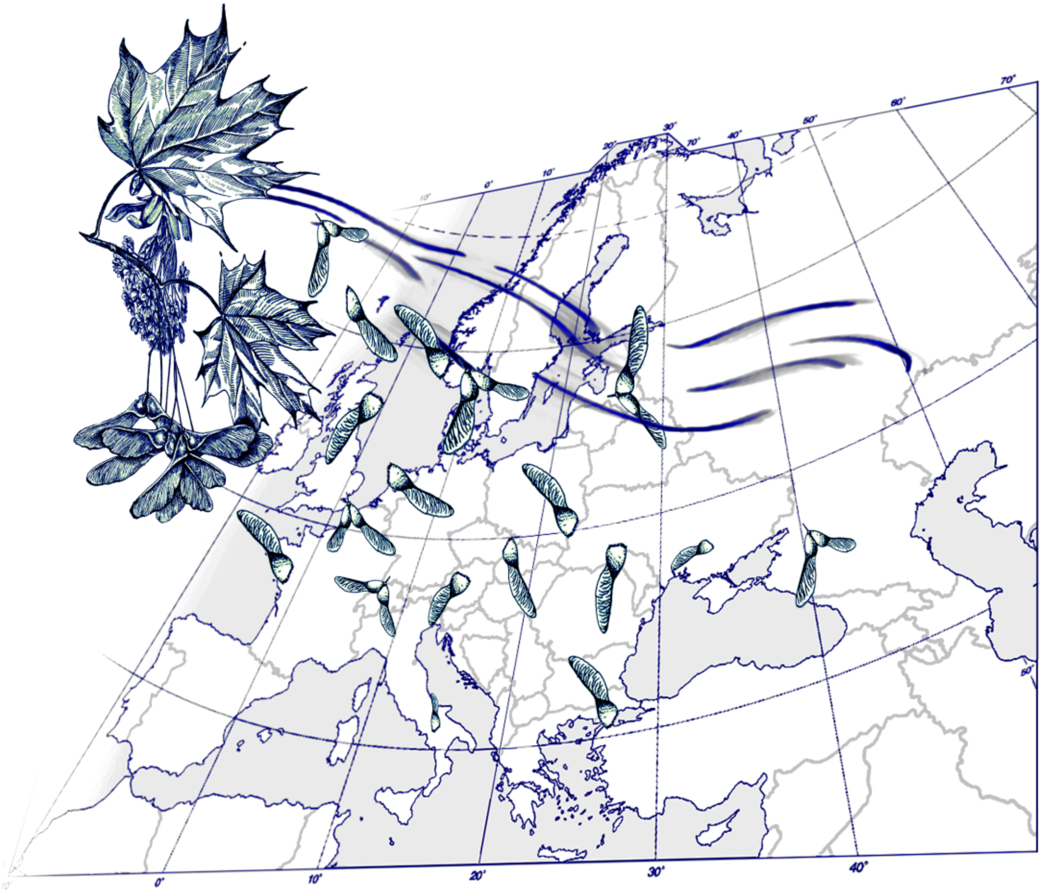
Regeneration From Seeds of Two Acer Species in the Face of Climate Change



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Regeneration from seeds of two *Acer* species in the face of climate change

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Dutch translation of the title:
De invloed van klimaatverandering op de verjonging van esdoorn (*Acer platanoides* en *A. pseudoplatanus*)

Illustrations on the cover:
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Nada grande puede hacerse sin alegría
Arturo Jaureche

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Content

Summary

The warming of the global system is an accepted phenomenon and it is considered unprecedented over decades to millennia. By the end of this century, depending on the concentration-driven scenario, an increase of the global mean temperature between 0.3 °C and 4.8 °C is projected. In Europe, the mean annual temperature will likely increase more in the northern than in central or southern regions. On the other hand, increased mean annual precipitation is expected in northern and central Europe, and decreases around the Mediterranean. Also the frequencies of rainfall will likely change, with more extreme rainfall events and longer dry intervals.

Impacts of contemporary climate change are already visible in several ecosystems around the world. Climate influences plant population dynamics in many ways: not only species' distributions and phenology are affected but also recruitment, growth and mortality. However, the sensitivity of the different life-cycle stages to climate change is expected to differ. One of the most sensitive phases is the sexual reproductive phase; therefore, this is a good indicator of species' sensitivity to environmental change, often better than the response of other aspects of the plant life cycle such as growth and mortality of mature plants.

Considering that the impacts of climate change on some key process of reproduction such as pollination, seed production, dormancy break, germination, seedling survival and subsequent seedling growth are not well known and are still being investigated, increasing our knowledge on this phase is very relevant. Moreover, models predicting changes in species' performance, distribution and community composition urgently need to incorporate reliable information on species' reproduction in the face of climate change. Considering that a compositional change due climate change is forecasted for European forests in favour of currently secondary tree species, increasing our understanding of the effects of climate change on these species' recruitment is key to better understand and forecast population and community dynamics in forests.

In this thesis we contribute to fill this knowledge gap by analysing the impacts of climate change on sexual reproduction of two important secondary forest tree species *Acer platanoides* (Norway maple) and *A. pseudoplatanus* (sycamore maple)

along a latitudinal gradient from Italy to Norway. We analysed an important part of the sexual reproduction of these species from seed production (nutrient concentration, morphology and physiology of seeds) until interannual seedling survival and seedling growth simulating a wide range of warming intensities and changes in precipitation and biotic interactions (in this case, competition).

We found that seed traits of *A. platanoides* were more influenced by the climatic conditions experienced by the mother tree than those of *A. pseudoplatanus*. Additionally, seed viability, germination percentage, and seedling biomass of *A. platanoides* were strongly related to the seed mass and seed nutrient concentrations. Additionally, A. *platanoides* seeds were more influenced by the environmental conditions (generally negatively affected by rising temperatures) than in *A. pseudoplatanus*, the former exhibited higher germination percentages and seedling biomass than *A. pseudoplatanus*.

After seed production, the seed bed conditions were important for seed germination and seedlings performance. With three complementary full factorial experiments we assessed the effects of moderate and strong warming (2.7 °C to 7.2 °C), drought (80% and 60% soil moisture reduction of the field capacity) and changes in precipitation frequency (one, two and three times per week) on germination, seedling survival and growth of seeds and seedlings of different provenances sampled along a latitudinal gradient. The germination of *A. platanoides* was negatively affected by drought but increasing the frequency of precipitation enhanced germination. Moreover, the survival of both species decreased under (particularly strong) warming. The growth variables of both species were generally negatively affected by warming and drought, while the precipitation frequency did not change growth. The joint manipulation of different factors resulted for some variables in additive effects while for others in partial compensation. Often, *A. platanoides* exhibited higher sensitivity than *A. pseudoplatanus* to the treatments applied and especially to its joint manipulation. Finally, across these experiments, the temperature experienced by the mother tree during seed production and the seed provenance showed to play an important role on seed germination and seedling performance. The germination was mostly negatively related to the temperature experienced by the mother tree with seeds from central and northern provenances germinating better. However, the southern provenances generally performed better under simulated climate change.

Finally, in an *in situ* transplant experiment, we observed that *Acer platanoides* was more sensitive to changes in competition with the other understorey plants while *A. pseudoplatanus* was affected by biotic changes and + 1°C warming and reduced precipitation. In general, competition reduced germination in *A. platanoides* and warming enhanced survival in *A. pseudoplatanus*. Reduced competition strongly increased the growth of *A. platanoides* seedlings. However, in this experiment, the seedlings' responses were independent from the conditions experienced by the mother tree during seed production and maturation.

Based on the findings in this work, we conclude that climate change will most likely have significant effects on the sexual reproduction of these two congeneric species. We clearly showed that the different aspects of plant reproduction (i.e., seed production, germination, seedling survival and growth) will be differently impacted by the projected changes in climatic conditions. Along this research often *A. platanoides* showed to be more responsive to the treatments applied than *A. pseudoplatanus*. Additionally, due the provenance specific responses observed it is possible that not only these two species will differently respond to climate change but also the impacts will be dissimilar across Europe. Future research with special focus on the subsequent life stages will be important to thoroughly predict the impacts of climate change on these species along their distribution range. Such information is key to soundly project changes in community's composition and inform forest management and policy decisions.

Samenvatting

De huidige opwarming van het klimaat is ongeëvenaard gedurende de laatste decennia tot millennia. Afhankelijk van het gebruikte scenario zal tegen het einde van deze eeuw de gemiddelde temperatuur op aarde stijgen met 0.3-4.8 °C. De stijging in temperatuur zal waarschijnlijk groter zijn in noord Europa dan in centraal of zuid Europa. Anderzijds wordt een stijging in de gemiddelde jaarlijkse neerslaghoeveelheid verwacht in noord en centraal Europa, en een daling rond het Middellandse zeegebied. Inzake neerslagfrequentie worden meer extreme regenbuien met langere droge intervallen tussenin voorspeld.

De impact van klimaatverandering is reeds zichtbaar in vele ecosystemen. Klimaat beïnvloedt de populatiedynamica van planten op verschillende manieren: niet enkel de verspreiding van soorten, maar ook de fenologie, reproductie, groei en mortaliteit zijn gevoelig. Echter, niet elke levensfase is even gevoelig voor klimaatverandering. Een van de meest gevoelige fases is de seksuele reproductie van plantensoorten: dit is een goede indicator van de gevoeligheid van soorten voor veranderingen in globale milieuomstandigheden, vaak beter dan de respons in termen van groei en mortaliteit van volwassen planten.

De effecten van klimaatverandering op de belangrijkste fases van het reproductieproces zoals bestuiving, zaadproductie, dormantie, kieming, en zaailingenoverleving en –groei zijn echter nog niet goed gekend. Modellen die de toekomstige verspreiding van soorten voorspellen zouden evenwel sterk verbeteren indien zulke informatie geïncorporeerd kan worden. Men verwacht, als gevolg van klimaatverandering, een verschuiving in de samenstelling van Europese bossen, met een sterk negatief effect op enkele primaire boomsoorten zoals beuk en fijnspar. Daarom is het belangrijk om onze kennis te vergroten omtrent de respons van andere, momenteel secundaire boomsoorten, waarvan voorspeld wordt dat ze door de klimaatverandering een meer prominente plaats zullen krijgen in de Europese bossen.

In dit doctoraat gingen we na wat het effect is van klimaatverandering op de seksuele reproductie van twee belangrijke, momenteel secundaire, boomsoorten,

zijnde gewone esdoorn (*Acer pseudoplatanus*) en Noorse esdoorn (*Acer platanoides*), langsheen een latitudinale gradiënt tussen Italië en Noorwegen. We analyseerden verscheidene fases van de reproductie, van zaadproductie en zaadkwaliteit (nutriëntenconcentraties, morfologie en fysiologie) tot de overleving van de zaailingen en hun groei. We simuleerden een aantal mogelijk toekomstige scenario's voor wat betreft de temperatuur (opwarming), veranderingen in neerslag (frequentie en totale hoeveelheid) en biotische interacties (competitie).

We vonden dat de zaden van Noorse esdoorn meer beïnvloed werden door het klimaat waarin de moederboom groeide dan bij gewone esdoorn. Het kiemingspercentage en de biomassa van zaailingen van Noorse esdoorn was sterk gerelateerd aan de massa en de nutriëntenconcentraties van de zaden en werd in grotere mate beïnvloed door de milieuomstandigheden dan bij gewone esdoorn. Stijgende temperaturen hadden vaak een negatief effect op de zaadkwaliteit van Noorse esdoorn, terwijl Noorse esdoorn wel een algemeen hogere kieming en zaailingenbiomassa had dan gewone esdoorn.

In experimenten waarin opwarming (controle, en temperatuurstijging van + 2.7 °C tot + 7.2 °C), droogte (bodem op 100 %, 80 % en 60 % van veldcapaciteit) en verandering in neerslagfrequentie (één, twee, of drie keer per week) werden gesimuleerd, bestudeerden we de effecten op kieming, overleving en groei van zaailingen van verschillende herkomsten bemonsterd langsheen de latitudinale gradiënt. De kieming van zaden van Noorse esdoorn werd negatief beïnvloed door droogte, maar toegenomen neerslagfrequenties hadden een positief effect. De overleving van beide soorten nam af onder stijgende temperaturen terwijl de groei negatief beïnvloed werd door opwarming en droogte. Veranderingen in de frequentie van de neerslag hadden geen effect op de groei. We vonden zowel additieve effecten als gedeeltelijke compensatie bij de gelijktijdige manipulatie van temperatuur en neerslag. Noorse esdoorn was meestal gevoeliger dan gewone esdoorn, vooral voor gecombineerde opwarming en droogte. De temperatuur die ervaren werd door de moederboom speelde meestal ook een grote rol in de respons van de zaailingen op de experimentele behandelingen. De zaadkieming was meestal negatief gecorreleerd met de door de moederboom ervaren temperatuur, waarbij zaden van centrale en noordelijke herkomst dus beter kiemden. Echter, de zuidelijke herkomsten presteerden beter onder het gesimuleerde toekomstige klimaat.

Tot slot incorporeerden we biotische interacties in onze experimenten omdat competitie in de kruidlaag met andere soorten waarschijnlijk ook zal toenemen. In een transplantatie-experiment vonden we dat Noorse esdoorn veel gevoeliger was voor competitie met andere soorten dan gewone esdoorn. Gewone esdoorn werd positief beïnvloed door opwarming. In dit experiment had de herkomst van de moederboom geen invloed op de respons van de zaailingen.

We concluderen dat klimaatverandering waarschijnlijk significante effecten zal hebben op de reproductie van beide onderzochte esdoorn-soorten. We toonden aan dat verschillende fases van de seksuele reproductie (zaadproductie, kieming, zaailingengroei en –overleving) vaak verschillend beïnvloed worden door gesimuleerde klimaatverandering, en dat de respons ook nog eens verschilt tussen de herkomsten die bemonsterd werden langsheen de latitudinale gradiënt van Italië tot Noorwegen. Noorse esdoorn was vaak gevoeliger voor de toegepaste behandelingen dan gewone esdoorn. Verder langetermijnonderzoek is nodig om de effecten van klimaatverandering op beide soorten beter te begrijpen en te kunnen voorspellen, om zo het Europese bosbeheer en -beleid correcter te informeren.

List of Abbreviations

(*)	$P < 0.10$
*	$P<0.05$
**	$P<0.01$
***	$P<0.001$
°C	Celsius degree
AIC	Akaike Information Criterion
CEU	Central Europe
cm^3	cubic centimetres
CMIP5	Coupled Model Intercomparation Project Phase 5
DJF	December, January, February
F	test statistic (F-value)
G	Grams
GDH	growing degree hours
Gls	generalized least squares
H	Hours
IPCC	Intergovernmental Panel on Climate Change
IR	Infrared
JJA	June, July, August
K	Potassium
km	Kilometres
km^2	square Kilometres
L	Litre
Lat	Latitude
Long	Longitude
LRT	Likelihood ratio test
M	Molar
m a.s.l.	meters above the see level
Max	maximum
MED	Mediterranean region
mg	milligrams
Min	minimum
ml	millilitres
mm	millimetres
N	sample size
NEU	Northern Europe
OTC	Open Top Chamber
P	signicance of statistical test (p-value)
Par.est	Estimated parameter
PCA	principal components analyses
pH	measure of acidity
r	Pearson correlation coefficient
Scaled dev.	Scaled deviance
SD	standard deviation
T	test statistic (t-value)
vol	Volume
X^2	likelihood ratio test statistic
Z	z test statistic (z-value)

List of Chemical Elements and Formulas

Al	Aluminium
Ca	Calcium
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
H_2SO_4	Sulphuric acid
HNO_3	Nitric acid
K	Potassium
Mg	magnesium
N	Nitrogen
NH_4	Ammonium
P	Phosphorus
S	Sulfur

1. Introduction

Global climate change

The increasing atmospheric greenhouse gas concentrations and the consequent Earth's temperature increase is now widely accepted among scientists (IPCC 2013a). The Intergovernmental Panel on Climate Change (IPPC) states that it is *extremely likely* (95–100% probability; comparable to the IPCC confidence levels of probability) that human influence has been the main cause of global warming since the mid-20th century. Moreover, even if all the necessary steps are taken to stop the CO_2 emissions, most aspects and impacts of climate change will persist for centuries (IPCC 2013a).

The warming of the climate system is unequivocal and unprecedented over decades to millennia. The Earth's surface temperature of the past three decades has been successively warmer than any of the previous decades measured instrumentally (IPCC 2013a). Between 1880 and 2012, the warming over the land and ocean together amounted to 0.85 °C, while the decade of the 2000's has been the warmest on record (IPCC 2013a). The warming is not only observable from changes in temperature but also from decreasing amounts of snow and ice and the rise of the global average sea level (IPCC 2013a).

The warming of the global climate is projected to continue in the coming decades. By the end of this century, the increase of the global surface temperature is *likely* (more than 66 % probability) to exceed 1.5 °C relative to the mean temperature between 1850 and 1900. This trend will likely continue beyond 2100 (IPCC 2013a). Moreover, warming will exhibit seasonal and interannual-to-decadal variability (IPCC 2013a). Under the different concentration-driven scenarios, the temperature increase is projected to be between 0.3 and 4.8 °C by the end of this century (**Table 1.1**) (Stocker et al. 2013).

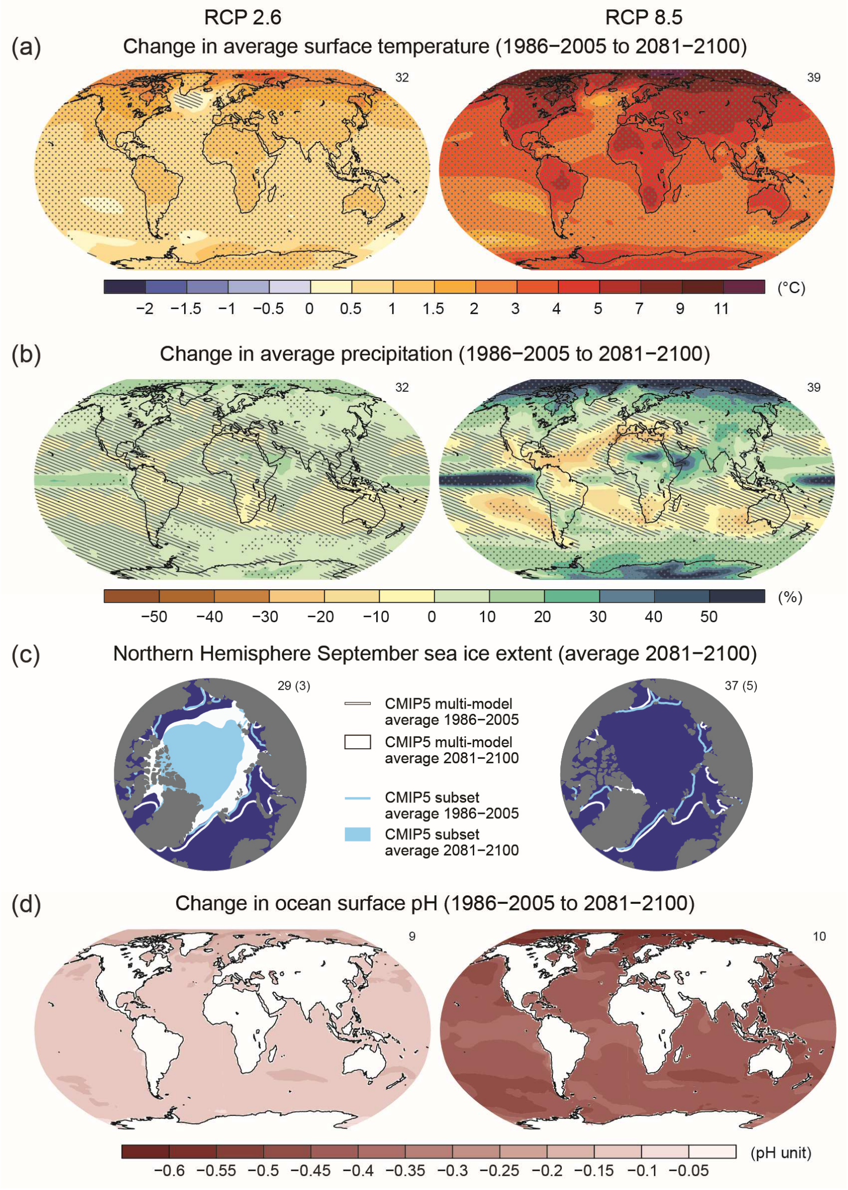

Fig. 1.1: Maps of projected late 21st century annual mean surface temperature change, annual mean precipitation change, Northern Hemisphere September sea ice extent, and change in ocean surface pH. Reprinted from IPCC, 2013: Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1–30, doi:10.1017/CBO9781107415324.004.

Table 1.1: Projected temperature increase of the global mean air temperature by the end of this century under different Representative Concentration Pathways 1(RCP). Adapted from "Technical Summary in Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change" by Stocker et al. 2013 Cambridge, UK, and New York, NY, USA.

	Global Mean Surface Temperature Change (°C)			
	2046 – 2065*		2081-2100*	
Scenario	Mean	Likely range	Mean	Likely range
RCP2.6	1.0	0.4 to 1.6	1.0	0.3 to 1.7
RCP4.5	1.4	0.9 to 2.0	1.8	1.1 to 2.6
RCP6.0	1.3	0.8 to 1.8	2.2	1.4 to 3.1
RCP8.5	2.0	1.4 to 2.6	3.7	2.6 to 4.8

*Range calculated from projections as 5–95% model ranges.

As a consequence of the global warming over the 21st century, the global water cycle is projected to alter as well; yet, not uniformly around the world. In general, it is forecasted that the contrast in precipitation between the wet and dry regions, and wet and dry seasons, will increase. Sites at high latitudes (e.g. Scandinavia) are likely to receive more precipitation, while many of the mid-latitude (e.g. the Mediterranean basin) and subtropical arid and semi-arid regions (e.g. North of Africa) will likely experience less precipitation than at this moment (Stocker et al. 2013). Moreover, seasonal differences will be observed. For example, Northern Eurasia and North America are projected to show larger changes in precipitation during the winter than in summer (Stocker et al. 2013).

Regional climate projections for Europe

Although climate models have improved in simulating aspects of regional climates, the spread in projections is still substantial. This is partly due to large amounts of natural variability, aerosol forcing and land cover and use changes, but also related to the inherent model deficiencies such as calibration of model output to match local observations and downscaling from global to local models (Christensen et al. 2013).

For the three European sub-regions i.e. Northern Europe (NEU), Central Europe (CEU) and the Mediterranean region (MED) (**Fig. 1.2**), the projections indicate warming in all seasons, while precipitation projections are more variable across both sub-regions and seasons (Christensen et al. 2013). The projections show that it is likely that the winter mean temperature will rise more in NEU than in CEU or MED, while the warming in summer will likely be more intense in MED and CEU than in NEU (Christensen et al. 2013). Moreover, the length, frequency,

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[1] Representative Concentration Pathways are scenarios that include time series of emissions and concentrations of the full suite of greenhouse gases, aerosols and chemically active gases, as well as land use and land cover (IPCC 2013a)

and/or intensity of warm periods or heat waves are very likely to increase in the whole region. On the other hand, the projections regarding precipitation changes are more variable than temperature projections. However, there is medium confidence that the mean annual precipitation will increase in NEU and CEU, while a decrease is *likely* in MED summer mean precipitation (Table 1.2) (Christensen et al. 2013).

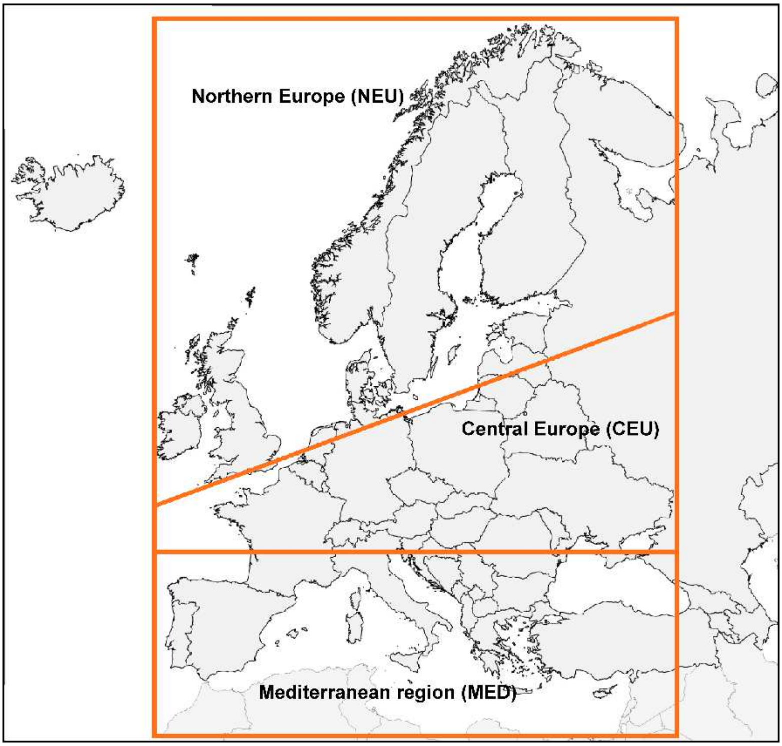

Fig. 1.2: European sub-regions used for the regional climate change models: Northern Europe (NEU), Central Europe (CEU) and the Mediterranean region (MED).

Impacts of climate change on plants

Current global warming is not only discernible from the changes in the abiotic factors mentioned above. Impacts of contemporary climate change are also visible in several ecosystems around the world (e.g. Pereira et al. 2010; Bellard et al. 2012; Fronzek et al. 2012). Considering the potential magnitude and velocity of climate change, analyses of its impacts on biodiversity have become a very active field of research (e.g. Dillon et al. 2010; Pereira et al. 2010; Bellard et al. 2012). The components of climate change are projected to affect all levels of biodiversity, from individual organisms to entire biomes (Bellard et al. 2012). Climate change will also alter the interactions at the community level, because the responses of some species to climate change can indirectly affect other species that depend or interact with them (Gilman et al. 2010; Angert et al. 2013; HilleRisLambers et al. 2013).

Table 1.2: Temperature and precipitation projections based on the Coupled Model Intercomparison Project Phase 5 (CMIP5). The 25th, 50th and 75th percentiles, and the lowest and highest response among 42 models, for temperature (°C) and precipitation (% change). The information is presented for December, January and February (DJF), June, July and August (JJA), and the annual mean. Adapted from "Climate Phenomena and their Relevance for Future Regional Climate Change in Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change" by Christensen et al. 2013. Cambridge, UK, and New York, NY, USA.

		Temperature (C°)					Precipitation (%)				
Sub-region	Month	Min	25%	50%	75%	Max	Min	25%	50%	75%	Max
Northern Europe	DJF	-3.2	2.6	3.4	4.4	6.0	2	7	11	14	25
	JJA	-1.1	1.6	2.2	3.0	4.7	-4	2	5	8	23
	Annual	-2.3	2.1	2.7	3.5	4.5	1	5	8	10	24
Central Europe	DJF	-0.8	2.0	2.6	3.4	5.1	-4	3	7	11	18
	JJA	0.4	2.0	2.7	3.0	4.6	-16	-6	0	5	13
	Annual	-0.3	2.0	2.6	3.1	4.0	-5	0	4	6	14
Southern Europe*	DJF	-0.2	1.5	2.0	2.4	3.0	-19	-7	-4	-1	9
	JJA	1.2	2.3	2.8	3.3	5.5	-28	-17	-11	-6	2
	Annual	0.6	2.0	2.3	2.7	4.0	-19	-10	-6	-3	4

*Mediterranean

To avoid or mitigate the effects of climate change, organisms can respond in several ways. Bellard et al. (2012) proposed to classify these possible mechanisms in three main categories: space, time and self (i.e. physiological changes) (**Fig. 1.3**). The three proposed directions of responses to climate change include moving in space (dispersing to areas with suitable conditions), shifting in time (adjusting life cycle events to match the new climatic conditions), or changing physiological life history traits to better cope with new climatic conditions (Bellard et al. 2012).

Climatic variables can influence plant population dynamics in many ways. For example, temperature and precipitation can limit species' distributions at the macro-scale. Moreover, these factors can condition growth, mortality, phenology and influence processes that govern regeneration, all important drivers of population dynamics (Baskin and Baskin 1998; Menzel et al. 2001; Goldblum and Rigg 2005; Reich and Oleksyn 2008; Walck et al. 2011; Carnicer et al. 2011). Furthermore, climate probably has a greater influence than species composition and historical population dynamics (i.e. recruitment and survival) (Adler and HilleRisLambers 2008). Therefore, it is clear that climate change will have a strong impact on plants across the globe (Hedhly et al. 2008; Walck et al. 2011). In the face of climate change, it is likely that species may find themselves in a climate regime that exceeds their environmental tolerances, resulting in impacts on species' performance, abundance and distributions (Normanda et al. 2007; Mok et al. 2012). To adapt to the effects of climate change, species can change their physiology and phenology. However, shifts in distribution ranges will be probably more difficult due the limited migration rates of many plant species (Svenning and Skov 2004; Normanda et al. 2007; Morin et al. 2008).

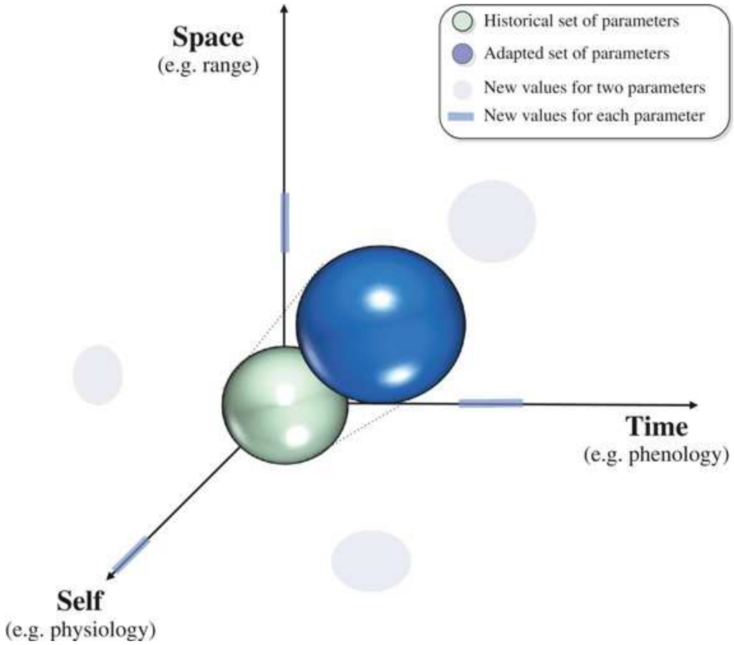

Fig. 1.3: Scheme of the three possible mechanisms how species can avoid or mitigate the impacts of climate change. Reprinted from "Impacts of climate change on the future of biodiversity" by Bellard *et al.* 2012. Ecology Letters 15: 365–377.

Furthermore, the intensity of impacts of climate change on different stages of the plant life cycle is expected to be dissimilar. The sexual reproductive phase is one of the most sensitive phases of the plant life cycle, making it a good indicator of the species sensitivity to environmental change, often better than the response of mature plants (Walck et al. 2011). Changes in environmental conditions including temperature, atmospheric vapour pressure and soil moisture can affect seed production, dormancy break, germination and subsequent establishment (Gibson and Bachelard 1986; Daws et al. 2004; Graae et al. 2009; Milbau et al. 2009; Shevtsova et al. 2009; De Frenne et al. 2011b; Mok et al. 2012; De Frenne et al. 2012; Sun et al. 2012). As a consequence, population size and structure can be affected in the long term (e.g. Gibson and Bachelard 1986; Lloret and Pen 2004; Svenning and Skov 2006; Adler and HilleRisLambers 2008).

Impacts of climate change on European forests

Given the potentially large influence of climate change on plant species, it is clear that forests, as many other ecosystems, will be also impacted by climate change. Currently, forests dominate many regions in the world and cover 31% of the world's total land area; (FAO and JRC 2012), including regions that are predicted to suffer the most intense changes of the climatic factors (Kremer et al. 2012).

In Europe, forests are generally intensively managed and are an important source of income for the economy through the timber industry (Hanewinkel et al. 2013). Therefore, climate change may not only have ecological impacts but also major economic impacts (Hanewinkel et al. 2013). The projected changes in temperature and precipitation will most likely cause an important loss in the value of European forests. A loss between 14 and 50% of the present value of the forest land in Europe is projected under climate change (Hanewinkel et al. 2013). Therefore, due to the increasing pressure of biotic and abiotic disturbances caused by climate change, some European countries are performing more climate change-directed management by replacing climate change-sensitive tree species such as Norway spruce (*Picea abies*) with more drought-tolerant species such as Douglas fir (*Pseudotsuga menziesii*) (Bolte et al. 2009). However not only management directed to a change in species composition can be performed, also selection of genotypes for breeding programs and successful reforestation can be performed and have been done with for instance *Larix ssp.*, *Picea abies*, and *Pseudotsuga menziesii* (Wang et al. 1994; Krabel and Wolf 2013).

Cold-adapted and mesic species such as Norway spruce (*Picea abies*), Beech (*Fagus sylvatica*) and Silver Fir (*Abies alba*), important commercial tree species in Europe (e.g. Norway spruce covers 30 million hectares across 17 European countries; Jansson et al. 2014), will eventually lose a big part of its distribution ranges (of almost 50% of its present area under an increase of the global temperature between 1.7 – 4.4 °C; Hanewinkel et al. 2012) in favour of more drought-adapted species such as oaks (*Quercus* spp.) (Attorre et al. 2011; Hanewinkel et al. 2013).

Despite its relevance, forecasting changes in species composition is not a simple task because climate change can drive changes in individuals' biology what may lead to shifts in the geographical range of the species (Lovejoy and Hannah 2005). Moreover, even closely related species can show divergent responses to climate change (e.g. Hong and Ellis 1990; Fay et al. 2002; Milbau et al. 2009). Despite the complexity of the predictive models, species range shifts have been predicted for a large variety of organisms and ecosystems during the last decade (Jorritsma et al. 2002). Additionally, the development of these predictive models has been shown to be helpful to elaborate plans to mitigate the potential consequences of climate change on biodiversity (Millennium Ecosystem Asessment 2005).

For trees, various types of process-based models are developed or are under development, such as gap models (see Bugmann 2001), fitness-based models (Chuine and Beaubien 2001) or landscape models (Higgins et al. 2000). However, due the nature of these models not all the factors of plant population dynamics can be included. The regeneration, for instance, contribute to the maintenance of species composition in forests and the migration of tree species across landscapes (Price et al. 2001), but this phase has not received enough attention by modellers.

Commonly, models incorporate the regeneration as an aggregation of different regeneration stages (seed production, dispersal, storage, germination and seedling establishment) (Price et al. 2001; Morin et al. 2008). However, different aspects of plants regeneration will not be equally impacted by climate change (Hedhly et al. 2008; Walck et al. 2011). This aggregation of phases that will be probably differently impacted by climate change as is one of the main limitations of some models (Price et al. 2001), however current models are improving its performance by including several aspects of the regeneration process (e.g Kint et al. 2014; Schlaepfer et al. 2014). Seed production (flowering and dormancy break), seed dispersal (interactions between seed dispersal and spatial variability in site conditions), establishment (drought as a factor influencing seedling survival and a vegetative growth) are key aspects of regeneration (Adler and HilleRisLambers 2008; Hedhly et al. 2008; Walck et al. 2011). To be able to incorporate these aspects into models, detailed information of forest regeneration is necessary. Therefore, specific areas for further research were recommended including ecophysiological studies of seed production and seedling establishment in a range of environmental conditions (Price et al. 2001). This information will allow to validate process models of seedling establishment, growth and mortality (Price et al. 2001).

Acer pseudoplatanus and *Acer platanoides* in the face of climate change

Acer platanoides (Norway maple) and *A. pseudoplatanus* (sycamore maple) are two currently secondary European forest tree species that normally do not cover more than three percent of the forest cover in Europe in pure stands (Spiecker et al. 2009). However, considering that a compositional change is forecasted for the European forest in the face of climate change, it is possible that these species might become more important in the future (Hanewinkel et al. 2013). Under climate change conditions it is expected that the most abundant, primary tree species - from a management point of view - such as beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) H. Karst.) will decrease in fitness and abundance and consequently retreat from parts of their distribution range (Kramer et al. 2010; Hanewinkel et al. 2013). For this reason, currently less abundant, secondary forest tree species (e.g. *Acer sp.* and *Tilia sp.*, *Quercus sp.)* may become more important and abundant (Lloret et al. 2005; Hanewinkel et al. 2013).

Acer pseudoplatanus, is naturally distributed along the West, Middle and South of Europe (Forest Ecology and Forest Management Group) (**Fig, 1.4**). It is commonly found in mixed stands together with other forest tree species such as *Fagus sylvatica*, *Fraxinus excelsior*, and *Quercus spp*. This species has moderate site requirements, being able to grow almost anywhere but prefers soils not too dry or too poor (Krabel and Wolf 2013). *Acer pseudoplatanus* is an ecologically important species associated with a number of insects species (e.g. bees and bumble bees) and it is a soil-improving species because its leaves are cherished by the earthworms (Krabel and Wolf 2013). Despite its valuable timber, this species is not yet as well accepted by both, producers and consumers, as other species such as Beech and Oak (Krabel and Wolf 2013). *Acer pseudoplatanus'* wood is mainly used for musical instruments, furniture and flooring while the poorer quality wood is used for firewood and paper production (Krabel and Wolf 2013).

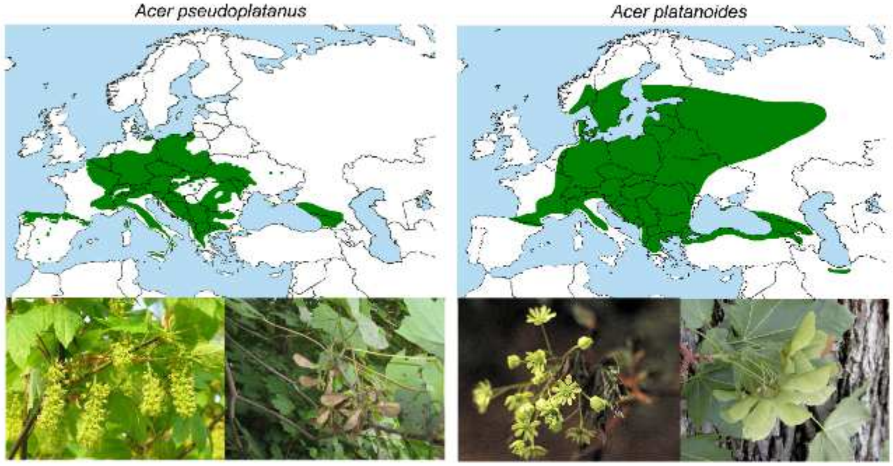

Fig. 1.4: Distribution range of *Acer pseudoplatanus* and *A. platanoides* in Europe; adapted from MPF and EUFORGEN 2009. The photos show the typical morphology of leaves, flowers and seeds; copyright by Leo Goudzwaard (Tree Database, Forest Ecology & Forest Management Group, Wageningen University)
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Second, *Acer platanoides* is a native species distributed across North Europe, Central Europe, the Kaukasus and Turkey, and is commonly found in forests and urban environments (**Fig. 1.4**) (Forest Ecology and Forest Management Group). *Acer platanoides* combines two important aspects: robust growth and high shade tolerance (Nowak and Rowntree 1990) making it a potentially important invasive species, especially in the North-eastern of United States from Maine to Wisconsin, south to Tennessee and Virginia and also in the Pacific Northwest (Martin 1999; Swearingen et al. 2010).

Acer platanoides can easily grow on loamy and clayey soils, is highly shade tolerant, but prefers nutrient rich soils (Forest Ecology and Forest Management Group). Moreover, this species is capable of dominating some forest stands, but this is not frequently observed (Kloeppel and Abrams 1995). However, as many other species, its current distribution could be limited by the dispersal capacity of the species (Martin and Marks 2006). This species can be used for timber production for furniture, cladding, flooring, musical instruments, and tools and can also be used as street and ornamental tree (Forest Ecology and Forest Management Group).

In the context of climate change it is likely that both species will show different responses to the changes in the environmental conditions. However, some common responses can be expected. For example, both species are expected to expand to northern regions and higher elevations than the area they occupy at present (Hemery et al. 2009). Moreover, it is likely that the susceptibility to pathogens will increase with stress for both species (Hemery et al. 2009). Nevertheless, considering that *Acer pseudoplatanus* is drought-intolerant while *A. platanoides* is relatively drought tolerant (Hemery et al. 2009) both species will likely not be equally impacted under projected dryer summer conditions (Hemery et al. 2009). Moreover, it is important to take into account that there is not a clear agreement about the drought tolerance of *A. platanoides* (see Table 1.3), making this species even more interesting to study in relation to climate change.

Both species will also be differently impacted by climate change in the recruitment phase. Considering that *A. platanoides* produce orthodox seeds (desiccation-tolerant) while *A. pseudoplatanus* recalcitrant seeds (desiccation sensitive) (Hong and Ellis 1990), changes in the soil moisture content will probably not affect the seeds and the seed bank of these species with the same intensity. Moreover, seeds of *A. platanoides* require more days of cold stratification than *A. pseudoplatanus* for successful dormancy break. Therefore, despite that probably the dormancy break of both species will be impacted by warming due to a reduction of the amount of chilling days in winter, it is likely that the impact will be larger in *A. platanoides*. Finally, the germination will be probably be impacted by global warming. Considering the threshold temperature for both species' germination (10-15 °C for *A. platanoides* and 25 °C for *A. pseudoplatanus* (Jensen 2001)), it is likely that *A. platanoides* will be more impacted by warming than *A. pseudoplatanus*. Only relatively scarce knowledge exists about the impacts of climate change on other sensitive phases of reproduction of these species (i.e. flowering, pollination and seed production) (Hedhly et al. 2008; Walck et al. 2011). Considering the similarities between the flowering time, the flower type and the time of seed ripen of both species, it is possible that these phases of reproduction will be similarly impacted by climate change (Table1.3).

Table 1.3: Morphological and physiological characteristics of Acer pseudoplatanus and A. platanoides. Adapted from Tree factsheet. Forest Ecology and Forest Management Group Wageningen University.

Characteristic	*Acer pseudoplatanus*	*Acer platanoides*
Flowering	April	April
Flowering plant	Monoecious	Monoecious
Flower	Hermaphrodite	Hermaphrodite
Flower diameter (cm)	0,5-1	1-1,2
Pollination	Insects	Insects
Fruit; length samara[2]	3-4 cm	4-7 cm
Fruit petiole (cm)	1-2	1-2
Seed length nut	0,8-1 cm	1-1,2 cm
Seed wing length (cm)	3	3-4
Weight of 1000 seeds (g)	70-125	70-125
Seeds ripen	September	September
Seed dispersal	Wind	Wind
Soil type	Sand, loam, clay	Sand, loam, clay
Soil fertility	Nutrient rich	Nutrient rich
Light requirements	Shade tolerant	Highly shade tolerant
#Shade tolerance: (0=no tolerance to 5=max. tolerance)	3.7	4.2
#Drought tolerance (0=no tolerance to 5=max. tolerance)	2.8	2.7
#Waterlogging tolerance (plant) (0=no tolerance to 5=max. tolerance)	1.1	1.5

The rankings of tolerance for a given species were estimated from average of tolerance rankings after detailed cross-calibration of the different data sets at different environmental conditions (Niinemets and Valladares 2006).

Methods to assess the impacts of climate change on trees

The impacts of global warming on plant population dynamics can be investigated using a variety of techniques (**Fig. 1.5**). However, when studying the impacts of climate change on plant species, the selection of the best method varies with the species characteristics such as life span and the specific aspect that is being investigated (reproduction, survival, growth, mortality etc.). For organisms with long life span such as trees, there are strong limitations for the applicability of some methods.

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[2] A samara is a simple dry fruit, a winged achene.

Each of the methods available to study the effects of climate warming on plant species have specific advantages and disadvantages that have to be considered when selecting the method to be used. The experimental methods can be categorized in active and passive warming methods (Aronson and McNulty 2009). The active methods include heat resistance cables, soil heating mats, infrared (IR) lamps and active field chambers, while the passive methods include nighttime warming and passive field chambers (Aronson and McNulty 2009). Passive nighttime warming is created by reflection of infrared radiation: automatically operated reflective curtains cover the vegetation at night to reduce heat loss to the atmosphere (Beier et al. 2004). One of the major advantages is that this method produces minimal disturbance but its use is limited by the canopy height). Field chambers (field-style greenhouses, tents and small plexiglas greenhouses; e.g. open-top chambers) are also common. However, they also present some limitations, including a non-constant temperature increase at different heights. Nevertheless, they are easy to use in remote locations where active methods are difficult to apply. Among the active methods, infrared (IR) lamps provide energy in the form of heat to the soil and vegetation, block relatively little solar radiation, and entail a low degree of disturbance (Kimball 2005). However, as nighttime heating, the height of the vegetation canopy can be, from a practical point of view, a limiting factor. Finally, heat-resistance cables and soil heating mats, are also commonly used. These methods allow a good control of the temperature, but produce a decoupling between the aboveground and belowground ecosystem components (only part of the system receives direct heat). Moreover, its installation produce high disturbance and the temperature increase is different according to the distance to the cables (higher close to the cables).

In addition, there are other methods that do not involve direct warming of the system such as latitudinal or altitudinal gradients. These methods allow researchers to study the long-term effects of temperature and precipitation on terrestrial organisms (Rustad 2008; De Frenne et al. 2013a) with a space-for-time substitution (*sensu* Fukami and Wardle 2005) using the environmental variation along natural gradients. This method constitutes a big advantage because it allows to study plant life stages that, in the case of tree species, are difficult to study through traditional warming methods such as flowering, pollination, seed production, mortality, etc. However, drawbacks include co-varying environmental factors and the difficulty of distinguishing between plastic responses (changes in phenotype in response to changes in the environment) and adaptive differentiation (adaptation to their home environment) between populations (De Frenne et al. 2013a). Moreover, the results obtained with this method are difficult to extrapolate temporally and spatially due to the confounding effects of factors such as local adaptation and the speed differences between historic, current and future climate change (Dunne et al. 2004). Therefore, it might be needed to determine how much of the observed phenotypic differences are due to plastic responses and adaptive differentiation between populations (De Frenne et al. 2013a). Given the considerations mentioned above, the integration of different techniques constitutes a promising way forward for a better understanding and forecasting of the potential impacts of climate change on plant populations and communities (Shaver et al. 2000; Fukami and Wardle 2005; De Frenne et al. 2013a)

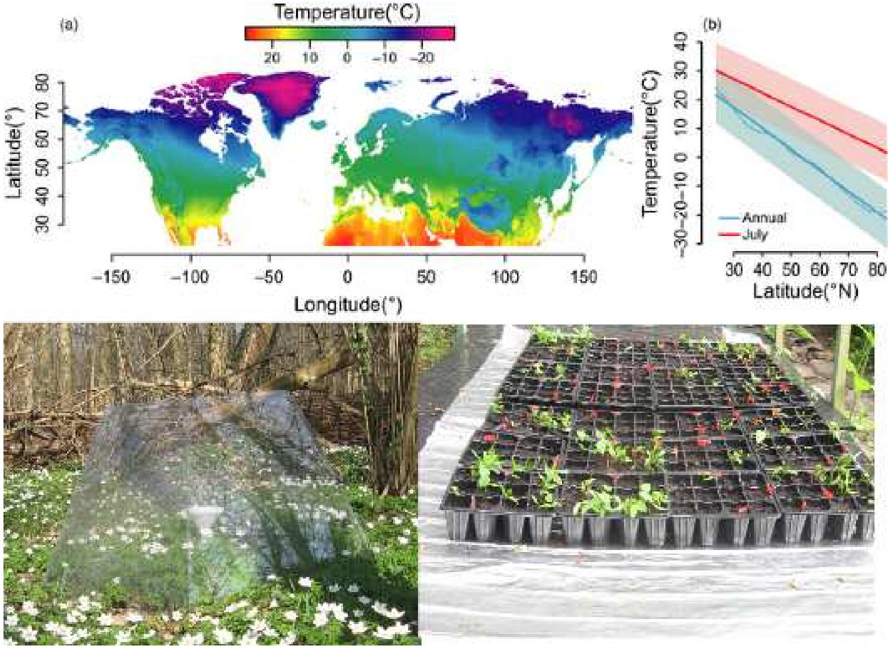


Fig. 1.5: Some of the most common methods for studying the impacts of climate change on plant populations were applied in this thesis: latitudinal gradients (top) reprinted from De Frenne P, Graae BJ, Rodríguez-Sánchez F, et al. (2013) Latitudinal gradients as natural laboratories to infer species' responses to temperature. J Ecol 101:493–501, open top chambers (below left) and soil heating mats (below right).

Objectives and outline of the thesis

Sexual reproduction is considered to be one of the major bottlenecks for plant recruitment (Walck et al. 2011). Moreover, considering that the impacts of climate change on some key processes of reproduction such as pollination, seed production, dormancy break, germination, seedling survival and subsequent growth are still being investigated (e.g. Hedhly et al. 2008; Hovenden et al. 2008; De Frenne et al. 2011; Walck et al. 2011), increasing our knowledge on this phase is very relevant.

Models that predict changes in species performance and distribution in the face of climate change urgently need to incorporate reliable information on species' reproduction (Price et al. 2001). Considering that the sexual reproduction allows to detect early species-specific reactions to climate change (Milbau et al. 2009) and that secondary forest tree species might win relevance in the face of climate change (Hanewinkel et al. 2013), increasing our knowledge on the regeneration of secondary forest tree species in the context of climate change is key.

The main objective of this thesis is to:

Assess the response of regeneration from seed and the early establishment of *Acer platanoides* and *A. pseudoplatanus*, two important secondary European forest tree species, to climate change.

In this study, using a combination of empirical methods, the impact of climate change on several important and very sensitive phases of plants' sexual reproduction are studied:

- Seed production and seed quality
- Seed germination and early seedling survival and growth (short term experiments)
- Seed germination and interannual seedling survival and growth (longer term experiment)

We specifically addressed the following hypotheses:

1. Both species show differences in seed production according to the environmental conditions experienced by the mother tree. *Acer platanoides* will be more sensitive to variation in climate than *A. pseudoplatanus* (see above).
2. The germination and seedling survival and growth of *A. platanoides* will be more impacted by drought, warming and competition than in *A. pseudoplatanus*.
3. Slightly warmer conditions and reduced competition will enhance germination and survival of both species, while drought will produce the opposite effect.
4. The interacting effects of temperature, precipitation reduction and competition will produce different impacts than the individual factors in both species regeneration.

In this thesis we cover several subsequent stages of the tree life cycle from seed production (**Chapter 2**) until interannual seedling survival and growth (**Chapter 5**) and perform comparisons of the response of these two very closely related species to changes in temperature, soil moisture/precipitation (amount and frequency) and biotic interactions (i.e. competition with the forest understorey vegetation).

A variety of techniques has been applied including latitudinal gradients, soil heating mats and open top chambers (OTCs) depending on the life stage under study and the simulated climate change conditions. However, in many cases a combination of techniques was used to better understand species' responses to climate change. Each chapter focuses on one of the phases (i.e. seed production, early establishment or interannual establishment) (**Fig. 1.6**) although in some chapters more than one phase is considered (i.e. seed production, germination and survival or germination, survival and growth). A range of climatic conditions is simulated, including warming up to 7.2 °C and a decrease in soil moisture content and precipitation of up to 40% of the soil field capacity.

Seed production and quality in relation to climatic conditions experienced by the mother tree was analysed with the use of latitudinal gradients through a space-for-time substitution (*sensu* Fukami and Wardle 2005) to evaluate the influence of the temperature and precipitation regime experienced by the mother tree on seed nutrient concentrations, morphology, viability, germination and seedlings performance (**Chapter 2**).

The seed germination and seedling survival and growth in response to warming and simulated drought was studied with the use of soil heating mats and controlled watering. This experimental set up was combined with a seed collection along the latitudinal gradient. The combination of techniques not only allows us to evaluate the impacts of climate change on species regeneration but also to link the response of the regeneration to the temperature experienced by the mother tree during seed production (**Chapter 3**).

The effects of projected warming combined with changes in the **frequency** of precipitation were studied on seed germination, seedlings early survival and growth. In this experimental set-up the influence of mother tree provenance (region of origin) was included in the analysis.(**Chapter 4**). In a final experiment, the effect of changes in both abiotic (temperature and precipitation) and biotic conditions (competition with surrounding vegetation) were evaluated. By using open top chambers, combined with reduced precipitation and competition with natural vegetation the regeneration from seed and seedlings' early and interannual survival and growth was evaluated over two growing seasons *in situ* in the field. This technique was chosen to be able to study the effects of climate change on the study species over a longer time period, with lower disturbance and under more natural and realistic conditions (**Chapter 5**).

Finally in the **Chapter 6**, an integrated analysis of the main findings of this research is presented and discussed and recommendations for further research are put forward.

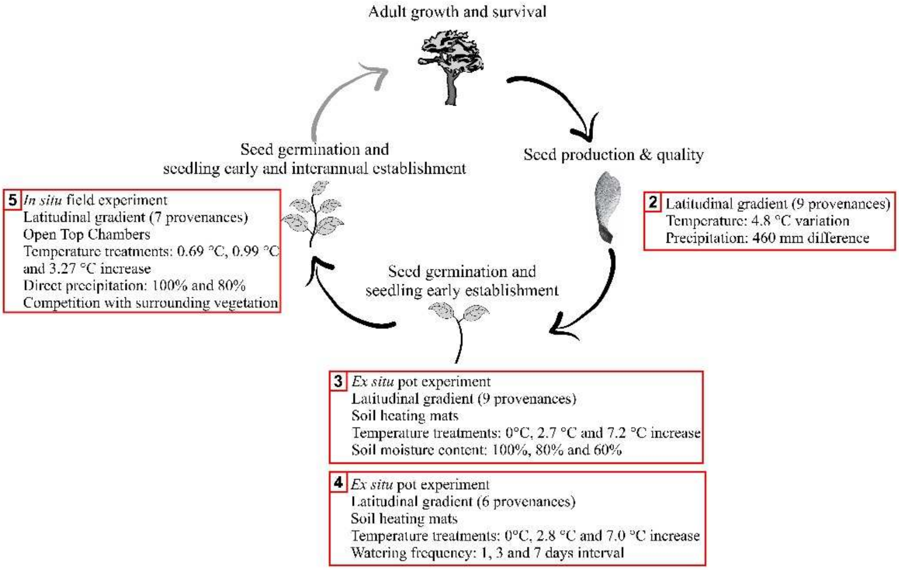


Fig. 1.6: Outline of this thesis. The chapter numbers are in red boxes as well as the main methods and treatments applied in each chapter. The black arrows indicate the consecutive phases studied in this thesis while the grey arrow indicates the plant life phases not assessed in this work

2. Latitudinal variation in seeds characteristics of *Acer platanoides* and *A. pseudoplatanus*

After: Carón MM, De Frenne P, Brunet J, et al. (2014a) Latitudinal variation in seeds characteristics of Acer platanoides and A. pseudoplatanus. Plant Ecol. doi: 10.1007/s11258-014-0343-x

Abstract

Climate change will likely affect population dynamics of numerous plant species by modifying several aspects of the life cycle. Because plant regeneration from seeds may be particularly vulnerable, here we assess the possible effects of climate change on seed characteristics and present an integrated analysis of seven seed traits (nutrient concentrations, samara mass, seed mass, wing length, seed viability, germination percentage, and seedling biomass) of *Acer platanoides* and *A. pseudoplatanus* seeds collected along a wide latitudinal gradient from Italy to Norway. Seed traits were analyzed in relation to the environmental conditions experienced by the mother trees along the latitudinal gradient. We found that seed traits of *A. platanoides* were more influenced by the climatic conditions than those of *A. pseudoplatanus*. Additionally, seed viability, germination percentage, and seedling biomass of *A. platanoides* were strongly related to the seed mass and nutrient concentration. While *A. platanoides* seeds were more influenced by the environmental conditions (generally negatively affected by rising temperatures), compared to *A. pseudoplatanus*, *A. platanoides* still showed higher germination percentage and seedling biomass than *A. pseudoplatanus*. Thus, further research on subsequent life-history stages of both species is needed. The variation in seed

quality observed along the climatic gradient highlights the importance of studying the possible impact of climate change on seed production and species demography.

Introduction

The production of seeds is an important aspect of plant population dynamics, directly linked with population persistence and the colonization of new areas (Hedhly et al. 2008). The impacts of climate change on seed characteristics such as seed set, maturation, predation and germination have been documented before (e.g. Meunier et al. 2007; Hovenden et al. 2008; Walck et al. 2011). However, other seed characteristics such as morphological, chemical and physiological aspects are influenced by temperature and precipitation (Wulff 1986; Fenner 1992; Conklin and Sellmer 2009) and will likely be affected by climate change. While variation in seed traits has been studied in different species (e.g. De Frenne et al. 2011; Sun et al. 2012), there is less information about the effects of climatic conditions on closely related species (e.g. same genus) that can exhibit either, similar or very different seeds characteristics (see Leishman and Westoby 1994; Green and Juniper 2004). However, this information may be very important to forecast community changes. Moreover, the effects of climate change on some aspects related to seeds have not been well documented (Walck et al. 2011) and a thorough, integrated analysis is especially lacking.

Seed mass is considered a key trait that plays a crucial role in interspecific interactions, by affecting seedling growth, buffering carbon losses (Foster and Janson 1985; Westoby et al. 1996) and herbivore damage (Dalling and Harms 1999; Green and Juniper 2004; Espelta et al. 2009), thereby influencing the competitive hierarchies between different species (Pérez-Ramos et al. 2010). It is a trait that tends to decrease towards higher latitudes both among and within species (Moles and Westoby 2003; De Frenne et al. 2013a). The variation in seed size can occur not only within populations, but also within individual plants, inflorescences and fruits (Fenner and Thompson 2005). The intraspecific variation in seed size seems to be the result of genetic differences between mother plants as well as the environmental conditions experienced during seed production (Wulff 1986; Baskin and Baskin 1988; Castro et al. 2008; Souza et al. 2010). However, seed mass is not the only aspect of seeds that plays an important role in the regeneration success of plants; many other seed characteristics affect dormancy breaking and germination (Webb and Wareing 1972) or modify the seed's susceptibility to insect and pathogens attack (Beckman and Muller-Landau 2011).

The seed nutrient concentration is another key trait, especially relevant for early seedling establishment, since seed reserves are important for the growth in the first stages of seedling development (Westoby et al. 1996; Pérez-Ramos et al. 2010) and may condition the capacity of seedlings to cope with environmental stress

(Fenner and Thompson 2005; Quero et al. 2007). It has been suggested that the nutrient concentration varies in time and space in relation to the maternal environmental conditions, such as temperature, water availability, incident radiation, and the amount and availability of soil nutrients (Drenovsky and Richards 2005; De Frenne et al. 2011b).

Considering the influence of the environmental conditions on seed production, studying the variation in seed characteristics developed under different environments is likely to provide with important information on future impacts of climate change on plant regeneration from seeds. There are several approaches to study the effect of climate change on vegetation including the use of temporal or environmental gradients (Koch et al. 1995; Lenoir et al. 2008; De Frenne et al. 2010a; De Frenne et al. 2013a). Among the latter, latitudinal gradients, through a space-for-time substitution (Fukami and Wardle 2005), offer the possibility to study seed characteristics because they allow researchers to analyse the effect of environmental conditions on plant traits that have evolved with the climate over centuries. By using a wide latitudinal gradient, it is possible to understand the relative role and contribution of environmental conditions such as temperature, precipitation and soil fertility (De Frenne et al. 2013a) on seed quality and the potential impacts of climate change on plant regeneration success.

Here we analyse seed traits of two currently secondary tree species, *Acer platanoides* and *A. pseudoplatanus*, to understand the effects of climate change in temperate regions species. We specifically address the following questions: i) is there variation in seed traits in species growing under different climatic conditions along a wide latitudinal gradient in Europe? ii) if so, does variation in seed traits influence seed viability and germination as well as early seedling growth? iii) do two congeneric species respond equally to climatic variation during seed production?

Materials and Methods

Study region and populations

In 2011, approximately 600 seeds of *Acer platanoides* L. and *A. pseudoplatanus* L. were collected from nine regions along a 2200 km long latitudinal gradient from Arezzo, Italy to Trondheim, Norway. The seed collection included both native (populations within the natural distribution range of the species) and non-native populations (outside the natural distribution range of the species) of the two species (**Table 2.1**). In each region, three forest patches within a landscape area of 40 x 40 km^2 were selected. In each forest patch, one vital, seed-bearing healthy mother tree was used for seed collection. Seeds were collected using water permeable nets or picked from the forest floor immediately after seed dispersal (**Table 2.1**).

Environmental characteristics

Temperature and precipitation data for the year 2011 were obtained from nearby weather stations (always less than 50 km from the sampled trees) made available through the European Climate Assessment and Dataset project (Klein Tank et al. 2002) and Centro Funzionale della Regione Toscana and Archivio CFS - Ufficio Territoriale per la Biodiversità di Pratovecchio. Precipitation was expressed in millimetres of rain per year, while the temperature experienced by the mother tree between April 1 and September 30 (average period between flowering and seed maturation) was given in growing degree hours (GDH). The GDH were calculated with a base temperature of 5 °C (Lindsey and Newman 1956):

$$If \quad Tmax_i \leq 5°\text{C} \rightarrow GDH_i = 0$$

$$If \quad Tmax_i > 5°\text{C}\ and\ Tmin_i > 5°\text{C} \rightarrow GDH_i = 24 \times (Tmin_i - 5) + 12 \times (Tmax_i - Tmin_i)$$

$$If \quad Tmax_i > 5°\text{C}\ and\ Tmin_i \leq 5°\text{C} \rightarrow GDH_i = 12 \times \frac{(Tmax_i - 5)^2}{Tmax_i - Tmin_i}$$

$$\text{GDH} = \sum_{April\ 1}^{September\ 30} \text{GDH}_\text{i}$$

Where GDH_i, $Tmax_i$ and $Tmin_i$ are the GDH, maximum temperature and minimum temperature for day i, respectively. After calculation, the GDH were standardized per location by dividing site-specific values by the overall mean (Graae et al. 2009). The Pearson correlation between latitude and GDH was $r = -0.72$ ($p < 0.0001$, $n = 9$), while the Pearson correlation between elevation and GDH was $r = 0.19$ ($p = 0.34$, $n = 27$).

We collected 15 mineral soil samples (0-20 cm depth) randomly located in a 20 m × 20 m area around each mother tree. The samples were pooled per mother tree, oven-dried (40 °C) for 48 hours and passed through a 2 mm sieve. The pH was determined from a solution of 14 ml soil and 70 ml $CaCl_2$ 0.01 M using a glass electrode. Additionally, 5 g of dry soil were used for the analysis of calcium (Ca), aluminum (Al), magnesium (Mg) and potassium (K) with atomic absorption spectrometry (SpecrAA- 220; Varian, Santa Clara, CA, USA); for this analysis, the sample was extracted in 100 ml ammonium lactate solution [9.01 ml lactic acid (88%) + 18.75 ml acetic acid (99%) + 7.75 ml NH4-acetate diluted to 1 l] according to the modified method of Enger et al. (1960). Phosphorus (P) was determined in a spectrophotometer (Cary 50; Varian) according to the malachite-green method, using malachite-green and molybdate as colour reagent. Finally, the percentages of carbon (C), nitrogen (N) and sulfur (S) were obtained from 0.250 g of soil with an element analyzer (elementar Vario Macro Cube, Germany) (**Table A1** in **Appendix A**).

Seed and seedling traits

From each mother tree, 30 apparently healthy samaras (seed and wing) were randomly selected for the determination of morphological variables. Each individual samara was scanned and air-dried at room temperature for approximately 72 hours, which was shown to be sufficient to obtain a stable mass during preliminary experiments. Each individual samara was weighed first with and then without the wing (i.e. seed mass), after which wing mass was determined as the difference between the two values. The scanned images were used to calculate the wing length using ImageJ software (Rasband 2012).

After the above analysis, the seeds were bisected through the embryo and submerged in a 0.5% Tetrazolium salt solution in distilled water (The tetrazolium Subcommittee of the Association of Official Seed Analysts 2000). After coloration (approximately 24 h), the seeds were analyzed under a microscope for viability determination. The seeds were categorized into viable and non-viable based on results from the Tetrazolium salt test, presence of necrotic tissue and/or absence of an embryo in the samaras.

To determine germination percentages, ten seeds from each mother tree were sown in plastic pots (25 cm^3 vol.) filled with standard potting soil (mean pH 6, nutrient ratio NPK 15:10:11, organic matter 20% and water holding capacity 80%).All seeds were cold stratified for dormancy break before testing germination. Initially the seeds were soaked in water until the desired level of humidity was reached (i.e., 48% and 38% for *A. pseudoplatanus* and *A. platanoides*, respectively). Afterwards the seeds were placed in controlled cold conditions (at 0 °C to 1 °C and 90-95 % humidity) for approximately two months until germination started. The stratification period depended on the species and the provenance, assuring that all the seeds were at the same development stage and at optimal conditions for germination. Seed lots that reached the correct development stage and started germinating were stored in a freezer at -2°C till the moment of seed sowing.

Table 2.1: Location, climatic characteristics (average temperature between April and September of 2011, average annual temperature for the period 1992-2011, GDH (growing degree hours between April and September 2011) and annual precipitation recorded in 2011, mother tree status (native or non-native) according to expert knowledge and the literature (Fitter and Peat 1994; EUFORGEN 2009), seed collection method (nets or forest floor) and seed collection date of *A. platanoides* and *A. pseudoplatanus*

Location	Species	Lat (°)*	Long (°)#	Average elevation (m above sea level)	Temperature April-September 2011 (°C)	Average Annual temperature (1992-2011) (°C)	GDH	Precipitation (mm)	Mother tree status	Collection method	Collection date
Arezzo,]Italy	*A. platanoides*	43.84	11.83	795	15.9	9.5	47952	1027	Native	Floor	25/10/11
	A. pseudoplatanus	43.83	11.84	955					Native	Floor	25/10/11
Amiens, France	*A. platanoides*	49.92	2.09	104	15.8	11.0	47700	565	Not native	Nets	08/09/11
	A. pseudoplatanus	49.83	2.15	124					Native	Nets	06/09/11
Brussels, Belgium	*A. platanoides*	50.98	3.80	103	15.6	10.7	49887	753	Not native	Nets	11/11/11
	A. pseudoplatanus	50.98	3.97	130					Native	Nets	11/11/11
Templin, Germany	*A. platanoides*	52.60	13.56	66	16.7	10.2	51037	587	Native	Nets	04/09/11
	A. pseudoplatanus	52.60	13.53	60					Native	Nets	07/09/11
Potsdam, Germany	*A. platanoides*	52.58	11.01	32	16.7	10.2	51037	587	Native	Nets	27/10/11
	A. pseudoplatanus	52.57	13.03	32					Native	Nets	27/10/1
Bremen, Germany	*A. platanoides*	53.11	8.84	18	15.3	9.7	44873	622	Not native	Floor	15/10/11
	A. pseudoplatanus	53.11	8.84	20					Not native	Floor	15/10/11
Lund, Sweden	*A. platanoides*	55.707	13.37	50	14.8	9.3	43090	799	Native	Nets	13/09/11
	A. pseudoplatanus	55.72	13.31	82					Not native	Nets	13/09/11
Stockholm, Sweden	*A. platanoides*	59.22	18.06	26	15.2	7.4	45257	949	Native	Floor	16/09/11
	A. pseudoplatanus	59.22	18.06	8					Not native	Floor	16/09/11
Trondheim, Norway	*A. platanoides*	63.42	10.41	27	11.9	6.2	32088	990	Not native	Floor	18/10/11
	A. pseudoplatanus	63.42	10.41	30					Not native	Floor	18/10/11

* Latitude
\# Longitude

Each seed lot was controlled every week until approximately 10% of the seeds started to germinate, this was considered as an indication that the stratification was completed and the time required was recorded. The pots were placed at the edge of a deciduous forest composed of *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus robur* and *Acer* sp. in Gontrode, Belgium (50°58'N, 3°48'E), installed under plastic roofs (70 cm above pots) to exclude natural precipitation and allow free air exchange. The soil moisture was held constant at field capacity by weighing each pot three times per week and adding the necessary amount of distilled water until field capacity (gravimetric soil moisture 65%). Germination was recorded three times per week and the total germination was assessed 36 days after sowing. At the end of the germination trial, the seedlings (above and belowground biomass) were carefully removed from the pots, washed, dried at 60 °C during 24 hours and weighed.

A subsample of 30 seeds (without wing) from each mother tree was randomly selected, oven-dried at 65 °C for 24 hours, milled and used for determination of seed concentrations of Ca, Mg, P and K.. First we decomposed 75 mg of the milled samples using acid digestion with HNO_3 and H_2SO_4. Then, Ca, Mg, and K were measured with atomic absorption spectrometry (SpecrAA- 220; Varian, Santa Clara, CA, USA). Seed concentrations of P, C and N were determined using the same methods as described above for the soil analyses. The concentrations of the above elements were also used to calculate C:N and N:P ratios.

Data analysis

To analyze the effects of soil variables (pH, C, N, P, K, Ca, Al and Mg) on the seeds traits. Principal components analyses (PCA) with VARIMAX rotation were performed, extracting the two first PCA axes in SPSS (version 21.0 IBM Corp. 2012). In both *A. platanoides* and *A. pseudoplatanus*, the first PCA axis was mainly correlated with P, K, N, C and S ($r > 0.532$ in all cases) and will be hereafter referred to as the soil nutrient axis, accounting for 39.8% and 40.2% of the variability, respectively. The second PCA axis was mainly correlated with pH, Mg, Ca and Al (all $r > 0.812$ in *A. platanoides*, $r > 0.583$ in *A. pseudoplatanus*) and explained 34.2% and 29.8% of the variability of soil variables, respectively. This axis will hereafter be referred to as soil pH axis.

Mixed-effect models using the *lme* and *lmer* functions in R version 3.0.0 (R Core Team 2013) were used to analyze the effects of environmental conditions on some seed traits i.e. samara mass, wing length, seed mass and wing mass over seed mass ratio (Gaussian error structure), as well as seed viability and germination (binomial error structure). The fixed effects were the GDH, annual precipitation during 2011, the two soil PCA axes and the mother tree status (native vs. not native), while *region* and *mother tree* were used as random effects to account for the hierarchical structure of the data.

The effects of environmental variables on seed nutrient concentration, stratification time (days) and seedling biomass for both species were analyzed with a generalized least squares (gls) model using the gls-function in the nlme-library in R with GDH, annual precipitation during 2011, the two soil PCA axes and the mother tree status (native vs. not native) as explanatory variables. For all the variables analyzed, the determination of the optimal random-effect structure, gls regressions (i.e. without random-effects) and *lmer* functions with *region* and/or *mother tree* as random effects, was performed by comparing the Akaike Information Criterion (AIC) values for each model and the model with the lowest AIC value was selected.

To analyze the effects of seed nutrient concentration on seed viability, stratification time (days), germination and seedling biomass, a PCA analysis with VARIMAX rotation was performed on the seed nutrient concentrations (P, K, Ca, Mg, Al, N, C) extracting again the two first axes for both species. For *A. platanoides,* the first PCA axis (mainly correlated with seed K, Ca, N and P, all $r > 0.588$) and the second PCA axis (mainly correlated with Mg and C, all $r > 0.620$) explained 47.7 and 25.9 % of the total variation of the chemical seed variables, respectively. In *A. pseudoplatanus*, the first PCA axis (mainly correlated with P, K, Mg and C, all $r > 0.712$) and the second PCA axis (mainly correlated with Ca and N, all $r > 0.527$) explained 40.4 % and 20.9 % of the total variation of the seed properties, respectively.

Seed viability and germination (binomial data) were analyzed using mixed-effect models with the two seed nutrient PCA components and the seed mass as fixed factors and *region* and *mother tree* as random effects. Finally, the stratification time (days) and seedling biomass were analyzed with a gls model (lower AIC value than the mixed effect models) using the same fixed factors.

In all cases, the model selection (gls vs. mixed effects model) was based on the AIC criteria. Afterwards the full model was fitted (all fixed effects included) and the model simplification was achieved by dropping one non-significant explanatory variable per time and each time a t-test was applied (Zuur et al. 2009).

To fulfill normality and homoscedasticity assumptions, some variables were transformed. In *A. platanoides*, seed K, Mg, N, C, Ca concentrations, wing mass, wing mass: seed mass and stratification time were log-transformed while C:N and C:P ratios were square root-transformed. In *A. pseudoplatanus*, Mg, N, C concentrations, wing mass: seed mass and stratification time were log transformed and Ca, C:N and C:P ratios square root-transformed.

Results

Seed and samara size characteristics

The seed mass of *A. platanoides* was negatively influenced by the accumulated temperature (GDH). Seeds produced under the warmest conditions were 33% lighter than those produced under the coldest condition (**Table 2.2, Fig 2.1** and **Table A2** in **Appendix A**). Additionally, the soil nutrient axis had a positive effect on samara mass. In *A. pseudoplatanus*, wing length and wing mass: seed mass ratio were positively affected by the soil pH axis. Samara mass, wing length and seed mass were influenced by the mother tree status; the samaras and seeds produced by non-native mother trees, transplanted north of the natural range of this species (locations with lower GDH), were 28% and 23% heavier than those produced by native mother trees within the natural range. Additionally, the wings were 18% shorter in native trees (**Table 2.2** and **Table A2** in **Appendix A**). Finally, there was a clear increase in wing mass with the increase in seed mass in *A. pseudoplatanus* samaras (**Fig.2.1**).

Table 2.2: Morphological seed characteristics as a function of the environmental conditions experienced by the mother tree: growing degree hours (GDH), annual precipitation during 2011, soil nutrient and soil pH axes and mother tree status (Native/Non-native)

Morphological trait	*Acer platanoides*			*Acer pseudoplatanus*		
	Predictor	Par.est	t-value	Predictor	Par.est	t-value
Samara mass	Soil nutrient axis	8.66E-3	2.42*	Mother tree status	0.04	4.14**
Wing length			n.s.	Mother tree status	8.39	3.74**
				Soil pH axis	2.24	2.66*
Seed mass	GDH	-0.06	-2.91*	Mother tree status	0.02	2.55*
Wing mass: seed mass ratio			n.s.	Soil pH axis	0.10	2.33*

P<0.001***, P<0.01**, P<0.05*

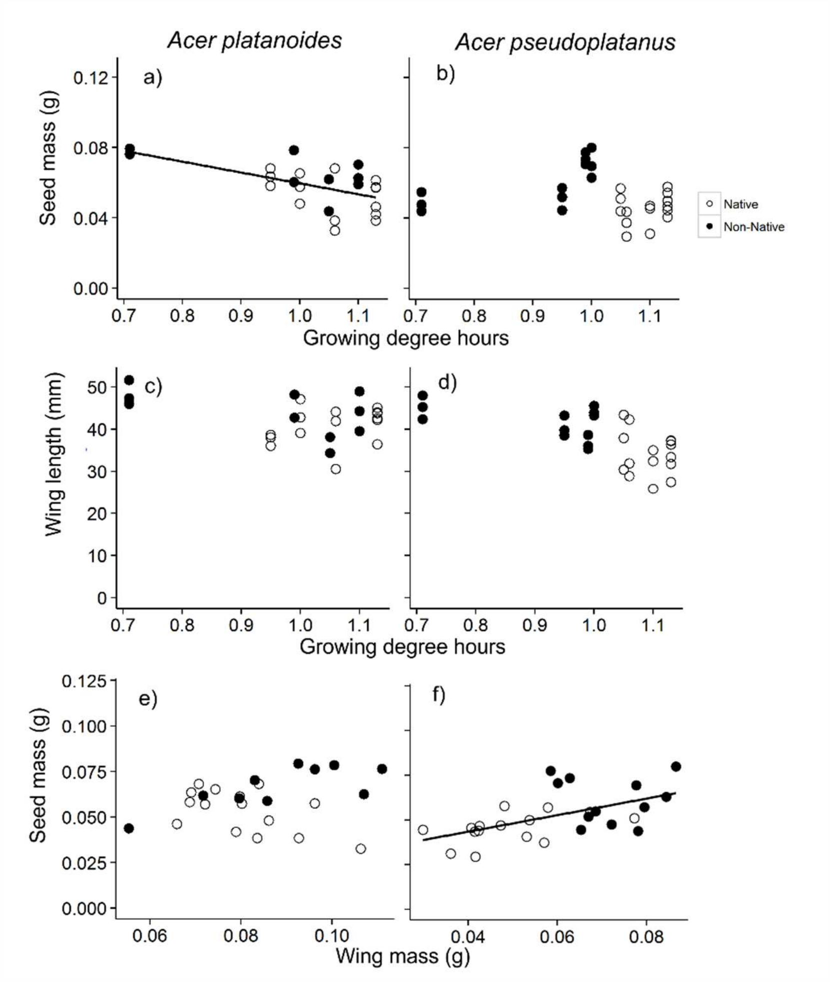


Fig 2.1: Seed traits as a function of the standardized growing degree hours (a-d), and relationship between seed mass and wing mass (e-f). Different colours denote the mother tree status

Chemical composition

The seed nutrient concentration in *A. platanoides* seeds was more influenced by the environmental conditions experienced by the mother tree than those in *A. pseudoplatanus* seeds (Table 2.3). In *A. platanoides* seeds, the seed N and K concentrations depended on GDH and precipitation, respectively. The seed N concentration was 13% lower in seeds developed under the warmest than in the coldest populations (difference of 4.8 °C between the warmest and coldest provenances), while the seed K concentration was 34% lower in seeds developed under the driest than under the wettest conditions.

Additionally, seed N, K, P, Mg, and Ca concentrations as well as the C:N and C:P ratio in *A. platanoides* seeds were affected by the soil characteristics. Seed P and Mg were positively related to the soil nutrient axis, while N, P, and K were negatively related to the soil pH axis. This positive relationship was especially evident for P due the positive correlation between seed P and soil P (**Table A4** in **Appendix A**), while the seed C:P ratio exhibited the opposite effect. In *A. platanoides*, the seed Ca concentration increased along the pH axis (positive correlation between soil Ca and seed Ca) (**Table 2.3** and **Table A4** in **Appendix A**).

Table 2.3: Influence of environmental conditions on seed chemical characteristics of *A. platanoides* and *A. pseudoplatanus*: standardized number of growing degree hours (GDH), annual precipitation recorded during 2011, soil nutrient axis and soil pH axis and mother tree status (Native/Non-native) on seed chemical characteristics

	Acer platanoides			*Acer pseudoplatanus*		
Seed nutrient	Predictor	par.est	t-value	Predictor	par.est	t-value
C			n.s.	Precipitation Soil nutrient axis	-6.3E-5 8.4E-3	-3.15** 2.37*
N	GDH Soil pH axis Precipitation	-0.71 -0.08 -3E-4	-3.95*** -3.92*** -2.48*			n.s.
P	Soil nutrients axis Soil pH axis	317.24 -467.68	2.18* -3.21**			n.s.
Mg	Soil nutrient axis mother tree status	0.07 -0.14	2.69* -2.73*			n.s.
K	Precipitation Soil pH axis	5E-4 -0.04	5.14*** -2.53*	Precipitation	10.56	3.56**
Ca	Soil pH axis	0.10	3.02**	Soil pH axis	3.23	2.91**
CN	GDH Precipitation Soil pH axis	1.35 6.0E-4 0.14	3.86*** 2.37* 3.67**			n.s.
NP	GDH Precipitation	-3.49 -3.1E-3	-2.34* -2.91**			n.s.
CP	Soil nutrients axis Soil pH axis	-0.20 0.29	-2.24* 3.22**			n.s.

P<0.001***, P<0.01**, P<0.05*

Conversely, the nutrient concentration in *A. pseudoplatanus* seeds was less influenced by the environmental conditions under which the seeds were produced than in *A. platanoides*. Seed C and K concentrations decreased and increased with precipitation, respectively. The seed K concentration was 14% lower in seeds produced under the driest conditions compared to the wettest (**Table A3** in **Appendix A**). Finally, the seed C and Ca concentrations were positively affected by the soil nutrient axis and soil pH axis, respectively. A clear positive correlation was observed between seed Ca and soil Ca concentrations (**Table 2.3** and **Table A4** in **Appendix A**).

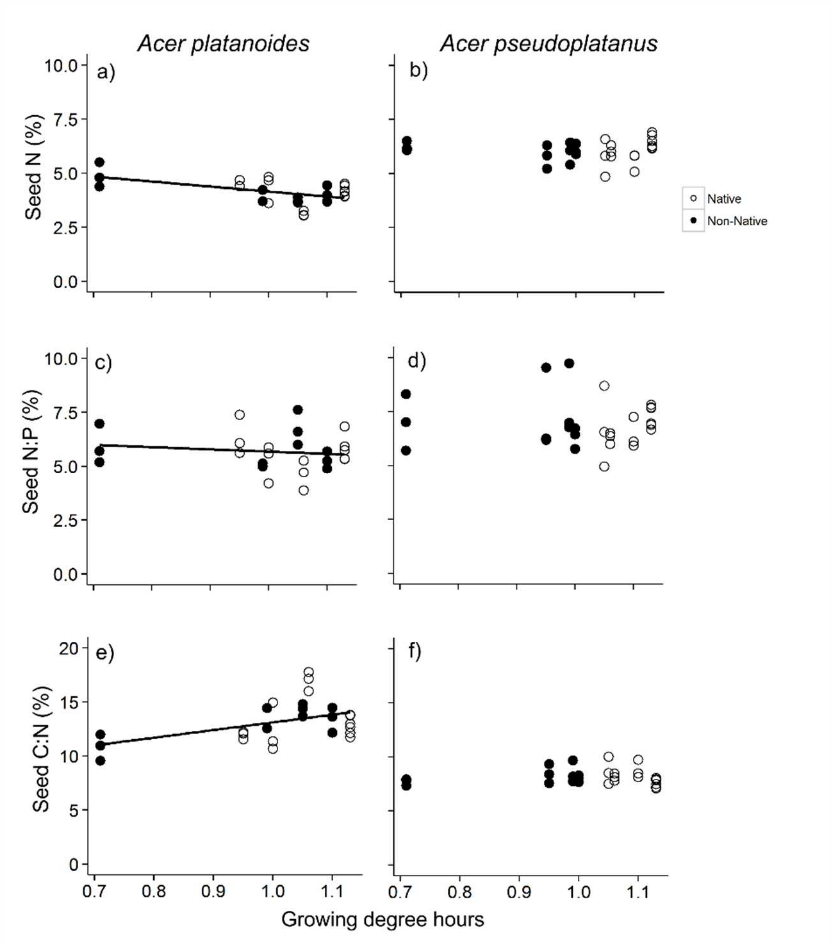


Fig 2.2: Seed nitrogen concentrations (N), nitrogen:phosphorus (N:P) and carbon:nitrogen (C:N) ratios of *A. platanoides* and *A. pseudoplatanus* along the latitudinal gradient from Norway to Italy as a function of the standardized growing degree hours. Different colours denote the mother tree status.

The seed N concentration and the N:P ratio in *A. platanoides* were negatively affected by the accumulated temperature (higher values were found at lower GDH). In contrast, the seed C:N ratio increased with the accumulated temperature (**Fig 2.2**) due to the latitudinal variation in nitrogen. The effect of the environmental conditions on seed chemical composition for *A. pseudoplatanus* was less noticeable than in *A. platanoides* seeds, as there was, for example, no correlation between nutrient concentration and accumulated temperature experienced by the mother tree during seed production (**Table A.4** in **Appendix A**).

Stratification, viability, germination and seedling growth

For both species, the cold stratification time appeared to be independent of the environmental conditions experienced by the mother tree during seed production. However, *A. platanoides* was 30% faster in concluding stratification in non-native mother trees. For this species, seed viability was negatively influenced by the accumulated GDH and the annual precipitation, while it was positively affected by the soil nutrient axis. Seed viability recorded under the warmest conditions was 38% lower than that recorded for seeds produced under the coldest conditions, and an additional reduction of viability was observed with the increase of precipitation (**Table 2.4** and **Table A5** in **Appendix A**). Germination in *A. platanoides* increased along the pH axis, and seedling biomass increased with increasing precipitation. Seedlings from seeds produced in the wettest conditions were 46% bigger than those produced under the driest conditions (**Fig. 2.3** and **Table A5** in **Appendix A**). Consistent with the results of the analysis of seed mass and size characteristics, in *A. pseudoplatanus*, nutrient concentrations, stratification time, viability, germination and seedling biomass were not influenced by the climatic conditions (**Table 2.4** and **Fig 2.3**). The seed viability of both species was positively influenced by the seed mass, indicating that the heavier seeds were more viable (**Fig. 2.3**). Seed germination and seedling biomass of *A. platanoides* were affected by the seed nutrient concentration as reflected in the seed PCA1 (K, Ca, N and P) (**Table 2.4**). Finally, in contrast to *A. platanoides*, seed germination and seedling biomass of *A. pseudoplatanus* did not depend on chemical composition (**Table 2.4**).

Table 2.4: Effects of the environmental conditions experienced by the mother tree during seed production (standardized number of growing degree hours (GDH), annual precipitation recorded during 2011, soil nutrient axis and soil pH axis, mother tree status (Native/Non-native) and seed mass and seed chemical characteristics on seed stratification time, seed viability, germination and seedling biomass. The z or t-statistics are presented according to the model used for each variable analysed (gls or mixed effects model).

	Acer platanoides			*Acer pseudoplatanus*		
Morphological trait	Predictor	par.est	t/z-value	Predictor	par.est	t/z-value
Environmental conditions						
Stratification time	mother tree status	-0.34	-2.89**			n.s.
Viability	GDH	-2.40	-2.08*			
	Precip	-2.3E-3	-2.77**			
	Soil nutrient axis	0.43	3.23**			
Germination	Soil pH axis	-0.41	-2.21*			n.s.
Biomass	Precip	1.2E-4	2.06*			n.s.
Seed traits						
Stratification time	SeedPCA2	0.15	2.33*			n.s.
Viability	seed mass	47.75	9.68***	seed mass	54.54	10.27***
Germination	SeedPCA1	0.66	2.81**			n.s.
Biomass	SeedPCA1	0.03	2.23*			n.s.

P<0.001***, P<0.01**, P<0.05*
Precip : Precipitation

Discussion

Seeds of *A. platanoides* and *A. pseudoplatanus* collected along a 2200 km long latitudinal gradient from Italy to Norway showed important variation in chemical, morphological and physiological traits. The effects of the environmental conditions on seed characteristics were variable among species, even in these two related species: *A. platanoides* was clearly more influenced by environmental variation than *A. pseudoplatanus*.

Seed mass, which represents the amount of resources available for the first stages of seedling establishment, was negatively influenced by GDH in *A. platanoides* but not in *A. pseudoplatanus*. However, due the limited number of sampling points in colder conditions used in this study, a better representation of colder conditions in the analysis could strengthen this relationship. It is clear that *A. platanoides* seeds produced under colder conditions are heavier than those produced in warmer environments. The production of heavier seeds at lower temperatures is likely due to slower seed ripening, which allows for the seed filling process to occur slowly allowing for greater total assimilation (Fenner and Thompson 2005). Moreover, it is important to point out that the mother tree was relevant in the statistical model and therefore is an important source of variation in seed mass. Additionally, considering that the seed size is conditioned by both genetic and environmental aspects (Fenner and Thompson 2005) further research on the genetic variation on seed mass can be relevant to understand the relative importance of each factor.

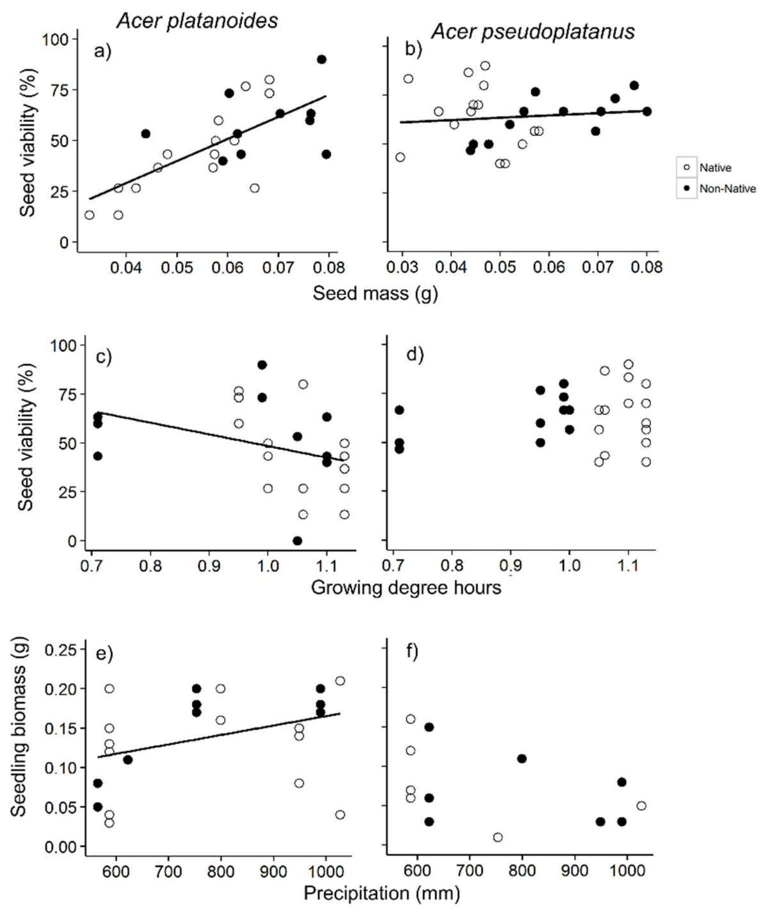


Fig 2.3: Seed viability, as a function of the seed mass and standarized growing degree hours (a-d) and seedling biomass as a function of precipitation (e,f) in *A. platanoides* and *A. pseudoplatanus*. Different colours denote the mother tree status

An important seed trait related to the dispersal capacity of these species is wing mass in relation to wing length. In *A. pseudoplatanus*, heavier seeds generally also had heavier and larger samaras, which might be related to the need for bigger structures to allow the wind dispersal of heavier seeds, while in *A. platanoides* seeds this relationship was not as clear. Additionally, in *A. pseudoplatanus*, the wing length was positively influenced by the native status of the mother tree. This relationship indicates that the populations transplanted north of their natural distribution range produce bigger wings with a higher dispersal capacity than the ones growing in their native range. Consequently, the seeds produced in relocated populations might have a larger dispersal capacity, which is considered to be one of the key factors related to the capacity of a species' ability to cope with climate change (Bellard et al. 2012).

The seed size variation in relation to the environmental conditions experienced by the mother tree, such as temperature, water availability, radiation and nutrient availability, has been previously reported (e.g. Valencia-Díaz and Montaña 2005; Baraloto and Forget 2007; Souza et al. 2010). However, the observed variation in seed nutrient concentrations due to environmental variation has received much less attention.

In *A. platanoides*, the seed nutrient concentration was affected by a combination of temperature, precipitation and soil nutrient concentration (De Frenne et al. 2011b; Sun et al. 2012). A clear negative relationship was found between the GDH experienced by the mother tree and the seed N concentration and N:P ratio. This observation is in contrast with the decrease in leaf N:P ratios with decreasing temperatures reported for 244 herb species (Reich and Oleksyn 2004). Similarly, a decrease in seed N concentrations and N:P ratios with increasing latitude and decreasing temperatures was previously reported for the forest understory herb *Anemone nemorosa* (De Frenne et al. 2011b). Our findings for *A. platanoides* are supported by similar observations by Sun et al. (2012) that found *Quercus variabilis* acorns show variation in nutrient composition in relation to climatic and soil conditions of the mother plants. Additionally, our observed decrease in N with increasing GDH could be caused by a possible stimulation of the accumulation of storage protein in northern locations (Piper and Boote 1999). Furthermore, as observed in both *Acer* species, precipitation may affect the concentration of other nutrients such as seed K and C. In contrast to *A. platanoides*, the seed nutrient concentration of *A. pseudoplatanus* was clearly less influenced by the environmental conditions. Moreover, an aspect not analyzed in this work is the possible dilution effect expected when analyzing seed production and seed nutrient concentration. It is known that in general the allocation of resources to reproduction, does not vary greatly, and it is frequent to observe a trade-off between seed number and seed size (Fenner and Thompson 2005). Therefore, this might be also a relevant aspect to take into account when analyzing the variation of seed nutrient concentration.

Related to seed nutrients concentrations, seed mass and seed size, a higher seed N concentration has been shown to increase seed predation and therefore require additional seed defense traits such as an increased dry seed mass allocation to the seed coat (Soriano et al. 2011). While we did not measure seed coat thickness directly, we observed a decrease in the seed N concentration and seed mass with increasing GDH. This pattern can potentially induce increases in seed predation in northern populations where larger seeds with higher N concentration are produced.

As observed for the other seed traits, the viability and germination *A. platanoides* seeds were influenced by the environmental conditions. This relationship was not observed in *A. pseudoplatanus*. The viability of *A. platanoides* seeds decreased from colder and wetter to warmer and dryer provenances. The difference between the viability and germination percentages (**Table A5** in **Appendix A**) highlights the relevance of both variables to determine potential reproductive success (Conklin and Sellmer 2009). Additionally, the difference between viability and germination can be related to a differential degree of maturity of the seeds associated, for example, with the temperature experienced by the mother tree (e.g. Graae et al. 2009). This difference can also be related to the fact that the seeds may have experienced different dormancy levels and some may have remained dormant after stratification (Conklin and Sellmer 2009). Moreover, it has been shown that the maternal environments can alter which genes are most important for germination having interesting implications for molecular population genetics (Donohue 2009). However, the analysis of these aspects are beyond the scope of this work.

For both *Acer* species, seed viability was clearly related to seed mass, indicating that larger seeds show higher viability. For both species, no relationship was found between seed mass and germination and seedling biomass, although it has been suggested that larger seeds produce bigger seedlings with greater probability of successful seedling establishment, higher fitness, access to deeper soil layers, and higher resistance to drought stress and other environmental hazards than small seeds (Westoby et al. 1996; Dalling and Hubbell 2002; Moles and Westoby 2006). Additionally, since the mother tree can modify the seed nutrient concentration (e.g. Drenovsky and Richards 2005; De Frenne et al. 2011) and because a clear relationship was found between seed nutrient concentration and germination and seedling biomass, seed nutrient concentration might be a more important factor than the seed mass *per se*.

Conclusions

The species-specific responses of seed nutrient concentrations, seed mass and size to environmental variation along a latitudinal-climatic gradient that we found here stresses the complex interactions between climate, seed characteristics and seedling establishment (Arnold et al. 1995; Marrush et al. 1998; Zerche and Ewald 2005). Our findings are especially relevant in the context of climate change because they further our understanding of the effect of climatic conditions on seed traits. The observed variation in seed quality along the environmental gradient highlights the possibility of a significant impact of climate change on seed characteristics with a consequent impact on the future demography of these species. Considering that a compositional change in temperate forests is expected, the information about species-specific variation on important seed traits can contribute to the understanding of how plant demography and communities structure may vary with future climatic changes, especially considering that novel communities might emerge in the context of climate change (Williams and Jackson 2007).

It is likely that under future warmer conditions, *A. platanoides* will produce smaller seeds, with lower N concentration and reduced viability. On the other hand, the projected dryer conditions for southern Europe may increase the viability and thereby partly compensate for the negative effect of warmer conditions. In contrast, *A. pseudoplatanus* is not expected to show important changes in nutrient concentration, seed mass and size and early establishment as climate changes. Despite the fact that *A. platanoides* seeds were more influenced by the environmental conditions, and were generally negatively affected by higher temperatures, it is important to note that *A. platanoides* had higher germination percentages and seedling biomass than *A. pseudoplatanus.* Thus, further research on subsequent life-history stages including studies of the genetic variability between and within populations of both species is needed to clarify the possible impacts of climate change on these co-generic species and to unravel the relative role of environmental and genetic aspects on seed and seedlings traits.

3. Interacting effects of warming and drought on regeneration and early growth of *Acer pseudoplatanus* and *A. platanoides*

After: Carón MM, De Frenne P, Brunet J, et al. (2014) Interacting effects of warming and drought on regeneration and early growth of *Acer pseudoplatanus* and *A. platanoides*. Plant Biol 1–11. doi: doi:10.1111/plb.12177

Abstract

Climate change is acting on several aspects of plant life cycles including the sexual reproductive stage, which is considered amongst the most sensitive life cycle phases. In temperate forests, it is expected that climate change will lead to a compositional change in community structure due to changes in the dominance of currently more abundant forest tree species. Increasing our understanding of the effects of climate change on currently secondary tree species' recruitment is therefore important to better understand and forecast population and community dynamics in forests. Here we analyse the interactive effects of rising temperatures and soil moisture reduction on germination, seedling survival and early growth of two important secondary European tree species, *Acer pseudoplatanus* and *A. platanoides*. Additionally, we analyse the effect of the temperature experienced by the mother tree during seed production by collecting the seeds of both species along a 2200 km long latitudinal gradient. For most of the responses, *A. platanoides* showed higher sensitivity to the treatments applied and especially to its joint manipulation, which for some variables resulted in additive effects while for others in partial compensation. In both species, germination and survival decreased with rising temperatures and/or soil moisture reduction while early growth decreased with declining soil moisture content. We conclude that, although *A. platanoides'*

germination and survival were more affected by the applied treatments, its initial higher germination and larger seedlings might allow this species to be relatively more successful than *A. pseudoplatanus* in the face of climate change.

Introduction

Models predict that the mean annual temperatures in Europe are likely to increase more than the global mean (Christensen et al. 2007; Hansen et al. 2010), while the changes in precipitation will differ geographically: increases are expected in northern Europe and declines in southern Europe (Christensen et al. 2007). Resulting decreases in soil moisture are expected in the Mediterranean and parts of central Europe, while for other European regions no consensus on the direction of soil moisture change exist (Wang 2005).

Considering that the climate has a large influence on plant population dynamics (e.g Woodward 1987), climate change can be expected to affect plant populations across the globe (Hedhly et al. 2008; Walck et al. 2011). It is known that climate change can modify several aspects of the life cycle of plants (Farnsworth et al. 1995; Norby et al. 2003), including the sexual reproductive stage (Hedhly et al. 2008).

For many plant species, seed germination and seedling establishment are considered a major bottleneck in their regeneration (Lloret et al. 2005; Fay and Schultz 2009; Dalgleish et al. 2010), but these phases also provide the plant with an opportunity to adapt to environmental changes (Hedhly et al. 2008). Increasing our understanding of the effect of climate change on regeneration is therefore particularly important (Jeltsch et al. 2008; Milbau et al. 2009) to better forecast population dynamics (Milbau et al. 2009).

Most of the previous research on the effects of climate change on plant reproduction has been performed on the individual effects of changes in temperature (Milbau et al. 2009; Klady et al. 2011; De Frenne et al. 2012) and precipitation (Lloret et al. 2005; Kos and Poschlod 2008; Fay and Schultz 2009). These approaches provide valuable information but analyses of the interactive effects of these two global-change drivers on tree species recruitment are largely lacking (Beier et al. 2012). Moreover, research about plant reproduction response to climate change has been mainly directed at tundra vegetation, boreal forest and tree line ecotones, with less emphasis on temperate regions (Walck et al. 2011).

Specifically in temperate forests, a compositional change based on changes in the dominance of currently dominant forest tree species due to climate change can be expected (Booth et al. 2012). The most abundant; primary tree species - from a management point of view - such as beech (*Fagus sylvatica L.*) and Norway spruce

(*Picea abies (L.) H.Karst.*) are expected to decrease in fitness and abundance and consequently retreat from parts of their distribution range (Kramer et al. 2010; Hanewinkel et al. 2013). For this reason, currently less abundant, secondary forest tree species (e.g. *Acer sp.* and *Tilia sp.*, *Quercus cerris L., Quercus petraea (Matt.) Liebl.*) may become more important (Lloret et al. 2005; Hanewinkel et al. 2013).

Here we investigate the performance of the regeneration from seed of two currently secondary tree species (*Acer pseudoplatanus* and *A. platanoides*) from nine regions along a 2200 km latitudinal gradient covering most of the distribution range of these species, including areas outside their natural range. We analyzed the germination, seedling survival and growth of these two species using two common garden experiments in which both temperature and soil moisture availability were manipulated. This set-up allowed evaluating the effects of the temperature that the mother tree experienced during seed production as well as the response of germination and seedling survival and growth to projected changes in climatic conditions. We specifically addressed: a) how differences in temperature and soil moisture content affect the germination, survival and growth of two congeneric *Acer* species; b) whether there is an interacting effect of the joint manipulation of temperature and soil moisture content, and c) whether the response to the experimental treatments depends on the temperature regime experienced by the mother trees during seed production.

Materials and Methods

Study region and populations

In 2011, we collected seeds of *Acer platanoides* L. and *Acer pseudoplatanus* L. along a wide (2200 km) latitudinal gradient in Europe to be able to study a wide range of temperature effects on the mother trees. We included both native (located in the natural distribution range of the species) and non-native (located outside the natural distribution range of the species) populations to fully represent possible temperatures experienced by the mother trees in Europe. These species were specifically selected since they (i) will likely become more important for European forestry in the context of future climate change (Hanewinkel et al. 2013), (ii) are characteristic for and abundant in broadleaved forests across Europe, (iii) have a large distribution range and occur along a wide latitudinal gradient in Europe, and (iv) tend to have high germination rates, easy dormancy break and similar phenology. We sampled populations located in nine regions from Italy (43°49'N) via France, Belgium, Germany, Sweden to Norway (63°26'N) (**Fig. 3.1**), more details about the seed collection are given in **Chapter 2**.

Fig. 3.1: Location of the sampled *A. pseudoplatanus* and *A. platanoides* populations along the latitudinal gradient from Italy to Norway.

Climatic data from weather stations close to the sampling point (< 50 km distance) and available in the European Climate Assessment and Dataset project (Klein Tank et al. 2002) were used for the calculation of the growing degree hours (GDH) experienced by the mother tree between the 1st of April and September 30 of 2011 (period between flowering and seed maturation). The GDH were calculated considering a base temperature of 5 °C and the formulas mentioned in **Chapter 2**. The GDH were standardized per location by dividing site-specific values by the overall mean across the gradient (Graae et al. 2009). The Pearson correlation between latitude and GDH was $r = -0.90$ ($n = 9$; p-value < 0.0001), between GDH (April and September 2011) and the mean annual GDH (average 1981-2011) was $r = 0.93$ ($n = 9$; p-value <0.001), between GDH (April and September 2011) and the average single-seed mass (without dispersal structures) calculated from 30 seeds per mother tree, was -0.73 ($n = 9$ provenances; p-value = 0.03) and -0.37 ($n = 9$; p-value = 0.32) for *A. platanoides* and *A. pseudoplatanus*, respectively.

Experimental design

We performed two full factorial soil heating and moisture manipulation experiments to simulate a total of nine climate change scenarios. For the design of our two experiments we considered the A1B-IPCC scenarios that projected an increase of the summer (June, July and August) temperature between +1.4°C and +5.0°C (average +2.7°C) for northern Europe. The expected summer (again June, July and August) temperature increase for southern Europe is between +2.7°C and +6.5°C (average +4.1°C) (Christensen et al. 2007). Concerning large-scale changes in precipitation, a change in summer precipitation between +16% to -21% is projected for northern Europe while a reduction between -3% and -53% is forecasted for southern Europe (Christensen et al. 2007). Moreover, the models that predict soil moisture are even more variable than precipitation projections; the reason behind this is mainly due the differences in land surface parameterization (Wang 2005). Nevertheless, we decided to express our precipitation treatments as soil moisture rather than millimetres of rainfall, since (i) this is as a stronger predictor of plant establishment than precipitation (Walck et al. 2011) and (ii) germination is highly dependent on available soil moisture (Kos and Poschlod 2008). It is important to mention that due the variability in precipitation and soil moisture change projections we decided to simulate a relative wide range of soil moisture conditions from relatively high moisture to relatively dry conditions.

Both experiments were performed with the collected seeds of *Acer platanoides* and *A. pseudoplatanus* from three mother trees (except *A. platanoides* from Bremen and Amiens with two mother trees), and 10 and 25 seeds per mother tree for the first and second experiment, respectively (see below). In total 27 *A. pseudoplatanus* and 25 *A. platanoides* mother trees were sampled. The seeds were randomly sowed in plastic pots (25 cm^3 vol. for the first experiment and 112 cm^3 vol. for the second experiment), filled with standard potting soil (mean pH 6, nutrient ratio NPK 15:10:11, organic matter 20% and water holding capacity 80%). Before sowing thethe seeds were cold stratified at 0 °C to 1 °C and 90-95 % humidity, (for a variable period, ca. two months until each seed lot started to germinate (see details in **Chapter 2**)

For both experiments, warming was achieved though soil heating mats (ACD Aluminum heating mats HMT-A, Oberweidbach Germany). The experiment was installed at the edge of a deciduous forest composed of *Fagus sylvatica, Fraxinus excelsior L., Quercus robur L.* and *Acer sp.* in Gontrode, Belgium (50°58'N, 3°48'E) under plastic roofs (70 cm above pots) to exclude the natural precipitation and allow free air exchange. The soil temperature in the pots was measured every minute, and averaged and logged every 15 minutes using Decagon data loggers Em50 ECH$_2$O LOGGER, while the soil moisture was controlled by weighing each pot three times per week and adding the necessary amount of distilled water until the desired moisture content was reached.

We assessed the regeneration in terms of germination time (emergence above the ground), germination amount, survival (living seedling at the end of the experiment) and seedling growth (number of leaves, biomass and height). The germination was recorded three times per week. At the end of the experiments, the seedlings were carefully removed from the soil, and for each seedling the number of leaves was recorded. The belowground and aboveground plant parts were separated, dried at 60 °C during 24 hours and weighed. In the second experiment only, the height reached by the seedlings was additionally measured.

First experiment

In the first experiment, which lasted for 36 days (starting on 16th July 2012), we sowed 10 seeds per mother tree per species per treatment. The manipulations of temperature were as follows: 1) Control at ambient temperature (the average for the whole period was 16.5°C ± SD 3.7°C); 2) Average temperature increase of 2.5°C (19.0°C ± SD 2.5°C), and 3) average temperature increase of 7.2°C (23.7°C ± SD 3.1°C). Additionally, the three soil moisture treatments were applied: soil moisture constantly at 100% of the field capacity (gravimetric soil moisture 65% and volumetric soil moisture 29%), 2) soil moisture at 80% of the field capacity (gravimetric soil moisture 52% and volumetric soil moisture 23%) and 3) soil moisture at 60% of the field capacity (gravimetric soil moisture 32% and volumetric soil moisture 17%).

The total amount of water added during the experiment to maintain the desirable level of soil moisture content under the different warming treatments was +0°C: 8.9 L (control), 5.6 L (80 %) and 4.3 L (60%); +2.5°C: 23.8 L, 14.9 L and 8.2 L; +7.2°C: 26.4 L, 15.2L and 13.1 L.

Second experiment

The objective of this experiment was to evaluate the possible changes in seedling establishment and growth over time using a longer experimentation period (more than two times longer). This experiment was a replication of the intermediate temperature treatment (+2.5°C) with the same three soil moisture content treatments as in the first experiment. The second experiment lasted for approximately three months (79 days, starting on the same day as the first experiment, 16th July 2012). In this experiment, we sowed 25 seeds per mother tree per treatment and the soil temperature was set at an average for the whole period of 19.0°C± SD 2.4°C, while three levels of soil moisture content were applied (same procedure as above, i.e., 100%, 80% and 60% of the field capacity). The total amount of water added along the experiment to maintain the desirable level of soil moisture content was 180L, 169L and 133L.

Data analysis

Mixed-effects models using the lme and lmer functions in R version 3.0.0 (R Core Team 2013) were applied. For the continuous variables (seedling height, total, aboveground, belowground biomass and root:shoot ratio), binomial data (germination and survival) and count data (germination time and number of leaves) we used Gaussian, Binomial and Poisson error structures, respectively. All the continuous data were log-transformed prior to the analyses to fulfill the requirements of normality and homoscedasticity, except for the total, aboveground, and belowground biomass of *A. platanoides* in the second experiment. As predictor variables, we included the temperature and soil moisture treatments and the standardized growing degree hours (GDH) experienced by the mother tree between the 1st of April and September 30 as fixed effects, while the mother tree and the seed provenance were included as nested random effects (to account for the hierarchical structure of the data). For each variable analyzed, first the maximal model was fitted and model simplification was achieved by comparing the deviance of the model dropping one interaction and explanatory variable per time.

Results

First experiment

The germination and survival of the species showed different responses in relation to the temperature and soil moisture treatments and the GDH experienced by the mother tree during seed production (**Table 3.1**). Both species showed different responses to the treatments applied regarding germination time: the germination was 24% earlier and 28% later, for *A. platanoides* and *A. pseudoplatanus* respectively, under the treatment (+7.2°C*80%) than at control moisture and temperature conditions (0C°*100%). Additionally, in *A. pseudoplatanus* the strong reduction of soil moisture content (60%) combined with GDH experienced by the mother tree affected the germination time: seedlings emerged on average 26% later under drier conditions than at the control treatment (**Table 3.1**).

A. platanoides showed to be more affected by the treatments applied and the GDH experienced by the mother tree during seed production. The germination in this species was affected by experimental changes in temperature, soil moisture content, GDH experienced by the mother tree and its joint manipulation. The strong reduction of soil moisture content (60%) produced an average reduction of 5% in the germination success. Moreover, the soil moisture reduction combined with rising temperature (+7.2°C) increased the germination success in comparison with the full moisture capacity at the same temperature (**Fig. 3.2**). Still for *A. platanoides*, the GDH experienced by the mother tree during seed production had a negative effect on the germination success. The seeds from provenances with the lowest GDH showed 53% higher germination success than the seeds from the warmest provenance at control temperature treatment (**Table 3.1** and **Table B1** in **Appendix B**). However, rising temperatures (+2.7°C and +7.2°C) increased the difference between seeds from the colder and warmer provenances (lower and higher values of accumulated GDH) to 73% and 79% (**Table 1** and **Table B1** in **Appendix B**). In *A. platanoides* the joint manipulation of temperature (+7.2°C) and soil moisture content reduction (80% and 60%) reduced survival by 2.4 and 1.9 times, respectively, compared with the control temperature and soil moisture conditions (**Table 3.1** and **Fig. 3.2**).

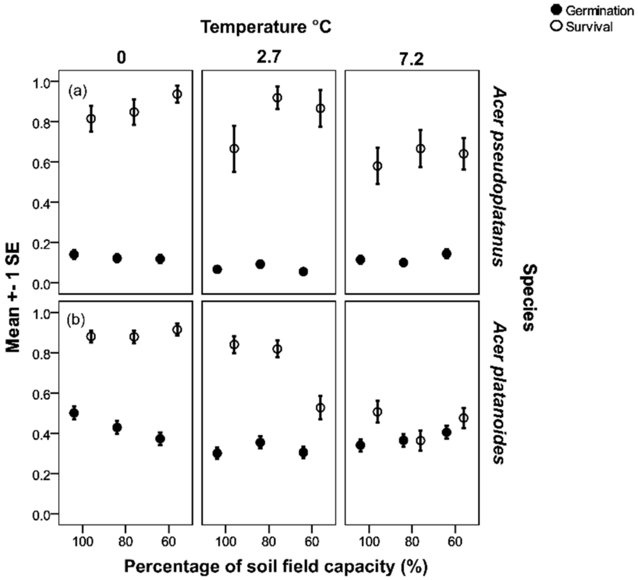


Fig. 3.2: *Acer pseudoplatanus* and *Acer platanoides* germination and survival as a function of the applied temperature and soil moisture treatments in the first experiment.

Table 3.1: Effects of warming (temperature increased by 2.7°C and 7.2°C), drought (soil moisture reduced to 80% and 60% compared to field capacity; M80% and M60%) and the standardized number of growing degree hours between April and September 2011 (GDH) on the germination, survival, number of leaves, and germination time for *Acer platanoides* and *A. pseudoplatanus* in the first experiment.

	Acer platanoides																			
	T +0°C	T +2.7°C	T +7.2°C	M 100%	M 80%	M 60%	GDH	T+0°C* M100%	T+2.7°C * M80%	T+7.2°C * M80%	T+2.7°C * M60%	T+7.2°C *M60%	T+2.7°C * GDH	T+7.2°C * GDH	M80% * GDH	M60% * GDH	T+2.7°C *M80% * GDH	T+7.2°C *M80% * GDH	T+2.7°C *M60% * GDH	T+7.2°C *M60% * GDH
	Germination (%)																			
mean	43	32	37	38	38	36		50	35	36	30	40								
SE	1.8	1.7	1.8	1.8	1.8	1.8		3.2	3.0	3.1	2.9	3.1								
z-value		0.3	0.6		-1.8 (*)	-3.1 **	-2.9 **		2.2 *	1.7 (*)	2.2 *	3.3 ***	-2.0 *	-1.9 *	✛	✛	✛	✛	✛	✛
	Survival (%)																			
mean	89	73	45	76	70	64		88	82	36	53	48								
SE	1.7	2.9	3.0	2.5	2.7	2.9		2.9	4.1	5.1	5.8	5.0								
z-value		0.9	-0.9		0.6	-0.8	0.7		-1.5	-2.3 *	-0.1	2.2 *	-1.0	-0.1	-0.6	0.9	1.51	2.2*	-0.6	-2.3 *
	Number of leaves																			
mean	3.10	3.8	3.5	4.2	3.3	2.5		3.9	3.8	2.9	2.3	3.2								
SE	0.10	0.15	0.17	0.13	0.13	0.10		0.19	0.23	0.30	0.20	0.28								
z-value		3.1 **	2.0 *		-4.2 ***	-7.6 ***	✛		✛	✛	✛	✛	✛	✛	✛	✛	✛	✛	✛	✛
	Germination time (days)																			
Mean	6.4	6.9	5.4	6.8	6.1	5.8		5.9	6.4	4.5	7.3	4.3								
SE	0.31	0.36	0.38	0.35	0.34	0.34		0.44	0.55	0.52	0.75	0.51								
z-value		1.4	6.6 ***		1.5	0.3	1.1		-2.1 *	-9.1 ***	-2.3 *	-4.7 ***	-0.8	-5.7 ***	-0.9	-0.5	1.2	7.6 ***	2.6 **	3.4 ***

	Acer pseudoplatanus																			
	T +0° C	T +2.7°C	T +7.2°C	M 100 %	M80 %	M60 %	G D H	T+0°C* M100%	T+2.7° C* M80%	T+7.2° C* M80%	T+2.7° C* M60%	T7.2°C *M60 %	T+2.7° C * GDH	T+7.2° C * GDH	M80% * GDH	M60% * GDH	T+2.7° C *M80% * GDH	T+7.2° C *M80% * GDH	T+2.7° C *M60% * GDH	T+7.2° C *M60% * GDH
	Germination (%)																			
mean	13	7	12	11	10	11		14	9	10	6	14								
SE	1.17	0.92	1.14.	1.09	1.08	1.08		2.12	1.77	1.83	1.39	2.14								
z-value		1.9 *	-0.8		☩	☩	-0.9		☩	☩	☩	☩	-2.6 *	0.7	☩	☩	☩	☩	☩	☩
	Survival (%)																			
mean	86	83	63	70	81	79		82	92	67	87	64								
SE	3.34	5.00	4..93	4.94	4.27	4.41		6.37	5.53	9.25	9.08	7.78								
z-value		-0.6	-3.5 ***		☩	☩	☩		☩	☩	☩	☩	☩	☩	☩	☩	☩	☩	☩	☩
	Number of leaves																			
mean	2.96	2.87	3.07	3.51	2.89	2.56		3.74	2.96	3.18	2.33	2.87								
SE	0.15	019	0.20	0.21	0.16	0.14		0.29	0.26	0.39	0.33	0.27								
z-value		☩	☩		-1.6	-2.8 **	☩		☩	☩	☩	☩	☩	☩	☩	☩	☩	☩	☩	☩
	Germination time (days)																			
mean	12.6	15.9	11.9	11.1	14.4	13.7		8.1	15.9	11.2	18.5	11.2								
SE	0.87	0.98	0.81	0.82	0.84	0.99		0.99	1.36	1.28	2.48	1.28								
z-value		1.5	0.2		1.5	3.3 **	-1.5		-1.5	-3.8 ***	-0.3	-0.9	-0.96	0.8	-0.6	-2.34 *	1.1	2.8 **	-0.1	-0.1

Results from mixed-effect models
Significance codes: P < 0.001***, P < 0.01**, P < 0.05*, P < 0.10 (*)
Mean and SE (standard error) are calculated on the untransformed variables
☩: Factors removed during model simplification

On the other hand, the germination in *A. pseudoplatanus* only showed significant differences due the experimental warming interacting with the GDH experienced by the mother tree during seed production. The temperature treatments significantly increased the difference between the germination of *A. pseudoplatanus* seeds from colder and warmer provenances (**Table 3.1** and **Table B.1** in **Appendix B**), while the survival was only affected by the temperature increase of +7.2°C (**Fig. 3.2**).

Regarding growth, both *Acer* species showed similar but not identical responses to the treatments applied. *A. platanoides* was sensitive to changes in temperature, moisture and the joint manipulation of these factors (**Table 3.2**). A small temperature increase had a positive effect on the biomass variables, but a higher increase of temperature (+7.2°C) reduced the total biomass by 35%, the aboveground biomass by 30% and the belowground biomass by 33% (**Table 3.2**). The strong soil moisture decrease (60%) produced an average reduction of 33% in the total and aboveground biomass (**Table 3.2**). The joint manipulation of temperature and soil moisture content (+2.7*60%) produced a stronger reduction of the biomass variables that the individual modification of both factors (**Table 3.2** and **Fig. 3.3**).

A. pseudoplatanus showed changes in the growth variables related to the changes in the temperature and soil moisture content but did not show significant differences caused by the joint manipulation of the temperature and moisture (**Table 3.2**). The temperature increase and the soil moisture content reduction produced a negative effect on most of the biomass variables. The total biomass was reduced by 21% when the temperature increased by 2.7°C and 33% when the soil moisture content was 60% of field capacity. The belowground biomass was 41% and 39% lower when the temperature increased by 2.7°C and 7.2°C respectively, while the soil moisture reduction decreased the belowground biomass by 20%. The above ground biomass was only affected by the moisture; the reduction was 23% and 32% for the 80% and 60% of field capacity treatments respectively (**Table 3.2**). The root:shoot ratio was reduced by 39% on average when the temperature increased by 2.7°C and 7.2°C and increased by 18% when the soil moisture was 80% of the field capacity (**Table 3.2** and **Fig 3.3**).

Table 3.2: Effects of warming (temperature increased by 2.7°C and 7.2°C) and drought (soil moisture reduced to 80% and 60% compared to field capacity; M80% and M60%) on the total, aboveground and belowground biomass and root:shoot ratio of *Acer platanoides* and *A. pseudoplatanus* in the first experiment. The effects of GDH were not significant and therefore excluded from the model.

	T+0°C	T +2.7°C	T +7.2°C	M100%	M80%	M60%	T+0°C* M100%	T+2.7°C * M80%	T+7.2°C * M80%	T+2.7°C * M60%	T+7.2°C * M60%
Acer platanoides											
Biomass (g)											
Mean	0.17	0.17	0.11	0.18	0.17	0.12	0.18	0.17	0.12	0.12	0.10
SE	0.005	0.007	0.007	0.007	0.007	0.005	0.018	0.020	0.020	0.019	0.014
t-value		2.20*	-4.46***		-0.09	-2.65**		-1.72	0.21	-2.73**	0.52
Below Biomass (g)											
Mean	0.06	0.06	0.04	0.06	0.07	0.04	0.06	0.06	0.04	0.04	0.04
SE	0.003	0.004	0.003	0.003	0.005	0.002	0.002	0.004	0.002	0.002	0.002
t-value		1.13	-4.84***		0.64	-1.87(*)		-1.83(*)	0.27	-2.56*	1.08
Above biomass (g)											
Mean	0.10	0.11	0.07	0.12	0.10	0.08	0.12	0.10	0.07	0.07	0.06
SE	0.004	0.004	0.005	0.004	0.003	0.006	0.004	0.003	0.003	0.002	0.003
t-value		2.32*	-3.95***		-1.29	-3.33***		-1.34	0.65	-2.41*	0.71
Ratio											
Mean	0.65	0.54	0.50	0.54	0.66	0.56	0.57	0.58	0.55	0.50	0.51
SE	0.034	0.027	0.068	0.019	0.052	0.046	0.025	0.054	0.085	0.031	0.160
t-value		-3.21**	-4.15***		2.34*	1.14		+	+	+	+
Acer pseudoplatanus											
Biomass (g)											
Mean	0.14	0.11	0.12	0.15	0.13	0.10	0.17	0.12	0.13	0.06	0.10
SE	0.108	0.080	0.060	0.010	0.009	0.007	0.016	0.012	0.018	0.013	0.012
t-value		-2.91**	-1.52		-0.63	-3.72***		+	+	+	+
Below Biomass (g)											
Mean	0.05	0.03	0.03	0.04	0.05	0.03	0.06	0.03	0.04	0.02	0.02
SE	0.004	0.003	0.003	0.004	0.005	0.003	0.006	0.004	0.009	0.005	0.004
t-value		-4.05***	-3.59***		0.83	-2.32*		+	+	+	+
Above biomass (g)											
Mean	0.09	0.08	0.08	0.10	0.08	0.07	0.11	0.07	0.08	0.06	0.07
SE	0.005	0.007	0.006	0.007	0.005	0.005	0.010	0.008	0.010	0.013	0.008
t-value		+	+		-2.35*	-3.49***		+	+	+	+
Ratio											
Mean	0.63	0.39	0.37	0.46	0.57	0.45	0.59	0.48	0.36	0.34	0.39
SE	0.045	0.027	0.031	0.052	0.044	0.032	0.095	0.040	0.048	0.031	0.063
t-value		-4.72***	-4.98***		3.50***	0.58		+	+	+	+

Results from mixed-effect models

Significance codes: P < 0.001***, P < 0.01**, P < 0.05*, P < 0.10 (*)

Mean and SE (standard error) are calculated on the untransformed variables

+: Factors removed during model simplification

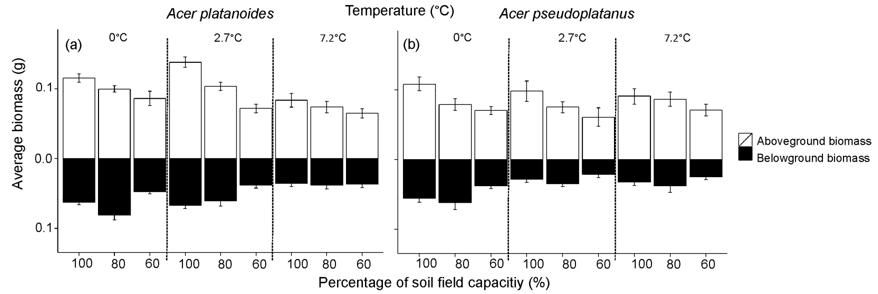


Fig. 3.3: Average aboveground and belowground biomass of *Acer pseudoplatanus* and *Acer platanoides.* Error bars indicate the standard errors.

Second experiment

In the second experiment, the number of GDH experienced by the mother tree reduced the germination success in *A. platanoides*, while the soil moisture content reduction narrowed the difference in germination time between the colder and warmest provenances (**Table 3.3**). For this species, the germination was 75% lower in seeds from higher GDH than in seeds produced under colder conditions (lower accumulated GDH). The germination time of *A. pseudoplatanus* increased by 18% and 26% when the soil moisture content was 80% and 60% of the field capacity, respectively (**Table 3.3**).

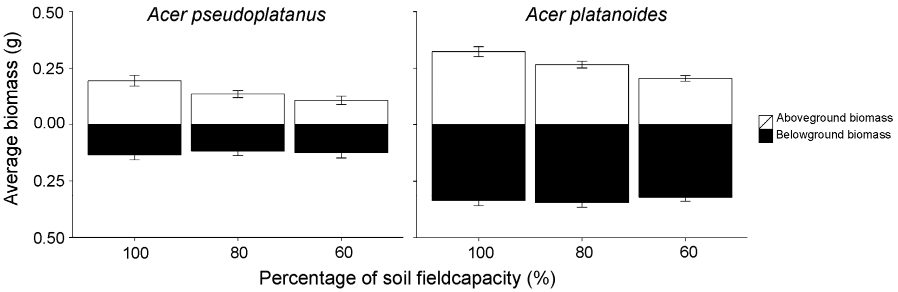


Fig. 3.4: Effects of drought treatments on aboveground and belowground biomass of *Acer pseudoplatanus* and *Acer platanoides* in the second experiment. Error bars denote standard errors.

The growth variables for both species were mainly affected by the soil moisture content. The drought negatively affected the total, aboveground biomass and height, while the soil moisture reduction increased the root:shoot ratio (**Table 3.4**).

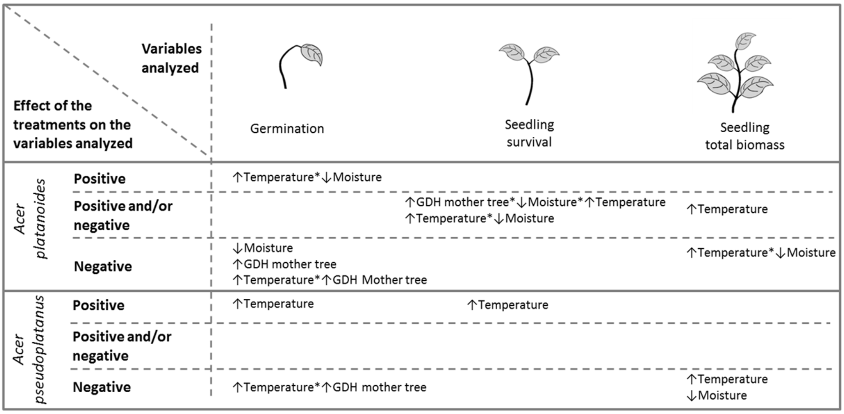
Table 3.3: Effects of drought (soil moisture content reduced to 80% and 60% compared to field capacity; M80% and M60%) and the standardized number of growing degree hours between April and September (GDH) on the germination, survival, number of leaves, and germination time for *Acer platanoides* and *A. pseudoplatanus* in the second experiment.

	Acer platanoides						***Acer pseudoplatanus***					
	M100%	**M80%**	**M60%**	**GDH**	**M80%* GDH**	**M60%* GDH**	**M100%**	**M80%**	**M60%**	**GDH%**	**M80%* GDH**	**M60%* GDH**
Germination (%)												
Mean	23	25	24				6	6	5			
SE	1.68	1.72	1.72				0.89	0.89	0.81			
z-value		‡	‡	-3.06**	‡	‡		‡	‡	-0.989	‡	‡
Survival (%)												
Mean	58	61	60				66	63	47			
SE	4.13	3.96	4.01				7.79	7.95	8.27			
z-value		‡	‡	0.85	‡	‡		0.779	-1.582	‡	‡	‡
Number leaves												
Mean	5.9	4.6	3.7				4.8	4.0	3.1			
SE	0.65	0.48	0.39				0.33	0.37	0.26			
z-value		-3.52***	-6.41***	‡	‡	‡		-1.16	-2.11*	‡	‡	‡
Germination time (days)												
Mean	9.3	9.6	11.3				14.6	17.9	19.9			
SE	0.77	0.78	0.93				1.27	1.02	1.38			
z-value		1.11	-1.43	0.51	-0.93	2.35*		2.19*	4.47***	‡	‡	‡

Results from mixed-effect models
Significance codes: P < 0.001***, P < 0.01**, P < 0.05*, P < 0.10 (*)
Mean and SE (standard error) are calculated on the untransformed variables
‡: Factors removed during model simplification

The soil moisture content at 60% of the field capacity reduced the total biomass by 20% and 30% for *A. platanoides* and *A. pseudoplatanus* respectively. For both species, the root:shoot ratio was higher at 60% of the field capacity than at field capacity (**Table 3.4** and **Fig. 3.4**). Additionally, the height reached by the seedlings at the end of the experiment was 28% and 25%, 18% and 17% lower under the treatments 80% and 60% of field capacity for *A. pseudoplatanus* and *A. platanoides*, respectively (**Table 3.4**).

Finally, the temperature (+2.7°C and +7.2°C) and the soil moisture reduction (80% and 60% of the field capacity) treatments applied produced different effects on germination, seedling survival and growth of both species. Along both experiments, *A. platanoides* was more susceptible than *A. pseudoplatanus* to the treatments applied (**Fig. 3.5**).



	Variables analyzed / Effect of the treatments on the variables analyzed	Germination	Seedling survival	Seedling total biomass
Acer platanoides	**Positive**	↑Temperature*↓Moisture		
	Positive and/or negative		↑GDH mother tree*↓Moisture*↑Temperature ↑Temperature*↓Moisture	↑Temperature
	Negative	↓Moisture ↑GDH mother tree ↑Temperature*↑GDH Mother tree		↑Temperature*↓Moisture
Acer pseudoplatanus	**Positive**	↑Temperature	↑Temperature	
	Positive and/or negative			
	Negative	↑Temperature*↑GDH mother tree		↑Temperature ↓Moisture

Fig. 3.5: Scheme of the main experimental outcomes. In the first column, the effects of the treatments on germination, survival and biomass are indicated (positive, negative and a combination of positive and negative). The small arrows indicate increases and decreases of the level of the treatments; the stars indicate the interaction between the treatments.

Table 3.4: Effects of drought (soil moisture content reduced to 80% and 60% compared to field capacity; M80% and M60%) and the standardized number of growing degree hours between April and September (GDH) on the total, aboveground and belowground biomass and root:shoot ratio of *Acer platanoides* and *A. pseudoplatanus* in the second experiment.

	Acer platanoides						*Acer pseudoplatanus*					
	M100%	M80%	M60%	GDH	M80%* GDH	M60%* GDH	M100%	M80%	M60%	GDH	M80%* GDH	M60%* GDH
Biomass (g)												
Mean	0.66	0.61	0.53				0.33	0.25	0.23			
SE	0.04	0.03	0.03				0.04	0.04	0.03			
t-value		-0.93	-2.58*	✛	✛	✛		-1.98(*)	-1.40	✛	✛	✛
Belowground biomass (g)												
Mean	0.34	0.35	0.32				0.14	0.12	0.13			
SE	0.03	0.02	0.02				0.02	0.02	0.02			
t-value		-0.10	1.96(*)	0.439	0.181	-2.134*		-1.49	-0.03	✛	✛	✛
Aboveground biomass (g)												
Mean	0.32	0.27	0.21				0.20	0.13	0.11			
SE	0.02	0.02	0.01				0.02	0.02	0.01			
t-value		-2.38*	-4.89***	✛	✛	✛		-2.34*	-3.02**	✛	✛	✛
Ratio												
Mean	1.06	1.33	1.77				0.65	0.76	1.32			
SE	0.03	0.05	0.12				0.04	0.07	0.144			
t-value		3.26**	7.32***	✛	✛	✛		0.41	3.95***	✛	✛	✛
Height (cm)												
Mean	8.21	6.8	6.13				7.67	6.30	5.56			
SE	0.32	0.24	0.19				0.44	0.33	0.26			
t-value		-3.41***	-4.84***	✛	✛	✛		-2.43*	-3.41***	✛	✛	✛

Results from mixed-effect models
Significance codes: $P < 0.001$***, $P < 0.01$**, $P < 0.05$*, $P < 0.10$ (*)
Mean and SE (standard error) are calculated onthe untransformed variables
✛: Factors removed during model simplification

Discussion

By simulating moderate and extreme warming and drought (Smith 2011; Chmura et al. 2011) combined with the effect of the temperature experienced by the mother tree along the latitudinal gradient (Johnsen et al. 2005a; De Frenne et al. 2013a), we were able to provide important information about the germination and seedling establishment of two currently secondary tree species in the face of climate change. The variable responses of germination, germination time and survival to the combination of temperature and soil moisture treatments indicates the relevance of jointly manipulating different climatic variables and highlights the need of more complex full factorial experiments (Shaver et al. 2000; Dieleman et al. 2012). Across both experiments, two congeneric species *Acer platanoides* and *A. pseudoplatanus* showed divergent responses. This species-specific behavior during the recruitment stage is relevant to better forecast and understands population dynamics (Milbau et al. 2009). Additionally, considering the temperature experienced by the mother tree, the genetic differences between and within populations and the nature of the populations, reliable information about the effects of climate change on early establishment, might be especially relevant to better forecast future forest compositional changes and develop adaptive management plans (Wang et al. 1994; Chmura et al. 2011; Hanewinkel et al. 2013).

Life-stage transitions that are important for plant population dynamics (germination and survival) were especially sensitive to changes in temperature, the interaction with soil moisture content and the GDH experienced by the mother tree. Previous research indicated that for species with dormant seeds, climate warming may be favourable for germination and recruitment as long as the stratification requirements are satisfied (Chmura et al. 2011). Additionally, warmer temperatures enhance plant biochemical and physiological processes as long as threshold temperatures are not exceeded and moisture content is adequate (Chmura et al. 2011). In these experiments, the stratification requirements were specifically satisfied before the beginning of the experiments and therefore excluded from our analyses. Regardless of whether future stratifications requirements will be met or not, our result suggest that the projected changes in temperature and soil moisture content will likely have negative effects on germination and/or survival of both *Acer* species. However, as indicated, it is likely to expect species specific responses dependent as well of the level of change of the climatic variables. This denote the complexity of understand and anticipate the effect of climate change in plants communities.

The effects of the experimental temperature and moisture treatments on recruitment depended not only on the level of change of the climatic variables but also on its interactions. For example, in *A. platanoides*, the soil moisture reduction negatively affected the germination. Yet, the negative effect of soil moisture content reduction on germination was partly compensated when interacting with a temperature increase. However, the germination under dry and warm conditions did not reach the level recorded under control conditions. Such partial compensation can be explained by the fact that at high temperatures and dryer conditions the seeds are less exposed to fungi and pathogens than under warm and wet conditions (Blaney and Kotanen 2001) and are stimulated to germinate by higher temperatures. For both species, warming, drought and the GDH experienced by the mother tree affected the germination time, an important factor in the potential regeneration of plants (Chmura et al. 2011). Under warm and wet conditions the germination was advanced, extending the growing season length and potentially improving the chance of seedlings to survive the following winter (Milbau et al. 2009; De Frenne et al. 2012). However, due the high early mortality under warm and wet conditions, the relative adventage of advanced germination might be suppressed and not provide advantages for the species analyzed.

For both species, soil warming reduced seedling survival. However, the negative effect of temperature on the survival of *A. platanoides* increased when the temperature and soil moisture content were jointly manipulated, while in *A. pseudoplatanus* survival was not affected by the soil moisture content. The high sensitivity of emerging seedlings to drought is well known, and because shoot growth continues later into the growing season, late season droughts may impact seedlings more than they affect mature trees (Dieleman et al. 2012). For this reason it is likely that drought-related regeneration failures will increase under future climatic regimes. However, explaining the different drought sensitivity observed, the effect of drought on survival depends on several factors including drought hardiness, evolutionary adaptation and drought acclimation (Abrams 1990; Abrams 1994; Martinez-Vilalta et al. 2004). As was observed in these two closely related species, differences in drought hardiness have been also found between species, varieties and populations which also points to the relevance of provenance-related tests (Martinez-Vilalta et al. 2004). In this work, we showed that two congeneric species exhibit different drought sensitivities. *A. pseudoplatanus* survival was not affected by the soil moisture, indicating this species is more drought tolerant than *A. platanoides*. As these two species can occur in the same sites, we can expect that under drought stress, *A. pseudoplatanus* will exhibit relative advantages.

The germination and seedling survival of *A. platanoides* from colder provenances (lower GDH) were much higher than from warmer provenances (higher GDH) when the seeds are placed under equal conditions. Thus, seeds from warmer provenances may be less provisioned (as indicated by the negative correlation between seed mass and GDH along the latitudinal gradient). The higher seed mass recorded under colder conditions might be caused by a lower rate of seed ripening and a longer seed filling process that allows a greater total assimilation (Fenner and Thompson 2005). Conversely, the effects of the populations being native vs. non-native had limited and no clear directional effects on the studied response variables (results not shown). This matches with observations of Skrøppa et al. (2010) who reported rapid adaptation in *Picea albies* regarding seedling timing of bud set at the end of the first growing season. They compared seedlings resulting from seeds from Central European and Norwegian parents producing seeds in Norway with seedlings from seeds from mother trees producing seeds at their geographical origins. They argued that the rapid change in this adaptive trait is related to the influence of day length and temperature conditions during embryo formation and maturation on the phenotypic performance of Norway spruce seedlings. Moreover, it is important to mention that despite that the mother tree was not considered in the analysis, the maternal environment can influence the gene expression influencing germination and even generation time and population growth rates (Donohue 2009).

The differences between colder and warmer provenances in germination and survival success increased under the treatments applied in our experiment, showing the importance of bigger seeds and, consequently, higher nutrient reserves on the early regeneration success under climate change. The seed size is the result of both environmental conditions and the mother tree genotype (Fenner and Thompson 2005). Moreover, the genetic differences between mother trees (not studied here) can be also relevant regarding the performance of seeds and seedlings under climate change conditions (Donohue 2009). Finally, it is important to mention that the response of tree survival to soil moisture reductions can change over longer time periods. For instance, it is possible that seedlings and saplings resulting from seeds from warmer regions perform better under drought stress as was reported before for other species (Atzmon et al. 2004; O'Brien et al. 2007).

Concerning the growth variables, warming and drought affected height and biomass growth of both species. The influence of the GDH of different provenances appears to be important only in the very beginning of the recruitment stage. However, after germination, the role of the provenance loses relevance in terms of growth that is mainly conditioned by the experimental conditions. Again, these results show species-specific responses especially relevant in terms of the population dynamic variables (germination and survival) and indicate how different aspects of the plant life can be differentially affected in congeners.

Drought and warming reduced the growth of both species (total, above and belowground biomass and height). The root:shoot ratio generally increased as drought intensified, as reported before for low-severity drought conditions (Kramer et al. 2010) and extreme drought (Dreesen et al. 2012). Our results thus equally confirm that under stress conditions there is a differential allocation of resources in favour of belowground biomass.

To conclude, our results suggest that increasing temperatures might negatively affect critical life stages such as germination and survival of *Acer pseudoplatanus* and *A. platanoides*. The negative impact of soil moisture was especially important in constraining biomass production and growth. However, especially the two global-change drivers acting together might strongly modify population dynamics of our study species. The germination success of *A. platanoides* was more than two times higher than in *A. pseudoplatanus*. An additional potential future advantage of this species is that under all the treatments applied the seedlings of *A. platanoides* were larger than the seedlings of *A. pseudoplatanus*, especially regarding the root biomass. This may allow this species to reach a bigger soil volume for nutrient and water uptake. Together, this suggests that *A. platanoides* might be better able to cope with changing climatic conditions, which is especially relevant since deciduous trees are gaining dominance, for instance, in several Central European forests, due to higher coniferous mortality caused by climate-change related drought stress (Gimmi et al. 2010). This is particularly important considering that potential novel regional communities might emerge in the context of climate change (Williams and Jackson 2007). However, it is necessary to stress that we focused only on the germination, survival and early growth of the seedlings and that information about the subsequent life stages is key to fully understand the impacts of climate change on these species' regeneration.

4. Impacts of warming and changes in precipitation frequency on the regeneration of two *Acer* species

Abstract

An increase of the global temperature between 0.3 °C and 4.8 °C is expected by the end of this century. Additionally, the projections indicate that more extreme rainfall events and longer intervening dry periods will take place in most areas of the world. Climate change will likely affect all phases of the life cycle of plants, however plant reproduction has been suggested to be especially sensitive. Here, using a combination of techniques (soil heaters and different provenances along a latitudinal gradient), we analyze the regeneration from seeds of two important secondary tree species, *Acer platanoides* and *A. pseudoplatanus*, in terms of germination, seedling survival and growth with a full factorial experiment including warming and changes in precipitation frequency. Both species responded to warming, precipitation and country of origin, with stronger (negative) effects of warming and country than of precipitation frequency. In general the central provenances performed better than the northernmost and southernmost provenances. We also detected interactive effects between warming, precipitation frequency and / or seed provenance. It is possible to suggest that both species will be similarly affected by changes in climatic factors (warming and precipitation frequency). However, due the frequent divergent responses of seeds and seedlings along the gradient, it is likely that climate change will not have homogeneous impacts across Europe. These results point out the relevance of performing experiments to test the performance of different provenances under simulated future climatic conditions, providing important information for forest adaptation in the context of climate change.

Introduction

The global mean surface temperature has steadily been increasing by 0.85 °C since the late 19th century (IPCC 2013b). Depending on the concentration-driven scenario considered (suite of greenhouse gases, aerosols and chemically active gases), a future increase between 0.3 °C and 4.8 °C is expected by the end of this century (IPCC 2013b). Extreme events of warming are also expected; the length, frequency, and/or intensity of warm periods are very likely to increase in many regions (IPCC 2013b). As a consequence of global warming, also the water cycle is expected to change non-uniformly around the world. Precipitation frequencies will be likely modified, with more extreme rainfall events and longer dry intervals in most areas of the world (Chaoyang et al. 2012; IPCC 2012; IPCC 2013b). However, not only the changes in individual factors will be important in the future. The interaction between different climatic factors can produce different impacts on terrestrial ecosystems than the individual factors; for example the combination of drought, extreme heat and/or low humidity, will clearly have stronger impacts than any factor alone (IPCC 2012; IPCC 2013b). Additionally, changes in climatic factors can induce changes in other abiotic environmental factors. For instance, soil moisture is the result of a combination of different factors such as precipitation, temperature, air moisture content, soil texture and organic matter content (Wang 2005; Chaoyang et al. 2012; Schneider et al. 2014).

Even though the changes in climatic drivers and other related abiotic aspects are expected to be larger at the end of the century, the effects of current global warming are already visible in several ecosystems (Hedhly et al. 2008; Van Mantgem et al. 2009) and affecting the ecology of many species, including their geographical distribution, phenology, biotic interactions and extinction risks (for a review Peñuelas et al. 2013). Temperature has been shown to influence seed production (Walck et al. 2011; Carón et al. 2014a), germination, establishment (Lewis et al. 1999; Jensen 2001) and growth of plants (Rapp et al. 2012). Rainfall amount or soil moisture content may affect plant distributions (North et al. 2005), seed germination (Fay and Schultz 2009; Carón et al. 2014b), growth (Fay and Schultz 2009; Dreesen et al. 2012), phenology (Seghieri et al. 2009), and mortality (Anderegg et al. 2013). However, different climate-change aspects (i.e. precipitation, warming, CO_2 concentration increase) have not received the same level of attention. For instance, the impacts of changes in precipitation frequency are rarely assessed (but see e.g. Fay et al. 2003, Chaoyang et al. 2012, Schneider et al. 2014). Precipitation frequency may change independently from rainfall totals when the total amount of rainfall over a certain period remains constant, but the amount of rainfall events decrease and the precipitation in each rainfall event is enhanced. Some of the few available studies on plants have shown that reduced rainfall frequencies can increase productivity, decrease root-to-shoot ratios or affect leaf senescence in grassland species (Fay et al. 2002; Fay et al. 2003; Schneider et al. 2014). Yet, more research is urgently needed to better understand the effect of changes in precipitation frequency on other plant growth parameters, functional groups, and ecosystems (Schneider et al. 2014).

Even though climate change will likely affect all plant life cycle phases, plant reproduction has been suggested to be especially sensitive (Hedhly et al. 2008; Walck et al. 2011). In many cases, warming has been shown to positively influence seed germination (Milbau et al. 2009; McCarragher et al. 2011) or to enhance seedling survival and growth (Piper et al. 2013). Moreover, reductions in soil moisture tend to negatively affected seed germination and seedling survival and growth (Fay and Schultz 2009; Shevtsova et al. 2009). Information about the impacts of climate change on regeneration from seeds is especially essential for tree species because it is the most common natural way through which forests regenerate around the globe. In practice the majority of tree species used for afforestation or reforestation in Europe are grown from seeds (den Ouden et al. 2010). Throughout Europe, young trees that are used for reforestation and afforestation generally come from a pool of recommended and/or autochthonous provenances (cf. the EU Directive 1999/105/EC on the marketing of forest reproductive material). However, only very limited information is available on how these recommended provenances will perform under future climatic conditions.

Here we analyze the effects of warming and changed precipitation frequency on regeneration from seeds of two important, currently secondary tree species, namely *Acer platanoides* and *A. pseudoplatanus*. We combined soil heating cables (Aronson and McNulty 2009; Carón et al. 2014b) with seed sampling along a latitudinal gradient (Fukami and Wardle 2005; De Frenne et al. 2013a). The combination of techniques constitutes an interesting step further, allowing to illuminate different aspects of the impacts of climate change on plant populations (De Frenne et al. 2013a).

We specifically ask i) how warming and changes in precipitation frequency affect seed germination, seedling survival and growth? ii) Is there an interacting effect of warming and precipitation frequency on tree species' regeneration? iii) Is there variation in the seed and seedlings response to changes in temperature and precipitation frequency according to the provenance of the seeds? We hypothesize that the seeds and seedlings from mother trees from southern provenances will be able to better cope with interactive warming and less frequent precipitation than the seeds and seedlings from mother trees growing under colder and wetter northerly conditions. Additionally, we expect that warming will enhance seedling survival and growth and that more frequent watering will stimulate germination.

Material and Methods

Experimental design

Seeds of *Acer platanoides* and *A. pseudoplatanus* were collected from four and five regions, respectively. The regions selected for this study were located along a 2200 km long latitudinal gradient, from Italy through Hungary, Poland, Denmark and Sweden to Norway (**Fig. 4.1**). Seeds were either (i) bought from a nursery which could provide exact provenance information (Poland, Denmark and Hungary), or (ii) collected from each of three forest patches in an area of 40 x 40 km^2 (Italy, Sweden and Norway). In the latter case, one seed-bearing healthy mother tree was used for seed collection in each forest patch and the seeds were picked from the forest floor immediately after seed dispersal (Italy, Mid Sweden and Norway).

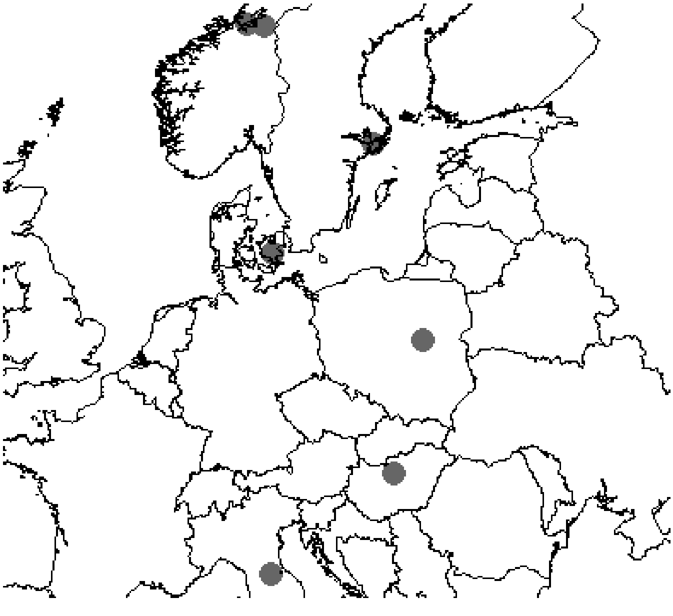


Fig. 4.1: Location countries/regions of seed provenances *A. platanoides* and *A. pseudoplatanus* along a latitudinal gradient

We performed a full factorial experiment with soil heating and precipitation frequency manipulation, to simulate a total of nine climate change scenarios. We based our experiment on the different representative concentration pathwayscenarios proposed by the Intergovernmental Panel on Climate Change by the end of this century (IPCC 2013). In Europe an increase of the temperature between 1.5 °C and 4.4 °C is projected, while the mean annual precipitation will likely increase in northern and central Europe and decrease in the Mediterranean area (IPCC 2013b). Moreover, considering the possible changes in precipitation frequency, three precipitation frequency treatments were applied. The amount of water was expressed as soil moisture content rather than millimetres of rainfall since (i) this is a stronger predictor of the impact on plant establishment than precipitation (Walck et al. 2011), (ii) germination and other early establishment variables are highly dependent on available soil moisture (Kos and Poschlod 2008), and (iii) it is easier to compare the results with previous studies that used this variable (Vicca et al. 2012; Carón et al. 2014b).

All seeds were cold stratified until dormancy break. The seeds were soaked in water until the desired level of humidity was reached, after which the seeds were placed under controlled cold conditions (0 °C to 1 °C and 90-95 % humidity) until germination started (See details in **Chapter 2**). Because the stratification time is highly dependent on species and provenance, and to ensure that all the provenances were at the same developmental stage at the start of the experiment, seed lots that started to germinate were stored in a freezer at -2°C until the start of the experiment (52 days of difference between the first and the last seed lot germination).

Four seeds of each species and region were randomly sown in each of 12 plastic pots (112 cm^3) (4 × 12 = 48 seeds) per treatment, filled with standard potting soil (mean pH 6, nutrient ratio NPK 15:10:11, organic matter 20 % and water holding capacity of 80 %). The manipulations of the soil temperature were: 1) Control at ambient temperature (the average for the whole period was 19.2 ± SD 3.2 °C measured every minute, and averaged and logged every 15 minutes using Decagon data loggers Em50 ECH2O LOGGER inside the soil of the pots at 5 cm depth); 2) warming with +2.8°C (average 22.0 ± SD 2.9 °C), and 3) extreme warming with +7.0 °C (26.2 ± SD 4.6 °C). Additionally, the precipitation frequency was manipulated by adding the same amount of water on each occasion, but in different intervals (one, two or three times per week). The necessary amount of water to be added every week was calculated by weighting three randomly chosen pots per warming treatment and calculating the amount of water to reach field capacity. Then the necessary amount of water was divided by the watering interval assuring that all the treatments received exactly the same amount of water per week but in different intervals. The average weekly amount of water added to each pot during the experiment under each treatment was: +0°C: 24 ml (control), +2.8°C: 25 ml, and +7.0°C: 29 ml.

For this experiment, warming was achieved though soil heating mats (ACD heating mats HMT-A, Oberweidbach Germany). The experiment was installed at the edge of a deciduous forest in Gontrode, Belgium (50°58'N, 3°48'E), with *Fagus sylvatica, Fraxinus excelsior*, *Quercus robur* and *Acer pseudoplatanus* dominating the canopy. The pots were placed on top of the soil heating mats, on wooden tables under plastic roofs (70 cm above the pots) to exclude the natural precipitation and allow free air exchange.

At the end of the experiment (*ca.* 3 months after sowing), we assessed the regeneration in terms of germination (number of emerged seedlings), survival (number of living seedlings at the end of the experiment) and seedling growth (height, aboveground and belowground biomass and total biomass). The germination was recorded once per week. At the end of the experiment, the seedlings were carefully removed from the soil, the aboveground and belowground parts separated, dried at 60 °C during 24 hours and weighed.

Data analysis

Generalized linear models (*glm*) in R version 3.1.0 (R Core Team 2013) were applied. For the binomial data (germination and survival) and continuous data (aboveground, belowground and total biomass, root: shoot ratio and height) we used binomial and Gaussian errors structures, respectively. To fulfil the requirements of normality and homoscedasticity, for both species, the aboveground, belowground, total biomass were log transformed and root: shoot ratio was square root-transformed. Additionally the height of *A. platanoides* was log-transformed prior to the analyses. The temperature treatments (+ 0 °C, +2.8 °C and +7 °C), the precipitation frequency treatments (one, two and three times weekly) and the country (Poland, Denmark, Sweden, Norway, Italy and Hungary) were used as fixed effects. The *a priori* data exploration showed that the seeds' origin (i.e. collected by us vs. purchased from tree nurseries) did not affect the results, therefore this factor was not included in the analyses. For each variable analysed, first the full model was fitted (all the fixed effects and their interactions), after which models were simplified by dropping first the least significant interactions and then the least significant individual variables at each step. The comparison between models was based on the likelihood ratio test or the scaled deviance for the binomial and Gaussian data respectively, until all remaining terms were significant (Zuur et al. 2009).

Results

Establishment variables

Both species responded to differences in temperature, precipitation frequency and the country of origin, with stronger effects of temperature and the country of origin than of precipitation frequency on seed germination and seedlings' performance (**Table 4.1**). Seed provenance significantly affected germination in both species; both species exhibited the highest germination approximately in the centre of the analysed distribution range (**Table 4.1** and **Fig. 4.2**). Moreover, the germination was 19 and 12% (*A. platanoides*) and 16 and 12% (*A. pseudoplatanus*) higher when the precipitation treatment was applied two and three times per week, respectively, compared to the least frequent precipitation (**Table 4.1** and **Fig. 4.2**). Additionally, for *A. pseudoplatanus*, the temperature increase reduced germination, being 17.3% lower with 2.8 °C warming than under the control conditions (**Table 4.1** and **Table C.2** in **Appendix C**).

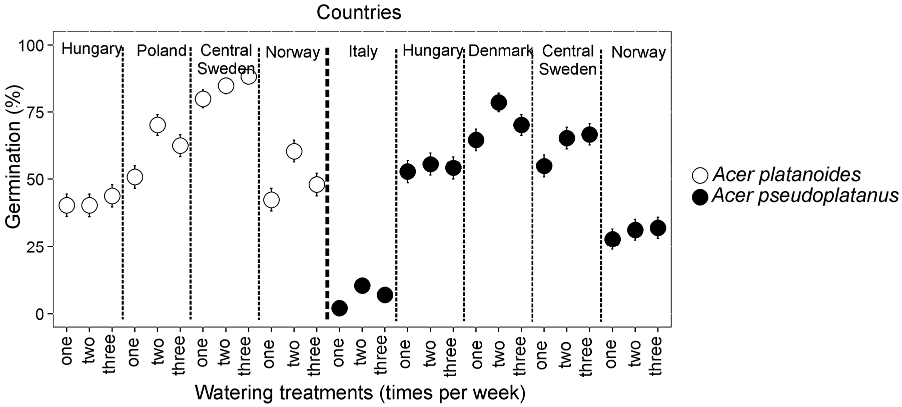


Fig 4.2: Germination of *Acer platanoides* and *A. pseudoplatanus* as a function of precipitation frequency (one, two and three time per week) and seed provenance. The error bars indicate standard errors

Significant differences were observed between the species in terms of seedling survival. In A. *platanoides*, survival decreased with warming, being 49.9% lower under warmer conditions than under control conditions (**Table 4.1**, **Fig. 4.3** and **Table C.1** in **Appendix C**). Survival of *A. pseudoplatanus*, on the other hand, responded differently to increases in temperature depending on the country of origin of the seeds (significant warming × country interaction, **Table 4.1** and **Fig. 4.3**). Survival of seedlings of seeds from Hungary and central Sweden increased with warming, while survival of seedlings of seeds from Denmark and Norway declined (**Fig. 4.3**). The joint manipulation of temperature and precipitation frequency differently affected seedling survival according to the provenance of origin of the seeds (**Table 4.1**). For example, the seedlings from Italian seeds, showed a higher germination with warming when watered one per week, but the same seedlings reduced the germination under warming when watered more often (3 times per week), while other provenances such as Central Sweden exhibit a rather constant increase of survival with warming across all the precipitation frequencies.

Table 4.1: Establishment and growth variables as a function of the country of provenance of the seeds and the experimental conditions: warming (temperature increased by 2.8 °C and 7.0 °C) and precipitation frequency (one, two and three times per week).

	Acer platanoides		***Acer pseudoplatanus***	
Establishment variables	**Predictor**	**LRT**	**Predictor**	**LRT**
Germination	Precipitation frequency	↑6.38*	Precipitation frequency	↑4.49*
	Seed provenance	183.69***	Seed provenance	545.79***
			Warming	↓10.46**
Survival	Warming	↓84.11***	Warming* Seed provenance	13.24*
			Warming*Precipitation frequency* Seed provenance	11.68*
Growth	**Predictor**	**Scaled dev**	**Predictor**	**Scaled dev**
Total biomass	Warming	↓99.38***	Warming	↓49.42***
	Seed provenance	36.58***	Seed provenance	71.99***
	Warming* Seed provenance	114.61***	Warming* Precipitation frequency	31.40***
Aboveground biomass	Warming	↓88.86***	Warming	↓43.87***
	Seed provenance	28.14***	Seed provenance	42.1***
	Warming: Seed provenance	103.22***	Warming: Precipitation frequency	25.00***
Belowground biomass	Warming	↓92.98***	Warming	↓35.54***
	Seed provenance	40.20***	Seed provenance	77.88***
	Warming: Seed provenance	106.17***		
	Water: Seed provenance	32.53***		
Root: shoot ratio	Warming	↓24.33***	Warming	↓10.76**
	Seed provenance	36.10***	Seed provenance	53.52***
Height	Warming	↓64.69***	Warming	↓139.72***
	Seed provenance	32.23***	Seed provenance	57.54***
	Warming* Seed provenance	89.69***		

*** P<0.001, ** P<0.01, * P<0.05
LRT: Likelihood ratio test
Scaled dev: Scaled deviance
The direction of the effect is indicated by arrows: ↑ and ↓ correspond to an increase and decrease of the variables analyzed, respectively

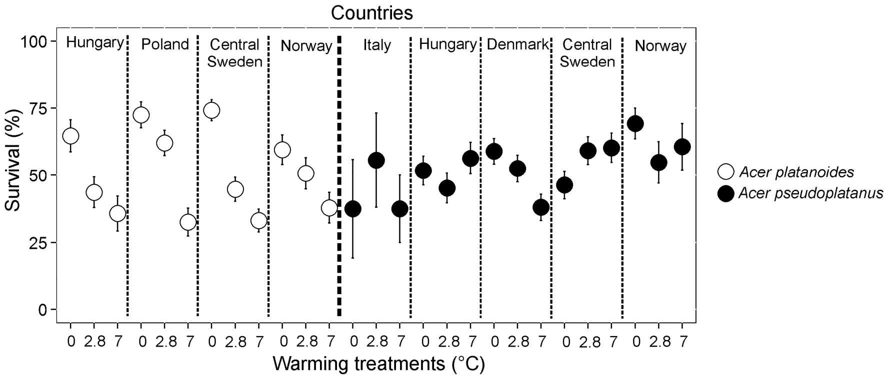


Fig. 4.3: Survival of *Acer platanoides* and *A. pseudoplatanus* as a function of temperature (ambient temperature and increases of 2.8°C and 7°C) and seed provenance. The error bars indicate standard errors

Growth variables

Regarding the biomass variables, both species showed similar responses to the manipulated factors (**Table 4.1**). However, it is important to mention that the seedlings of *A. platanoides* were much bigger than the seedlings of *A. pseudoplatanus* (**Fig. 4.4** and **Fig. 4.5**).

Warming negatively affected the aboveground, belowground and consequently the total biomass of both species. The total biomass was 53.5% (*A. platanoides*) and 37.9% (A. *pseudoplatanus*) lower in seedlings growing under the warmest condition (+7.0 °C) than under the control temperature treatment (+0 °C) (**Fig. 4.5, Table C.1** and **Table C.2** in **Appendix C**). Furthermore, the provenance of the seeds influenced the aboveground, belowground and the total biomass, which generally increased to the north, but the highest values were recorded in central Sweden (*A. platanoides*) and Denmark (*A. pseudoplatanus*). In addition, analyses revealed interactive effects for both species (**Table 4.1**). The aboveground, belowground and total seedling biomass of *A. platanoides* generally decreased with warming, but seedlings grown from seeds of middle latitudes (Poland and central Sweden) were smaller under a moderate warming (+2.8 °C) than under extreme warming (significant warming × provenance interaction, **Table 4.1**, **Fig. 4.4** and **Table C.1** in **Appendix C**). In *A. pseudoplatanus* the aboveground biomass and total biomass were was 38 and 46.3 % higher, respectively, when growing under un-warmed, less frequently watered conditions (control temperature and one time watered per week) compared with the warmest condition and the most frequent watering (significant warming x Precipitation frequency interaction, **Table 4.1** and **Table C.2** in **Appendix C**).

The root: shoot ratio of both species depended on the seed provenance and was affected by experimental warming (**Table 4.1**). Root: shoot ratios were highest in central Sweden (*A. platanoides*) and Denmark (*A. pseudoplatanus*) (**Fig. 4.4**, **Fig. 4.5**, **Table C.1** and **Table C.2** in **Appendix C**). Warming decreased the root: shoot ratio in both species (**Table 4.1**, **Fig. 4.4**, **Fig. 4.5**, **Table C.1** and **Table C.2** in **Appendix C**).

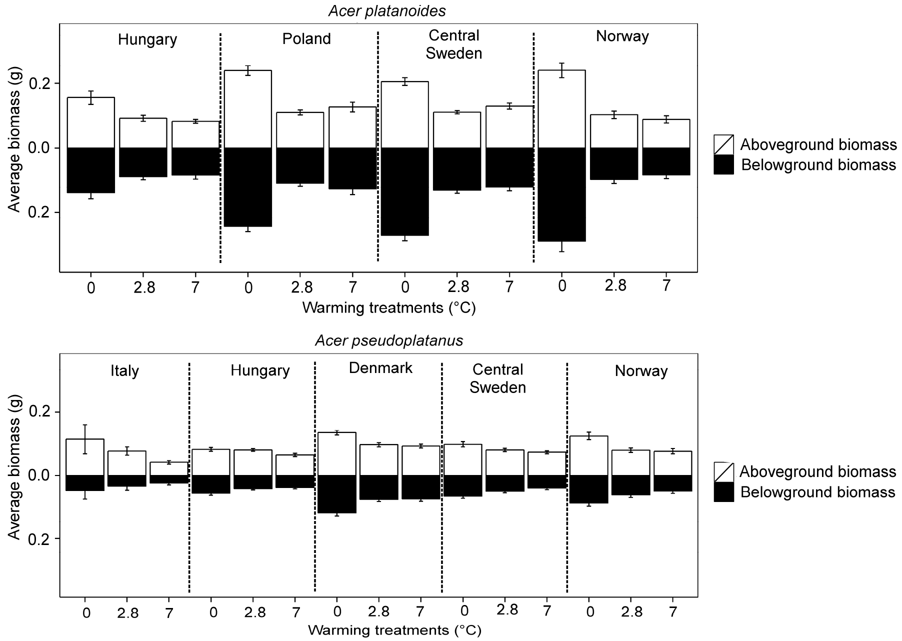


Fig. 4.4: Total biomass and biomass allocation (root vs. shoot) of *Acer platanoides* and *A. pseudoplatanus* under the different temperature treatments and in relation to the country of origin of the seeds. Error bars indicate standard errors.

Finally, the height of both species was negatively affected by warming. The seedlings of *A. platanoides* and *A. pseudoplatanus* were 18.3% and 24.2% shorter under the warmest conditions compared to the control (**Table 4.1**, **Table C.1** and **Table C.2** in **Appendix C**). Finally, seedling height appeared to be provenance-specific; seedlings grown from seeds collected in Poland (*A. platanoides)* or Central Sweden (*A. pseudoplatanus*) were tallest. Finally, for *A. platanoides* the northern and southern provenances (Hungary and Norway) showed a strong reduction of seedling height under warming (2.8°C and 7.0°C), while that was not the case for the central provenances (**Table 4.1** and **Table C1** in **Appendix C**).

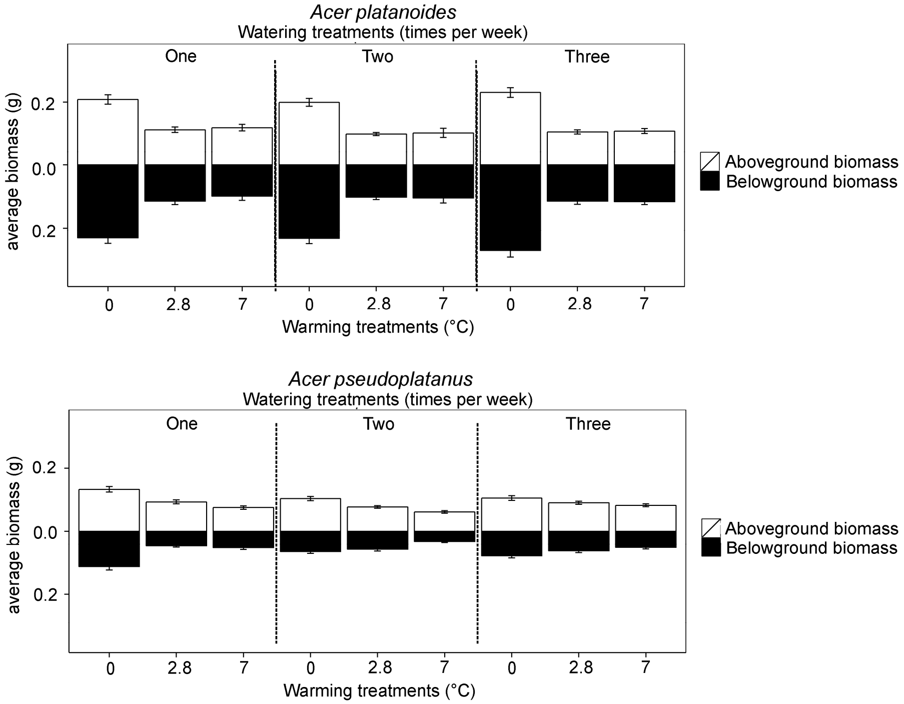


Fig. 4.5: Total biomass and biomass allocation (root and shoot) of *Acer platanoides* and *A. pseudoplatanus* as a function of the warming and precipitation frequency treatments. Error bars indicate standard errors.

Discussion

Germination, seedling survival and growth of two closely related tree species responded to warming and precipitation frequency treatments. However, we not only observed differences between species, but also among the life phases analysed. In both species, germination was strongly influenced by the precipitation frequency and the country of origin of the seeds, while warming was the main factor affecting seedling growth. We also detected interactive effects between warming, precipitation frequency and / or seed provenance, but these effects were less important than initially expected. Overall, temperature and the country of origin of the seeds appeared to be the most important factors affecting the regeneration of these species.

The frequency of precipitation affected the seed germination of both species. Indeed, the precipitation amount and soil moisture content may strongly affect germination success (Shevtsova et al. 2009; Carón et al. 2014b). Here we demonstrated the importance of the precipitation frequency on seed germination. Precipitation frequency has been shown to affect soil moisture variability (Fay and

Schultz 2009), which in turn may influence germination patterns. Our results, consistent with the observations of Fay & Schultz (2009), show that cycles of hydratation and dehydratiation (i.e lower amounts, but more frequent precipitation) tend to enhance germination. It is interesting to notice that despite that both species showed different germination percentages according to the seed provenance, contradicting our hypothesis, there was not a different response to the treatments applied according to the seed provenance. Germination generally appeared to be higher in seeds from more northerly provenances, probably due to higher northerly seed mass (Carón et al. 2014a), that might be cause by a slower seed ripening at lower temperatures, slowly seed filling process and therefore a greater total assimilation (Fenner and Thompson 2005). Warming, however, only affected germination of one of the species, *A. pseudoplatanus*, with germination decreasing at increased temperatures. This was not caused by a corresponding negative effect on dormancy break, because the stratification process was completed before the beginning of the experiment. It is likely that the experimental warming reached a level close to the threshold temperatures for the species' germination (Chmura et al. 2011).

Treatment effects on seedling survival differed more between the two species than their effects on germination, apart from the effects of warming on seed germination that were clearly species-specific. Seedling survival of both species decreased under warmer conditions. It is known that warmer conditions tend to enhance physiological processes, thereby also increasing the chances of survival (Chmura et al. 2011), but probably the warming applied was excessive for these species. Moreover, the survival of *A. pseudoplatanus* was influenced by a complex interaction of warming and precipitation frequency. Under extreme warming (+7.0 °C), more frequent precipitation enhanced survival. It is likely that extremely warm conditions intensify the evapotranspiration. Consequently, more frequent watering tends to conserve the water in the higher layers of the soil avoiding the extreme heat at the bottom of the pot caused by the direct contact with the soil heaters. This not only highlights the relevance of multiple interacting factors (Shaver et al. 2000), but also points to some of the problems (i.e. not-uniform warming of the soil column in the pot, and not uniform warming of the mats surface; warmer closer to the cables) associated with the methods utilized for studying the effects of climate change on plants. To clarify the relevance and role of both these aspects and to unravel the effect of temperature and precipitation frequency, more experiments using different methods can be performed using, for example, growth chambers (e.g. Deines *et al.* 2007; De Frenne *et al.* 2012). Moreover, *A. pseudoplatanus* also showed provenance-specific responses to increases in temperature. The survival of seedlings grown from seeds collected in Hungary and central Sweden was higher under warmer conditions, while survival of seedlings grown from seeds collected in Denmark and Norway showed an opposite response. This is likely related to the environmental conditions experienced by the mother tree during seed production (Johnsen et al. 2005a; Johnsen et al. 2005b; Carón et al. 2014a). While we lack detailed information on environmental conditions experienced by all the mother trees, previous studies suggest that the observed patterns (different survival according to the seed provenance under changing environmental conditions) are likely related to the temperature experienced during seed production (Johnsen et al. 2005a; Carón et al. 2014b; Carón et al. 2014a). Nevertheless, our results clearly point out the influence of the seed provenance on seedling responses to climate change (Atzmon et al. 2004; Thiel et al. 2014). Additionally, the possibility of the presence of local adaptations, should be analysed (Kawecki and Ebert 2004), through the use of, for instance, transplant experiments.

Regarding seedling growth, the total biomass was much more affected by warming than by the changes in precipitation frequency. The absence of effects of precipitation frequency is surprising given that previous experiments demonstrated effects of precipitation amount (e.g. Dreesen et al. 2012, Carón et al. 2014a) and of soil moisture variability (e.g. Fay *et al.* 2002, 2012) on growth. However, it is possible that the high organic matter content (20%) of the potting soil used in this experiment induced a high water retention capacity, therefore, irrespective of the treatments applied, the seedlings never experienced strong drought stress related to differences in watering frequency. Conversely, increases in temperature negatively affected seedling growth in both species, although effects were not of the same magnitude. Growth of *A. platanoides* was strongly reduced in both warming treatments (+2.8 °C and 7.0 °C), while growth of *A. pseudoplatanus* showed a more progressive reduction of biomass under warmer conditions. However, under warmer conditions the physiological processes including photosynthesis and respiration increase as long as the water content is adequate (Chmura et al. 2011). In a previous study, similar results were obtained, with both species not showing a positive growth response to warming (Carón et al. 2014b). Moreover, considering that emerging seedlings are known to be more sensitive than mature trees to drought (Dieleman et al. 2012), the effect of warming can be partially linked to drought stress due a higher evapotranspiration under warmer conditions.

Warming also reduced belowground and aboveground biomass. However, the higher effect of warming on belowground biomass resulted in a reduction of the root: shoot ratio under warmer conditions. The opposite pattern has been observed in many other plant species; under drought stress the belowground allocation is generally favoured (Weltzin et al. 2000; Dreesen et al. 2012). However, as mentioned before, it is possible that the seedlings did not experience strong drought stress driven by warming or changes in precipitation frequency changes due the high water retention capacity of the potting soil. Moreover, the relationship between temperature and precipitation frequency was especially clear in *A. pseudoplatanus* seedlings where the interaction of warming and precipitation frequency was important for the biomass growth. Conversely, it is interesting to note that *A. pseudoplatanus* did not show provenance-specific growth responses to the treatments applied. This indicates that, independent of the provenance of *A. pseudoplatanus* seeds, the seedlings are equally affected in terms of growth. However, *A. platanoides* showed provenance-specific responses in the biomass growth as observed earlier for other tree species such as *Fagus sylvatica* where the marginal provenances showed a more stable performance under drought but apparently there was a trade-off between drought tolerance and growth (Thiel et al. 2014). Despite a general decrease of seedling biomass with warming, some seedlings performed better under extreme warming than under moderate warming, probably related to the environmental conditions experienced by the mother tree. (Johnsen et al. 2005a; Johnsen et al. 2005b; Skrøppa et al. 2010). It is possible that the seedlings from seeds produced under warmer conditions perform better under warmer conditions than the seedlings produced under colder conditions.

Considering the results of this study, it is possible to suggest that both species will be similarly affected by changes in some climatic factors (warming and precipitation frequency). However, due the frequent divergent responses of seeds and seedlings along the gradient analysed, it is likely that the impact will not be homogenous across Europe.

Our results highlight that different climatic factors may have divergent effects on different phases of plant recruitment, and that effects may differ both in terms of direction and magnitude. We found strong effects of warming, while precipitation frequency appeared to be less important. The influence of the tree provenance on the response of seeds and seedlings to changes in climatic conditions was observable in many of the variables analysed. In general, the more central European provenances performed better irrespective of the fact that some seeds were collected from trees growing in natural forests or were purchased from nurseries. These results point out the relevance of performing more experiments where different provenances are tested under simulated future climatic conditions, including transplant experiments to analyse the presence of local adaptation and to deliver urgently-needed information on the development of strategies of forest adaptation in the context of climate change (Pereira et al. 2010; Bellard et al. 2012).

5. Divergent tree recruitment responses to direct abiotic and indirect biotic effects of climate change

Abstract

Changing temperature and precipitation can strongly influence plant reproduction. However, also biotic interactions might indirectly affect the reproduction and recruitment success of plants in the context of climate change. Information about the interactive effects of changes in abiotic and biotic factors is essential, but still largely lacking, to better understand the potential effects of a changing climate on plant populations. Here we analyze the regeneration from seed of *Acer platanoides* and *A. pseudoplatanus*, two currently secondary forest tree species from seven regions along a 2200 km-wide latitudinal gradient in Europe. We assessed the germination, seedling survival and growth over two years in a common garden experiment including warming, precipitation and competition treatments. *Acer platanoides* was more sensitive to changes in biotic conditions while *A. pseudoplatanus* was affected by both abiotic and biotic changes. In general, competition reduced (in *A. platanoides*) and warming enhanced (in *A. pseudoplatanus*) germination and survival, respectively. Reduced competition strongly increased the growth of *A. platanoides* seedlings. Seedling responses were independent of the conditions experienced by the mother tree during seed production and maturation. Our results indicate that, due to the negative effects of competition on the regeneration of *A. platanoides*, it is likely that under stronger competition (projected under future climatic conditions) this species will be negatively affected in terms of germination, survival and seedling biomass. Climate-change experiments including both abiotic and biotic factors constitute a

key step forward to better understand the response of tree species' regeneration to climate change.

Introduction

Climate has a strong influence on plant reproduction (Walck et al. 2011). First; the prevalent climatic conditions experienced by the mother tree may influence seed size, quality, germination and seedlings performance (De Frenne et al. 2011b; González-Rodríguez et al. 2011; Carón et al. 2014a). Second, early establishment and seedlings' growth are also highly dependent on the seed bed conditions such as temperature and precipitation (e.g. Milbau et al. 2009, Fay and Schultz 2009). Temperature affects plants biochemical and physiological processes such as photosynthesis, respiration, and transpiration (Wan et al. 2004; Chmura et al. 2011; Carón et al. 2014b), while precipitation is an important factor for the mobilization of soil nutrients and plant growth (e.g. Scharnweber et al. 2011; Dreesen et al. 2012). Additionally, interspecific and intraspecific plant-plant interactions might affect the reproduction and recruitment success of plants (Adler and HilleRisLambers 2008; George and Bazzaz 2014). Seedlings are more susceptible to the interaction with dominant plants than more established plants. Positive impacts of such interactions on the recruitment stage (i.e. germination), but negative for survival, have been identified (Callaway and Walker 1997). These impacts were often linked to abiotic factors. Positive interactions can be caused by a higher soil moisture under plant canopies, whereas negative interactions may involve competition for water in deeper soil layers (Adler and HilleRisLambers 2008).

In the context of climate change, information about the effects of temperature (Hedhly et al. 2008; De Frenne et al. 2011a) and precipitation changes (Abrams 1990; Scharnweber et al. 2011) on plant communities has rapidly built up in recent years. Unfortunately, there is less knowledge about the effects of interacting climate-change factors (but see Garten et al. 2009; Bai et al. 2010; Dreesen et al. 2012). Moreover, reliable information about the joint effect of changes in abiotic and biotic factors is essential to better understand the potential impact of a changing environment on plants populations (Adler and HilleRisLambers 2008; HilleRisLambers et al. 2013).

It is known that the effects of individual environmental factors (e.g. temperature, precipitation and soil conditions) on tree populations can differ from the effects observed when these factors are jointly manipulated. For instance, in one experiment conducted by Paradis et al. (2014) with *Betula glandulosa* seedlings, it was proven that seedlings exposed to nutrient addition had greater phosphorus concentrations in their leaves. However, when nutrient addition was combined with enhanced precipitation, phosphorus availability declined while this did not affect seedling biomass (Paradis et al. 2014). Furthermore, changes in abiotic conditions (levels of soil moisture and light) altered biotic interactions between *Quercus macrocarpa* and *Q ellipsoidalis* seedlings and the surrounding vegetation such that drought enhanced competition intensity and reduced seedling establishment success, while increased soil water content decreased competition for water with herbaceous vegetation (Davis et al. 1998).

Due to the variability of results obtained under contrasting environmental conditions, the impacts of interacting abiotic and biotic factors in the context of climate change are still difficult to predict (HilleRisLambers et al. 2013). Given the important link between the structure and composition of the understory vegetation and tree regeneration due to its influence on microhabitat conditions, competition, facilitation, etc. (George and Bazzaz 2014), it is important to also consider the indirect biotic effects of climate change on trees reproduction. Yet, a relatively small number of climate change experiments jointly manipulated abiotic and biotic factors (but see Davis et al. 1998; Paradis et al. 2014). Nevertheless, it was shown that warming enhanced survival, drought decrease germination and reduced growth with a differential allocation of resources in favour of belowground biomass (Scharnweber et al. 2011; Dreesen et al. 2012), while reduced competition generally enhanced early establishment and increased growth (Davis et al. 1998).

The impacts of different aspects of climate change on plants populations can be studied with the use of several techniques. Experimental warming techniques include the use of facilities such as infrared heaters (Dreesen et al. 2012), soil heating cables (Carón et al. 2014b) and open top chambers (OTCs) (De Frenne et al. 2011a; Klady et al. 2011). Precipitation can be manipulated using controlled watering (Fay and Schultz 2009; Carón et al. 2014b) or by installing rainout shelters (Grime 2000; Heisler-White et al. 2008). Through the use of climatic gradients, e.g. those across elevations or latitudes, the effects of several environmental characteristics such as temperature, precipitation, soil conditions, etc. (Koch et al. 1995; De Frenne et al. 2013a) on plants can be examined. However, the combination of techniques is an important step forward because that allows to illuminate different aspects of the impacts of climate change that are not easily analysed through the use of only one technique (e.g. seed production, seedling and sapling establishment and growth and mortality), especially when studying tall species with a long life span such as trees.

Here we analyze the regeneration from seed of *Acer platanoides* and *A. pseudoplatanus* from seven regions along a 2200 km-wide latitudinal gradient in Europe. The species are two currently secondary forest tree species that might gain importance in European forests due to the compositional change projected under climate change conditions (Kramer et al. 2010; Hanewinkel et al. 2013). We specifically addressed the following questions i) is there an effect of the temperature experienced by the mother tree during seed production on seed germination and seedling performance?, ii) do experimental warming, reduced precipitation and interspecific competition impact germination, seedling survival and growth?, iii) do temperature, precipitation and competition interact in affecting germination, seedling survival and growth? We hypothesized that seed germination and seedling survival will show a latitudinal pattern in relationship with the temperature experienced by the mother tree during seed production. It is expected that seedlings resulting from seeds produced under warmer conditions can better cope with warming than seedlings resulting from seeds produced under colder conditions. Additionally, we expect that competition and reduced precipitation will reduce seed germination, seedling survival and growth, while warming will produce the opposite effect.

Materials and Methods

Study region and populations

In 2011, seeds of *Acer platanoides* and *A. pseudoplatanus* were collected in seven regions located along a latitudinal gradient in Europe from Arezzo, Italy to Trondheim, Norway (**Fig. 5.1**). The seed collection included both native (located in the natural distribution range of the species) and non-native (located outside the natural distribution range of the species) populations (**Table D.1** in the **Appendix D**). In each region, one landscape window of 40 × 40 km^2 was selected. In each window, three forest patches (with similar canopy cover of ca. 85-95% and mesotrophic soil) and one seed-bearing healthy mother tree per forest patch were selected. Seeds were collected using permeable nets (Amiens, France; Brussels, Belgium; Templin, Germany and Lund, Sweden) or picked directly from the forest floor immediately after seed dispersal (Arezzo, Italy; Stockholm, Sweden and Trondheim, Norway) (**Table D.1** in the **Appendix D**).

Climatic data from weather stations close to the sampling point (< 50 km apart) available through the European Climate assessment and Dataset project (Klein Tank et al. 2002) and Centro Funzionale della Regione Toscana and Archivio CFS - Ufficio Territoriale per la Biodiversità di Pratovecchio were used to calculate the growing degree hours (GHD) experienced by the mother tree during the mean reproductive period, between flowering and seed maturation (1st of April until 30th of September 2011). The GDH were selected for the analysis because of the established relationships with plant development (e.g. Diekmann (1996) and were calculated with a base temperature of 5°C following the formulas presented in **Chapter 2**). For both species, the GDH values were subsequently standardized per location, by dividing site-specific values by the overall mean across the gradient

(Graae et al. 2009). The correlation coefficient between GDH and latitude was r = -0.75 (n = 42, p < 0.001).

Fig. 5.1: Location of the sampled *A. pseudoplatanus* and *A. platanoides* populations along the latitudinal gradient from Italy to Norway.

Experimental design

A full factorial experiment, with seeds from seven provenances along a latitudinal gradient and additional manipulation of temperature, precipitation and competition with natural understory vegetation, was installed in the experimental area of the Aelmoeseneie forest in Gontrode - Belgium (50°58'N 3°49'E and 11–21 m a.s.l.). The mean annual precipitation in the area is 726 mm, evenly distributed throughout the year, while the mean annual temperature is 9.5°C, with a mean monthly minimum and maximum temperature of 2.5°C and 16.8°C, respectively (average for the period 1981-2010) (Royal Meteorological Institute of Belgium). The experimental area is located in a mixed deciduous forest dominated by ash (*Fraxinus excelsior*) and pedunculate oak (*Quercus robur*) with sycamore maple (*A. pseudoplatanus*) in a lower proportion. The forest understory is composed mainly of *Anemone nemorosa*, co-ocurring with *Lamiastrum galeobdolon*, *Oxalis acetosella*, *Polygonatum multiflorum*, *Hedera helix* (De Frenne et al. 2010b).

To simulate warming, 20 hexagonal open-top chambers (OTCs) were randomly located in a ca. 0.5 ha zone. The OTCs are small plexiglas greenhouses with inclined walls to passively heat a small plot of vegetation (Wan et al. 2004; Dermody et al. 2007; De Frenne et al. 2010b). The OTCs were 60 cm high, covering a total surface of 1.15 m^2. Additionally, 20 hexagonal control plots (same area as the open top chambers) were randomly located within the same experimental area. During the experiment (Oct 2011 – July 2013), the mean temperature recorded in the control plots was 12.12 °C, 11.35 °C and 11.04 °C at 20 cm above the soil surface, at the soil surface below the litter layer and -5 cm below the soil surface, respectively. On the other hand, the temperature recorded inside the OTCs was 12.81 °C, 12.34 °C, and 14.31 °C at 20 cm above the soil surface, at the soil surface below the litter layer and -5 cm below the soil surface, respectively. Therefore, the heating inside the OTCs amounted to 0.69 °C in the air at 20 cm height, 0.99°C at the soil surface below the litter layer and 3.27°C at -5 cm below the soil surface, respectively (average of 2 Type T miniature thermocouples (TC Direct, Nederweert, NL).

Next, removable transparent plastic rainout shelters (0.84 m^2) were installed above half of the open top chambers and half of the control plots approximately 1.5 m above ground. The rainout shelters were removed and re-installed every two weeks to reduce the direct natural precipitation by approximately 50%. However, due to the effect of wind and other factors the effective reduction of the precipitation was, in average, of 18% (14%, 25% and 16% for the years 2011, 2012, 2013 respectively). The precipitation was measured by using bucket rain gauges with a funnel diameter of 14 cm and collected every two to three weeks depending on the period of the year. The amount of precipitation collected during the experimental period in the plots without rainout shelters was 136.17 mm, 510.92 mm and 296.39 mm (average of eight collectors), while in the plots with rainout shelters the precipitation was 117.18, 384.11 and 249.05 mm (average of eight collectors) in Oct.-Dec. 2011, Jan.-Dec. 2012 and Jan.-Jul. 2013, respectively. The amount of precipitation recorded in an open-field weather station close to the forest at *ca.* 1 km distance was 209.4, 976.5 and 452.3 mm during the same period in 2011, 2012 and 2013, respectively (i.e. *ca.* 40% reduction in the forest due to canopy interception, comparable with the results obtained by Staelens et al. (2006)).

Finally, for the competition treatments, the natural vegetation was systematically removed in spring and summer by clipping away all other plants to a height of 1 - 2 cm above the soil surface in half of the open top chambers and half of the control plots allowing a full factorial design with 5 replications of each combination of treatments (temperature*precipitation*competition).

In the centre of each experimental plot (combination of temperature, precipitation and competition treatments) 7-9 plastic rings (12 cm diameter) were placed approximately 5 centimeters in the soil leaving one centimeter above the forest floor for easy identification of the sown individuals (the variable number of rings was due to the installation of rain collectors in some of the plots to quantify the direct precipitation). In each ring, 20 seeds from each mother tree, region and species were randomly sown in Nov. 2011, making sure that only one mother tree per provenance per species was sowed in each experimental plot (**Fig. 5.2**), (i.e. 2 species × 7 provenances × 3 mother trees).

Between March and June 2012 (first growing season), the germination rate was recorded (maximum number of germinated seeds). In June 2012, the seedlings were randomly weeded and only a maximum of four seedlings per ring remained to avoid excessive competition between seedlings. In late August of the same year, the number of surviving seedlings (i.e., early survival), the number of leaves and the height reached by the seedlings were recorded. In July 2013 (the second growing season) the number of surviving seedlings (i.e., interannual survival), the number of leaves and the height of the seedlings were recorded. Next, each individual seedling was carefully collected and the belowground and aboveground plant parts were separated, dried at 60 °C during 24 hours and weighed.

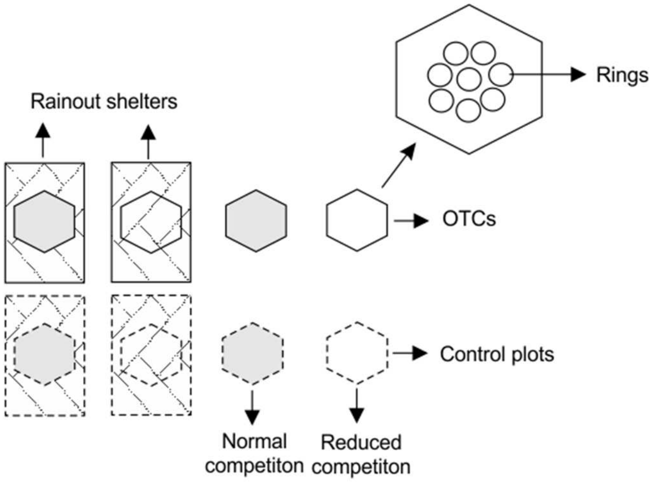


Fig. 5.2: Scheme of the experimental setup

Data analysis

For each species separately, mixed-effects models using the *glmer* and *lmer* functions, lme4 and nlme libraries in R version 3.0.0 (R Core Team 2013) were applied. These models combine fixed and random components into a single model (Zuur et al. 2009). For the binomial data (germination rate, early survival and interannual survival), count data (number of leaves at the end of the first and second growing season) and continuous data (height at the end of the first and second growing season, total biomass and root:shoot ratio) we used binomial, Poisson and Gaussian errors structures, respectively. To fulfil the requirements of normality and homoscedasticity, the aboveground, belowground and total biomass were log-transformed prior to the analyses.

The temperature (OTCs vs. control plots), precipitation (with and without rainout shelter) and competition treatments (natural competition vs. reduced natural competition) as well as the standardized growing degree hours (GDH) experienced by the mother tree between the 1st of April and 30th of September were used as fixed effects. We found no significant effects of the status of the mother tree (native vs. non-native) on the germination, survival and growth of the seedlings (except for the height of *A. platanoides* in both growing seasons; results not shown). For the model selection of each species, we followed the modelling framework recommended by Zuur et al. (2009) for nested data. We started by defining the optimal variance structure of the random component from the "beyond optimal model" containing all fixed effects and the possible two ways interaction terms. The random structure selection was based on the Akaike's Information Criterion (AIC) comparing models with different random structures, including mother tree, region and plot number; considering nested (i.e. mother tree nested in country) and not nested structures, and selecting the model with the lower AIC value. For the continuous data the resulting random structure included the mother tree nested in region and the plot number, while for the binomial and count data, only plot number was used as random-effect. Afterwards the full model was fitted (all fixed effects and possible interactions): for *A. platanoides* the full model was based on the standardized GDH, the individual factors and all the possible two way interactions between temperature, precipitation and competition treatments, while for *A. pseudoplatanus* the full model was the same as for *A. platanoides* but without the interaction between temperature and competition due a lack of recorded data under this combination of treatments. For each variable analyzed, first the full model was fitted and model simplification was achieved by dropping first the less significant interactions and then the less significant variables at each step based on likelihood ratio tests (Zuur et al. 2009).

Results

Establishment variables

The study species differentially responded to the temperature, precipitation and competition treatments. Establishment (germination, early survival and interannual survival) of *A. platanoides* was affected most by competition, while the main factor affecting the seedling establishment of *A. pseudoplatanus* was warming (**Table 5.1**). The tested interactions were not significant. The germination of *A. platanoides* was negatively affected by competition; the germination in plots where the competition with the natural vegetation was eliminated was 35% higher than in plots with natural competition, while the germination rate of *A. pseudoplatanus* was independent of all the treatments applied (**Table 5.1** and **Fig. 5.3A**).

Table 5.1: Effects of the GDH, temperature, precipitation and competition on establishment and growth variables of *A. platanoides* and *A. pseudoplatanus* over two growing seasons

	Acer platanoides		*Acer pseudoplatanus*	
Variable	Predictor	Chi square	Predictor	Chi square
Establishment variables				
Germination rate	Reduced Competition	↑3.70*		n.s.
Early survival	Reduced Competition	↑12.41***	Warming	↑3.69*
			Reduced Competition	↑7.74**
Inter annual survival	Reduced Competition	↑6.04*	Warming	↑15.51***
Growth first year (2012)				
Number of leaves		n.s.		n.s.
Height	Reduced precipitation	↑5.72*	Reduced precipitation	↑5.27*
			Reduced competition	↓10.08**
	Reduced precipitation*Reduced competition	↑5.23*	Reduced precipitation*Reduced competition	↑10.95***
			Warming*reduced competition	↓7.03**
Growth second year (2013)				
Number of leaves	GDH mother tree	↓5.03*		n.s.
Height	Reduced precipitation	↑6.79**	Reduced competition	↓6.27*
	Reduced precipitation*Reduced competition	↑4.77*	Reduced precipitation*Reduced competition	↑7.14**
Total biomass	Reduced competition	↑3.70*		n.s.
Aboveground biomass		n.s.		n.s.
Belowground biomass	Reduced competition	↑5.12*	Reduced precipitation	↑4.44*
Root: shoot ratio	Reduced precipitation	↓6.11*		n.s.
	Reduced competition	↑5.62*		

Values are X^2-values and significances from likelihood ratio tests of mixed-effect models. The direction of the effect is indicated by arrows: ↑ and ↓ correspond to an increase or decrease of the variables analyzed, respectively (ns: $P>0.05$; $*P<0.05$; $**P<0.01$; $***P<0.001$

For both species, competition reduced the survival at the end of the first growing season (**Table 5.1**). The early survival was 17% and 44% higher in plots where the vegetation was periodically eliminated in *A. platanoides* and *A. pseudoplatanus* seedlings, respectively (**Fig. 5.3B** and **Table D.2** in **Appendix D**). Additionally, the early survival of *A. pseudoplatanus* increased under warmer conditions. Under this treatment, the early survival was 27% higher than in un-warmed plots (**Fig. 5.3B**). Still in *A. pseudoplatanus*, the temperature also increased the interannual survival of seedlings, being 91% higher in warmed plots than in un-warmed plots (**Fig. 5.3C**). Conversely, the interannual survival of *A. platanoides* was 30% higher in plots without natural competition than in plots with competition (**Fig. 5.3C** and **Table D.2** in **Appendix D**).

The GDH experienced by the mother tree did not influence seed germination and seedling survival (**Table 5.1**). Additionally, the treatments applied did not unidirectionally affect germination along the latitudinal gradient (**Fig. 5.4**).

Growth during the first growing season

For both species, the number of leaves developed by the seedlings during the first growing season was independent on treatments applied and the GDH experienced by the mother tree during seed production. Conversely, the height reached by the seedlings of both species during the first growing season was positively affected by reduced precipitation: *A. plantanoides* and *A. pseudoplatanus* seedlings were 12% and 10% taller when growing under reduced precipitation than under full precipitation (**Table 5.1**, **Fig. 5.5**, **Table D.3** and **Table D.4** in **Appendix D**). Additionally, competition positively affected seedling height: *A. pseudoplatanus'* seedlings were 22% shorter under reduced competition than under full competition (**Table 5.1**, **Fig. 5.5**, and **Table D.4** in **Appendix D**). Still, in *A. pseudoplatanus,* warming intensified the effects of competition on seedling height: the seedlings growing under warmer conditions and competing with the surrounding vegetation were 21.5% larger than the seedlings growing in unheated plots competing with the surrounding vegetation (**Table 5.1** and **Table D.4** in **Appendix D**). Finally, for both species, reduced precipitation increased the positive effect of natural competition on seedling height (**Fig. 5.5**).

Growth during the second growing season

At the end of the second growing season, only the number of leaves of *A. platanoides* seedlings was negatively affected by the GDH (**Table 5.1**). The height of the seedlings of both species was still affected by some of the same factors as in the first growing season. However, in *A. pseudoplatanus* some factors that were important during the first growing season lost relevance (i.e. reduced precipitation and the interaction between competition and warming) (**Table 5.1**). During the second growing season, the positive effect of reduced precipitation in *A. platanoides* seedlings and the negative effect of reduced competition in *A. pseudoplatanus* seedlings were very similar to those of the first growing season (**Fig. 5.5**, **Table D.3** and **Table D.4** in **Appendix D**). During the second growing season, the seedlings growing under reduced precipitation and reduced competition were 13% and 4% taller than the ones growing under full precipitation and competition for *A. platanoides* and *A. pseudoplatanus* respectively (**Table 5.1** and **Fig. 5.5**). For the seedling height of *A. pseudoplatanus*, reduced precipitation counteracted the negative effects of reduced competition (**Table 5.1**).

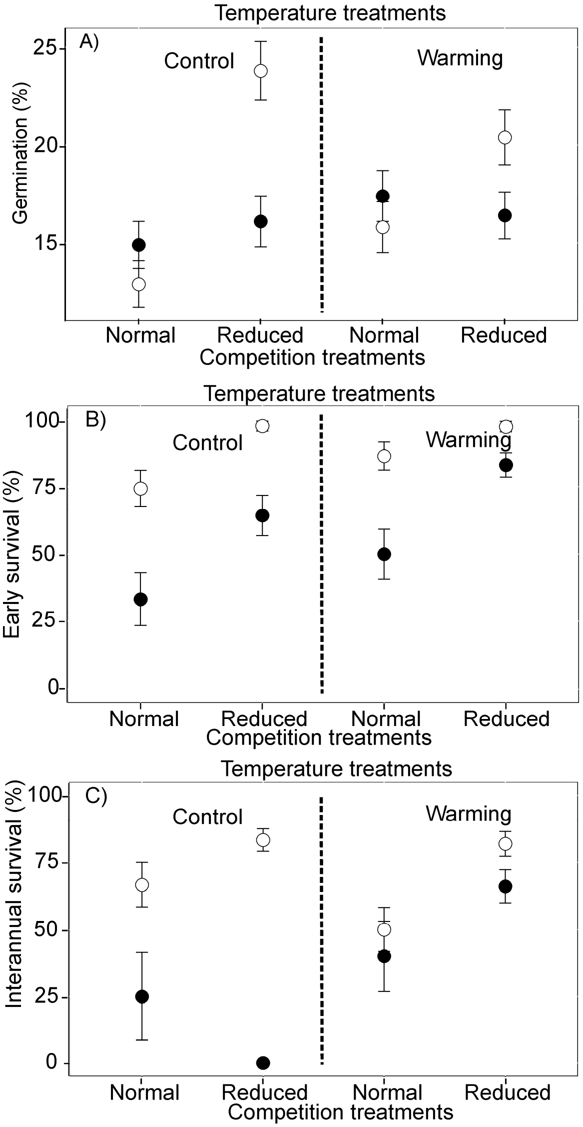


Fig. 5.3: Germination (A), early survival (B) and interannual survival (C) as a function of the joint manipulation of temperature and competition. Open circles denote *A. platanoides* and closed circles represent *A. pseudoplatanus*.

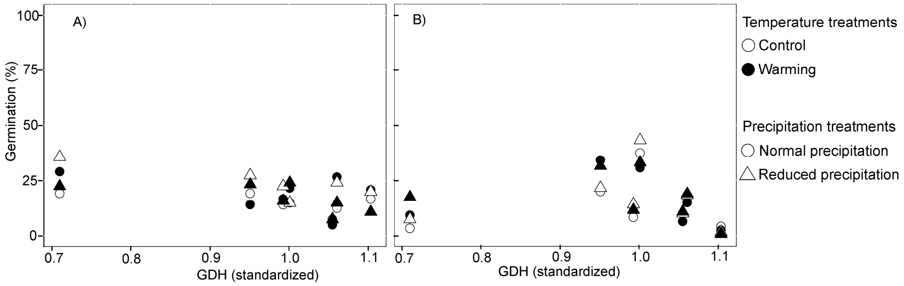


Fig. 5.4: Seed germination as a function of the standardized growing degree hours experienced by the mother tree and the experimental temperature and precipitation treatments for *A. platanoides* (A) and *A. pseudoplatanus* (B).

The biomass variables were influenced less by the experimental treatments than the height. *A. platanoides* seedlings growing without the natural vegetation competition were 31% heavier than those surrounded by natural forest understory vegetation (**Table 5.1**, **Fig. 5.6A** and **Table D.3** in **Appendix D**). Moreover, the root: shoot ratio of *A. platanoides* increased in seedlings growing surrounded by the understory vegetation. Reduced competition and precipitation decreased the root: shoot ratio with 19 % and 14%, respectively (**Table 5.1** and **Fig. 5.6B**).

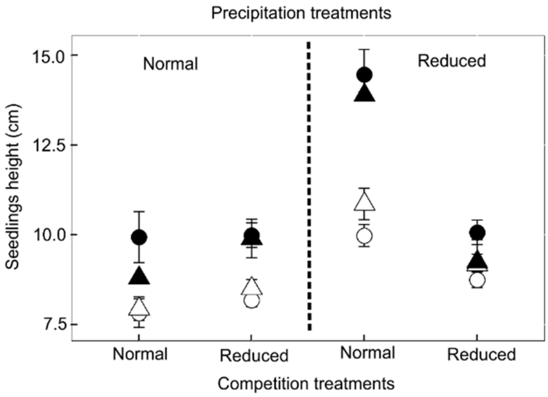


Fig. 5.5: Height reached by the seedlings of *A. platanoides* (white) and *A. pseudoplatanus* (black) and the end of the first (circles) and the second growing season (triangles) under a combination of precipitation and competition treatments.

Discussion

The experimental assessment of direct abiotic effects of climate change (i.e. temperature and precipitation changes), the influence of the temperature experienced by the mother tree along the latitudinal gradient, and the impact of two levels of competition, allowed us to assess the effects of changing environmental conditions on early tree establishment and growth. We focused on two currently secondary *Acer* tree species which are predicted to become more important in European forests with climate change (Hanewinkel et al. 2013). Species-specific responses to changes in abiotic and biotic conditions during the recruitment stage were observed (e.g. Adler and HilleRisLambers 2008; Milbau et al. 2009; Shevtosova et al. 2009). However, both species showed to be more susceptible to changes in biotic (i.e. interspecific competition) than abiotic factors (i.e. reduced precipitation and warming).

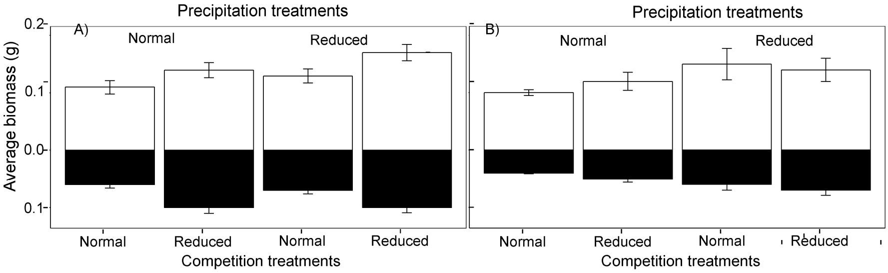


Fig. 5.6: Total, aboveground and belowground biomass of *A. platanoides* (A) and *A. pseudoplatanus* (B) under normal and reduced precipitation and competition. The white bars represent the aboveground biomass and the black bars the belowground biomass.

With regard to establishment (germination and survival), *A. platanoides* was mainly influenced by competition, while *A. pseudoplatanus* responded to changes in both biotic and abiotic conditions. The higher germination of *A. platanoides* recorded in plots where the surrounding vegetation was eliminated can be related to pulses of light (George and Bazzaz 2014). However, despite the fact that the germination of some *Acer* species (*A. pseudoplatanus* and *A. rubrum*) is light sensitive (Webb and Wareing 1972), *A. platanoides'* germination was expected to be equal under darker and lighter conditions (Baskin and Baskin 1998). The effect of the elimination of most aboveground plant-plant competition on germination can also be related to a reduction of insect predation due to decreased amount of vegetation cover (Jinks et al. 2006) and to soil desiccation due to water uptake by the surrounding vegetation. The regular weeding probably reduced the water uptake by the understory vegetation, favouring higher soil moisture content and nutrient availability, thereby providing the seeds with better conditions to germinate (Baskin and Baskin 1998) and reducing mortality risks (Jinks et al. 2006). However, the explanation of a potential positive effect of reduced competition due to lower soil desiccation is partially contradicted by the absence of reduced precipitation effects on germination. Yet, it is possible that lateral belowground flow of infiltrated water reduced the effects of the reduced precipitation treatment. On the other hand, *A. pseudoplatanus* was unresponsive in terms of germination to the treatments applied. This indicates that, in terms of germination, this species will likely be less influenced by slightly warmer conditions, reduced precipitation and changes in competition, than *A. platanoides* (see also **Chapter 3**). Moreover, the germination of both species was unresponsive to the temperature treatment, however it is important to consider that the warming simulated in this work was low compared to other studies that showed significant effects of warming on recruitment (e.g. Carón et al. 2014b). The stratification requirements of these species under slightly warmer conditions (ca. 0.99 °C increase at the soil surface below the litter layer and 3.27 °C at -5 cm below the soil surface in warmed plots) were apparently still met and germination not affected. Moreover, the germination percentages recorded were similar to those observed in seed lots exposed to controlled stratification in a tree nursery (Carón et al. 2014b).

The survival of both species during the first growing season and the seedling survival of *A. platanoides* during the second growing season were negatively affected by competition. This indicates that the periodical elimination of the surrounding vegetation provided the seedlings with pulses of resources (water, light and nutrients) (Craine 2005), facilitating growth and allowing the seedlings to better survive until the end of the first growing season and the following winter (Adler and HilleRisLambers 2008; Milbau et al. 2009). This result is especially relevant in the context of climate change because under warmer conditions some herbaceous understory plants might enhance their growth, thereby increasing competition intensity (De Frenne et al. 2011a). However in this research, no interactive effects of warming and competition were observed in relation to the establishment variables. On the other hand, *A. pseudoplatanus'* survival was influenced by both, abiotic and biotic conditions. Seedlings of *A. pseudoplatanus* were negatively influenced by competition during the first growing season, when the seedlings are still less prepared to compete for resources. However, the effects of competition decreased during the second growing season, while warming enhanced seedling survival in both growing seasons. The different responses observed during the first and the second growing season imply that interactions with the understory vegetation have stronger effects on younger seedlings than on more established plants (Adler and HilleRisLambers 2008). Moreover, the positive effects of warming might be related to lower exposure to freezing during winter when OTCs in forests are more effectively warming due to the open deciduous tree canopies (De Frenne et al. 2011a).

Contrasting with the findings on the seedling establishment, growth was only influenced by the competition and the precipitation amount. Surprisingly, warming did not affect seedling growth despite the fact that warming enhances plant biochemical and physiological processes such as photosynthesis, respiration, and transpiration (Wan et al. 2004; Chmura et al. 2011). An interesting pattern emerging was that seedling height of both species increased with reduced precipitation, probably due a reduction in light intensity under the rainout shelters. Previously, a positive effect of reduced precipitation on growth of some species has been reported (Dreesen et al. 2012). However, the positive effect of the rainout shelters was partially cancelled out due to negative effects of reduced competition on seedling height. It is possible that under normal competition, the seedlings enhanced their height growth more than when growing without competition to have more access to light. The different results observed under interacting abiotic and biotic factors demonstrates, once again, the relevance of its joint manipulation.

For *A. platanoides* seedlings, the reduced precipitation enhanced belowground biomass, reducing the root: shoot ratio. A similar pattern was observed for low intensity reduced precipitation and extreme drought in herbs (*Malva neglecta*, *Myosotis arvensis* and *Solanum nigrum*) and tree seedlings (*Acer platanoides* and *A. pseudoplatanus*) (Dreesen et al. 2012; Carón et al. 2014b). Additionally, the total biomass and the root: shoot ratio of *A. platanoides* was negatively affected by competition, probably linked to the lack of pulses of resources when the surrounding vegetation was not eliminated. The differential influence of the understory on seedling growth influenced the size structure among seedlings that might ultimately influence the outcome of future tree-tree competition.

Finally, the temperature experienced by the mother tree during seed production along the latitudinal gradient did not affect seed germination and seedling performance, except for the number of leaves recorded in the second growing season. Moreover, the native vs. non-native status of the mother trees did not influence any of the variables analyzed except for the height of *A. platanoides* in both growing seasons (results not shown). This means that, irrespective of the seed origin and the conditions in which the seeds were produced, the responses to the treatments applied are very similar.

The lack of a pattern related with the temperature experienced by the mother tree, is surprising because generally the environmental conditions experienced by the mother tree affect early establishment and growth variables, including seed mass, viability and seed germination and seedling biomass (see Verheyen et al. 2009; González-Rodríguez et al. 2011). Moreover, the influence of the temperature experienced by the mother plant is species-specific (Graae et al. 2009) and, in some species, the influence of the conditions experienced by the mother tree on seed germination and seedlings growth can change over time (Carón et al. 2014b).

These experimental results suggest that *A. platanoides* might be less influenced by changes in the climatic conditions (warming and reduced precipitation) than *A. pseudoplatanus*. However, considering the strong negative effect of competition on the regeneration of *A. platanoides*, it is likely that with potentially enhanced competition intensity in forest understories due to the positive effect of warming on the growth of some understory plants (De Frenne et al. 2011b) this species may be less successful than *A. pseudoplatanus* despite its originally higher germination, survival and biomass. Moreover, it is important to consider that other co-occurring species might show different responses to climate change and affect these species' recruitment, especially when co-occurring with seedlings of more (climate change) resistant tree provenances (Thiel et al. 2014). The relevance of the interacting factors and the influence of abiotic factors on biotic interactions (i.e. competition) is clear, especially related to the growth variables.

In sum, our results highlight the complexity of understanding and anticipating the impact of climate change on forests. Climate-change experiments inferring the effects of direct abiotic and indirect biotic drivers constitute a step forward to better understand the response of tree species' regeneration to climate change. This information is essential to better comprehend and forecast changes in the structure and composition of plant communities and, consequently, develop adaptive management plans (Adler and HilleRisLambers 2008; Chmura et al. 2011; Hanewinkel et al. 2013; George and Bazzaz 2014; Gilliam 2014). Additionally, the information presented here is relevant for forestry regarding the selection of the best adapted species and provenances in the face of climate change. Finally, these results are very relevant for species distribution modelling which is currently pointing at the importance of incorporating biotic interactions into species distribution models to improve future predictions on the impact of climate change on biodiversity (Wisz et al. 2013). The effects of temperature, precipitation and competition treatments not only depended on each individual factor but also on their interactions and were highly species-specific. Moreover, species interactions may influence population dynamics more than the direct effects of changes in climatic variables (Suttle et al. 2007). Nevertheless, further research is needed to understand the effect of changing climatic conditions as well as biotic interactions over longer temporal scales.

6. General discussion

In this thesis, we analyzed the response of two currently secondary forest tree species to warming, changes in precipitation amount and frequency, and competition. Using observational and experimental empirical approaches we were able to study an important part of the regeneration process of the two forest tree species *Acer platanoides* and *A. pseudoplatanus*.

Several authors already reviewed the impacts of climate change on seed banks, seed dormancy, germination and seedling establishment under a variety of climate change conditions (see e.g. Walck et al. 2011). However, the main focus of most of the studies so far has been on primary forest tree species such as beech, oak, pine and spruce. For instance, *Fagus sylvatica* recruitment was studied (survival and growth) under drought and biotic interactions considering different provenances (Baudis et al. 2014). These authors found that the site had a significant impact while no indication of adaptation to drought of the different provenances was reported. The leaf damage and growth of *Fagus sylvatica* seedlings was also analysed under drought, comparing the performance of central and marginal provenances (Thiel et al. 2014) and reporting provenances specific responses. In general stable performance under drought of the marginal provenances compared with the central ones were observed. Moreover, Goisser et al. (2013) studied young *Fagus* trees exposed to extreme and repeated summer drought along a natural gradient of light availability and reported progressive acclimation to water and light limitation which led to a decreased drought and shade sensitivity of diameter growth. Furthermore, for *Quercus ilex*, Pérez-Ramos et al. (2013) reported that different stages of plant reproduction (i.e. seed maturation, seed survival, seedling emergence and seedling survival) will likely be differently by drought. The most sensitive aspects of this species reproduction under decreased rainfall were the seed maturation, seedling emergence and survival and, to a lesser extent, post-dispersal seed survival. But also the interaction between drought and light was relevant.

To the best of our knowledge, this is the first time that a deep and complete analysis of the regeneration process, from seed production to interannual survival, of two congeneric secondary forest tree species is studied under a wide range of climatic conditions.

The seed collection along the latitudinal gradient, through the use of a space-for-time substitution procedure (Fukami and Wardle 2005), allowed us to analyze the effects of varying temperatures, precipitation and soil characteristics on seed production (**Chapter 2**). Moreover, with a combination of techniques, i.e. seed collection along the latitudinal gradient and different experimental set ups including soil heating mats and watering treatments, we analyzed the impacts of potential warming and precipitation changes on seed germination, seedlings survival and growth. The effects of the temperature experienced by the mother tree during seed production on the response of seeds and seedlings to the experimental manipulation of climatic factors was incorporated as well (**Chapters 3** and **Chapter 4**). Finally, a longer term experiment performed under forest conditions enabled us to analyse the impacts of warming, reduced precipitation and competition on the regeneration from seeds collected along a latitudinal gradient over two growing seasons (**Chapter 5**). In this final chapter, we summarize the main findings of the possible future impacts of climate change on the successive phases of *Acer* spp. recruitment. We present the possible implications and relevance of these findings, and discuss the limitations of this study thereby suggesting options for further research.

Impacts of climate change on seed production

All the phases of seed production from gamete development, the progamic phase from pollination to fertilization and the postzygotic early embryo development as well as subsequent stages such as seed and fruit maturation, dormancy break and germination rates are all affected by temperature (Hedhly et al. 2008; Walck et al. 2011). Moreover, information about the impacts of climate change on regeneration from seeds is essential when considering that this is the most common natural way through which trees regenerate. Additionally, most of the tree species used for afforestation or reforestation in Europe are grown from seeds (den Ouden et al. 2010) from a relatively small pool of provenances (cf. the EU Directive 1999/105/EC on the marketing of forest reproductive material) but limited information is available about how these provenances will perform under climate change. Moreover, global change could also alter the relative fitness of present genotypes leading to evolutionary changes in natural plant populations (Wang et al. 1994).

We showed that the seeds of *A. platanoides* and *A. pseudoplatanus* exhibited an important variation in chemical, morphological and physiological traits due to the environmental conditions experienced by the mother tree. However, as expected, these effects were variable among species: seed characteristics of *A. platanoides* were clearly more influenced by environmental variation experienced by the mother tree than those of *A. pseudoplatanus*. Therefore, seed characteristics of the first species will probably be more influenced by the environmental changes projected in the context of climate change. However, the mother tree genotype and its response to environmental changes can be also a key factor for the response of seeds and seedlings to climate change.

The mass of a seed is considered a key aspect of plant regeneration because this trait reflects the amount of resources available for the first stages of seedling establishment (Leishman and Westoby 1994; Fenner and Thompson 2005; Osunkoya et al. 2014). For *A. platanoides,* the seed mass was negatively influenced by GDH. Seeds of *A. platanoides* produced under colder conditions were heavier than the seeds of the same species produced in warmer environments. Therefore, global warming will likely induce the production of smaller seeds with less resources available for early establishment. Conversely, we did not detect a relationship between the seed mass and the temperature experienced by the mother tree in *A. pseudoplatanus*. However, *A. pseudoplatanus* showed an increase of the wing mass with seed mass. This positive relationship may compensate, to some extent, the negative effect of seed mass on wind dispersion and potential species migration distances. Moreover, seeds of *A. pseudoplatanus* sampled in non-native mother trees had heavier wings possibly indicating that seeds produced in relocated populations might have a larger dispersal capacity, which is considered a key strategy to cope with climate change (Bellard et al. 2012).

In both *Acer* species, the seed nutrient concentration varied mainly with the differences in precipitation and soil characteristics, but the temperature also played a relative important role (see De Frenne et al. 2011a, Sun et al. 2012). In general, the seed nutrient concentration in *A. platanoides* was much more influenced by the environmental conditions experienced by the mother tree than *A. pseudoplatanus*. For instance, we inferred that, under warmer conditions, the N concentration of *A. platanoides* seeds might decrease in the future. Our findings for *A. platanoides* are supported by similar observations by Sun et al. (2012) in *Quercus variabilis*. This observed relationship can be caused by a possible stimulation of the accumulation of storage protein at northern latitudes (Piper and Boote 1999) and can have important implications for seedling survival as seedlings resulting from seeds with higher N concentration show generally higher survival and can perform better under more stressful environmental conditions (Parrish and Bazzaz 1985; Stock et al. 1990). Conversely, other important nutrients such as P, Ca, K, Mg were more affected by changes in soil nutrient concentration and precipitation.

As was observed for the other seed traits, the physiological aspects of *A. platanoides* seeds were influenced by the environmental conditions experienced by the mother tree during seed production, while this was not the case for *A. pseudoplatanus* seeds. However, for both species, the viability was positively related to the seed mass. Considering that the seeds mass of *A. platanoides* showed the same trend as the viability (i.e. seed mass and viability decrease with increasing GDH), global warming might induce the production of smaller, less viable seeds in *A. platanoides*. Conversely, the two species did not show any relationship between seed mass, germination and seedling biomass. The lack of a relationship between seed mass and seedling biomass in these species is surprising. According to many studies, larger seeds tend to produce bigger seedlings with greater probability of successful establishment, access to a larger volume of soil and higher resistance to environmental hazards than smaller seedlings resulting from small seeds (Westoby et al. 1996; Dalling and Hubbell 2002; Moles and Westoby 2006). However, it is important to consider that genetic differences between mother trees also influence seed size and the relative role of environmental and genetic factors are not completely clear (Wang et al. 1994). This was, however, beyond the scope of this work. Finally, considering that the environmental conditions experienced by the mother tree can affect the seed nutrient concentration (e.g. Drenovsky & Richards 2005; De Frenne *et al.* 2011) and because of the clear relationship between seed nutrient concentration and germination and seedling biomass, this work points out that seed nutrient concentration might be a more important factor than the seed mass *per se.* However, the influence of climate and climate change on seed nutrient concentration is one of the less studied aspects of the impacts of climate change on plants' regeneration (but see De Frenne et al. 2011a, Soriano et al. 2011, Sun et al. 2012).

We found strong impacts of the environmental conditions experienced by the mother tree on the morphological, chemical and physiological aspects of the seeds of both species but these impacts were clearly stronger in *A. platanoides* seeds (**Chapter 2**). To the best of our knowledge, this is the first time that a wide variation in several environmental characteristics, with special focus on climatic conditions, were related to a wide range of tree seed quality traits (that is, morphological, chemical and physiological characteristics). The results obtained in this work show that the seed quality varies according to the environmental conditions, and therefore, subsequent stages such as germination, survival and growth are also be influenced by these factors (**Chapter 3, Chapter 4** and **Chapter 5**). The results of this work support the earlier findings of Johnsen et al. (2005a&b) about the influence of the conditions experienced by the mother tree on the performance of the offspring of *Picea abies*. However, it is interesting to notice that both species showed clear differences. *A. platanoides* will probably be more negatively affected by climate change in terms of seed quality than *A. pseudoplatanus*.

Impacts of climate change on seed dormancy break, seed germination and seedling survival

The conditions experienced by the mother tree during seed production are important factors affecting seed production and seedling performance (Graae et al. 2009; De Frenne et al. 2011b). However, also the local conditions (i.e., seed bed conditions) strongly affect seed dormancy break, seed germination and seedling performance (Fenner 1992; Baskin and Baskin 1998).

Using two different approaches, we compared the germination of the seeds after controlled stratification and field conditions (**Chapter 2** and **Chapter 5**). Using the germination as a proxy of dormancy break, it is possible to indicate that, apparently, under forest conditions, even under slightly warmer conditions in the OTCs (*ca.* 1 °C) (**Chapter 5**), the stratification was probably equally achieved, due the similarities between germination percentages. However, this results should be cautiously used because it is known that seeds can break dormancy but not germinate if the environmental conditions are not appropriate (Thompson & Ooi 2010). These results suggests that certain global warming conditions might not affect the dormancy break of these two *Acer* species. However, more research is needed to unravel the level of warming that will start affecting the stratification process of these species by reducing the number of cold days, which probably, as observed by other physiological traits (i.e. viability) will be highly variable between the species. This expected species-specific response is especially clear when considering that the required cold days for dormancy break are significantly different for both species (i.e. *A. platanoides* requires 105 days of cold stratification while *A. pseudoplatanus* only 49 to 63 days; Baskin & Baskin 1998). However, it is important to indicate that the recorded stratification time during this research was significantly different to the time reported by Baskin & Baskin (1998); in our case an average of 63 ± 18.7 and 87 ± 13.6 days were needed for *A. platanoides* and *A. pseudoplatanus*, respectively. The differences between the stratification time required for dormancy break recorded in this study and reported by Baskin and Baskin (1998) can be linked to the specific conditions under which the dormancy break was performed and to the origin of the seeds. Moreover, an aspect not studied in this research is the way in which the maternal genotypes control dormancy and link this to the germination timing. The seed coat, a maternal tissue, imposes mechanical constraints on germination and acts, in many cases, as an environmental filter (Donohue 2009). Therefore, this might be a relevant aspect of sexual regeneration that should be studied in the context of climate change in future experiments.

We found that life-stage transitions that are important for plant population dynamics such as germination and survival were the result of a complex combination of temperature, competition, soil moisture content/precipitation frequency but were also influenced by the temperature experienced by the mother tree. Moreover, the interaction between factors showed to be important in some cases, but not as frequently as originally expected. For example, partial compensation was observed when warming is combined with soil moisture reduction. Reduced soil moisture negatively affected the germination of *A. platanoides*. Yet, when soil moisture reduction interacted with warming the negative effect was partially compensated. This pattern might be linked with the fact that at high temperatures and drier conditions the seeds are less exposed to fungi and pathogens than under warm and wet conditions (Blaney and Kotanen 2001) (**Chapter 3**).

The germination of both species was not equally influenced by the temperature experienced by the mother tree and by the changes in the seed bed conditions (**Chapter 3, Chapter 4 and Chapter 5**). In general, as predicted, *A. platanoides* was more affected than *A. pseudoplatanus* to the changes in the local conditions (temperature, precipitation and competition). For example, the reduction of the soil moisture content negatively affected the germination in *A. platanoides* (**Chapter 3**). Increasing the precipitation frequency pointed out that cycles of hydration and dehydration (i.e. less abundant but more frequent precipitation) tend to enhance the germination of both species (**Chapter 4**) as indicated for other plants species of grass and forbs (Fay and Schultz 2009). Thus, diverse aspects of precipitation that are predicted to change in the future (i.e. precipitation amount and frequency; IPCC 2013) might contrastingly affect seed germination. Moreover, the lower germination of *A. platanoides* recorded when competing with the surrounding vegetation can be explained by multiple reasons. It is possible that pulses of light, caused by the systematic elimination of the vegetation (George and Bazzaz 2014) in the competition-free treatment stimulated germination. However, *A. platanoides*' germination was expected to be equal under dark vs. light conditions (Baskin and Baskin 1998). Therefore, an alternative hypothesis is that the effect of the elimination of most aboveground plant-plant competition on germination is related to a reduction of insect and slug predation due to decreased amount of vegetation cover (Jinks et al. 2006) and to a reduction of the soil desiccation related to water uptake by the surrounding vegetation. The regular weeding probably favoured a higher soil moisture content and nutrient availability, thereby providing the seeds with better conditions to germinate (Baskin and Baskin 1998). However, less vegetation can also dry out soils, but this depends on many factors such as soil texture, colour, water retention capacity but also variables related with the vegetation (e.g. water requirements). These results stress the influence of abiotic factors on biotic interactions, and vice versa. On the other hand, the warming of the seed bed did not have a unidirectional impact on seed germination and did not equally affect both species. Slightly warmer conditions (+1.5 °C) induced a positive effect on germination, while significantly warmer conditions (+2.7 °C and 7.2 °C in **Chapter 3** and 2.8 °C and 7.0 °C in **Chapter 4**) resulted in decreased germination in *A. pseudoplatanus* but not in *A. platanoides* (**Chapter 3 and Chapter 4**). Additionally, in some occasions the response of the species to the seed bed conditions depended on the temperature experienced by the mother tree (**Chapter 3**). Apparently, this is an important factor that can affect germination and seedling performance under climate change conditions (Johnsen et al. 2005a; Skrøppa et al. 2010; De Frenne et al. 2012). But also the mother tree genotype might play a relevant role on seed germination and seedlings performance that deserve attention.

Finally, the germination time differed among the provenances. For both species, warming, soil moisture reduction and the GDH experienced by the mother tree affected the germination time, an important factor in the potential regeneration of plants (Chmura et al. 2011). Under warm and wet conditions the germination was advanced, extending the growing season length and potentially improving the chances of seedlings to survive the following winter (Milbau et al. 2009; De Frenne et al. 2012).

Slightly warmer conditions (**Chapter 5**) had a positive effect on seedlings survival, probably because enhanced physiological processes and growth, therefore, increasing the chances of survival (Chmura et al. 2011), while intense warming (**Chapter 3 and Chapter 4**) had a negative effect possibly caused by exceeding the physiological temperature threshold for these species. Moreover, the seedling survival of *A. platanoides* constantly showed to be more susceptible than the survival of *A. pseudoplatanus*, indicating that if the level of warming reaches the threshold of this species (probably ≥2.8 °C, but still more research in need it) it will possibly be more affected than *A. pseudoplatanus*.

Moreover, changes in soil moisture content or precipitation frequency had a very small role in these species' seedling survival. Only when interacting with warming this was relevant for seedling survival. For example, the negative effect of temperature on the survival of *A. platanoides* increased when the temperature and soil moisture content were jointly manipulated, probably inducing stronger drought effects. The high sensitivity of emerging seedlings to drought is well known. For this reason, it is likely that drought-related regeneration failures will increase under future climatic regimes, especially when low precipitation co-occurs with warming (Allen et al. 2010). However, different drought sensitivities were observed. Again, *A. platanoides* was, in general, more negatively affected than *A. pseudoplatanus*. However, it is known that the effect of drought on survival depends on several factors including drought hardiness, evolutionary adaptation and drought acclimation (Abrams 1990; Abrams 1994; Martinez-Vilalta et al. 2004). Therefore, a deeper study of the drought sensitivity is needed. Provenance specific responses to changes in soil moisture content were not observed for these species (but see Martinez-Vilalta *et al.* 2004). Conversely, *A. pseudoplatanus* exhibit provenance specific responses to warming (**Chapter 4**). Survival of *A. pseudoplatanus* seedlings was enhanced with warming when the seeds were collected at southern provenances, while the northern provenances showed the opposite behaviour (**Chapter 4**). This pattern might be related to the environmental conditions experienced by the mother tree during seed production (Johnsen et al. 2005a; Johnsen et al. 2005b). However, the limited information about the environmental conditions experienced by the mother tree available for this study (**Chapter 4**) do not allow us to indicate which factor triggers this provenance-specific behaviour. Yet, based on previous results (**Chapter 2**) the temperature experienced by the mother tree during seed production is probably one of the key factors.

Finally, competition also played an important role on seedling survival (**Chapter 5**). The survival of both species was negatively affected by competition. The periodical elimination of the surrounding vegetation eliminate the direct aboveground and belowground competition. Moreover, this response suggest that the elimination of vegetation provided the seedlings with pulses of resources (water, light and nutrients) (Craine 2005), facilitating growth and allowing the seedlings to better survive until the end of the first growing season and the following winter (Adler and HilleRisLambers 2008; Milbau et al. 2009). This is a very relevant result because it is predicted that under warmer conditions some herbaceous understory plants might enhance growth thereby increasing the competition intensity (De Frenne et al. 2011a).

Impacts of climate change on seedlings' growth

This work showed that all the variables that affected seedling establishment will likely affect seedling growth as well. However, the influence of the environmental conditions experienced by the mother tree during seed production was not as relevant for growth as for the establishment variables (i.e. germination and survival). The seedling biomass, evaluated through the use of different techniques, showed to be mainly influenced by warming, while precipitation and the provenances or the GDH experienced by the mother tree during seed production were less important (**Chapter 3, Chapter 4 and Chapter 5**).

Warming and reduced soil moisture content negatively influenced seedling growth (total, aboveground and belowground biomass and height). Warming negatively affected seedling growth for both species. However, this trend was only observable with moderate (2.7 °C, **Chapter 3** and 2.8 °C, **Chapter 4**) and strong warming (7.0 °C, **Chapter 4** and 7.2°C, **Chapter 3**) and not under slightly warmer conditions. The reduced biomass, even under moderate warming, was surprising because warmer conditions enhance physiological processes including photosynthesis, respiration and transpiration (Wan et al. 2004; Chmura et al. 2011). Moreover, the precipitation amount showed to be a more important factor for the growth of these species than the precipitation frequency, which showed a limited influence on growth (**Chapter 4**). This prevailing role of precipitation amount over frequency, contradicts previous studies where significant effects of soil moisture variability were observed on the growth of grasses and forbs species (e.g. Fay et al. 2002, 2012). Additionally, both factors, precipitation amount and warming can, under certain conditions, lead to drought which it is known as a key factor for emerging seedlings (Dieleman et al. 2012).

The aboveground biomass decreased with warming and reduced precipitation. However, the belowground biomass was more sensitive than the aboveground biomass to the treatments applied. Reduced precipitation reduced or enhanced the belowground biomass, depending on the level or manipulation of this factor (**Chapter 3 and Chapter 5**). Additionally, warming reduced the belowground allocation, especially extreme warming (7.0 °C and 7.2 °C). Moreover, the biomass growth not only depended on abiotic factors: the reduced competition also enhanced belowground ground allocation probably due to the pulses of resources (Craine 2005), indicating that biotic interactions are also an important factor for seedling growth (**Chapter 5**).

The root: shoot ratio increased as drought intensified, as reported before for low-severity drought conditions (Kramer et al. 2010) and extreme drought (Dreesen et al. 2012). Our results thus equally confirm that under water stress there is a differential allocation of resources in favour of belowground biomass. Moreover, warming reduced the root: shoot ratio (**Chapter 3 and Chapter 4**). Moreover, these results show that under stress the response of the seedlings varied and the allocation of resources is different depending on the stressor.

A consistent pattern observed in this research is the reduction of the seedlings' height growth due to warming. As mentioned before, this reduction of seedling growth (i.e. height) is possibly linked to a negative effect of warming due to the exceedance of the threshold temperature of the species for optimal growth, for instance triggering an excessive evapotranspiration which might be detrimental for growth. Moreover, the competition with surrounding vegetation enhanced seedling growth in height (**Chapter 5**). It is possible that under normal competition, the seedlings enhanced their height growth more than when growing without competition to have more access to light, due to the unidirectional nature of this factor. The different results observed under interacting abiotic and biotic factors demonstrate, once again, the relevance of its joint manipulation (Adler and HilleRisLambers 2008).

General conclusion, recommendations for further research and forest management

The two closely related secondary forest tree species studied here, *Acer platanoides* and *A. pseudoplatanus,* play an important role in terms of ecological processes and environmental services (Krabel and Wolf 2013). Despite the currently limited relevance of *Acer platanoides* and *A. pseudoplatanus* in terms of the percentage of forest cover (< 3% in pure stands; Spiecker *et al.* 2009), these species are still ecologically relevant and relatively abundant in mixed forest where, *A. pseudoplatanus* for example has a frequency of 24 % (De Frenne et al. 2013b). Moreover, considering that non-analog novel communities (compositionally different from the ones found today) occurred in the past (110, 000 years ago during the late-glacial period) and will likely develop in the future due to climate change (Williams and Jackson 2007) in relation with changes in fitness and abundance of primary and secondary forest tree species (Hanewinkel et al. 2013). Increasing our knowledge on these species regeneration in the face of climate change is thus important and this research assists in filling this knowledge gap.

The differential influence of climatic factors on seed dormancy break, seed germination and seedling establishment and growth showed the complexity of understanding and predicting the effects of climate change on forest tree species regeneration. This research clearly showed that two closely related tree species can show strongly divergent responses to climate change (Abrams 1990; Milbau et al. 2009; Fay and Schultz 2009). In general, *A. platanoides* showed to be more susceptible than *A. pseudoplatanus* to the projected environmental changes (i.e. temperature, precipitation and competition) (IPCC 2013a). The temperature experienced by the mother tree during seed production affected seed mass and viability but with species-specific responses. After that, seed germination was unresponsive to slightly warmer conditions (**Chapter 5**). However, the seedling survival of *A. pseudoplatanus* will probably be positively affected by slightly warmer conditions. Yet, more intense warming, as simulated in **Chapter 3** and **Chapter 4**, tend to reduce seedling survival of both species, especially when combined with drought. Moreover, intense warming and drought will negatively affect seedling survival and growth. Finally, it is important to consider the combined effects of changes in abiotic and biotic conditions (**Chapter 5**). Slightly warmer conditions, reduced precipitation and changed interactions among species (i.e. competition), become key factors.

Recommendations for further research

Based on our results several recommendations can be done for future research. We showed that warmer conditions will affect these species' reproduction in terms of seed mass, viability (**Chapter 2**) and germination (**Chapter 3 and Chapter 4**).

However, these trend can change over time (probably be intensified) when considering the cumulative effect of a minor increase in temperature on adult trees. Therefore, considering the influence of the temperature experienced by the mother tree during seed production on regeneration, we suggest, for studying the recruitment stage, take advantage of provenance-specific trails. These trials have been established since the beginning of the 20th century for a variety of tree species throughout Europe for silvicultural purposes including, occasionally, pan-continental seed collections (Reich and Oleksyn 2008) and can help to understand the performance of offsprings and adult trees from different provenances in the face of climate change. Additionally, genotypic variation is present among naturally occurring populations from widely distant locations and between nearby populations from contrasting sites. Information about the genotypes might be a key aspect to help to unravel the relative role of environmental, genetic and environment*genotype interactions in terms of seed production, seed dormancy beak, germination, survival and subsequent stages. We also strongly recommend to include in future researches more co-occurring species and biotic interactions in climate change research. Ideally, the species selection should include primary and secondary co-occurring forest tree species, which can set the base for empirical assessments of the predicted compositional changes for European forests in the context of climate change (Hanewinkel et al. 2013). Moreover, longer experiments are needed to be able to complement the information presented in this thesis, because it is possible that susceptibility to environmental conditions can change over time. This information will be especially important for the development of more accurate models that can predict the compositional and/or distributional changes of European temperate trees (Wisz et al. 2013) and to develop adaptive management plans and policy decisions in the context of climate change.

Of course, several questions remain after having performed these experiments. For example, which level of warming will start affecting the dormancy break of both species? Which is the threshold temperature that change the direction of the effect of warming on seedling survival from positive to negative? How will seedlings respond to increasing competition with the understory vegetation? Will the observed species-specific responses change the composition at the community level? What is the role of the mother tree genotype on the response to environmental changes including climate change? To answer some of these questions a field experiment can be proposed with the use of infrared heaters or soil heating cables over a long time period. A complete range of temperatures should be simulated from moderate (~2 °C) to extreme warming (~7 °C), including heat waves, changes in precipitation amount and frequency, and simulated competition with the main forest understory species and other co-occurring forest tree species. The germination, survival and growth should be analysed over several years in response to a wide spectrum of warming, precipitation and competition treatments and related with the temperature experienced by the mother tree during seed production. Moreover, including different provenances of the same species in such experiment and with complementary studies such as deep genetic analysis will be possible to unravel the genetic signature of adaptation to the projected environmental changes and to better recommend more adapted provenances in the context of climate change.

Implications for forest management

As mentioned, the species can exhibit different strategies to cope with climate change (i.e. time, space and self; **Chapter 1**) (Bellard et al. 2012). However it is possible that some species will be able to respond *in situ* by changing their physiology or phenology, but other species will have to move in space to be able to cope with climate change (Bellard et al. 2012).

The projected shift in range of these two *Acer* species, as many others, is the result of the colonization of new areas in the northern and colder locations and extinction at southern warmer locations (Hemery et al. 2009). However, future species' distribution are frequently projected based on the relation between the past or current geographical distribution of a species and some climatic variables. It is often assumed that the habitat is homogeneous, and the geographical response of the species are only limited by the shift of temperature, and, for instance, not influenced by the landscape configuration (Opdam and Wascher 2004). However, in reality, natural or semi-natural ecosystems are embedded in a matrix of unsuitable habitats, and therefore, populations are spatially fragmented, especially in the most densely populated parts of the world (i.e. Europe and North America) (Opdam and Wascher 2004). Moreover, climate change can reduce habitat suitability and fragmentation can prevent individuals from reaching alternative suitable habitats (Opdam and Wascher 2004; Hoegh-Guldberg et al. 2008; Gregory et al. 2014). Therefore, probably the expansion of species ranges will be fastest in regions with high connectivity and will lag behind in regions where the landscape is fragmented. In the attempt to mitigate the impacts of climate change on plant populations one of the possible alternatives is to create new corridors reconnecting isolated populations to facilitate species migration to suitable areas (Tewksbury et al. 2002; Gregory et al. 2014). The reconnection of isolated fragments is expected to reduce the risk of population extinction by increasing dispersal, facilitating genetic admixing and species access to suitable habitat in the context of climate change. Moreover, the connection between fragments will also facilitate some interactions between plants and animals such as pollination and seed dispersal what will have a positive impact on plant populations and communities (Tewksbury et al. 2002; Damschen et al. 2014; Gregory et al. 2014). The influence of corridors on wind dispersed species such as *Acer platanoides* and *A. pseudoplatanus*, is not yet well studied. However, Damschen *et al.* (2014) showed that the increasing connectivity has a positive impact on species richness of wind-dispersed plant species and showed that the wind-driven dispersal depends on how wind is oriented relative to landscape features and the corridors. The most effective corridors for promoting long distance dispersal of seeds should be aligned with the prevalent winds (Damschen et al. 2014). However, in the design of corridors it is important to consider that other factors, such as topography, the heterogeneity of canopy height, the dimensions of corridor, and species-specific seed traits (e.g., size, shape). Therefore, and based on the reported negative effect of warming and drought on these species' regeneration, it is possible that the development of new south-north corridors connecting forest patches along Europe might facilitate species migration to the northern and colder regions as is projected to happen in response to climate change (Hemery et al. 2009). Considering that the two *Acer* species studied here are very often planted, improving the connectivity between forest patches to facilitate species migration might, however, be less relevant than for other species such as typically slow colonizing herbaceous understory species that will need to independently migrate to new suitable areas.

In addition, it is important to consider that some species, especially some animals, will be able to move fast enough to track climate change (Warren et al. 2001; Menéndez et al. 2006). In these cases it may be sufficient to improve the connectivity between landscapes patches. However, in other cases more extreme action, such as assisted colonization might be needed, but a thorough evaluation is necessary to decide which practice is the best in each case (Hoegh-Guldberg et al. 2008). If the rate of migration of the species is too low to track climate change, resource managers and policymakers must consider moving species to the north, possibly even to sites where they do not currently occur or have not been known to occur in recent history (Hoegh-Guldberg et al. 2008). However, due to the possible negative impacts of such actions (transmission of diseases, invasion risk, etc.), it is advised to assess habitat requirements and distributions of some species to identify low-risk situations where the assisted colonization can be done (Hoegh-Guldberg et al. 2008). Moving individuals from warmer locations to historically colder locations may increase the probability of subsequent adaptation as the climate changes (Hoegh-Guldberg et al. 2008). This might be an option for these two *Acer* species, if the rate of "natural" migration is proven to not be fast enough to track climate change. Considering that we show that seedlings from seeds produced under relative warmer and drier conditions (in general southern or central provenances) performed relatively better under the simulated climate change conditions. These provenances should be further analysed to prove if they are, in fact, the best option for new afforestation projects. Therefore, forest managers looking for more climate-change adapted trees could select such provenances (e.g. the ones from Poland and Hungary). New afforestation in northern locations using seeds produced in southern or central regions can also be considered as a form of assisted colonization, even though this term has been frequently used to refer to the establishment of new populations in locations where they did not occur and for species that are not as intensively managed as forest tree species.

Moreover, the information provided in this thesis can be complemented with other sources of information to better select the provenances to be recommended to use under climate change conditions. The delineation of seed zones or provenances within which plant species can be transferred has to be very carefully performed to ensure good adaptation to their new location. Moreover, a wrong seed transfer guideline can negatively impact restoration success. Therefore, to better assess the provenances to be used for afforestation or reforestation purposes, the information presented here can be combined with other practices such as the proposed time-effective population genomics approach (De Kort et al. 2014), to better assist foresters and policy makers in the elaboration of guidelines of seed sourcing and provenances.

A correct seeds provenance selection can be complemented with some general recommendations for the management of natural regeneration of these species in the face of climate change. Taking into account that both species' germination, survival and growth were, in general, negatively affected by warming and drought, the regeneration practices could be located in forest areas with a moderate to high soil moisture content and under a dense forest canopy. A dense forest canopy can maintain the forest soil at lower temperatures and with higher moisture content compared than in forest gaps by avoiding intense solar radiation and high evaporation. Moreover, due to the negative effects of competition on both species recruitment success, but especially on *A. platanoides'* regeneration, this species could preferentially be regenerated in forest areas with low density of understory plant species. However, because in this research the canopy cover, competition with the shrub layer, and herbivory were not evaluated, further research is needed to complement these findings and to better recommend specific management actions.

Appendix A

Table A1: Mean and standard deviation of soil nutrient concentration of the surrounding area of the sampled *A. platanoides* and *A. pseudoplatanus* trees along the latitudinal-climatic gradient

Acer platanoides

Country	**Temperature (April-September 2011) (°C)**	**GDH***	**GDH (April-September 2011)**	**Forest status**	**Precip# (mm)**		**pH ($CaCl_2$)**	**C (%)**	**N (%)**	**S (%)**	**P (mg/kg)**	**Mg (mg/kg)**	**K (mg/kg)**	**Ca (mg/kg)**	**Al (mg/kg)**
Tuscany, taly	15.9	1.06	47952.29	Native	1027	Mean	6.97	5.93	0.46	0.04	8.53	529.00	209.00	26165.33	100.67
						SD	0.58	0.67	0.03	0.01	1.39	184.52	35.37	22099.83	22.03
Amiens, France	15.8	1.05	47700.01	Not Native	565	Mean	7.43	6.63	0.51	0.04	22.02	398.33	181.33	75714.00	57.00
						SD	0.04	2.61	0.13	0.00	5.60	115.11	41.65	40032.18	29.70
Brussels, Belgium	15.6	1.10	49887.05	Not Native	753.1	Mean	4.54	2.97	0.26	0.04	71.61	143.67	166.33	1457.00	250.67
						SD	1.80	0.40	0.03	0.01	54.41	187.38	161.97	2060.43	208.01
Templin, Germany	16.7	1.13	51037.32	Native	587	Mean	4.04	2.39	0.22	0.03	10.34	37.67	38.00	556.67	244.67
						SD	0.40	0.43	0.04	0.00	7.13	15.04	7.55	508.64	75.08
Potsdam, Germany	16.7	1.13	51037.32	Native	587	Mean	3.73	2.65	0.25	0.03	12.74	42.00	35.00	533.33	158.67
						SD	0.31	0.09	0.04	0.00	1.16	13.00	11.79	281.02	30.62
Bremen, Germany	15.3	0.99	44873.83	Not Native	622.2	Mean	5.28	4.87	0.38	0.07	132.51	265.50	180.50	2532.00	194.00
						SD	0.09	1.16	0.08	0.01	12.52	45.96	16.26	67.88	45.25
Lund, Sweden	14.8	0.95	43090.53	Native	798.7	Mean	4.72	3.21	0.33	0.04	14.83	134.67	188.00	1805.33	261.33
						SD	1.49	0.71	0.08	0.01	5.11	129.48	110.01	2499.69	173.37
Stockholm, Sweden	15.2	1.00	45257.52	Native	948.6	Mean	3.84	7.17	0.47	0.05	38.02	108.33	167.33	657.33	522.67
						SD	0.24	3.03	0.11	0.01	22.86	30.86	14.05	174.68	29.01
Trondheim, Norway	11.9	0.71	32088.06	Not Native	989.5	Mean	5.21	4.76	0.49	0.06	67.70	315.00	198.00	2543.00	326.67
						SD	0.64	2.98	0.28	0.03	38.59	232.44	140.04	1916.44	170.06

Acer pseudoplatanus

Country	**Temperature (April-September 2011) (°C)**	**GDH***	**GDH (April-Septembe 2011)**	**Forest status**	**Precip# (mm)**		**pH ($CaCl_2$)**	**C (%)**	**N (%)**	**S (%)**	**P (mg/Kg)**	**Mg (mg/Kg)**	**K (mg/Kg)**	**Ca (mg/Kg)**	**Al (mg/Kg)**
Tuscany, taly	15.9	1.06	47952.29	Native	1027	Mean	6.46	5.55	0.41	0.04	15.36	596.00	225.33	21986.67	266.67
						SD	1.59	2.52	0.13	0.00	6.10	399.68	8.14	22804.28	330.37
Amiens, France	15.8	1.05	47700.01	Not Native	565	Mean	5.55	2.87	0.31	0.05	35.79	199.00	153.33	3120.00	243.33
						SD	0.51	1.04	0.10	0.01	35.56	113.53	17.04	1308.37	87.64
Brussels, Belgium	15.6	1.10	49887.05	Not Native	753.1	Mean	3.61	4.52	0.36	0.05	29.10	61.33	129.00	454.33	310.67
						SD	0.08	2.37	0.16	0.01	18.30	35.13	56.72	325.78	140.00
Templin, Germany	16.7	1.13	51037.32	Native	587	Mean	4.39	1.87	0.18	0.03	15.56	34.00	49.33	663.67	196.67
						SD	0.46	0.46	0.04	0.01	9.38	14.73	16.62	532.72	95.21
Potsdam, Germany	16.7	1.13	51037.32	Native	587	Mean	3.67	2.35	0.21	0.03	11.28	39.00	30.67	508.67	150.00
						SD	0.30	0.33	0.02	0.00	4.09	22.61	3.79	404.49	32.74
Bremen, Germany	15.3	0.99	44873.83	Not Native	622.2	Mean	4.63	5.37	0.45	0.06	95.62	204.00	114.33	2009.33	106.00
						SD	0.95	3.92	0.31	0.03	134.72	203.93	78.52	2127.57	61.61
Lund, Sweden	14.8	0.95	43090.53	Native	798.7	Mean	3.72	4.11	0.34	0.04	23.22	61.33	103.00	331.67	519.33
						SD	0.15	0.89	0.06	0.00	6.31	11.59	46.16	101.93	103.35
Stockholm, Sweden	15.2	1.00	45257.52	Native	948.6	Mean	5.08	4.17	0.37	0.04	100.62	223.00	391.67	2102.67	347.33
						SD	0.85	1.09	0.02	0.01	130.80	49.87	266.65	656.45	156.90
Trondheim, Norway	11.9	0.71	32088.06	Not Native	989.5	Mean	5.64	4.88	0.47	0.05	88.81	318.33	161.33	3544.33	266.00
						SD	0.48	3.18	0.28	0.03	97.37	222.62	53.89	2072.73	14.00

Table A.2: Mean and standard deviation (SD) of seed and samara size characteristics of *A. platanoides and A. pseudoplatanus* along the latitudinal gradient

Country	**Tuscany Italy**		**Amiens France**		**Brussels Belgium**		**Templin Germany**		**Potsdam Germany**		**Bremen Germany**		**Lund Sweden**		**Stockholm Sweden**		**Trondheim Norway**	
Latitude	43.84		49.92		50.98		52.6		52.58		53.11		55.71		59.22		63.42	
Temperature (April-September)(°C)	15.9		15.8		15.6		16.7		16.7		15.3		14.8		15.2		11.9	
GDH (April-September)	47952.29		47700.01		49887.05		51037.32		51037.32		44873.83		43090.53		45257.52		32088.06	
GDH (standardized)	1.06		1.05		1.1		1.13		1.13		0.99		0.95		1.00		0.71	
Precipitation (mm)	1027		565		753		587		587		622.2		799		949		990	
Forest status	Native		Not Native		Not Native		Native		Native		Not Native		Native		Native		Not Native	
Acer platanoides	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Samara mass (g)	0.14	0.01	0.12	0.02	0.16	0.01	0.11	0.01	0.13	0.01	0.15	0.03	0.13	0.00	0.14	0.01	0.18	0.01
Samara length (mm)	38.86	7.29	36.22	2.65	44.22	4.70	40.98	4.02	43.67	1.44	43.18	3.83	37.56	1.37	42.99	4.01	48.29	2.94
Seed mass (g)	0.05	0.02	0.05	0.01	0.06	0.01	0.04	0.00	0.06	0.00	0.07	0.01	0.06	0.01	0.06	0.01	0.08	0.00
Wing mass: seed mass	2.30	1.02	1.21	0.07	1.45	0.26	1.83	0.38	1.32	0.07	1.26	0.03	1.10	0.07	1.53	0.35	1.29	0.15
Forest status	Native		Native		Native		Native		Native		Not Native		Not Native		Not Native		Not Native	
Acer pseudoplatanus	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Samara mass (g)	0.08	0.01	0.11	0.02	0.08	0.01	0.11	0.01	0.09	0.02	0.13	0.00	0.12	0.01	0.15	0.01	0.12	0.00
Samara length (mm)	34.35	7.03	37.25	6.53	31.08	4.73	36.95	0.51	30.85	3.06	36.67	1.73	40.49	2.44	44.20	1.18	45.17	2.83
Seed mass (g)	0.04	0.01	0.05	0.01	0.04	0.01	0.05	0.01	0.05	0.01	0.07	0.00	0.05	0.01	0.07	0.01	0.05	0.01
Wing mass: seed mass	1.30	0.30	1.16	0.31	1.02	0.13	1.21	0.12	0.80	0.12	0.82	0.06	1.38	0.09	1.18	0.14	1.52	0.26

Table A.3: Mean and standard deviation of seed nutrient concentration of *A. platanoides* and *A. pseudoplatanus* seeds along the latitudinal-climatic gradient

Acer platanoides

Country	Temperature (April-September) (°C)	GDH*	GDH (April-September)	Forest status	Precip# (mm)		C (%)	N (%)	P (mg/Kg)	Mg (mg/Kg)	K (mg/Kg)	Ca (mg/Kg)	C:N	N:P	C:P
Tuscany, taly	15.9	1.06	47952.29	Native	1027	Mean	53.07	3.13	6867.33	3460.33	22093.0	7972.7	16.98	4.62	78.03
						SD	1.46	0.11	945.86	448.97	2380.1	3149.3	0.89	0.70	8.09
Amiens, France	15.8	1.05	47700.01	Not Native	565	Mean	53.48	3.75	5599.33	2935	14579.6	10389.3	14.29	6.74	96.10
						SD	0.50	0.12	546.31	184.62	4742.3	3426.3	0.57	0.81	9.06
Brussels, Belgium	15.6	1.10	49887.05	Not Native	753.1	Mean	53.88	4.04	7648.33	2826	20984.3	7062.3	13.42	5.28	70.69
						SD	0.45	0.38	567.61	134.01	1332.5	964.4	1.18	0.40	4.81
Templin, Germany	16.7	1.13	51037.32	Native	587	Mean	53.15	4.27	7999.33	3329.33	20628.7	6898.3	12.46	5.34	66.55
						SD	0.56	0.21	370.89	166.26	1896.5	479.2	0.66	0.01	3.44
Potsdam, Germany	16.7	1.13	51037.32	Native	587	Mean	54.07	4.10	6649.67	3601.67	19766.7	7017.0	13.23	6.18	81.34
						SD	0.39	0.28	188.74	353.28	1466.8	1146.9	0.95	0.59	1.78
Bremen, Germany	15.3	0.99	44873.83	Not Native	622.2	Mean	53.27	3.97	7820.50	3577.5	21263.0	7235.0	13.50	5.07	68.31
						SD	0.24	0.37	572.05	181.73	1732.4	274.4	1.34	0.11	5.30
Lund, Sweden	14.8	0.95	43090.53	Native	798.7	Mean	53.42	4.58	7502.67	3218	23248.0	6648.7	11.66	6.20	72.28
						SD	0.55	0.16	759.90	410.41	791.2	1876.3	0.34	0.92	9.01
Stockholm, Sweden	15.2	1.00	45257.52	Native	948.6	Mean	52.66	4.27	8410.00	4041.33	25528.7	6822.0	12.53	5.09	62.61
						SD	1.26	0.66	189.43	489.44	1217.8	1173.7	2.30	0.90	0.46
Trondheim, Norway	11.9	0.71	32088.06	Not Native	989.5	Mean	52.61	4.90	8243.00	3583	25425.0	6163.0	10.84	5.96	63.88
						SD	0.13	0.57	296.47	153.80	1534.82	689.71	1.21	0.91	2.50

Acer pseudoplatanus

Country	Temperature (April-September) (°C)	GDH*	GDH (April-September)	Forest status	Precip# (mm)		C (%)	N (%)	P (mg/Kg)	Mg (mg/Kg)	K (mg/Kg)	Ca (mg/Kg)	C:N	N:P	C:P
Tuscany, Italy	15.9	1.06	47952.29	Native	1027	Mean	49.03	6.04	9584.00	3701.33	35907.7	7432.00	8.13	6.30	51.19
						SD	0.16	0.25	325.06	418.39	1459.7	1756.28	0.32	0.26	1.60
Amiens, France	15.8	1.05	47700.01	Native	565	Mean	49.24	5.76	8738.67	2919.00	30799.0	7294.67	8.68	6.74	57.00
						SD	0.47	0.87	1103.02	143.95	1575.9	1161.11	1.26	1.87	7.82
Brussels, Belgium	15.6	1.10	49887.05	Native	753.1	Mean	48.73	5.58	8704.33	3116.00	36673.0	7027.33	8.78	6.44	56.32
						SD	1.18	0.43	740.04	633.02	3513.8	1030.85	0.84	0.71	5.92
Templin, Germany	16.7	1.13	51037.32	Native	587	Mean	48.78	6.29	9193.67	3331.67	34860.7	6340.33	7.76	6.84	53.08
						SD	0.09	0.18	225.23	194.32	2115.6	829.22	0.23	0.15	1.42
Potsdam, Germany	16.7	1.13	51037.32	Native	587	Mean	49.47	6.66	8605.33	3610.33	31279.7	6891.67	7.45	7.74	57.62
						SD	0.98	0.33	428.90	230.02	1772.7	1659.27	0.53	0.07	3.98
Bremen, Germany	15.3	0.99	44873.83	Not Native	622.2	Mean	50.67	5.97	7765.33	3173.67	31294.7	6209.00	8.55	7.83	66.25
						SD	1.52	0.52	1165.28	518.31	3485.4	776.78	1.02	1.65	10.09
Lund, Sweden	14.8	0.95	43090.53	Not Native	798.7	Mean	48.48	5.79	8210.33	3718.00	36978.7	5454.00	8.44	7.33	61.79
						SD	0.64	0.54	2024.62	511.48	2531.6	430.57	0.88	1.91	16.77
Stockholm, Sweden	15.2	1.00	45257.52	Not Native	948.6	Mean	48.67	6.09	9645.33	3365.33	36989.0	6836.33	8.01	6.33	50.54
						SD	0.41	0.26	491.70	205.16	3131.4	628.61	0.30	0.49	2.36
Trondheim, Norway	11.9	0.71	32088.06	Not Native	989.5	Mean	48.07	6.25	9080.33	3108.00	36074.7	6316.00	7.70	7.01	53.78
						SD	0.23	0.22	1438.17	149.41	1427.2	347.29	0.31	1.30	7.96

* standardized GDH
\# Precipitation

Table A.4: Pearson correlation values between chemical seed characteristics and environmental characteristics experienced by the mother tree during seed production, n indicates the number of observations

	GDH	**Precipitation**	**Soil.pH**	**Soil P**	**Soil K**	**Soil Mg**	**Soil Ca**	**Soil Al**	**Soil N**	**Soil C**	**Soil S**
Acer platanoides n=25											
Seed P	n.s.	n.s.	n.s.	**0.397**	n.s.	n.s.	**-0.629**	**0.399**	n.s.	n.s.	n.s.
Seed K	**-0.576**	**0.703**	n.s.	n.s.	n.s.	n.s.	n.s.	**0.418**	n.s.	n.s.	n.s.
Seed Mg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.505**	n.s.
Seed Ca	n.s.	n.s.	**0.515**	n.s.	n.s.	**0.523**	**0.453**	**-0.407**	n.s.	n.s.	n.s.
Seed N	**-0.511**	n.s.	**-0.490**	n.s.	n.s.	**-0.375**	**-0.471**	**0.504**	n.s.	n.s.	n.s.
Seed C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**-0.392**	**-0.539**	
Seed C:N	n.s.	n.s.	**0.504**	n.s.	n.s.	**0.410**	**0.455**	**-0.476**	n.s.	n.s.	n.s.
Seed N:P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Seed C:P	n.s.	n.s.	n.s.	n.s.	n.s.	**0.145**	**0.629**	**-0.409**	n.s.	n.s.	n.s.
Acer pseudoplatanus n=27											
Seed P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Seed K	n.s.	**0.580**	n.s.	n.s.	**0.439**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Seed Mg	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Seed Ca	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.489**	**-0.444**	n.s.	n.s.	n.s.
Seed N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Seed C	n.s.	**-0.403**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.393**
Seed C:N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.390**
Seed N:P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Seed C:P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Significant Pearson correlation coefficients (r) are shown ($P < 0.05$). n.s.: $P > 0.05$

Table A.5: Mean and standard deviation (SD) of # stratification days, viability, germination and seedling biomass of *A. platanoides* and *A. pseudoplatanus* along the latitudinal-climatic gradient

Country	**Tuscany, Italy**		**Amiens, France**		**Brussels, Belgium**		**Templin, Germany**		**Potsdam, Germany**		**Bremen Germany**		**Lund Sweden**		**Stockholm Sweden**		**Trondheim Norway**	
Latitude	43.84		49.92		50.98		52.6		52.58		53.11		55.71		59.22		63.42	
Temperature (April-September)(°C)	15.9		15.8		15.6		16.7		16.7		15.3		14.8		15.2		11.9	
GDH (April-September)	47952.29		47700.01		49887.05		51037.32		51037.32		44873.83		43090.53		45257.52		32088.06	
GDH (standardized)	1.06		1.05		1.1		1.13		1.13		0.99		0.95		1.00		0.71	
Precipitation (mm)	1027		565		753		587		587		622.2		799		949		990	
Forest status	Native		Not Native		Not Native		Native		Native		Not Native		Native		Native		Not Native	
Acer platanoides	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
# Stratification days	70.0	NA	34.5	12.0	49.0	0.0	77.0	9.9	56.0	12.1	63.0	0.0	76.7	24.8	83.7	13.5	56.0	12.1
Viability (%)	40.0	35.3	35.6	30.8	48.9	12.6	25.6	11.7	43.3	6.7	81.7	11.8	70.0	8.8	40.0	12.0	55.6	10.7
Germination (%)	26.7	37.9	45.0	7.1	50.0	10.0	56.7	40.4	33.3	5.8	55.0	7.1	60.0	10.0	66.7	15.3	56.7	20.8
Seedling biomass (g)	0.13	0.12	0.07	0.02	0.18	0.02	0.15	0.04	0.07	0.07	0.13	0.00	0.19	0.02	0.12	0.04	0.18	0.02
Forest status	Native		Native		Native		Native		Native		Not Native		Not Native		Not Native		Not Native	
Acer pseudoplatanus	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
# Stratification days	97.0	NA	72.0	24.1	84.0	NA	79.0	15.6	90.0	0.0	97.0	NA	80.0	14.1	86.0	3.5	97.0	0.0
Viability (%)	65.6	21.7	54.4	13.5	81.1	10.2	50.0	10.0	68.9	11.7	73.3	6.7	62.2	13.5	63.3	5.8	54.4	10.7
Germination (%)	6.7	11.5	0.0	0.0	3.3	5.8	43.3	40.4	13.3	11.5	33.3	5.8	10.0	17.3	6.7	5.8	10.0	10.0
Seedling biomass (g)	0.05	NA	NA	NA	0.01	NA	0.14	0.03	0.07	0.01	0.08	0.06	0.11	NA	0.03	NA	0.06	0.04

Appendix B

Table B.1: Provenance-specific responses to the temperature treatments applied (Control, 2.7°C and 7.2°C) and the moisture treatments (100 % field capacity, reduction to 80% and 60% compared to field capacity; M100%, M80%, M60%) on germination and survival probabilities and biomass

Acer platanoides	Lat*	Long#	GDH	Native status	Control	2.7° C	7.2°C	Control (M100 %)	M80 %	M60 %	0°C* 100 %	0°C* M80 %	0°C * M60 %	2.7°C * M100 %	2.7°C * M80 %	2.7°C * M60 %	7.2°C* M100 %	7.2°C * M 80%	7.2°C * M 60%
Italy	**43.84**	**11.83**	**1.544**	**Native**															
Germination (%)					28	18	11	18	18	21	27	23	33	10	27	17	17	3	13
Survival (%)					92	81	40	81	75	79	100	71	100	67	88	80	60	0	25
Biomass (g)					0.15	0.17	0.09	0.18	0.17	0.11	0.19	0.14	0.12	0.27	0.20	0.09	0.08	NA	0.13
France	**49.92**	**2.09**	**0.972**	**Not native**															
Germination (%)					37	23	27	38	27	22	45	30	35	35	25	10	35	25	20
Survival (%)					95	86	38	83	88	46	100	100	86	100	100	0	43	60	0
Biomass (g)					0.12	0.19	0.06	0.15	0.12	0.12	0.11	0.14	0.12	0.22	0.14	NA	0.07	0.06	NA
Belgium	**50.98**	**3.80**	**1.017**	**Not native**															
Germination (%)					46	40	40	51	40	34	50	60	27	47	30	43	57	30	33
Survival (%)					0.90	61	56	83	75	45	87	94	88	93	67	23	71	44	40
Biomass (g)					0.21	0.21	0.13	0.19	0.19	0.19	0.21	0.21	0.19	0.19	0.21	0.31	0.16	0.08	0.12
Germany Müncheberg	**52.60**	**13.56**	**1.040**	**Native**															
Germination (%)					23	6	34	27	19	18	57	7	7	3	13	0	20	37	47
Survival (%)					90	100	42	79	76	31	88	100	100	100	100	NA	50	64	21
Biomass (g)					0.19	0.23	0.09	0.19	0.13	0.09	0.19	0.20	0.12	0.33	0.21	NA	0.15	0.08	0.07
Germany Potsdam	**52.58**	**11.01**	**1.040**	**Native**															
Germination (%)					33	21	21	23	27	26	33	40	27	23	13	27	13	27	23
Survival (%)					97	79	58	86	88	70	100	92	100	86	100	63	50	75	43
Biomass (g)					0.16	0.15	0.11	0.15	0.17	0.11	0.13	0.20	0.14	0.21	0.12	0.12	0.04	0.15	0.04
Germany Bremen	**53.11**	**8.84**	**0.914**	**Not native**															
Germination (%)					52	47	47	45	48	52	55	45	55	45	50	45	35	50	55
Survival (%)					87	64	54	67	90	52	64	100	100	78	90	22	57	80	27

Biomass (g)					0.14	0.16	0.13	0.17	0.15	0.09	0.16	0.17	0.10	0.20	0.15	0.06	0.14	0.14	0.08
S-Sweden	**55.707**	**13.37**	**0.878**	**Native**															
Germination (%)					54	39	60	49	58	47	60	60	43	37	52	30	50	63	67
Survival (%)					84	71	43	68	62	64	83	89	77	73	80	56	47	21	60
Biomass (g)					0.17	0.16	0.09	0.18	0.16	0.10	0.20	0.17	0.11	0.23	0.15	0.10	0.08	0.14	0.08
N-Sweden	**59.22**	**18.06**	**0.922**	**Native**															
Germination (%)					59	30	39	44	41	42	67	57	53	30	30	30	37	37	43
Survival (%)					89	78	34	65	65	79	85	82	100	67	100	67	27	9	62
Biomass (g)					0.16	0.16	0.12	0.17	0.17	0.12	0.16	0.18	0.13	0.22	0.15	0.10	0.13	0.18	0.11
Norway	**63.42**	**10.41**	**0.654**	**Native**															
Germination (%)					59	66	53	49	66	63	57	60	60	47	80	70	43	57	60
Survival (%)					85	75	42	80	51	77	94	78	83	93	67	71	46	0	78
Biomass (g)					0.19	0.17	0.12	0.20	0.19	0.13	0.22	0.19	0.15	0.21	0.19	0.11	0.12	NA	0.12

*Latitude
#Longitude

Acer pseudoplatanus	Lat *	Long #	GDH	Native status	Control	2.7°C	7.2 °C	Control M 100%	M80 %	M60%	0°C* 100%	0°C* M80%	0°C * M60%	2.7°C * M100%	2.7°C* M 80%	2.7°C* M 60%	7.2°C * M 100%	7.2°C* M 80%	7.2°C* M 60%
Italy	**43.83**	**11.84**	**1.544**	**Native**															
Germination (%)					7	1	11	8	4	7	7	7	7	3	0	0	13	7	13
Survival (%)					67	100	40	43	50	67	50	50	100	100	NA	NA	25	50	50
Biomass (g)					0.20	0.14	0.10	0.20	0.15	0.10	0.27	0.21	0.15	0.14	NA	NA	0.19	0.09	0.06
France	**49.83**	**2.15**	**0.972**	**Native**															
Germination (%)					6	7	9	11	6	4	0	7	10	10	10	0	23	0	3
Survival (%)					100	33	25	30	60	75	NA	100	100	33	33	NA	29	NA	0
Biomass (g)					13	0.18	0.20	0.18	0.13	0.15	NA	0.10	0.15	0.15	0.20	NA	0.20	NA	NA
Belgium	**50.98**	**3.97**	**1.017**	**Native**															
Germination (%)					4	0	0	1	1	2	3	3	7	0	0	0	0	0	0
Survival (%)					100	NA	NA	100	100	100	100	100	100	NA	NA	NA	NA	NA	NA
Biomass (g)					14	NA	NA	0.03	0.06	0.24	0.03	0.06	0.24	NA	NA	NA	NA	NA	NA
Germany Müncheberg	**52.60**	**13.53**	**1.040**	**Native**															
Germination (%)					20	0	18	16	10	12	43	7	10	0	0	0	3	23	27
Survival (%)					83	NA	56	86	78	45	85	100	67	NA	NA	NA	100	71	38
Biomass (g)					0.16	NA	0.18	0.17	0.19	0.14	0.16	0.18	0.13	NA	NA	NA	0.26	0.19	0.14
Germany Potsdam	**52.57**	**13.03**	**1.040**	**Native**															
Germination (%)					14	7	4	6	8	12	13	13	17	0	7	13	3	3	7
Survival (%)					92	83	100	100	86	91	100	75	100	NA	100	0.75	100	100	100
Biomass (g)					0.12	0.10	0.09	0.13	0.12	0.10	0.16	0.16	0.08	NA	0.07	0.12	0.05	0.11	0.11
Germany Bremen	**53.11**	**8.84**	**0.914**	**Not native**															
Germination (%)					39	24	29	27	36	30	33	43	40	17	33	23	30	30	27
Survival (%)					86	95	81	71	94	93	70	92	92	80	100	100	67	89	88
Biomass (g)					0.14	0.11	0.11	0.15	0.14	0.08	0.19	0.16	0.09	0.16	0.12	0.05	0.10	0.13	0.08
S-Sweden	**55.72**	**13.31**	**0.878**	**Not native**															

Germination (%)					10	9	17	12	13	10	10	17	3	13	10	3	13	13	23
Survival (%)					89	75	73	82	75	78	100	80	100	50	100	100	100	50	71
Biomass (g)					0.16	0.07	0.09	0.13	0.10	0.08	0.19	0.13	0.16	0.07	0.09	0.04	0.11	0.06	0.08
N-Sweden	**59.22**	**18.05**	**0.922**	**Not native**															
Germination (%)					8	9	16	11	11	10	7	10	7	13	13	0	13	10	23
Survival (%)					86	88	57	70	80	67	50	100	100	75	100	NA	75	33	57
Biomass (g)					0.12	0.12	0.12	0.13	0.12	0.11	0.14	0.15	0.07	0.11	0.12	NA	0.14	0.05	0.13
Norway	**63.42**	**10.41**	**0.654**	**Not native**															
Germination (%)					7	8	4	6	6	8	10	3	7	3	10	10	3	3	7
Survival (%)					83	86	50	0.80	60	86	100	0	100	100	100	67	0	0	100
Biomass(g)					14	0.10	0.10	0.19	0.13	0.06	0.18	NA	0.09	0.20	0.13	0.03	NA	0.13	0.08

Appendix C

Table C.1: Germination, survival, aboveground, belowground, total biomass and height of *Acer platanoides* seedlings as a function of the treatments applied and the seed provenance

Acer platanoides	**Treatments**	**Germination (%)**		**Survival (%)**		**Aboveground biomass (g)**		**Belowground biomass (g)**		**Total biomass (g)**		**Height (cm)**	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Country	Hungary	43.75	2.39	48.24	3.55	0.12	0.01	0.11	0.01	0.22	0.02	6.00	0.19
Country	Poland	61.11	2.35	56.47	2.98	0.16	0.01	0.17	0.01	0.33	0.02	6.99	0.13
Country	C-Sweden	84.26	1.75	50.93	2.58	0.16	0.01	0.20	0.01	0.37	0.02	6.51	0.10
Country	Norway	50.23	2.41	49.56	3.32	0.16	0.01	0.17	0.02	0.32	0.03	6.29	0.17
Precip frequency	1/week	53.30	2.08	52.00	2.78	0.15	0.01	0.16	0.01	0.31	0.02	6.54	0.12
Precip frequency	2/week	65.63	1.98	47.80	2.54	0.15	0.01	0.17	0.01	0.32	0.02	6.48	0.12
Precip frequency	3/week	60.59	2.04	55.16	2.60	0.16	0.01	0.19	0.01	0.34	0.02	6.52	0.13
Temp	0 °C	59.03	2.05	68.8	2.45	0.21	0.01	0.25	0.01	0.46	0.02	7.27	0.12
Temp	2.8 °C	64.06	2.00	50.52	2.55	0.11	0.00	0.11	0.01	0.22	0.01	5.88	0.08
Temp	7°C	56.42	2.07	34.42	2.59	0.11	0.01	0.11	0.01	0.21	0.01	5.94	0.11
Temp*Precip	0 °C *1 /week	52.60	3.61	63.3	4.64	0.21	0.01	0.23	0.02	0.44	0.03	7.11	0.21
Temp*Precip	0 °C *2/week	68.23	3.37	65.19	4.12	0.20	0.01	0.23	0.02	0.43	0.03	7.20	0.19
Temp*Precip	0 °C *3/week	56.25	3.59	78.26	3.86	0.23	0.02	0.27	0.02	0.49	0.03	7.47	0.22
Temp*Precip	2.8 °C *1/week	57.81	3.57	57.26	4.59	0.11	0.01	0.12	0.01	0.23	0.02	6.15	0.14
Temp*Precip	2.8 °C *2/week	65.63	3.44	53.49	4.41	0.10	0.01	0.10	0.01	0.2	0.01	5.83	0.14
Temp*Precip	2.8 °C *3/week	68.75	3.35	42.03	4.22	0.11	0.01	0.11	0.01	0.22	0.02	5.65	0.15
Temp*Precip	7.0 °C *1/week	49.48	3.62	33.33	4.76	0.12	0.01	0.10	0.01	0.21	0.02	6.07	0.19
Temp*Precip	7.0 °C *2/week	63.02	3.49	22.76	3.80	0.10	0.01	0.10	0.02	0.2	0.03	5.83	0.26
Temp*Precip	7.0 °C *3/week	56.77	3.58	47.83	4.68	0.11	0.01	0.12	0.01	0.22	0.02	5.92	0.14
Temp*Country	0 °C *Hungary	42.36	4.13	93.85	3.00	0.15	0.02	0.14	0.02	0.29	0.04	6.88	0.35
Temp*Country	2.8 °C *Hungary	52.78	4.17	97.44	1.80	0.09	0.01	0.09	0.01	0.18	0.02	5.28	0.23
Temp*Country	7.0 °C *Hungary	36.11	4.02	92.86	3.47	0.08	0.01	0.08	0.01	0.16	0.02	5.48	0.17
Temp*Country	0 °C *Poland	57.64	4.13	95.4	2.26	0.24	0.02	0.24	0.02	0.48	0.03	7.93	0.22
Temp*Country	2.8 °C *Poland	70.83	3.80	94.44	2.21	0.11	0.01	0.11	0.01	0.22	0.02	6.30	0.12

Temp*Country	7.0 °C *Poland	54.86	4.16	95.18	2.37	0.13	0.01	0.13	0.02	0.24	0.03	6.31	0.24
Temp*Country	0 °C * C-Sweden	84.72	3.01	95.31	1.88	0.20	0.01	0.27	0.02	0.48	0.03	6.91	0.17
Temp*Country	2.8 °C * C-Sweden	83.33	3.12	97.56	1.40	0.11	0.01	0.13	0.01	0.24	0.01	5.93	0.11
Temp*Country	7.0 °C * C-Sweden	84.72	3.01	98.39	1.14	0.13	0.01	0.12	0.01	0.25	0.02	6.36	0.15
Temp*Country	0 °C *Norway	51.39	4.18	93.67	2.76	0.24	0.02	0.29	0.03	0.51	0.06	7.40	0.29
Temp*Country	2.8 °C *Norway	49.31	4.18	94.67	2.61	0.10	0.01	0.10	0.01	0.20	0.02	5.64	0.22
Temp*Country	7.0 °C *Norway	50.00	4.18	97.3	1.90	0.09	0.01	0.08	0.01	0.17	0.02	5.30	0.22
Precip*Country	1/week*Hungary	40.28	4.10	47.46	6.56	0.12	0.02	0.10	0.02	0.22	0.04	6.52	0.39
Precip*Country	2/week *Hungary	40.28	4.17	50.70	5.98	0.11	0.01	0.12	0.02	0.23	0.03	5.67	0.29
Precip*Country	3/week *Hungary	43.75	4.15	46.38	6.05	0.12	0.02	0.11	0.01	0.22	0.03	5.94	0.32
Precip*Country	1/week *Poland	50.69	4.18	53.16	5.65	0.16	0.02	0.16	0.02	0.32	0.03	6.95	0.24
Precip*Country	2/week *Poland	70.14	3.83	52.88	4.92	0.15	0.01	0.16	0.02	0.31	0.03	7.07	0.20
Precip*Country	3/week *Poland	62.50	4.05	63.16	4.98	0.18	0.02	0.18	0.02	0.36	0.03	6.94	0.24
Precip*Country	1/week * C-Sweden	79.86	3.35	55.37	4.54	0.17	0.01	0.19	0.01	0.36	0.02	6.51	0.14
Precip*Country	2/week * C-Sweden	84.72	3.01	43.9	4.49	0.16	0.02	0.21	0.02	0.38	0.04	6.55	0.22
Precip*Country	3/week * C-Sweden	88.19	2.70	53.44	4.37	0.16	0.01	0.20	0.02	0.36	0.03	6.48	0.17
Precip*Country	1/week *Norway	42.36	4.13	48.48	6.20	0.13	0.02	0.12	0.03	0.25	0.05	6.09	0.28
Precip*Country	2/week *Norway	60.42	4.09	44.94	5.30	0.15	0.02	0.16	0.02	0.31	0.04	6.32	0.27
Precip*Country	3/week *Norway	47.92	4.18	56.16	5.85	0.18	0.03	0.24	0.04	0.39	0.06	6.42	0.34

Table C.2: Germination, survival, aboveground, belowground, total biomass and height of *Acer pseudoplatanus* seedlings as a function of the treatments applied and the seed provenance

Acer pseuoplatanus	Treatments	Germination (%)		Survival (%)		Aboveground biomass (g)		Belowground biomass (g)		Total biomass (g)		Height (cm)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Country	Italy	6.48	1.19	42.42	8.74	0.07	0.01	0.03	0.01	0.10	0.02	5.64	0.38
Country	Hungary	54.17	2.40	50.83	3.23	0.08	0.00	0.05	0.00	0.12	0.01	6.49	0.15
Country	Denmark	71.06	2.18	50.00	2.85	0.11	0.00	0.09	0.01	0.20	0.01	7.06	0.12
Country	C-Sweden	62.27	2.33	54.98	3.03	0.08	0.00	0.05	0.00	0.13	0.01	7.87	0.15
Country	Norway	30.32	2.21	62.86	4.10	0.10	0.01	0.07	0.01	0.17	0.01	7.09	0.19
Precip frequency	1/week	40.42	1.83	51.96	2.86	0.10	0.00	0.08	0.01	0.18	0.01	7.41	0.14
Precip frequency	2/week	48.19	1.86	48.56	2.68	0.09	0.00	0.06	0.00	0.14	0.01	7.09	0.12
Precip frequency	3/week	45.97	1.86	58.88	2.68	0.09	0.00	0.06	0.00	0.15	0.01	6.95	0.13
Temp	0 °C	49.03	1.86	55.25	2.62	0.11	0.00	0.08	0.00	0.20	0.01	8.24	0.11
Temp	2.8 °C	45.00	1.86	52.91	2.76	0.09	0.00	0.06	0.00	0.14	0.01	6.63	0.11
Temp	7 °C	40.56	1.83	50.83	2.88	0.08	0.00	0.05	0.00	0.12	0.01	6.25	0.11
Temp*Precip	0 °C *1/week	49.58	3.23	51.18	4.45	0.13	0.01	0.11	0.01	0.25	0.02	8.6	0.17
Temp*Precip	0 °C *2/week	51.25	3.23	59.35	4.45	0.10	0.01	0.07	0.01	0.17	0.01	7.92	0.17
Temp*Precip	0 °C *3/week	46.25	3.23	55.36	4.72	0.11	0.01	0.08	0.01	0.18	0.01	8.25	0.23
Temp*Precip	2.8 °C *1/week	39.17	3.16	51.04	5.13	0.09	0.01	0.05	0.00	0.14	0.01	6.94	0.19
Temp*Precip	2.8 °C *2/week	45.42	3.22	57.80	4.75	0.08	0.00	0.06	0.01	0.13	0.01	6.53	0.15
Temp*Precip	2.8 °C *3/week	50.42	3.23	50.00	4.55	0.09	0.01	0.06	0.01	0.15	0.01	6.48	0.22
Temp*Precip	7.0 °C *1/week	32.50	3.03	54.22	5.50	0.07	0.01	0.05	0.01	0.13	0.01	6.20	0.22
Temp*Precip	7.0 °C *2/week	47.92	3.23	28.45	4.21	0.06	0.00	0.03	0.00	0.09	0.01	6.28	0.30
Temp*Precip	7.0 °C *3/week	41.25	3.18	73.08	4.37	0.08	0.00	0.05	0.00	0.13	0.01	6.26	0.14
Temp*Country	0 °C *Italy	5.56	1.92	37.50	18.3	0.11	0.05	0.05	0.03	0.16	0.07	7.10	0.87
Temp*Country	2.8 °C *Italy	5.56	1.92	55.56	17.57	0.08	0.01	0.03	0.01	0.11	0.03	5.88	0.33
Temp*Country	7.0 °C *Italy	8.33	2.31	37.50	12.5	0.04	0.01	0.02	0.01	0.06	0.01	4.52	0.37

Temp*Country	0 °C *Hungary	59.03	4.11	51.72	5.39	0.08	0.01	0.06	0.01	0.14	0.01	7.38	0.21
Temp*Country	2.8 °C *Hungary	56.25	4.15	45.12	5.53	0.08	0.00	0.04	0.00	0.12	0.01	6.38	0.25
Temp*Country	7.0 °C *Hungary	47.22	4.17	56.34	5.93	0.07	0.01	0.04	0.00	0.10	0.01	5.55	0.21
Temp*Country	0 °C *Denmark	74.31	3.65	58.88	4.78	0.14	0.01	0.12	0.01	0.25	0.02	8.06	0.17
Temp*Country	2.8 °C *Denmark	70.14	3.83	52.48	4.99	0.10	0.01	0.08	0.01	0.17	0.01	6.29	0.16
Temp*Country	7.0 °C *Denmark	68.75	3.88	38.00	4.88	0.09	0.01	0.07	0.01	0.17	0.01	6.47	0.18
Temp*Country	0 °C *N-Sweden	65.97	3.96	46.32	5.14	0.10	0.01	0.07	0.01	0.16	0.01	9.53	0.20
Temp*Country	2.8 °C *N-Sweden	64.58	4.00	59.14	5.13	0.08	0.01	0.05	0.01	0.13	0.01	7.33	0.20
Temp*Country	7.0 °C *N-Sweden	56.25	4.15	60.24	5.40	0.07	0.00	0.04	0.00	0.11	0.01	7.01	0.19
Temp*Country	0 °C *Norway	40.28	4.10	69.23	5.77	0.12	0.01	0.09	0.01	0.21	0.02	8.16	0.23
Temp*Country	2.8 °C *Norway	28.47	3.77	54.76	7.77	0.08	0.01	0.06	0.01	0.14	0.01	6.26	0.25
Temp*Country	7.0 °C *Norway	22.22	3.48	60.61	8.64	0.08	0.01	0.05	0.01	0.13	0.01	5.70	0.22
Precip*Country	1/week *Italy	2.08	1.19	71.43	18.44	0.05	0.01	0.02	0.01	0.07	0.01	5.48	0.49
Precip*Country	2/week *Italy	10.42	2.55	18.75	10.08	0.07	0.02	0.03	0.02	0.11	0.04	5.17	0.59
Precip*Country	3/week *Italy	6.94	2.13	60.00	16.33	0.08	0.03	0.04	0.02	0.12	0.04	5.98	0.73
Precip*Country	1/week *Hungary	52.78	4.17	51.28	5.70	0.09	0.01	0.05	0.01	0.14	0.01	6.93	0.29
Precip*Country	2/week *Hungary	55.56	4.16	46.25	5.61	0.07	0.00	0.04	0.00	0.10	0.01	6.2	0.23
Precip*Country	3/week *Hungary	54.17	4.17	54.88	5.53	0.08	0.00	0.05	0.00	0.12	0.01	6.34	0.22
Precip*Country	1/week *Denmark	64.58	4.00	41.49	5.11	0.12	0.01	0.12	0.01	0.24	0.02	7.34	0.24
Precip*Country	2/week *Denmark	78.47	3.44	46.02	4.71	0.11	0.01	0.08	0.01	0.19	0.01	7.07	0.17
Precip*Country	3/week *Denmark	70.14	3.83	62.38	4.84	0.11	0.01	0.09	0.01	0.20	0.01	6.88	0.21
Precip*Country	1/week * C-Sweden	54.86	4.16	51.9	5.66	0.09	0.01	0.05	0.01	0.14	0.01	8.14	0.27
Precip*Country	2/week * C-Sweden	65.28	3.98	52.13	5.18	0.07	0.01	0.04	0.00	0.12	0.01	8.01	0.25
Precip*Country	3/week * C-Sweden	66.67	3.94	60.2	4.97	0.09	0.01	0.06	0.01	0.14	0.01	7.57	0.24
Precip*Country	1/week *Norway	27.78	3.75	70.83	6.63	0.12	0.01	0.09	0.01	0.21	0.02	7.40	0.26
Precip*Country	2/week *Norway	31.25	3.88	62.22	7.31	0.09	0.01	0.06	0.01	0.15	0.02	6.79	0.27
Precip*Country	3/week *Norway	31.94	3.9	55.32	7.33	0.09	0.01	0.06	0.01	0.15	0.02	7.02	0.43

Appendix D

Table D.1: Location, weather and climatic characteristics (average temperature between April and September of 2011, average annual temperature for the period 1992-2011, growing degree hours between April and September 2011 standardized by dividing site-specific values by the overall mean across the latitudinal gradient), status of the population (native - non-native; according to expert knowledge and the literature; Fitter & Peat 1994; EUFORGEN 2009), seed collection method (with nets or directly from the forest floor) and the seed collection dates of *A. platanoides* and *A. pseudoplatanus* in the experiment

Location	**Species**	**Latitude (°N)**	**Longitude (°E)**	**Temperature April-September 2011 (°C)**	**Average Annual temperature (1992-2011) (°C)**	**Growing degree hours**	**Status**	**Collection method**	**Collection date**
Arezzo, Italy	*A. platanoides*	43.84	11.83	15.9	9.5	1.06	Native	Floor	25/10/11
	A. pseudoplatanus	43.83	11.84				Native	Floor	25/10/11
Amiens, France	*A. platanoides*	49.92	2.09	15.8	11.0	1.05	Not native	Nets	08/09/11
	A. pseudoplatanus	49.83	2.15				Native	Nets	06/09/11
Brussels, Belgium	*A. platanoides*	50.98	3.80	15.6	10.7	1.10	Not native	Nets	11/11/11
	A. pseudoplatanus	50.98	3.97				Native	Nets	11/11/11
Templin, Germany	*A. platanoides*	52.60	13.56	15.7	10.2	1.13	Native	Nets	04/09/11
	A. pseudoplatanus	52.60	13.53				Native	Nets	07/09/11
Lund, Sweden	*A. platanoides*	55.707	13.37	14.8	9.3	0.95	Native	Nets	13/09/11
	A. pseudoplatanus	55.72	13.31				Not native	Nets	13/09/11
Stockholm, Sweden	*A. platanoides*	59.22	18.06	15.2	7.4	1.00	Native	Floor	16/09/11
	A. pseudoplatanus	59.22	18.057				Not native	Floor	16/09/11
Trondheim, Norway	*A. platanoides*	63.42	10.41	11.9	6.2	0.71	Not native	Floor	18/10/11
	A. pseudoplatanus	63.42	10.41				Not native	Floor	18/10/11

Table D.2: Seed germination and early and interannual survival of the seedlings as a function of the treatments applied

	Acer platanoides						*Acer pseudoplatanus*					
Treatments	**Germination rate (%)**		**Early survival (%)**		**Interannual survival (%)**		**Germination rate (%)**		**Early survival (%)**		**Interannual survival (%)**	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Warm	18.1	0.9	93.2	2.3	70.9	4.4	16.9	0.9	73.3	4.4	60.8	5.7
Control	18.3	0.9	89.4	2.8	78.4	3.9	15.5	0.9	53.0	6.2	5.7	4.0
Precip 100%	17.0	0.9	89.3	3.1	78.5	4.3	15.2	0.9	58.2	5.6	52.2	7.4
Precip 50%	19.4	1.0	92.8	2.2	71.9	4.0	17.2	0.9	71.6	4.8	36.5	6.1
Comp 100%	14.3	0.9	80.5	4.3	57.7	5.9	16.1	0.9	42.6	6.8	34.8	10.2
Comp 0%	22.1	1.0	97.4	1.3	82.7	3.1	16.2	0.9	76.1	4.0	45.3	5.4
Warm *Comp(100%)	15.8	1.3	86.4	5.2	50.0	8.2	17.4	1.3	50.0	9.3	40.0	13.1
Warm *Comp(0%)	20.4	1.4	97.3	1.9	81.9	4.6	16.4	1.2	83.1	4.5	66.1	6.2
Control*Comp(100%)	12.9	1.2	74.4	6.7	66.7	8.3	14.9	1.2	33.3	9.8	25.0	16.4
Control*Comp(0%)	23.8	1.5	97.5	1.8	83.3	4.2	16.1	1.3	64.3	7.5	0.0	0.0
Warm *Precip(100%)	19.2	1.4	94.1	3.3	79.2	5.9	16.0	1.2	72.9	6.5	65.7	8.1
Warm *Precip(50%)	17.0	1.3	92.5	3.2	64.5	6.1	17.8	1.3	73.6	6.1	56.4	8.0
Control*Precip(100%)	14.9	1.2	84.6	5.1	77.8	6.3	14.3	1.2	35.5	8.7	9.1	9.1
Control*Precip(50%)	21.8	1.4	93.0	3.1	78.8	5.1	16.7	1.3	68.6	8.0	4.0	4.2
Precip(100%)*Comp(100%)	11.4	1.1	70.6	7.9	76.0	8.7	15.8	1.3	28.6	9.7	25.0	16.4
Precip(100%)*Comp(0%)	22.6	1.4	98.6	1.4	79.4	4.9	14.6	1.2	74.5	6.2	57.9	8.1
Precip(50%)*Comp(100%)	17.3	1.3	86.8	4.7	47.8	7.4	16.4	1.3	57.7	9.9	40.0	13.1
Precip(50%)*Comp(0%)	21.5	1.4	96.5	2.0	85.4	3.9	18.0	1.3	77.4	5.4	35.4	7.0

Table D.3: Number of Leaves (2012 and 2013), seedlings height (2012 and 2013), aboveground, belowground biomass, total biomass and root: shoot ratio of seedlings (2013 only) of *Acer platanoides* as a function of the treatments applied

Treatments	**Leaves 2012**		**Leaves 13**		**Height 12**		**Height 13**		**Aboveground biomass**		**Belowground biomass**		**Total biomass**		**Root: shoot ratio**	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mea n	SE	mean	SE
OTC	2.23	0.10	2.77	0.14	8.71	0.20	9.48	0.25	0.18	0.01	0.11	0.01	0.29	0.02	0.65	0.04
Control	2.20	0.09	2.31	0.09	8.74	0.19	8.63	0.24	0.12	0.01	0.07	0.00	0.19	0.01	0.65	0.04
Precip 100%	2.04	0.09	2.38	0.10	8.09	0.18	8.36	0.20	0.13	0.01	0.09	0.01	0.22	0.02	0.71	0.04
Pricip 50%	2.34	0.09	2.64	0.12	9.19	0.18	9.58	0.26	0.16	0.01	0.09	0.01	0.25	0.02	0.61	0.04
Comp 100%	2.03	0.09	2.41	0.16	9.22	0.27	9.51	0.36	0.12	0.01	0.06	0.00	0.18	0.01	0.55	0.04
Comp 0%	2.30	0.09	2.56	0.10	8.49	0.15	8.88	0.20	0.16	0.01	0.10	0.01	0.26	0.02	0.68	0.03
OTC*Comp(100%)	1.97	0.14	2.32	0.23	9.21	0.37	9.40	0.48	0.12	0.02	0.07	0.01	0.19	0.02	0.58	0.07
OTC*Comp(0%)	2.36	0.13	2.92	0.16	8.44	0.22	9.51	0.29	0.20	0.02	0.13	0.01	0.33	0.03	0.67	0.04
Control* Comp(100%)	2.10	0.11	2.50	0.22	9.23	0.40	9.60	0.54	0.12	0.01	0.06	0.01	0.18	0.13	0.53	0.03
Control*Comp(0%)	2.24	0.11	2.25	0.10	8.54	0.20	8.30	0.25	0.12	0.01	0.07	0.00	0.19	0.01	0.69	0.05
OTC*Precip(100%)	2.15	0.14	2.55	0.15	8.19	0.29	8.87	0.32	0.16	0.02	0.11	0.01	0.28	0.03	0.73	0.06
OTC*Precip(50%)	2.29	0.14	2.98	0.22	9.11	0.25	10.07	0.36	0.20	0.02	0.11	0.01	0.31	0.04	0.58	0.03
Control* Precip(100%)	1.93	0.11	2.20	0.11	7.98	0.19	7.81	0.20	0.10	0.01	0.06	0.01	0.17	0.01	0.68	0.05
Control* Precip(50%)	2.38	0.12	2.38	0.13	9.27	0.27	9.20	0.36	0.13	0.01	0.07	0.01	0.20	0.01	0.63	0.06
Precip(100%)* Comp(100%)	1.84	0.13	2.32	0.15	7.82	0.39	7.94	0.33	0.11	0.01	0.06	0.01	0.16	0.02	0.62	0.06
Precip(100%)* Comp(0%)	2.12	0.11	2.41	0.12	8.18	0.19	8.51	0.24	0.14	0.01	0.10	0.01	0.24	0.02	0.74	0.05
Precip(50%)* Comp(100%)	2.14	0.12	2.50	0.26	9.98	0.31	10.86	0.44	0.13	0.01	0.07	0.01	0.20	0.02	0.50	0.05
Precip(50%)* Comp(0%)	2.44	0.13	2.69	0.14	8.75	0.22	9.17	0.29	0.17	0.01	0.10	0.01	0.26	0.02	0.64	0.04

Table D.4: Number of Leaves (2012 and 2013), seedlings height (2012 and 2013),, aboveground, belowground biomass, total biomass and root: shoot ratio of seedlings (2013 only) of *Acer pseudoplatanus* as a function of the treatments applied

Treatments	**Leaves 2012**		**Leaves 2013**		**Height 2012**		**Height 2013**		**Aboveground biomass**		**Belowground biomass**		**Total biomass**		**Root: shoot ratio**	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
OTC	2.23	0.17	2.18	0.10	10.68	0.33	10.10	0.43	0.13	0.01	0.06	0.01	0.19	0.02	0.48	0.03
Control	1.68	0.11	2.00	0.00	10.57	0.43	11.20	2.30	0.08	0.03	0.04	0.01	0.11	0.03	0.55	0.13
Precip 100%	1.93	0.15	2.29	0.14	9.98	0.30	9.81	0.49	0.11	0.02	0.05	0.01	0.16	0.02	0.45	0.04
Pricip 50%	2.29	0.22	2.05	0.12	11.14	0.39	10.52	0.69	0.15	0.02	0.07	0.01	0.22	0.02	0.52	0.03
Comp 100%	2.05	0.20	1.88	0.13	12.89	0.69	12.63	1.13	0.14	0.02	0.06	0.01	0.19	0.03	0.45	0.04
Comp 0%	2.12	0.16	2.24	0.11	10.03	0.24	9.63	0.40	0.13	0.01	0.06	0.01	0.19	0.02	0.49	0.03
OTC* Comp(100%)	2.07	0.27	1.83	0.17	13.93	0.65	13.10	1.35	0.16	0.02	0.06	0.01	0.22	0.03	0.42	0.03
OTC* Comp(0%)	2.27	0.20	2.24	0.11	9.83	0.30	9.62	0.40	0.13	0.01	0.06	0.01	0.19	0.02	0.49	0.03
Control* Comp(100%)	2.00	0.00	2.00	0.00	10.94	1.34	11.20	2.30	0.08	0.03	0.04	0.01	0.11	0.03	0.55	0.13
Control* Comp(0%)	1.57	0.14	NA	NA	10.46	0.41	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
OTC* Precip(100%)	2.00	0.18	2.30	0.15	9.96	0.35	9.85	0.51	0.11	0.02	0.05	0.01	0.16	0.02	0.45	0.04
OTC* Precip(50%)	2.48	0.28	2.05	0.13	11.34	0.53	10.38	0.70	0.15	0.02	0.07	0.01	0.22	0.02	0.51	0.03
Control* Precip(100%)	1.67	0.17	2.00	NA	10.05	0.64	8.90	NA	0.10	NA	0.04	NA	0.15	NA	0.41	NA
Control* Precip(50%)	1.70	0.15	2.00	NA	10.81	0.55	13.50	NA	0.05	NA	0.03	NA	0.08	NA	0.68	NA
Precip(100%) *Comp(100%)	2.00	0.00	2.00	0.00	9.94	0.71	8.80	0.10	0.10	0.01	0.04	0.00	0.14	0.01	0.42	0.01
Precip(100%) *Comp(0%)	1.92	0.18	2.32	0.15	9.99	0.34	9.90	0.53	0.12	0.02	0.05	0.01	0.17	0.02	0.45	0.04
Precip(50%)* Comp(100%)	2.08	0.29	1.83	0.17	14.47	0.70	13.90	0.10	0.15	0.03	0.06	0.01	0.21	0.00	0.46	0.05
Precip(50%)* Comp(0%)	2.39	0.29	2.13	0.16	10.07	0.34	9.25	0.62	0.14	0.02	0.07	0.01	0.22	0.03	0.54	0.04

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Scientific publications

Publications in international peer-reviewed journals (IF: 2013 impact factor)

Carón M.M., De Frenne P., Brunet J., Chabrerie O., Cousins S.A.O., De Backer L., Decocq G., Diekmann M., Heinken T., Kolb A., Naaf T., Plue J., Selvi F., Strimbeck G.R., Wulf M., Verheyen K. (2014) Interacting effects of warming and drought on regeneration and early growth of *Acer pseudoplatanus* and *A. platanoides*. Plant Biology. DOI: 10.1111/plb.12177. (IF: 2.405)

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