerows towards new habitats at higher altitudes and latitudes as strategies to increase connectivity of isolated forest patches (Baudry *et al.* 2000; Tewksbury *et al.* 2002) might increase in the future. Sitzia (2007) and Wehling & Diekmann (2009) clearly indicated that hedgerows are suitable surrogate habitats for many understorey plants. Tewksbury *et al.* (2002) also showed higher fruit set and seed dispersal in connected versus in unconnected forest habitat patches and emphasised the utility of dispersal corridors for interpatch movements. Thus, the conservation and protection of, for example, hedgerows connected to source populations in ancient forests can constitute a mitigation option of climate change to facilitate migration should colonisation rates of perennial understorey plants effectively increase in the future. At least, increased connectivity definitely assists the migration of the more mobile (plant) species (Chapter 9).

The assisted translocation of populations, species or ecosystems towards higher altitudes and latitudes might be another option to mitigate the effects of climate change and preserve biodiversity (Hoegh-Guldberg *et al.* 2008; Richardson *et al.* 2009; Willis *et al.* 2009; Schlaepfer *et al.* 2009; Loss *et al.* 2011). Vanderveken (2008), for instance, argued that understorey plants could be particularly suited candidates to be assisted in their colonisation process since (i) they are slow colonising, (ii) likely of little threat to the new forest ecosystem, (iii) there are many candidate translocation sites in post-agricultural forests and (iv) translocating forest plants is convenient from a practical point of view (*cf.* also Hunter 2007). Here we show that ecotypes of understorey plants that are transplanted at contrasting latitudes might experience bottlenecks during their growth and recruitment and thus our findings of locally adapted ecotypes of understorey species cast some doubt on assisted migration as a mitigation strategy to save populations, species or ecosystems from extinction. For instance, the plant height and flowering probability of *A. nemorosa* and *M. effusum* plants of southern origin and transplanted at northern latitudes was lower than home transplants, and vice versa (Chapter 7). Conversely, Willis *et al.* (2009) successfully translocated two British butterfly species 35–65 km northward beyond their current distribution range edge. Hence, when there is a risk of moving maladapted ecotypes, a thorough monitoring of the introduced populations is necessary when assisted migration across latitudes is a management option (Minteer & Collons 2010; Vitt *et al.* 2010). It should be noted, however, that the majority of slow colonising understorey plants are not at instantaneous risk of extinction and thus, following the framework of Hoegh-Guldberg *et al.* (2008), managers should focus on conventional conservation techniques such as increasing the landscape connectivity or improving the genetic resilience of populations (see also Loss *et al.* 2011 and Dawson *et al.* 2011). At last, Vanderveken *et al.* (2008) calculated that hundreds of native European plant species are already sold hundreds to thousands kilometers north of their natural range limits in plant nurseries, which provides these species with an unintentional head start on climate change.

Finally, we stress the need to preserve ancient forests as refugia for slow colonising understorey plants (emergent group *small perennials with heavy seeds* from Chapter 9) in contemporary landscapes. An important outcome of Chapter 9 was that small perennial forest herbs strongly depend on temporal habitat continuity, occurring predominantly as relict populations in ancient forests or hedgerows. These species were most likely to be missing from the floristic community of post-agricultural forests. Thus, we stress the need to avoid ancient forest clearance to preserve the typical woodland flora with an intrinsically low recovery rate since these species generally fail to colonise post-agricultural forests. Additionally, to facilitate migration of forest understorey plants into post-agricultural sites, new forest establishment should focus on those sites that are adjacent to ancient forests (see also Honnay *et al.* 2002a).

10.3 Perspectives for further research

Based on the methodology and results from this thesis, a number of suggestions to further our understanding of the possible effects of climate change on forest plant dynamics are suggested.

Firstly, a major limitation of this thesis is the limited number of species involved. Therefore, our analyses could be extended to a much larger set of herbaceous forest plant species to be able to generalise among phenological syndromes, functional types or growth forms. We are far from understanding how understorey species with a different phenological syndrome, growth form and occurring close or far from their distribution edge, react to temperature changes. For instance, with respect to the observed latitudinal clines in plant height and seed nitrogen (Chapters 2–3), quantifications of the intraspecific variation in functional traits for many species across the globe would be worthwhile (see e.g. Moles & Westoby 2003 for a synthesis of interspecific and intraspecific seed mass data). Large global georeferenced traitbases such as `TRY` (`http://try-db.org/`) are a major leap forward. Moreover, additional *in situ* climate manipulation experiments could be initiated in temperate deciduous forests.

Secondly, we here hypothesized clear positive effects of climate warming on growth and recruitment of the characteristic understorey species *A. nemorosa*. It remains unclear, however, whether reproduction and recruitment of *A. nemorosa* will also benefit in warm and dry habitats within its distribution range (e.g. the Mediterranean region). Such habitats were not included in the present study. It is known that post-dispersal seed drying negatively affects the life span of *A. nemorosa* seeds (Ali *et al.* 2007) and that drought is a major cause of seedling death (Moles & Westoby 2004b). The seed and seedling dessication risk can be higher in warm and dry habitats. Likewise, precipitation changes and periods of extreme droughts, as predicted by the IPCC (2007), may negatively affect growth and recruitment of understorey forest plants (although vernal species can escape dry summer periods due to their specific phenology). Moreover, N deposition and climate warming could interactively affect the seed N concentration (Chapter 3 and 7). Extending this line of thought, we advocate the necessity to study the complex effects of multiple environmental changes on plants. Interactions of multiple global change drivers such as changing N deposition, climate warming, precipitation changes and elevated CO_2 concentration should therefore be a focus of future research (Zavaleta *et al.* 2003; Reich 2009; Baeten *et al.* 2010a).

Thirdly, the significance of increased seed production, quality and seedling establishment on dispersal and colonisation rates of forest plants remains to be quantified. It is far from clear that better recruitment of understorey species will lead to increased population growth rates and better colonisation. This issue could partly be assessed by, for instance, building matrix models with demographic data of different life stages of understorey plants (e.g. Jacquemyn & Brys 2008) collected for several consecutive years along a climatic gradient in ambient conditions and under experimental warming.

Finally, particularly little is known on how the interactions between understorey plants and other organisms such as pollinating, herbivorous or dispersing insects, symbiotic fungi as mycorrhiza, pathogens or belowground nematodes will be affected by climate change. Especially the impact of rematched timings between these organisms (both positive and negative for the plant, e.g. resulting in less fungal attack or pollination, respectively) and understorey plants are still largely unclear (see e.g. Memmott *et al.* 2007).

A

Study regions along the latitudinal gradient

Table A.1: Location and long-term (1961-1990) climatic characterization of the study regions along the latitudinal gradient and an overview of the study regions that are included in each chapter (indicated with ⊗)

Country or region	Closest city	Lat.	Long.	MAT[a]	MAP[b]	PET[c]	PET:	Included in Chapter						
		(°N)	(°E)	(°C)	(mm)	(mm)	MAP[d]	2	3	4	5	6	7	8
northern France	Amiens	49.8	2.1	9.9	671	571	0.85	⊗	⊗	⊗	⊗		⊗	⊗
Belgium	Brakel/Gontrode	50.9	3.8	9.7	821	580	0.71	⊗	⊗	⊗	⊗	⊗	⊗	⊗
northeastern Germany	Potsdam	52.6	13	8.7	585	642	1.1	⊗	⊗	⊗	⊗		⊗	⊗
northwestern Germany	Bremen	53.3	9.2	8.3	761	557	0.73	⊗	⊗	⊗	⊗		⊗	⊗
southern Sweden	Lund/Alnarp	55.6	13.3	7.9	732	545	0.74	⊗	⊗	⊗	⊗		⊗	⊗
Estonia	Tartu	58.4	26.7	4.8	585	553	0.95		⊗					
central Sweden	Stockholm	59.2	17.5	6.6	535	606	1.13	⊗	⊗	⊗	⊗		⊗	⊗
northern Sweden	Umeå	63.8	20	3.4	603	474	0.79	⊗	⊗		⊗		⊗	⊗
northern Sweden	Abisko	68.4	18.8	1.8	305	318	1.03	⊗					⊗	⊗

[a] MAT: mean (1961-1990) annual temperature; [b] MAP: mean annual precipitation; [c] PET: potential evapotranspiration; [d] PET:MAP: PET divided by MAP, aridity index (F.A.O. 2005)

B

Anthesis, seed collection dates and seed traits of *A. nemorosa* in 2009

Table B.1: Anthesis, seed collection dates (in days following anthesis) and mean seed trait values (± S.E.) across all collection dates of 15 *Anemone nemorosa* populations in eight regions along a latitudinal gradient in 2009 for the analyses described in Chapter 3

	France	**Belgium**	**Germany**	**Germany**	**Sweden**	**Estonia**	**Sweden**	**Sweden**
	Amiens	Brakel	Potsdam	Bremen	Lund	Tartu	Stockholm	Umeå
Anthesis (Julian day)	78	78	85 & 88[a]	84	95	117	105	136
First SCD	39	35	34	35	35	28	31	27
Final SCD	60	60	50	50	60	53	59	53
Total no. SCDs	6	6 & 7[a]	3 & 4[a]	3 & 4[a]	6	5	6	6
Seed mass (mg)	2.1 (0.29)	3.05 (0.43)	1.87 (0.17)	2.41 (0.11)	2.02 (0.22)	2.14 (0.19)	2.06 (0.27)	1.55 (0.29)
Germination (%)	38.25 (9.74)	45.61 (8.29)	77.14 (5.06)	65.95 (4.88)	28.69 (9.26)	58.3 (10.0)	26.05 (8.99)	40.77 (15.4)
Seed P (ppm × 1000)	3.91 (0.15)	4.25 (0.17)	4.04 (0.38)	5.25 (0.15)	3.87 (0.08)	4.41 (0.21)	3.84 (0.08)	3.90 (0.12)
Seed N (%)	3.33 (0.22)	3.47 (0.19)	3.27 (0.15)	3.82 (0.11)	3.16 (0.06)	2.84 (0.09)	2.55 (0.03)	2.51 (0.06)
Seed C (%)	49.22 (0.66)	49.85 (0.86)	53.44 (0.83)	50.93 (0.72)	50.94 (1.05)	50.77 (1.40)	48.47 (1.39)	50.57 (1.90)
Seed K (ppm × 1000)	27.96 (0.42)	24.90 (1.55)	20.65 (1.33)	26.04 (1.65)	18.12 (0.93)	20.10 (0.86)	22.56 (0.81)	28.17 (1.84)
Seed Ca (ppm × 1000)	5.24 (0.45)	4.15 (0.30)	3.32 (0.32)	3.63 (0.45)	2.09 (0.29)	2.45 (0.47)	4.18 (0.49)	3.50 (0.60)
Seed Mg (ppm × 1000)	3.07 (0.05)	2.89 (0.13)	2.56 (0.17)	3.16 (0.16)	2.76 (0.14)	2.70 (0.12)	2.60 (0.09)	3.03 (0.21)
Seed C:N	15.36 (0.8)	14.82 (0.76)	16.54 (0.89)	13.40 (0.53)	16.14 (0.32)	18.12 (0.97)	19.06 (0.64)	20.08 (0.60)
Seed N:P	8.41 (0.45)	8.12 (0.20)	8.34 (0.44)	7.27 (0.06)	8.20 (0.23)	6.52 (0.22)	6.68 (0.14)	6.46 (0.19)

[a] Due to small differences in phenology between the two populations

C

Microclimatic data from the common gardens

Legend to **Table C.1** (next page)

GDH	growing degree-hours [°C.hour] above 5 °C calculated according to Lindsey & Newman (1956) between 1 January and 15 June
NA	not applicable since both species were already harvested in July in Belgium
[a]	Data from 17 Sept. 2008 onwards in Belgium, 6 Sept. 2008 in south Sweden and 4 Sept. 2008 in north Sweden
[b]	Data until 15 June 2010 in Belgium
[c]	Data until 10 July 2010 in south Sweden and 8 July 2010 in north Sweden

Table C.1: Monthly and average microclimatic data (relative humidity, RH [%]; temperature, T [°C]; and growing degree-hours, GDH [°C.hour]) in the three common gardens and in the open-top chambers in the southernmost common garden (average of two sensors; described in Chapter 7)

	Belgium, ambient				Belgium, warmed				southern Sweden, ambient				northern Sweden, ambient			
	RH	T	T	T	RH	T	T	T	RH	T	T	T	RH	T	T	T
At height→	10cm	10cm	0cm	-5cm	10cm	10cm	0cm	-5cm	10cm	10cm	0cm	-5cm	10cm	10cm	0cm	-5cm
Sept-08[a]	88.1	11.3	10.5	11.7	90.7	11.6	11.2	12.5	82.1	12.6	11.4	10.7	81.0	8.2	7.8	7.8
Oct-08	94.3	8.2	7.8	9.7	96.3	8.6	8.4	10.7	87.0	10.4	9.3	8.5	88.9	4.3	4.5	4.5
Nov-08	96.2	5.0	4.9	7.0	97.7	5.4	5.7	8.2	89.2	6.2	5.7	5.2	90.2	-1.7	0.4	0.5
Dec-08	94.7	1.4	1.7	4.0	96.4	1.8	2.7	5.4	93.7	2.8	2.8	2.5	92.8	-2.0	0.1	0.4
Jan-09	92.3	0.4	0.7	2.1	94.4	0.6	1.2	3.6	90.6	0.7	0.3	0.0	89.2	-5.6	-0.2	-0.6
Feb-09	93.3	3.5	3.6	3.5	93.2	3.7	3.6	4.8	89.7	0.4	0.4	-0.1	92.8	-6.6	-0.4	-0.2
Mar-09	83.0	6.8	6.8	6.0	83.1	7.5	6.9	7.3	84.8	4.0	2.9	1.9	94.3	-2.1	0.0	0.4
Apr-09	86.7	10.4	10.3	10.1	87.1	10.8	10.4	10.8	66.6	10.5	8.7	7.3	82.6	2.4	1.2	1.7
May-09	84.6	13.6	12.3	12.0	84.3	13.6	12.6	12.3	73.6	12.3	10.7	9.1	64.4	10.9	9.0	8.9
Jun-09	85.2	15.1	14.0	13.6	85.4	15.1	14.2	13.5	76.2	14.4	12.3	10.8	66.9	13.8	13.0	11.7
Jul-09	89.0	16.8	15.4	15.5	89.1	16.7	16.0	15.4	77.9	18.5	16.1	14.6	82.1	15.3	15.6	14.4
Aug-09	85.9	16.7	15.1	15.2	86.4	16.8	16.1	15.9	75.9	18.2	15.9	14.6	84.0	14.8	15.5	14.2
Sep-09	86.8	14.9	12.8	12.7	87.6	14.9	14.3	14.6	79.0	15.0	13.3	12.4	81.7	10.9	11.9	11.2
Oct-09	92.0	11.0	9.0	9.6	92.6	10.9	10.8	12.2	84.4	7.8	6.6	6.8	86.3	1.2	3.8	4.0
Nov-09	93.6	9.4	7.1	8.5	94.1	9.2	9.2	10.8	92.0	7.3	5.7	5.7	94.4	1.4	3.7	3.3
Dec-09	94.6	2.7	2.1	4.1	95.1	2.6	3.7	6.7	92.4	1.3	1.5	2.5	91.0	-5.8	1.2	0.7
Jan-10	94.7	0.3	-0.3	1.3	94.8	0.2	1.1	3.9	87.6	-3.1	-2.1	-1.1	94.9	-5.3	3.3	0.4
Feb-10	92.9	2.5	1.2	2.2	92.8	2.5	2.9	4.6	91.0	-1.0	-1.1	-1.0	95.5	-4.4	3.9	0.4
Mar-10	82.1	6.8	4.1	4.1	81.3	7.2	6.1	7.3	84.6	3.3	1.4	1.1	97.6	-1.7	2.9	0.4
Apr-10	74.4	10.2	8.6	8.0	73.5	10.9	9.5	10.2	74.6	8.2	5.9	5.8	79.9	2.2	2.6	1.2
May-10	82.3	10.9	9.0	9.8	82.1	11.0	9.8	11.3	79.5	10.9	8.8	8.5	71.4	10.3	10.2	7.8
Jun-10[b]	86.6	15.4	10.6	11.1	86.6	15.3	14.3	14.2	79.7	14.8	12.2	11.6	72.5	12.0	12.1	10.2
Jul-10[c]	NA	NA	NA	NA	NA	NA	NA	NA	70.9	19.2	16.0	15.0	86.0	16.2	16.4	13.8
Average	88.3	8.6	7.5	8.1	88.7	8.8	8.5	9.6	83.1	8.1	6.9	6.4	85.2	3.5	5.7	4.8
GDH 2009		16850	15175	12502		19625	15514	14471		13652	10475	7549		7925	5768	5241
GDH 2010		16250	11195	8455		17885	13853	13895		11750	7793	6914		7307	7095	4074

D

Seed collection dates in 2008 and 2009 and supplementary figures

Table D.1: Seed collection dates of the *in situ* (2008) and *ex situ* (2009) seeds (in Julian days) along the latitudinal gradient for the experiments described in Chapter 8

Region	Lat. (°N)	Seed collection dates *in situ*		Seed collection dates *ex situ*
		A. nemorosa	*M. effusum*	*M. effusum*
northern France	49.8	140	157	
Belgium	50.9	130	157	162
northeastern Germany	52.6	136 & 137	162 & 164	
northwestern Germany	53.3	142	165	
southern Sweden	55.6	141	170 & 171	173
central Sweden	59	157	198	
northern Sweden	63.8	176	187 & 198	195
northern Sweden	68.4	-	235 & 239	

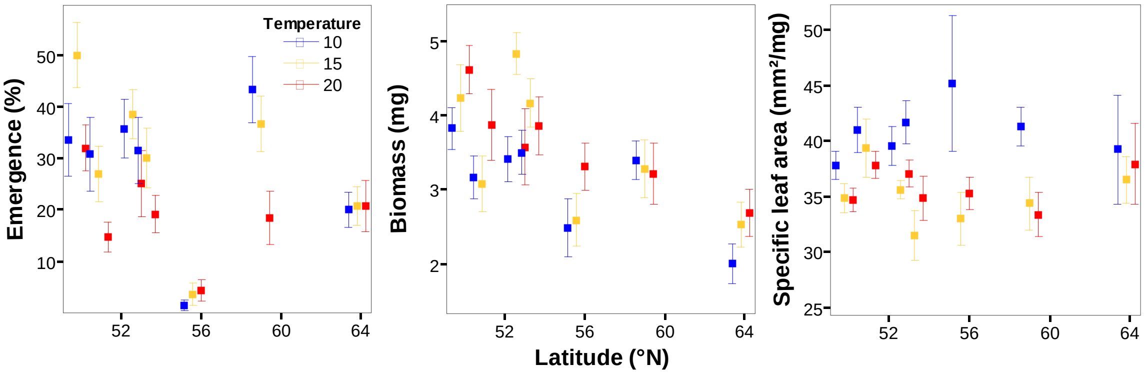


Figure D.1: Response of seedling traits of *Anemone nemorosa* resulting from *in situ* collected seeds to three spring-temperature scenarios along a latitudinal gradient (blue: 10 °C, yellow: 15 °C, red: 20 °C)

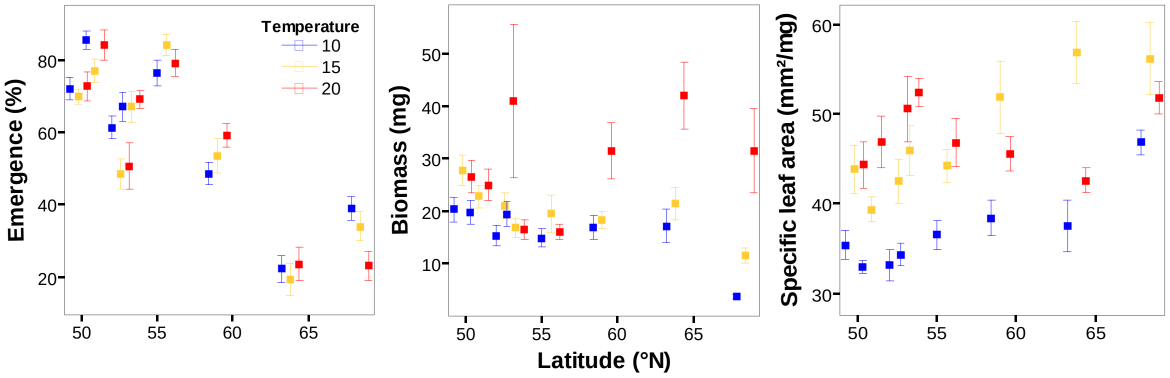


Figure D.2: Response of seedling traits of *Milium effusum* resulting from *in situ* collected seeds to three spring-temperature scenarios along a latitudinal gradient (blue: 10 °C, yellow: 15 °C, red: 20 °C)

E

Studies, emergent groups and species used in the meta-analysis

Table E.1: Overview of the 18 studies used in the meta-analysis (from north to south) described in Chapter 9. The numbers of the publications in this table refer to **Table 9.1** and **Fig. 9.1**.

ID	Publication	Size study area (km^2)	TF[a] (%)	AF[b] (%)	Mean CT[c]	Max CT[c]	MAT (°C)[d]	Aridity index[e]	mN[f]	mR[f]	mF[f]	mL[f]
1	Cousins and Eriksson (2008)	28	38	2	50	60	6.6	0.89	2.8	2.7	3.1	4.9
2	Graae (2000)	1029	15	5.6	135	200	7.67	1.28	4.5	3.4	4.1	3.7
3	Petersen (1994)	16[g]	8.7	0.9	53	100	8.21	0.74	5.8	4.7	4.6	3.5
4	Brunet (2007)	36	28	13	86	200	7.94	1.34	5.1	4.8	4.7	3.1
5	Kolb & Diekmann (2004)	365	13	3	130	240	8.3	1.37	4.5	3.6	4.9	4.2
6	Peterken & Game (1984)	930	5.6	2.8	201	370	9	1.13	5	3.6	4.6	4.7
7	Wulf (2003)	4090	22	15	58	100	7.94	0.93	4.9	4	4.8	4
8	Grashof-Bokdam & Geertsema (1998)	4.67[g]	50.7	10.7	80	150	8.94	1.45	3.6	3.3	3.9	3.9
9	Zacharias (1994)	3729[g]	23.8	21.2	79	90	8.67	0.97	4.7	4.3	4.3	4.6
10	Jakubowska-Gabara & Mitka (2007)	127[g]	45.2	18.5	115	160	7.74	0.87	4.1	3.6	4.4	3.8
11	Honnay *et al.* (1998)	8658[g]	8.5	6	94	120	9.57	1.23	4.5	4.3	4.7	4.6
12	Orczewska (2009)	3047	36	27	24	54	8.23	1.06	4.7	3.6	5.2	4.8
13	Jacquemyn *et al.* (2001)	80	15	6	70	200	9.68	1.38	4.3	3.3	4.3	4.5
14	Verheyen *et al.* (2003)	3.6	42	4.2	98	195	9.68	1.38	5.4	4.9	6	3.8
15	Dzwonko & Loster (1989)	11.7	10.1	10	70	141	7.71	1.88	4.5	4	4.5	3.5
16	Jamoneau (2010)	25	5.4	4.1	79	127	10.17	0.94	4.6	4.4	4.1	4.9
17	Sciama *et al.* (1999, 2009)	417	46	21	85	170	9.52	2.58	4.2	4.5	3.9	4.2
18	De Sanctis *et al.* (2010)	800	20	16	90	130	14.97	0.81	2.5	3.4	2.6	3.2

[a] Total forest cover. [b] Ancient forest cover. [c] Mean and maximum colonization time (CT) of the post-agricultural stands. [d] Mean annual temperature (1961-1990; F.A.O. 2005). [e] Aridity index calculated by dividing the mean annual precipitation by the potential evapotranspiration (1961-1990; F.A.O. 2005). [f] Average indicator value for nutrients (mN), reaction (mR), moisture (mF) and light (mL) (Ellenberg *et al.* 1992) for the ancient forest stands. [g] The size of the study area was estimated from a map (with scale) provided in the original publication in these cases. In all other cases, the exact study area was available.

Table E.2: Overview of the relative value of the life-history trait values per trait for the different emergent groups (after Verheyen *et al.* 2003c)

(a) Herbs	Sign.	EG1	EG2	EG3	EG4
Seed mass (mg)	***	b	b	a	b
Seed size (mm)	(ns)	a	a	a	a
Seed shape (length/width ratio)	***	ab	b	b	a
No. seeds per plant	***	a	a	b	a
Max. height (m)	***	b	b	b	a
Vegetative spread (absent, intermediate, strong)	***	a	c	b	c
Germination requirements (immediate, intermediate, difficult)	(ns)	a	a	a	a
Age of first reproduction (year)	***	a	b	b	b
Seed bank (strictly transient to strictly persistent)	***	ab	a	b	a
Dispersal type[a,b]	***	2	2	1	3
Life cycle[a,c]	***	1	3	3	3
Flowering phenology[a,d]	***	3	2	2	1

(b) Graminoids	Sign.	EG1	EG2	EG3
Seed mass (mg)	(ns)	a	a	a
Seed shape (length/width ratio)	**	ab	a	b
No. seeds per plant	(ns)	a	a	a
Max. height (m)	*	b	a	ab
Vegetative spread (absent, intermediate, strong)	***	a	b	ab
Germination requirements (immediate, intermediate, difficult)	**	b	a	ab
Age of first reproduction (year)	(ns)	a	a	a
Seed bank (strictly transient to strictly persistent)	*	a	ab	a
Dispersal type[a,b]	(*)	2	2	3
Flowering phenology[a,d]	***	2	2	1

The emergent groups (EG) for the herbs were: EG1: short-lived herbs, EG2: tall perennials with heavy seeds, EG3: tall perennials with light seeds, and EG4: small perennials with heavy seeds. For the graminoids the three groups were EG1: large, summer flowering graminoids, EG2: small, summer flowering vegetatively spreading graminoids, and EG3: early flowering graminoids. An (a) always depicts the lowest value for that trait, (b) and (c) respectively the second and third ranked value (from low to high). Significance values from Kruskal-Wallis tests or Pearson χ^2-association tests. [a]'1' depicts that mainly dispersal type '1', life cycle class '1' or flowering phenology class '1' occurs in that emergent group. Similar for '2' and '3'. [b]Dispersal type subdivided in three classes: 1:anemochory, 2: endo- & exozoochory, 3: ballisto-, myrmecochory & unassisted. [c]Life cycle subdivided in three classes: 1 annual, 2 biennial and 3 perennial. [d]Flowering phenology subdivided in three classes: 1 spring flowering, 2 mid summer flowering, 3 spring-mid summer flowering.

Table E.3: Mean recovery rate (RR) and coefficient of interregional variation (CIV_{RR}) for the 90 species included in this meta-analysis. The species are arranged from slow (negative RR) to fast colonizers (positive RR). Also the number of publications in which the species was cited (No. publ.; out of 18 studies) and the emergent group (Verheyen *et al.* 2003c) of each species is given.

No.	Species	RR	CIV_{RR}	No. publ.	EG herbs[a]	EG graminoids[a]
1	*Luzula pilosa*	-2.29	0.39	12		3
2	*Viola riviniana*	-1.56	0.44	17	4	
3	*Carex pallescens*	-1.56	0.60	7		3
4	*Geum rivale*	-1.50	0.52	5	4	
5	*Melica uniflora*	-1.49	0.47	13		3
6	*Sanicula europaea*	-1.42	0.51	10	4	
7	*Galium odoratum*	-1.41	0.61	10	4	
8	*Convallaria majalis*	-1.35	0.55	14	4	
9	*Mercurialis perennis*	-1.24	0.54	12	4	
10	*Hypericum hirsutum*	-1.23	0.44	5	3	
11	*Allium ursinum*	-1.21	0.44	7	4	
12	*Digitalis purpurea*	-1.20	0.50	5	3	
13	*Potentilla sterilis*	-1.13	0.61	6	4	
14	*Campanula trachelium*	-1.12	0.87	10	3	
15	*Carex sylvatica*	-1.10	0.72	13		3
16	*Dactylorhiza maculata*	-1.05	0.62	5	3	
17	*Valeriana officinalis*	-1.01	0.45	6	2	
18	*Oxalis acetosella*	-1.01	0.42	15	4	
19	*Primula elatior*	-1.01	0.78	8	4	
20	*Holcus mollis*	-0.98	0.54	8		2
21	*Maianthemum bifolium*	-0.98	0.45	12	4	
22	*Veronica montana*	-0.98	0.48	6	4	
23	*Lamium galeobdolon*	-0.95	0.41	14	4	
24	*Scrophularia nodosa*	-0.93	0.52	13	3	
25	*Ajuga reptans*	-0.89	0.52	11	4	
26	*Veronica officinalis*	-0.88	0.56	8	4	
27	*Anemone nemorosa*	-0.88	0.37	17	4	
28	*Lysimachia vulgaris*	-0.87	0.58	6	2	
29	*Stellaria holostea*	-0.87	0.46	14	4	
30	*Carex pilulifera*	-0.83	0.56	7		3
31	*Carex remota*	-0.82	0.77	10		3
32	*Circaea lutetiana*	-0.78	0.59	9	2	
33	*Aegopodium podagraria*	-0.77	0.76	9	2	
34	*Senecio sylvaticus*	-0.71	0.95	5	1	
35	*Elymus caninus*	-0.71	0.64	5		1
36	*Milium effusum*	-0.71	0.61	15		3
37	*Deschampsia cespitosa*	-0.65	0.61	10		2
38	*Melampyrum pratense*	-0.63	0.90	10	1	
39	*Deschampsia flexuosa*	-0.63	0.39	8		2
40	*Epilobium angustifolium*	-0.62	0.25	6	3	

No.	Species	RR	CIV$_{RR}$	No. publ.	EG herbs[a]	EG graminoids[a]
41	*Anthriscus sylvestris*	-0.61	1.06	5	2	
42	*Moehringia trinervia*	-0.60	0.68	13	1	
43	*Epilobium montanum*	-0.58	1.03	9	3	
44	*Rumex sanguineus*	-0.56	0.54	9	2	
45	*Ranunculus auricomus*	-0.55	0.52	10	4	
46	*Arum maculatum*	-0.54	0.64	6	4	
47	*Paris quadrifolia*	-0.54	0.51	12	4	
48	*Adoxa moschatellina*	-0.53	0.67	14	4	
49	*Primula veris*	-0.53	0.46	5	4	
50	*Agrostis capillaris*	-0.52	0.47	5		2
51	*Poa nemoralis*	-0.50	0.34	14		1
52	*Heracleum sphondylium*	-0.50	0.97	6	2	
53	*Orchis mascula*	-0.47	1.16	6	3	
54	*Hypericum perforatum*	-0.46	0.36	7	3	
55	*Glechoma hederacea*	-0.45	0.81	11	4	
56	*Mycelis muralis*	-0.44	0.68	10	3	
57	*Brachypodium sylvaticum*	-0.42	0.44	13		1
58	*Solidago virgaurea*	-0.39	1.17	8	3	
59	*Fragaria vesca*	-0.37	0.83	11	4	
60	*Silene dioica*	-0.34	0.74	7	3	
61	*Cirsium vulgare*	-0.34	0.17	5	1	
62	*Stachys sylvatica*	-0.31	0.31	13	2	
63	*Listera ovata*	-0.30	0.94	10	3	
64	*Veronica chamaedrys*	-0.27	0.63	9	4	
65	*Ranunculus ficaria*	-0.25	0.51	13	4	
66	*Epipactis helleborine*	-0.19	1.00	9	1	
67	*Teucrium scorodonia*	-0.12	1.42	6	2	
68	*Dactylis glomerata*	-0.12	0.37	7		1
69	*Carex muricata*	-0.09	0.86	6		3
70	*Impatiens parviflora*	-0.05	1.40	7	1	
71	*Solanum dulcamara*	-0.02	0.21	6	2	
72	*Poa trivialis*	-0.01	0.48	6		3
73	*Geum urbanum*	0.02	0.56	15	4	
74	*Veronica hederifolia*	0.03	0.66	7	1	
75	*Molinia caerulea*	0.03	0.85	5		3
76	*Galeopsis tetrahit*	0.04	0.33	6	1	
77	*Filipendula ulmaria*	0.09	0.63	6	2	
78	*Alliaria petiolata*	0.10	1.09	9	1	
79	*Rumex acetosella*	0.12	1.12	6	4	

No.	Species	RR	CIV$_{RR}$	No. publ.	EG herbs[a]	EG graminoids[a]
80	*Urtica dioica*	0.14	0.41	9	2	
81	*Torilis japonica*	0.17	0.86	7	1	
82	*Stellaria media*	0.17	1.41	7	1	
83	*Festuca gigantea*	0.18	1.14	11		1
84	*Lapsana communis*	0.20	0.91	10	1	
85	*Ranunculus repens*	0.22	0.90	7	4	
86	*Geranium robertianum*	0.24	1.37	13	1	
87	*Viola odorata*	0.29	0.47	7	4	
88	*Chaerophyllum temulum*	0.40	1.19	9	1	
89	*Lysimachia nummularia*	0.45	0.59	7	2	
90	*Galium aparine*	0.46	0.47	10	1	

[a] Emergent groups (Verheyen *et al.* 2003c): 1. short-lived herbs, 2. tall perennial herbs with heavy seeds, 3. tall perennial herbs with light seeds, and 4. small perennial herbs with heavy seeds. For the graminoids the three groups are: 1. large, summer flowering graminoids, 2. small, summer flowering vegetatively spreading graminoids, and 3. early flowering graminoids

F

Captions of the photos used between each chapter

p. xx	*Anemone nemorosa* population in 't Burreken, Brakel, Belgium
p. 14	Inflorescence of *Milium effusum* in Umeå, Sweden (above) and flowering ramets of *A. nemorosa* in Gontrode, Belgium (below)
p. 30	*A. nemorosa* seed and flowering adult individual in Gontrode, Belgium
p. 44	*Paris quadrifolia* in Abisko, Sweden
p. 60	*A. nemorosa* flower in Gontrode, Belgium
p. 76	Open-top chambers in subarctic tundra in Abisko, Sweden and in a deciduous forest in Gontrode, Belgium
p. 92	Radiation shields minimizing solar radiation and maximizing airflow over the temperature and relative humidity sensors in the common garden experiment in Belgium
p. 112	Germinating seeds of *M. effusum* with emerging radicle (below left) and shoot
p. 126	An isolated forest patch in a typical contemporary northwestern European landscape
p. 142	*Primula elatior* and *A. nemorosa* in the background

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Curriculum vitae

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Scientific publications

Publications in international peer-reviewed journals (IF: 2009 impact factor)

- Ampoorter E, **De Frenne P**, Hermy M, Verheyen K. Effects of soil compaction on growth and survival of tree saplings: a meta-analysis. *Basic and Applied Ecology*, major revisions (IF 2.422).

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- Verheyen K, Baeten L, **De Frenne P**, Verstraeten G (2011) It's more than nitrogen deposition: driving factors behind the eutrophication signal in temperate forest understorey plant communities. Abstract of oral presentation given by K Verheyen at the Annual Symposium of the British Ecological Society (Cambridge, UK; March, 28–30, 2011)

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- **De Frenne P**, Graae BJ, Verheyen K, Hermy M (2010) Phenotypic plasticity across latitude: A buffer against climate warming in forest herbs? Abstract of oral presentation at 40th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GFÖ) (Giessen, Germany; August, 30 – September, 3, 2010)

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- **De Frenne P**, Graae BJ, Kolb A, Hermy M, Verheyen K *et al.* (2009) Significant effects of temperature on the sexual reproduction of the clonal forest herb *Anemone nemorosa* L. Abstract of oral presentation at 9th Clonal Plant Workshop: beyond the patterns - ecological and evolutionary dynamics of asexual reproduction (Leuven, Belgium; July, 1–4, 2009)

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- Verheyen K, De Schrijver A, Staelens J, Baeten L, **De Frenne P**, Adriaenssens S, Verstraeten G, Ampoorter E, Van Nevel L, Demey A, Wuyts K, Gruwez R (2010) Pilootstudie naar kwantificering van de relaties tussen de achteruitgang van biodiversiteit en chronische overschrijding van kritische lasten. Studie in opdracht van het Instituut voor Natuur- en Bosonderzoek (Natuurrapportering).

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MSc thesis

- **De Frenne P** (2007) Vernatting van grasland: de agronomische impact. MSc thesis, Ghent University, Ghent, Belgium.

Scientific activities

Participation in congresses, symposia or workshops

Participation with oral presentation

- **De Frenne P**, Brunet J, Shevtsova A, Graae BJ, Verheyen K – Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. Annual Symposium of the British Ecological Society (Cambridge, UK; March, 28–30, 2011)

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- Co-organisation (co-chair) of session *Biodiversity along altitudinal and latitudinal gradients* at 40th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GFÖ) (Giessen, Germany; August, 30 – September, 3, 2010)

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- **De Frenne P**, Kolb A, Verheyen K, Hermy M, Graae BJ *et al.* – Unravelling the influence of temperature, latitude and local environment on the reproduction of six forest herbs along a latitudinal gradient. IUFRO Conference on Adaptation of Forests and Forest Management to Changing Climate (Umeå, Sweden; August, 25–28, 2008)
- Verheyen K, **De Frenne P**, Van Der Veken B – Gevolgen van klimaatverandering voor bosplantensoorten: adaptatie, migratie en extinctie. Presentation given by K Verheyen at seminar *Bos en Klimaat Studiedag* (Agentschap voor Natuur- en Bos) (Brussels, Belgium; September, 30, 2008)

Participation with poster presentation

- **De Frenne P**, Brunet J, Shevtsova A, Graae BJ, Hermy M, Verheyen K – Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Starters in het Bosonderzoek* conference (Brussels, Belgium; March, 17, 2011)

- **De Frenne P**, Kolb A, Verheyen K, Hermy M, Graae BJ *et al.* – Unravelling the effect of temperature, latitude and local environment on the reproduction of six forest herbs along a latitudinal gradient. *Aardse Zaken* symposium - K.U. Leuven (Leuven, Belgium; May, 30, 2008)

Participation without presentation

- Workshop *Bos en Klimaat Studiedag* (Agentschap voor Natuur- en Bos; Brussels, Belgium; September, 30, 2008)
- Second Generation Bio-fuels Workshop (Ghent Bio-energy Valley; Ghent, Belgium; April, 10, 2008)
- NecoV-wintersymposium Timeless Ecology: from seconds to centuries (Dutch and Flemish Ecological Society; Antwerp, Belgium; February, 7-8, 2008)
- 37th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GFÖ) (Marburg, Germany; September, 11-12, 2007)

Supervision of MSc thesis students

- 2010-2011: Justin Willaert: De mogelijke invloed van klimaatverandering op twee bosplanten. Supervisors: Prof. dr. ir. Kris Verheyen, Prof. dr. Martin Hermy
- 2008-2009: Rob D'hondt: Variatie in de kenmerken van bosplantenpopulaties langsheen een latitudinale gradiënt. Supervisors: Prof. dr. ir. Kris Verheyen, Prof. dr. Martin Hermy

Review tasks for international journals

- American Journal of Botany
- Australian Journal of Botany
- Global Change Biology
- Journal of Forest Research
- Trees - Structure and Function