

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

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**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.

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## Introduction

Climate is a key determinant of plant population dynamics across the globe (Woodward 1987). Therefore, climate change will likely affect population dynamics by modifying several aspects of the plant life cycle (Farnsworth et al. 1995; Norby et al. 2003; Hedhly et al. 2008; Walck et al. 2011). The sexual reproductive phase may be particularly vulnerable to climate change (Adler and HilleRisLambers 2008; Hedhly et al. 2008). Consequently, population size and structure, competitive interactions, distributional ranges, species richness, and diversity might be affected (e.g., Lloret and Pen 2004; Svenning and Skov 2006; Adler and HilleRisLambers 2008). Due to decreasing fitness of currently more abundant species such as beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) H.Karst.), a compositional change is forecast in temperate forests in favor of currently secondary species such as *Acer* sp., *Tilia* sp., and *Quercus* sp. (Lloret and Pen 2004; Kramer et al. 2010; Hanewinkel et al. 2012). In this context, understanding the effect of climate change on the regeneration of secondary forest tree species is especially relevant.

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), but 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 toward higher latitudes both among and within species (Moles and Westoby 2003; De Frenne et al. 2013). 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 1998; 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

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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 concentrations vary in time and space in relation to the maternal environmental conditions, such as temperature, water availability, incident radiation, and the amount and availability of nutrients (Drenovsky and Richards 2005; De Frenne et al. 2011).

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. 2010; 2013). 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 analyze the effect of environmental conditions on plant traits that have evolved with the climate over centuries. 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. 2013) on seed quality and the potential impacts of climate change on plant regeneration success.

Here, we analyze seed traits of two currently secondary tree species, *Acer platanoides* and *A. pseudoplatanus*, to understand the effects of climate change in temperate regions. 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, seeds of *Acer platanoides* L. and *A. pseudoplatanus* L. were collected from nine regions along a

2,200 km long latitudinal gradient from Arezzo, Italy to Trondheim, Norway (Table 1). 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 1). In each region, three forest patches within a landscape area of  $40 \times 40 \text{ km}^2$  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 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 (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  $5 \text{ }^\circ\text{C}$  (following Graae et al. 2012). 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  $r = -0.72$  ( $p < 0.0001$ ,  $n = 9$ ).

We collected 15 mineral soil samples (0–20 cm depth) randomly located in a  $20 \text{ m} \times 20 \text{ m}$  area around each mother tree. The samples were pooled per mother tree, oven-dried ( $40 \text{ }^\circ\text{C}$ ) for 48 h and passed through a 2 mm sieve. The pH was determined from a solution of 14 ml soil and 70 ml  $\text{CaCl}_2$  0.01 M using a glass electrode. Additionally, 5 g of dry soil was 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  $\text{NH}_4$ -acetate diluted to 1 l] according to the modified method of Enger et al. (1960).

**Table 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	Latitude (°)	Longitude (°)	Temperature April–September 2011 (°C)	Average Annual temperature (1992–2011) (°C)	GDH	Precipitation (mm)	Mother tree status	Collection method	Collection date
Arezzo, Italy	<i>A. platanoides</i>	43.84	11.83	15.9	9.5	47,952	1,027	Native	Floor	25/10/11
	<i>A. pseudoplatanus</i>	43.83	11.84					Native	Floor	25/10/11
Amiens, France	<i>A. platanoides</i>	49.92	2.09	15.8	11.0	47,700	565	Not native	Nets	08/09/11
	<i>A. pseudoplatanus</i>	49.83	2.15					Native	Nets	06/09/11
Brussels, Belgium	<i>A. platanoides</i>	50.98	3.80	15.6	10.7	49,887	753	Not native	Nets	11/11/11
	<i>A. pseudoplatanus</i>	50.98	3.97					Native	Nets	11/11/11
Templin, Germany	<i>A. platanoides</i>	52.60	13.56	16.7	10.2	51,037	587	Native	Nets	04/09/11
	<i>A. pseudoplatanus</i>	52.60	13.53					Native	Nets	07/09/11
Potsdam, Germany	<i>A. platanoides</i>	52.58	11.01	16.7	10.2	51,037	587	Native	Nets	27/10/11
	<i>A. pseudoplatanus</i>	52.57	13.03					Native	Nets	27/10/11
Bremen, Germany	<i>A. platanoides</i>	53.11	8.84	15.3	9.7	44,873	622	Not native	Floor	15/10/11
	<i>A. pseudoplatanus</i>	53.11	8.84					Not native	Floor	15/10/11
Lund, Sweden	<i>A. platanoides</i>	55.707	13.37	14.8	9.3	43,090	799	Native	Nets	13/09/11
	<i>A. pseudoplatanus</i>	55.72	13.31					Not native	Nets	13/09/11
Stockholm, Sweden	<i>A. platanoides</i>	59.22	18.06	15.2	7.4	45,257	949	Native	Floor	16/09/11
	<i>A. pseudoplatanus</i>	59.22	18.06					Not native	Floor	16/09/11
Trondheim, Norway	<i>A. platanoides</i>	63.42	10.41	11.9	6.2	32,088	990	Not native	Floor	18/10/11
	<i>A. pseudoplatanus</i>	63.42	10.41					Not native	Floor	18/10/11

Phosphorus (P) was determined in a spectrophotometer (Cary 50; Varian) according to the malachite-green method, using malachite-green and molybdate as color 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).

### Seed 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 h, 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 Image J 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<sup>3</sup> vol.) filled with standard potting soil (mean pH 6, nutrient ratio NPK 15:10:11, organic matter 20 % and water holding capacity 80 %) after the seeds had been cold stratified at 0–1 and 90–95 % humidity, (for a variable period, ca. two months until each seed lot started to germinate. 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; for additional details see Carón et al. 2014). 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 h and weighed.

A subsample of 30 seeds (without wing) from each mother tree was randomly selected, oven-dried at 65 °C for 24 h, and milled, which was then used for determination of seed concentrations of Ca, Mg, P and K, 75 mg. First, we decomposed the milled samples using acid digestion with HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>. Then, Ca, Mg, and K were measured with atomic absorption spectrometry (SpectrAA-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

#### *Environmental variables*

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. Afterward, 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

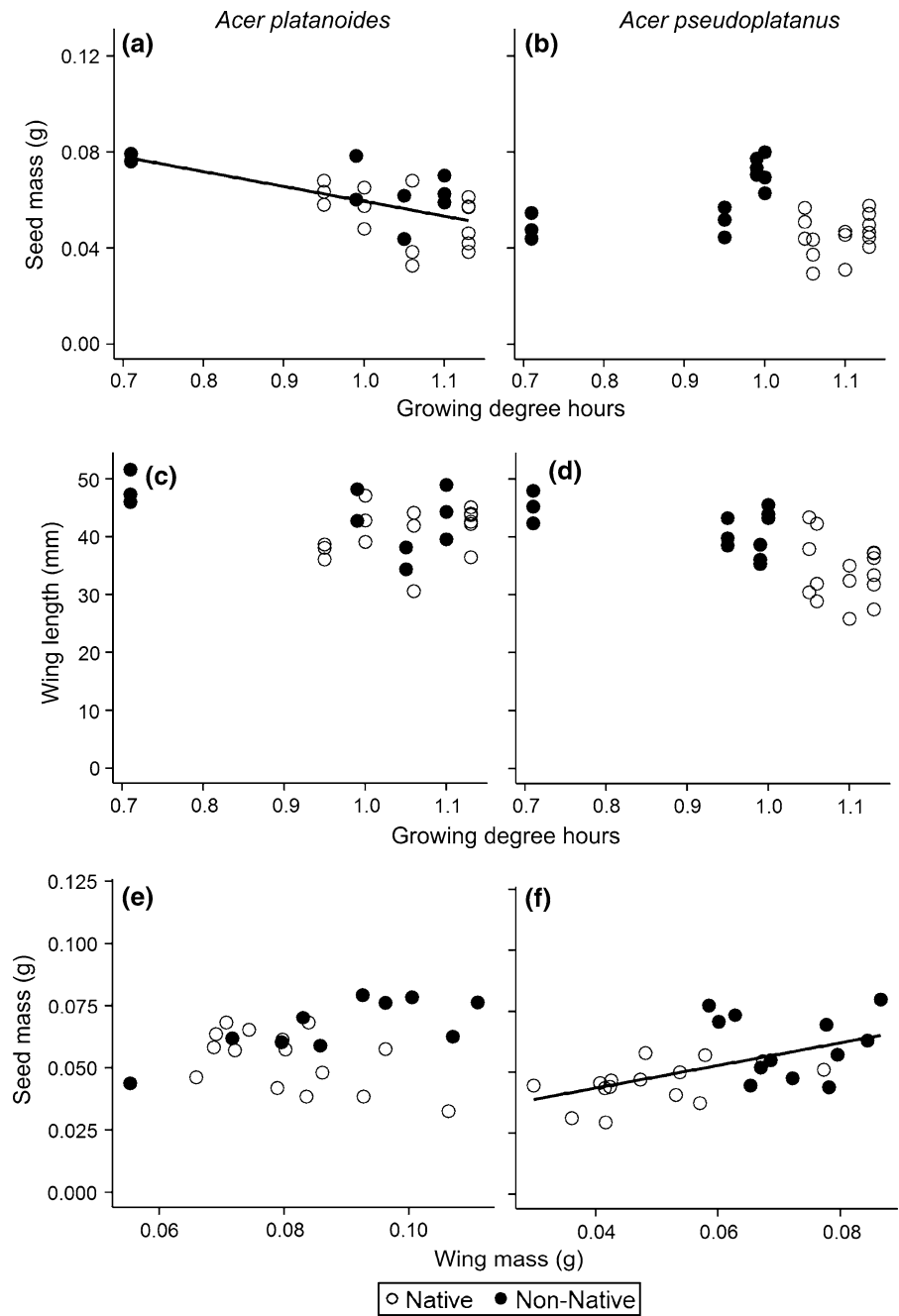
**Table 2** Morphological seed characteristics as a function of the environmental conditions experienced by the mother tree: growing degree hours (GDH), annual precipitation during 2011, soil nutrient and soil pH axes and mother tree status (Native/Non-native)

Morphological trait	<i>Acer platanoides</i>			<i>Acer pseudoplatanus</i>		
	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. 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 colors denote the mother tree status



33 % lighter than those produced under the coldest condition (Table 2; Fig. 1 and Online Resource1). 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 and Online Resource1). Finally, there was a clear

**Table 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

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

\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ 

increase in wing mass with the increase in seed mass in *A. pseudoplatanus* samaras (Fig. 1).

#### 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 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.

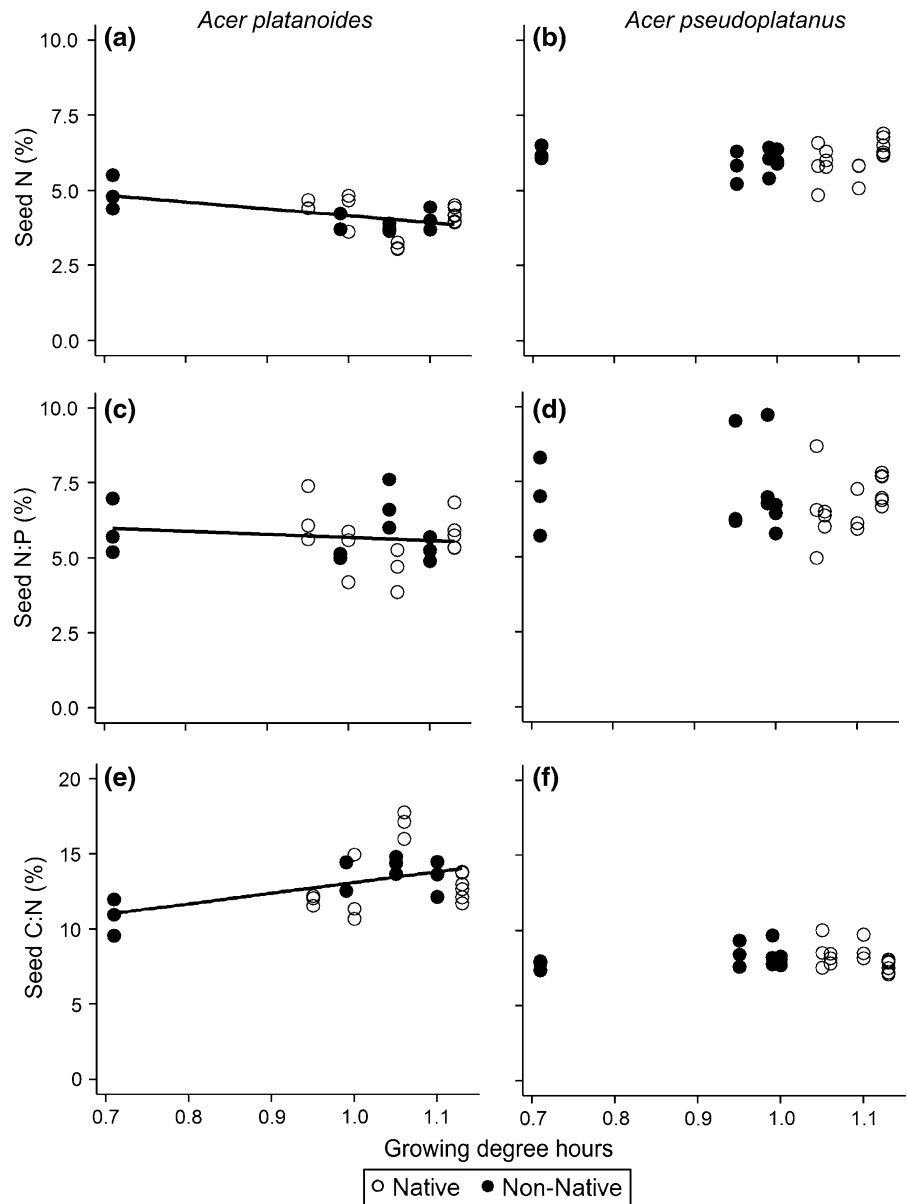
Additionally, seed N, K, P, Mg, and Ca concentrations as well as the C:N and C:P ratio in *A. platanoides* seeds were affected by the soil characteristics. Seed P and Mg were positively related to the soil nutrient axis,

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 (Online Resource 3), 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 3 and Online Resource 3).

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 (Online Resource 2). 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 3 and Online Resource 3).



**Fig. 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 colors 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) 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 (Online Resource 3).

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

**Table 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 analyzed (gls or mixed-effects model)

Morphological trait	<i>Acer platanoides</i>			<i>Acer pseudoplatanus</i>		
	Predictor	par.est	t/z-value	Predictor	par.est	t/z-value
	<i>Environmental conditions</i>			<i>Environmental conditions</i>		
Stratification time	Mother tree status	-0.34	-2.89**			n.s.
Viability	GDH	-2.40	-2.08*			
	Precipitation	-2.3E - 3	-2.77**			
Germination	Soil nutrient axis	0.43	3.23**			
	Soil pH axis	-0.41	-2.21*			n.s.
Biomass	Precipitation	1.2E - 4	2.06*			n.s.
	<i>Seed traits</i>			<i>Seed traits</i>		
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.

\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$

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 4 and Online Resource 4). 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. 3 and Online Resource 4). 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 4 and Fig. 3) The seed viability of both species was positively influenced by the seed mass, indicating that the heavier seeds were more viable (Fig. 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 4).

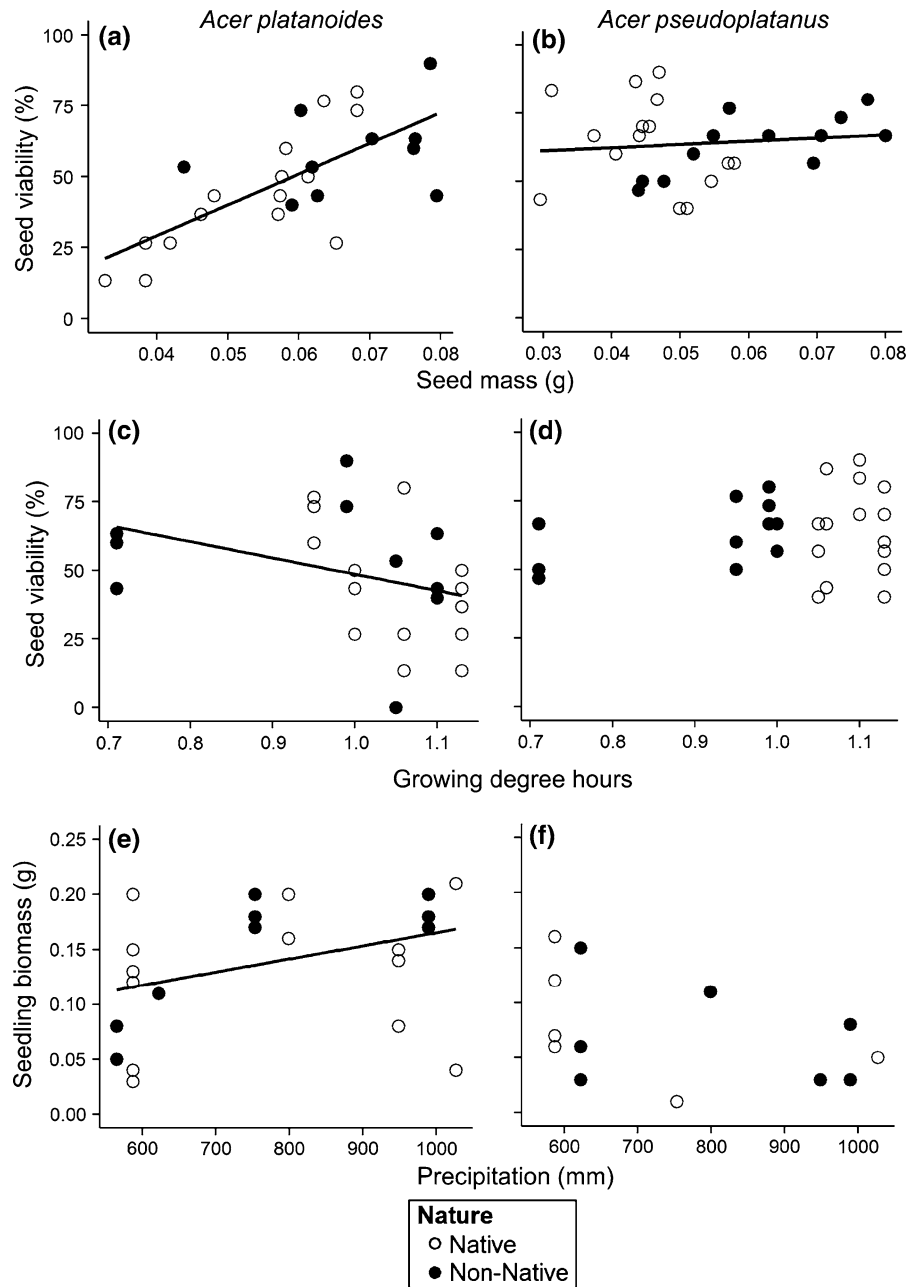
Finally, in contrast to *A. platanoides*, seed germination and seedling biomass of *A. pseudoplatanus* did not depend on chemical composition (Table 4).

## Discussion

Seeds of *A. platanoides* and *A. pseudoplatanus* collected along a 2,200 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

**Fig. 3** Seed viability, as a function of the seed mass and standardized growing degree hours (a–d) and seedling biomass as a function of precipitation (e, f) in *A. platanoides* and *A. pseudoplatanus*. Different colors denote the mother tree status



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).

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. 2011; 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. 2011). 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.

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 (Online Resource 4) 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).

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 stress 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 of both species is needed.

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