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Climatic control of forest herb seed banks along a latitudinal gradient

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ABSTRACT

Aim Seed banks are central to the regeneration strategy of many plant species. Any factor altering seed bank density thus affects plant regeneration and population dynamics. Although seed banks are dynamic entities controlled by multiple environmental drivers, climatic factors are the most comprehensive, but still poorly understood. This study investigates how climatic variation structures seed production and resulting seed bank patterns.

Location Temperate forests along a 1900 km latitudinal gradient in north-western (NW) Europe.

Methods Seed production and seed bank density were quantified in 153 plots along the gradient for four forest herbs with different seed longevity: *Geum urbanum*, *Milium effusum*, *Poa nemoralis* and *Stachys sylvatica*. We tested the importance of climatic and local environmental factors in shaping seed production and seed bank density.

Results Seed production was determined by population size, and not by climatic factors. *G. urbanum* and *M. effusum* seed bank density declined with decreasing temperature (growing degree days) and/or increasing temperature range (maximum–minimum temperature). *P. nemoralis* and *S. sylvatica* seed bank density were limited by population size and not by climatic variables. Seed bank density was also influenced by other, local environmental factors such as soil pH or light availability. Different seed bank patterns emerged due to differential seed longevities. Species with long-lived seeds maintained constant seed bank densities by counteracting the reduced chance of regular years with high seed production at colder northern latitudes.

Main conclusions Seed bank patterns show clear interspecific variation in response to climate across the distribution range. Not all seed banking species may be as well equipped to buffer climate change via their seed bank, notably in short-term persistent species. Since the buffering capacity of seed banks is key to species persistence, these results provide crucial information to advance climatic change predictions on range shifts, community and biodiversity responses.

Keywords

Climate change, interspecific variation, plant–climate interaction, seed longevity, seed production, temperate deciduous forest, temperature.

INTRODUCTION

Seed banks form a key part of the regeneration strategy of many plant species as they offer a possibility to bridge extended periods of unfavourable conditions (Ooi, 2012). Moreover, bet-hedging across stored long-lived seed cohorts enables species to even out both spatial and temporal environmental heterogeneity (Venable & Brown, 1988). As a result, persistent seed banks play a pivotal role in plant population dynamics and population persistence (Stöcklin & Fischer, 1999; Rees *et al.*, 2002), forming an important recruitment source for future generations (Kalisz & McPeck, 1992; Toräng *et al.*, 2010) and facilitating population re-establishment (Van der Veken *et al.*, 2007). Through the seed banks' storage effect, seed banks enhance species coexistence in plant communities (Chesson, 2000; Facelli *et al.*, 2005), accounting for a sizeable part of plant community diversity given the compositional vegetation – seed bank dissimilarity (Thompson & Grime, 1979). Consequently, any factor modifying seed bank dynamics will in turn profoundly affect plant regeneration, population dynamics, species persistence and ultimately, community composition and dynamics.

Climate is probably the most overarching driver of seed bank dynamics (Pakeman *et al.*, 1999; Ooi *et al.*, 2009; Middleton & McKee, 2011). The amount of persistent seeds stored in the soil at a single point in time results from multiple interacting biotic and environmental factors, most of which are governed by climatic conditions such as temperature and moisture (reviewed by Walck *et al.*, 2011). Temperature influences seed bank inputs via species-dependent effects on seed production (Molau & Shaver, 1997; Cummins & Miller, 2002) and germinable seed output (De Frenne *et al.*, 2009) by altering, e.g. plant–pollinator interactions (Kudo *et al.*, 2004) or seed predation rates (McKone *et al.*, 1998). Seed losses by germination are, next to nitrate, mainly regulated via temperature and moisture controls on dormancy-breaking and germination cues (Baskin & Baskin, 1998). Beside local abiotic conditions limiting seed survival (pH, C:N ratio; Pakeman *et al.*, 2011), complex climatic and environmental factors control other species-specific drivers of seed losses such as seed viability and seed senescence rates. Ultimately, all these stochastic processes together preset a species' seed longevity, i.e. the time between a seeds entry into the soil and its death. Precisely because of this intricate control, the response to climate of seed longevity may be species-dependent (Cavieres & Arroyo, 2001; Hill & Vander Kloet, 2005; Ooi, 2012), although some general patterns emerge. Elevated temperatures may decrease seed viability (Ooi *et al.*, 2009, but see Leishman *et al.*, 2000), and seed mortality is enhanced via fungal attack under moist soil conditions (Pakeman *et al.*, 2011; Mordecai, 2012).

Although mechanisms governing seed bank processes seem relatively well known at least qualitatively, direct quantitative studies linking seed banks with prevailing climatic conditions remain scarce (Walck *et al.*, 2011; Ooi, 2012). Evidence gained from temperature manipulation experiments (Akinola *et al.*, 1998; Leishman *et al.*, 2000) from studies conducted along altitudinal gradients (Cavieres & Arroyo, 2001; Cummins & Miller,

2002) and other observational work (Pakeman *et al.*, 1999; Middleton & McKee, 2011) suggests that cool, damp storage conditions in mild climates promote seed longevity due to low embryonic metabolic activity (Murdoch & Ellis, 1992) and less fungal activity (Burdon, 1987), enabling seed bank build-up, even if seed inputs decline with decreasing temperature (Cummins & Miller, 2002). Once prevailing climatic conditions deviate from the above, interspecific variation in seed bank responses is substantial, with no unidirectional pattern emerging. Seed bank density in many species remains indifferent to changing climatic conditions (Akinola *et al.*, 1998; Middleton & McKee, 2011), although seed bank density may also increase or decrease (e.g. Leishman *et al.*, 2000; Hill & Vander Kloet, 2005), without any identifiable cause behind this interspecific variation (Middleton & McKee, 2011). These different seed bank responses carry the potential to substantially alter population and community dynamics (Ooi *et al.*, 2009; Toräng *et al.*, 2010), significantly modulating species' chances to adapt to environmental and climatic change (Toräng *et al.*, 2010). With the buffering capacity of seed banks recognized as being key to species persistence (Kalisz & McPeck, 1992; Stöcklin & Fischer, 1999; Toräng *et al.*, 2010), it is crucial to investigate seed banks across species' climatic ranges to advance climatic change predictions on range shifts, as well as community and biodiversity responses (Middleton & McKee, 2011).

Here, we studied seed bank patterns in four forest herbs (*Geum urbanum*, *Milium effusum*, *Poa nemoralis* and *Stachys sylvatica*) along a 1900-km latitudinal gradient, from the core of their distribution range towards their respective northern range edges. First, we aimed to unravel how total seed production and seed bank density in each species were related to climatic and other environmental factors. We expect to find declining total seed production in cooler climatic conditions counteracted by improving conditions for seed storage resulting in constant numbers of banked seeds (cf. Cummins & Miller, 2002). Secondly, we asked whether variation in total seed production and seed bank density was species-dependent or unidirectionally climate-dependent. More specifically, we hypothesized that species with long-lived seeds might be better at maintaining constant numbers of banked seeds, as they could more easily compensate the lack of regular seed production in cooler climatic conditions.

MATERIALS AND METHODS

Study species

The importance of seed banking in forest herbs is generally dismissed. However, multiple independent studies have demonstrated seed banking to be more common than hitherto thought (Warr *et al.*, 1994; HilleRisLambers *et al.*, 2005; Kaeser & Kirkman, 2012; Plue *et al.*, 2012). Despite low seed bank density, low seed survival rates and short-term persistence (Kaeser & Kirkman, 2012), seed banks still substantially enhance seedling recruitment in any given year (HilleRisLambers *et al.*, 2005). Specifically in a temporally and spatially heterogeneous forest

Northern Range Edges
 - - - - - *Milium effusum*
 *Poa nemoralis*
 - - - - - *Stachys sylvatica*
 ——— *Geum urbanum*

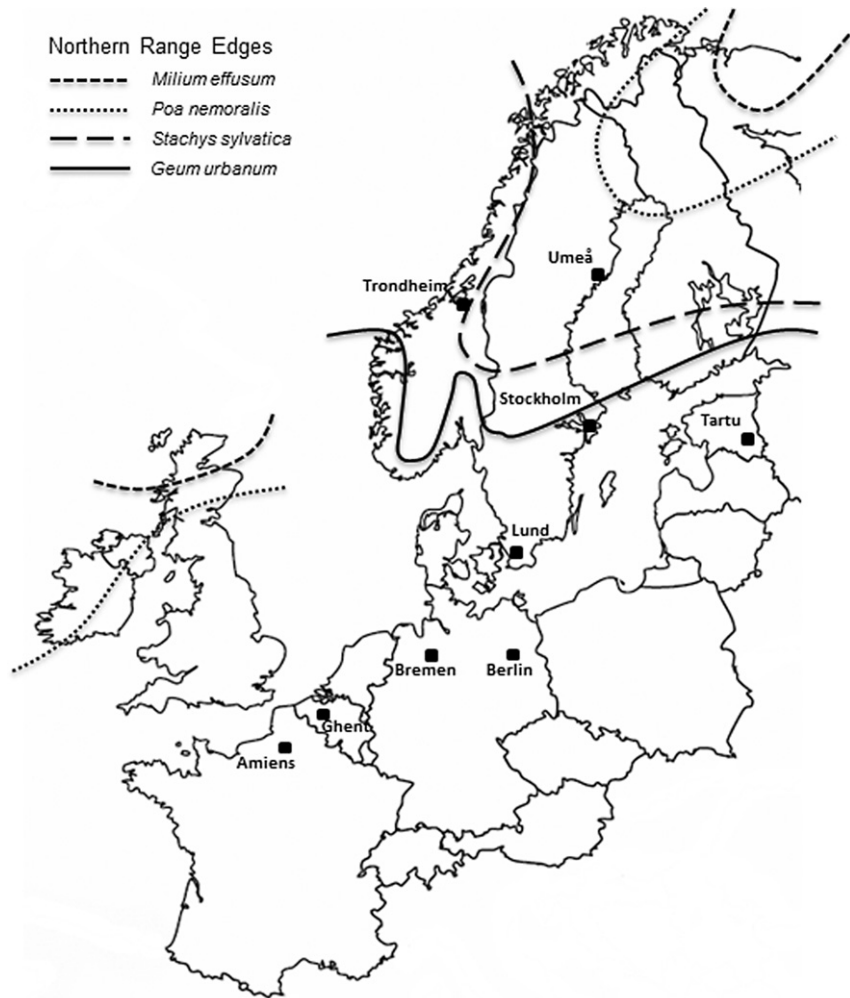


Figure 1 Location of the nine sampling regions along a 1900 km latitudinal gradient in north-western (NW) Europe. Seed production and seed bank density were sampled for *Geum urbanum*, *Milium effusum*, *Poa nemoralis* and *Stachys sylvatica* in five plots per species and region (except in Trondheim and Umeå where only *Poa nemoralis*/*Stachys sylvatica* and *Milium effusum* were sampled, respectively). The northern range limits are based on Hultén & Fries (1986). See Appendix S1 for more detailed information.

environment, seed banks enable forest species to capitalize on rare temporally unpredictable opportunities for successful recruitment, notably when seed production is low (HilleRisLambers *et al.*, 2005). As seed banks present clear benefits to forest species' persistence, we deliberately selected forest species for this study.

Species were selected based on their seed longevity index (SLI, Thompson *et al.*, 1998). The SLI ranges between 0 (strict transient seed bank) and 1 (strict persistent seed bank), as the ratio of all seed bank studies in which a species resided in the persistent seed bank over all seed bank studies in which the species resided in either the transient or persistent seed bank. Suitable species had an SLI > 0. Four forest understorey herbs with contrasting SLI were selected: *G. urbanum* L. [SLI = 0.05, northern distribution range limit (NRL, Hultén & Fries, 1986) = 55°N], *M. effusum* L. (SLI = 0.53, NRL = 71°N), *P. nemoralis* L. (SLI = 0.39, NRL = 70°N) and *S. sylvatica* L. (SLI = 0.31, NRL = 66°N). *G. urbanum* and *S. sylvatica* need a cold stratification to germinate, peaking in March (Taylor, 1997) and March–April (Taylor & Rowland, 2010), respectively. *M. effusum* seeds may germinate the autumn after seed shed in warmer climates, although winter stratification is required in colder cli-

mates to trigger early spring germination (Thompson, 1980). Light promotes germination in *G. urbanum* (Slade & Causton, 1979), *M. effusum* and *P. nemoralis* (Jankowska-Blaszczuk & Daws, 2007) but not in *S. sylvatica* (Jankowska-Blaszczuk & Daws, 2007). We found no information on the germination phenology of *P. nemoralis*.

Plot selection

We used eight regions along a 1900 km latitudinal gradient from northern France to Scandinavia, where five populations of up to four species were sampled within one 50 km × 50 km landscape window per region (Fig. 1). The forests in which populations were sampled met a number of criteria to assure sampled populations experienced a similar set of environmental conditions, minimizing the variation in driving factors other than climate. Sampled populations were restricted to mixed deciduous forests with > 50% canopy cover. Most sampled populations were located in ancient forest, i.e. forest with a long history of continuous forest cover, based on the earliest local cartographic material available. *M. effusum* and *P. nemoralis* populations were sampled in 40 plots (eight regions), *S. sylvatica* populations were

sampled in 38 plots (eight regions), and *G. urbanum* populations were sampled in 35 plots (seven regions). Detailed information on species-plot distributions across sampling regions is available in Table S1 in Supporting Information.

Seed production and seed bank

Plot size was 2 m × 2 m for every species to optimize the seed bank sampling design (Plue & Hermy, 2012). Each plot was randomly placed in one population of a species, assuring the presence of at least five individuals of the focal species. Between May and June 2011, 50 soil samples (3.5 cm diameter, 10 cm deep, litter removed) were collected once per plot. Both litter removal and timing sampling before new seed production and after the germination peak (cf. species' germination phenology) aimed to assure that most seeds were recovered from the persistent seed bank and not solely from last years' seed production. Of the 50 soil core samples, the first 25 samples were systematically collected on the grid nodes of a 50 cm × 50 cm grid within the 2 m × 2 m plot; the second set of 25 samples was collected within a *ca.* 20 cm radius around the mother plants to assure the highest chance in retrieving persistent seeds (Plue & Hermy, 2012). All 50 samples were pooled per plot. Once the mixed sample was concentrated after wet sieving (Ter Heerdt *et al.*, 1996), the samples were spread out in 25 cm × 25 cm containers on top of standard potting soil. The sample soil layer was always < 0.5 cm deep. In July 2011, these containers were immediately placed in a greenhouse in Stockholm (central Sweden) with a natural sunlight regime, with day- and night-time temperatures generally between 15–30 °C and 5–20 °C, respectively. From late November 2011 until the experiment ended, greenhouse temperatures were kept constant at 21 °C by additional heating while additional lighting provided a summer sunlight regime (14 h light–8 h darkness). These temperatures provide good conditions for germination in all four species (Thompson, 1980; Taylor, 1997; Graae *et al.*, 2009; Ter Brink *et al.*, 2012). Seed bank samples were not subjected to additional artificial winter stratification, since spring-early summer sampling should have assured natural stratification. Identified seedlings of all vascular plant species were counted and removed. The germination trial was ended after 39 weeks when no new seedlings had been recorded for 2 consecutive weeks.

Total seed production is used as a proxy of the potential annual seed inputs into the seed bank. We quantified total seed production per plot by clipping all inflorescences of each focal species at the time of seed maturity [i.e. when the first seeds reached maturity (July–September 2011) to assure that the majority of all produced seeds were collected]. Total seed production was estimated by counting all seeds per sample in *G. urbanum*, *M. effusum* and *S. sylvatica*. In *P. nemoralis* (which produced up to 80,000 seeds per 4 m² plot), we counted all seeds in 20 randomly selected inflorescences per population, and calculated seed production as the total number of inflorescences per plot times the average number of seeds per inflorescence.

Population and environmental descriptors

Since plant population size constrains total seed production, the number of individuals (*M. effusum*, *G. urbanum*), ramets (*S. sylvatica*) or tussocks (*P. nemoralis*) as well as the number of inflorescences were counted in each plot. We estimated species cover (in %) in the plot, and classified species population size in the area surrounding a plot into size categories based on the number of individuals, ramets or tussocks (1: < 50, 2: 50–100, 3: 101–250, 4: 251–500, 5: 501–1000, 6: > 1000). As species density, species cover, the number of inflorescences and population size were highly correlated in each species (all correlations per species: spearman rank correlation at least > 0.41 and $P < 0.002$), the four variables for each species were replaced by a single population descriptor PC1. PC1 was the first axis of a principal components analysis (PCA) with varimax rotation on the four population characteristics, using *prcomp()* from the base R functions. PC1 captured 91%, 79%, 99% and 87% of the variation in population size descriptors of *G. urbanum*, *M. effusum*, *P. nemoralis* and *S. sylvatica*, respectively. In each species, the first PCA axis correlated positively and significantly with each of the four population descriptors. PC1 will be further referred to as 'population size', as each underlying population characteristic related to a plots' population size.

Daily average temperature data for each sampling location were first extracted from the web application *FetchClimate* (<http://fetchclimate.cloudapp.net/>), which compiles climatic data from weather stations nearest to the sampled plots. To analyse the effects of temperature on seed production, we calculated the growing degree days above a base temperature T_{base} of 5 °C $\left(GDD = \sum_{k>0} k \text{ with } k = (T_{average} - T_{base}) \right)$ from 1 January 2011 until the seed collection date (De Frenne *et al.*, 2009). To analyse effects of temperature on the seed bank, daily average temperature was replaced by a longer-term (2001–11) daily average $\left(GDD_{10} = \sum_{k>0} k \text{ with } k = (T_{average10} - T_{base}) \right)$ to account for temperature effects on seed production in the years preceding sampling which may have accumulated in the seed bank. We calculated the average temperature range as the annual maximum temperature minus the minimum temperature, averaged for the period 2001–11. Hill & Vander Kloet (2005) showed its importance in explaining seed bank patterns of *Vaccinium* species in relation to climate. Secondly, since light availability affects seed germination in understorey plants (Jankowska-Blaszczuk & Daws, 2007), overstorey tree and shrub canopy cover (in %) and understorey herbaceous plant cover (in %) were visually estimated in each plot. Third, soil moisture and soil pH control seed longevity (Pakeman *et al.*, 2011). Soil moisture was estimated by assigning a plot to one of four different classes (1: dry, 2: fresh, 3: moist, 4: wet soil conditions following De Frenne *et al.*, 2009). In each plot, five soil samples (3.5 cm diameter, 10 cm deep, litter removed) were collected, mixed, oven-dried at 105 °C for 24 h and sieved through a 2 mm mesh. pH KCl (5 g soil/20 mL 1 M KCl) was measured with a glass electrode. Fourth, latitude was included as an overarching predictor

variable that should capture additional variation not sampled along the latitudinal gradient such as variation in precipitation, rainfall distribution or photoperiod. Latitude was negatively related to GDD_{10} in *S. sylvatica* (Spearman correlation coefficient $\rho = -0.54$, $n = 38$, $P < 0.001$), *P. nemoralis* ($\rho = -0.66$, $n = 40$, $P < 0.001$) and *G. urbanum* ($\rho = -0.35$, $n = 35$, $P < 0.05$) and positively related to the temperature range in *M. effusum* ($\rho = 0.75$, $n = 40$, $P < 0.001$), *S. sylvatica* ($\rho = 0.46$, $n = 38$, $P < 0.003$), *P. nemoralis* ($\rho = 0.43$, $n = 40$, $P = 0.006$) and *G. urbanum* ($\rho = 0.67$, $n = 35$, $P < 0.001$). A last binary environmental descriptor was included to account for the populations' location inside each forest patch (0: forest interior; 1: < 10 m from the forest edge).

Data analysis

To investigate the effects of environmental and population variables on total seed production and seed bank density (i.e. the number of germinated seedlings), we modelled both variables using generalized estimating equations (GEE) with a Poisson error distribution (Koper & Manseau, 2009; Zuur *et al.*, 2009). The tested predictor variables were (1) population size (PC1), (2) seed production (only in the seed bank density model), (3) temperature (as expressed by GDD and GDD_{10}), (4) climatic variation (as expressed by temperature range), (5) latitude, (6) canopy cover, (7) soil pH, (8) soil moisture and (9) forest edge/interior variable. Our modelling approach involved adding a single predictor variable into a null model on a one-by-one basis to avoid multicollinearity problems. The null model only included *region* as a random effect. The GEE correlation structure specifying the within-region correlation was set to 'exchangeable' (Zuur *et al.*, 2009). The Wald test statistic was used to compare the null model with the model which included one predictor variable to evaluate the significance of adding that individual predictor to the null model (Zuur *et al.*, 2009). In a second step, we investigated the additive effects of the remaining significant predictor variables when population size (PC1) proved significant, as we *a priori* assumed that population size (PC1) would be the strongest predictor of seed production and seed bank density (see Table 1). This meant including a single significant predictor variable into a model that contained PC1 as a main effect and *region* as a random effect. Predictor variables were added into the model on a one-by-one basis to avoid multicollinearity. Again, Wald test statistics were used to evaluate the significance of the additive effect of the predictor variables. Finally, as population size (PC1) was a strong predictor of seed production (Table 1), we wished to exclude that an underlying relation between population size (PC1) and the climatic predictor variables (latitude, GDD_{10} and temperature range) would bias the relationship between seed bank patterns and climatic variables along the gradient. However, no such relation was established in any of the four studied species, using the same GEE modelling approach (detailed results in Table S2). All analyses were performed in R 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria), using the *geepack* package for GEE modelling (Højsgaard *et al.*, 2006).

RESULTS

Seed production and seed bank density

Total seed production was highly variable between populations. *G. urbanum* and *M. effusum* produced a median of 418 seeds per m^2 (range 25–3461 seeds per m^2) and 497 seeds per m^2 (37–3212), respectively, while *S. sylvatica* only attained a median seed production of 175 seeds per m^2 (2–4140). *P. nemoralis* had the highest seed production with a median of 2527 seeds per m^2 (2–21164). Each study species was present in the seed bank samples of at least one plot per region. During the seed bank germination trial, 88 *G. urbanum* (in 23 out of 35 plots), 119 *M. effusum* (in 22 out of 40 plots), 1869 *P. nemoralis* (in 39 out of 40 plots) and 510 *S. sylvatica* (in 35 out of 38 plots) seedlings were recorded, translating into mean seed densities of 332, 416, 3992 and 1248 seeds per m^2 in the seed bank, respectively.

Environmental control on seed production

In all four species, seed production significantly increased with population size (PC1). Increasing canopy cover decreased seed production in *P. nemoralis*, and this effect was additive to population size (PC1, Table 1). Soil moisture did not affect seed production in any species, and soil pH significantly increased seed production only in *G. urbanum* (additive to the effect of population size; Table 1). Proximity to the forest edge influenced seed production, being higher in *M. effusum* and lower in *S. sylvatica* closer to the forest edge.

Neither growing degree days nor temperature range significantly affected seed production in any of the four study species. Seed production in *G. urbanum* decreased towards the north, even after correction for population size (significant additive effect in PC1 + Latitude, Table 1; Fig. 2). Latitude did not have significant effects on seed production in any of the three other species (Fig. 2).

Environmental control on seed bank density

Seed bank density in *G. urbanum* and *M. effusum* was independent from population size or seed production. In *P. nemoralis* and *S. sylvatica*, seed bank density increased with increasing population size (PC1). In *S. sylvatica*, larger seed production increased seed bank density, although this effect was entirely incorporated within the effect of population size (Table 1). While soil pH had no effects on seed bank density in any species, seed bank density in *G. urbanum* and *M. effusum* increased and *P. nemoralis* seed bank density decreased with increasing soil moisture (not additive in *P. nemoralis*). Soil moisture did not explain seed bank density patterns in *S. sylvatica*. Proximity to the forest edge decreased seed bank density in *M. effusum* and *S. sylvatica*. Increasing canopy cover significantly increased *G. urbanum*'s seed bank density.

Latitudinal effects on seed bank density were recorded in *G. urbanum* and *M. effusum*, with decreasing density towards

Table 1 Effects of environmental and population variables on seed production and seed bank density (number of germinated seedlings) in four herbaceous forest species along a latitudinal gradient from France to Scandinavia (Fig. 1). The direction of the relationship and the results are shown for individual predictor variables (comparison to null model with *region* as random term) and additive predictor variable effects (comparison to model with PC1 as fixed and *region* as random term). Significance of model comparison based on Wald test statistics (χ^2).

	<i>G. urbanum</i>				<i>S. sylvatica</i>					
	Seed production χ^2	P	+/-	Seed bank density χ^2	P	+/-	Seed production χ^2	Seed bank density χ^2	P	+/-
Population size (PC1)	17.2	< 0.001	+	0.6	0.45		214.0	54.1	< 0.001	+
Soil pH	4.6	0.03	+	2.7	0.10		0.1	0.4	0.71	
Soil moisture	0.0	0.93		9.5	0.002	+	0.4	2.0	0.51	
Canopy cover	1.8	0.18		5.9	0.02	+	0.0	0.7	0.85	
Forest edge	2.0	0.16		0.0	0.99		12.3	17.4	< 0.001	-
GDD	0.2	0.64					0.4		0.53	
GDD ₁₀				7.2	0.007	+		0.4		0.52
Temperature range	0.6	0.46		10.5	0.001	-	0.0	0.0	0.90	0.96
Latitude	14.7	< 0.001	-	12.7	< 0.001	-	0.9	0.6	0.34	0.45
Seed production				0.2	0.64			7.1	0.007	+
PC1 + seed production								0.3	0.60	
PC1 + latitude	14.8	< 0.001	-							
PC1 + soil pH	128.0	< 0.001	+							
PC1 + forest edge							17.4	130.0	< 0.001	-

	<i>M. effusum</i>				<i>P. nemoralis</i>					
	Seed production χ^2	P	+/-	Seed bank density χ^2	P	+/-	Seed production χ^2	Seed bank density χ^2	P	+/-
Population size (PC1)	52.6	< 0.001	+	1.8	0.18		49.1	6.2	< 0.001	+
Soil pH	-0.1	0.13		0.3	0.60		0.0	2.3	0.84	
Soil moisture	0.0	0.90		5.5	0.02	+	1.1	3.4	0.30	
Canopy cover	0.0	0.24		0.4	0.54		3.4	0.6	0.06	-
Forest edge	4.2	0.04	+	11.4	< 0.001	-	0.7	1.2	0.42	
GDD	0.0	0.82					0.1		0.77	
GDD ₁₀				0.7	0.41			0.0		0.99
Temperature range	1.6	0.20		6.1	0.01	-	0.1	0.1	0.72	0.79
Latitude	0.1	0.30		5.3	0.02	-	1.7	0.1	0.19	0.77
Seed production				0.4	0.51			0.0		0.93
PC1 + forest edge	2.6	0.11								
PC1 + canopy Cover							11.9	0.3	< 0.001	-
PC1 + soil moisture										0.61

+/- indicates the direction of the relationship between the tested variables; GDD, growing degree days in 2011, calculated from a 5 °C base temperature; GDD₁₀, average growing degree days in the last decade (2001–11); PC1, the first PCA axis, comprehensive population descriptor based on recorded species density, species cover, number of inflorescences and population size. Bold values indicate a significant effect of the predictor variable.

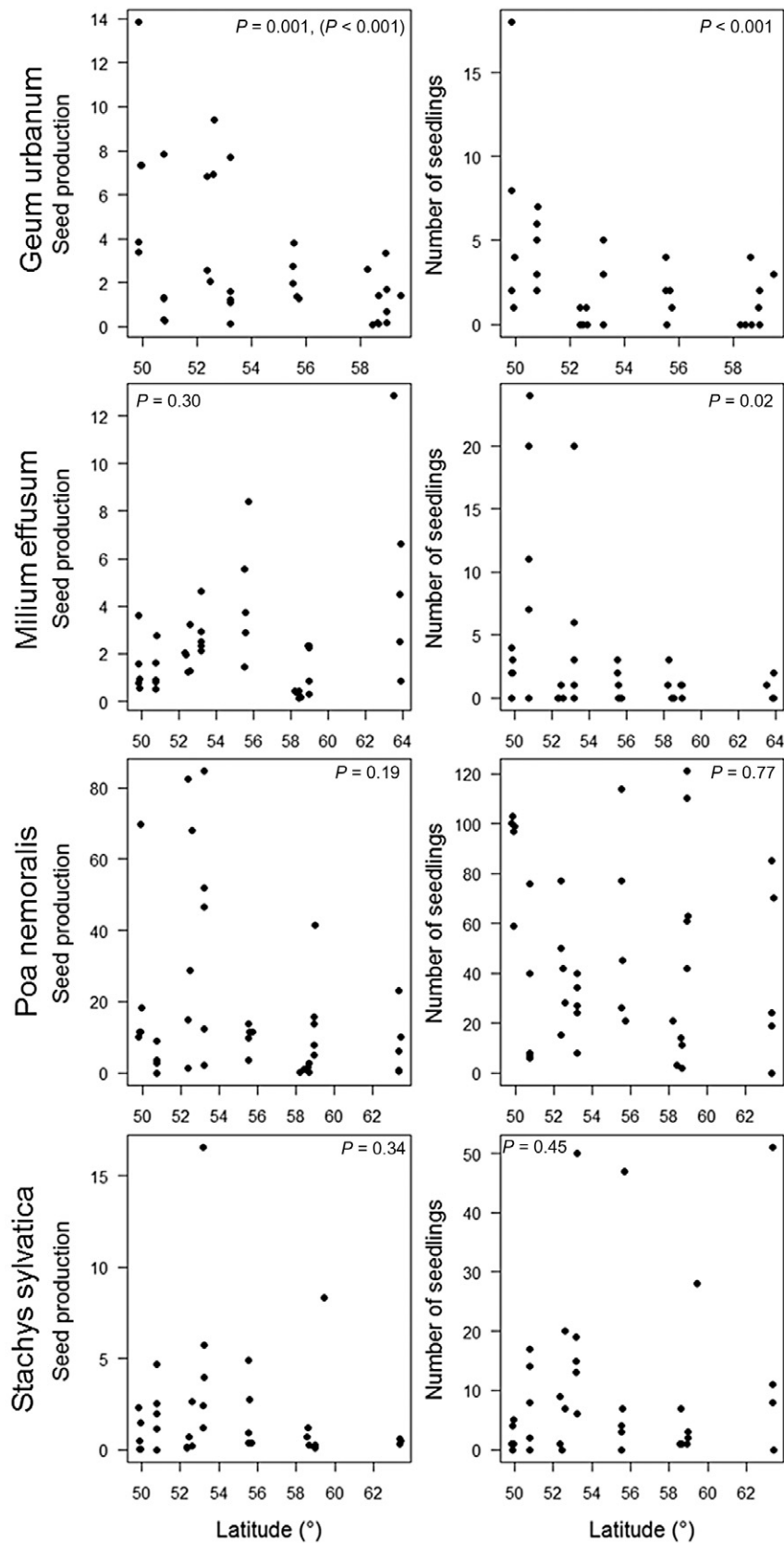


Figure 2 Variation in seed production ($\times 10^3$) and seed bank density (number of germinated seedlings) as a function of latitude. The *P*-value presents the direct significant effect of latitude on seed production or seed bank density, the *P*-value between parentheses presents the significance of latitude after correcting for population size, if both were significant during modelling (see Table 1).

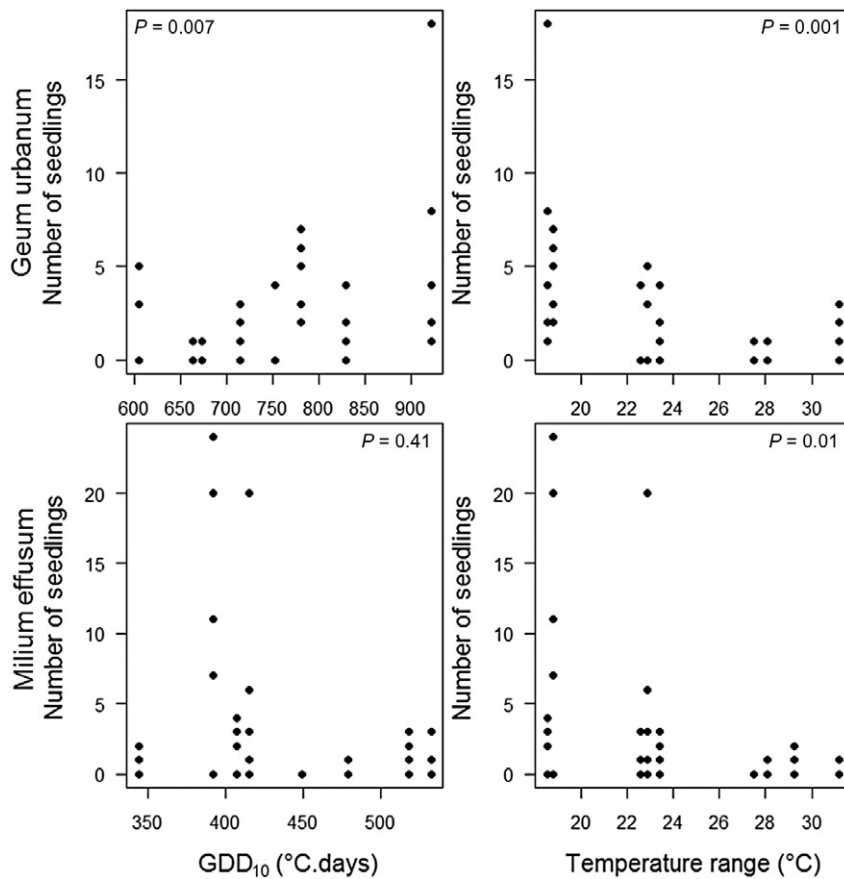


Figure 3 Variation in seed bank density (number of germinated seedlings) as a function of growing degree days (GDD₁₀) and temperature range in *Geum urbanum* and *Milium effusum* (see Table 1).

northern latitudes (Fig. 2). *G. urbanum* seed bank density was higher in warmer regions. As temperature range increased, *G. urbanum* and *M. effusum* seed bank density declined (Fig. 3). Neither temperature range, GDD₁₀ nor latitude explained any variation in seed bank density patterns of *P. nemoralis* or *S. sylvatica*.

DISCUSSION

Climate and seed banks

Large-scale climatic constraints on species' seed bank densities have been shown before in *Calluna vulgaris* (Pakeman *et al.*, 1999) and *Vaccinium* spp. (Hill & Vander Kloet, 2005). However, our large-scale study is the first to link prevailing climatic conditions with the amount of seeds a species banks in the soil. For the four investigated species, two distinct climatic responses emerged: either seed bank density responded strongly to temperature and latitude (*G. urbanum* and *M. effusum*) or seed bank density remained indifferent to latitude and temperature (*P. nemoralis* and *S. sylvatica*, Table 1). Seed banks are highly dynamic entities, with seed bank density being a function of seed input, of storage conditions controlling seed losses and of the innate seed persistence ability of a species (Hill & Vander Kloet, 2005). Despite the complex interactive nature among governing seed bank processes, we present a

strong framework which offers a plausible explanation on the formation of the observed seed bank patterns, by combining and supporting our results with existing knowledge on these individual processes.

We found no proof of climatic effects on total seed production in any of our study species (Table 1; Pigott & Huntley, 1981; Garcia *et al.*, 2000) as the observed latitudinal decline in *G. urbanum* seed production probably related to soil pH (*G. urbanum* seed production vs. latitude: Spearman's rho = 0.42, $n = 35$, $P = 0.02$). The reason for the lack of climatic control might be related to the fact that we assessed total seed production. Total seed production encompasses all seeds, irrespective whether some are viable and germinable, might be aborted or maybe not reach maturity. The two latter mechanisms diminish germinable seed production towards species' range edges (Pigott & Huntley, 1981; Garcia *et al.*, 2000), suggesting climate-independent total seed production and climatic control on germinable seed production (Garcia *et al.*, 2000; De Frenne *et al.*, 2009). In other words, each population along the gradient holds the inherent potential to produce an amount of seeds solely dependent on population size, if weather conditions in a given year are suitable. Any remaining species-dependent variation would then relate primarily to local environmental factors such as light availability or soil pH (Table 1; De Frenne *et al.*, 2009). From a seed bank perspective, it is sensible to focus on total seed production which might be achieved in favourable years (Pigott

& Huntley, 1981), when germinable seed production may approach total seed production. Seed banks have indeed been considered the cumulative result of favourable years with high germinable seed production, when adequate long-term storage of excess germinable seed production compensates for less germinable seed production in 'normal' years (Rees *et al.*, 2002).

Yet despite the climate-independent population control on total seed production in all four species, seed bank patterns still differed markedly between species. In accordance with our hypothesis, *S. sylvatica* and *P. nemoralis* banked more seeds per m² in larger populations, irrespective of any climatic or latitudinal gradients (Table 1). Growing season length decreases with latitude, limiting plant development times and resource allocation to reproduction (Hill & Vander Kloet, 2005). This gradually reduces the likelihood of northerly populations annually producing a large mature seed crop (McKone *et al.*, 1998), which appears more fundamental to seed bank build-up than regular yet smaller germinable seed production in 'normal' years (Rees *et al.*, 2002). Increasing seed survival rates due to improving soil storage conditions may promote seed longevity (Cavieres & Arroyo, 2001; Hill & Vander Kloet, 2005), counteracting the reduced chance of seed inputs. Seed bank density would then primarily relate to population size, independent from climatic variation as observed in *S. sylvatica* and *P. nemoralis* (Table 1), or *C. vulgaris* (Cummins & Miller, 2002). However, cooler temperatures towards the northern range edges likely improve soil storage conditions similarly for all four species, and thus cannot explain that seed bank patterns in *G. urbanum* and *M. effusum* appear to be climatically constrained (Fig. 3; Table 1). Even with a latitudinal pH gradient in total *G. urbanum* seed production, pH did not influence *G. urbanum* seed bank density directly (Table 1). Their northerly decline might therefore be related to either temperature range, accumulated temperature (GDD₁₀) or both (Fig. 3; Table 1). Remaining variation in seed bank density of all four species can still be substantial (Fig. 2, Pakeman *et al.*, 1999), when environmental factors such as soil pH, light quality and quantity or soil moisture steer seed losses through control over germination (Jankowska-Błaszczuk & Daws, 2007) or fungal activity (Pakeman *et al.*, 2011; Mordecai, 2012).

Larger seed banks are more commonly reported in cooler temperate climates (Pakeman *et al.*, 1999; Hill & Vander Kloet, 2005). However, we observed declining seed bank density in *G. urbanum* and *M. effusum* towards cooler climatic conditions without a clear mechanism which could explain this pattern. There are no obvious parallels among life history traits (seed size, seed mass, etc.), phenology or distributional range which could clarify why these paired species responded similarly. Even the SLI, which we specifically selected for, did not prove consistently similar between species sets: *G. urbanum* (SLI = 0.05) and *M. effusum* (SLI = 0.53) versus *P. nemoralis* (SLI = 0.39) and *S. sylvatica* (SLI = 0.31). As the SLI has been critiqued for its unreliability (Saatkamp *et al.*, 2009; Plue *et al.*, 2012), we adapted the seed accumulation index (SAI, Hölzel & Otte, 2004) to re-evaluate longevity in our four species. Using all plots from the full 153 plot dataset where a species occurred, we calculated

the SAI as the ratio of all plots where a species was solely present in the seed bank over all plots where the species was present in the seed bank, vegetation or both. This ratio proved to be 0.07 and 0.03 for respectively *G. urbanum* and *M. effusum* versus 0.15 and 0.20 for respectively *P. nemoralis* and *S. sylvatica*. Thus, *G. urbanum* and *M. effusum* mostly reside in the seed bank only when parent individuals are present. This implies both species possess only limited seed longevity, rendering them heavily reliant upon regular seed inputs to maintain a persistent seed bank (see HilleRisLambers *et al.*, 2005), or at least more so than *P. nemoralis* and *S. sylvatica*. Assuming germinable seed production in all four species to be equally susceptible to climatic factors, the long-term seed longevity in *P. nemoralis* and *S. sylvatica* can more effectively bridge the time between years with high germinable seed production, as such maintaining constant seed bank density. The short-term longevity in *G. urbanum* and *M. effusum* may be too short to bridge such longer unfavourable periods, causing the northerly decline in seed bank density. Hence, the temperature signal picked up in *G. urbanum* and *M. effusum* seed bank density seems to directly relate to climatic control on germinable seed production (Garcia *et al.*, 2000; De Frenne *et al.*, 2009). This is most clear in *G. urbanum*, where the temperature signal implies that more seeds may reach maturity at the southern most sampled latitudes, attaining regular and higher germinable seed inputs into the seed bank (Molau & Shaver, 1997), confirming our rationale on how these seed bank patterns may have formed. Moreover, the lack of population control on *G. urbanum* and *M. effusum* seed bank density (Table 1) may be explained by their limited seed longevity as high seed losses may erase the link between population size and seed bank density.

Ecological consequences of climate-controlled seed bank patterns

Climate sets boundaries to many geographical species distributions (Woodward, 1990), interacting further with life history traits to outline the range edge (Van der Veken *et al.*, 2007). Seed longevity is an important mechanism in explaining wide latitudinal distributions, due to the rescue effect provided by the seed bank (Van der Veken *et al.*, 2007). From our findings, seed banks appear to help plants adapt to increasingly unfavourable climatic conditions (Venable & Brown, 1988; Ooi, 2012), which become most notable towards the range edges. However, this implicitly assumes seed banks to be constant or at least large enough along climatic gradients to facilitate population persistence in suboptimal climatic conditions, where seedling losses during recruitment may become too high to enable successful establishment (Stanton-Geddes *et al.*, 2012). Although climate-independent seed bank patterns in support of the seed banks' rescue effect were confirmed in *P. nemoralis* and *S. sylvatica* (see also Cummins & Miller, 2002), climate seems to set boundaries to seed bank patterns in at least *G. urbanum* and *M. effusum* (cf. Pakeman *et al.*, 1999; Hill & Vander Kloet, 2005). Thus, it appears that seed banks in some species cannot fulfil their functional role towards their distribution edges, exposing the resid-

ing populations more directly to a higher extinction risk caused by climatic stochasticity, due to the increased likelihood of regeneration failure from banked seeds. Whether seed bank patterns may (1) be due to pure climatic control on germinable seed inputs, (2) have become genetically determined or (3) form a combination of both, remains uncertain. However, if the pattern reflects mere direct climatic control, seed bank build-up in these species should track climate change, building up seed banks at higher latitudes due to higher and more regular germinable seed inputs (Molau & Shaver, 1997). Local genetic adaptation is a common, well-documented phenomenon (Atkins & Travis, 2010). Suitable climatic conditions could become too infrequent due to marginal range edge conditions, leading to selection of genotypes which favour vegetative survival (Dorken & Eckert, 2001), reflected by, e.g. higher levels of seed abortion, decreased germinable seed production (Garcia *et al.*, 2000) and lower seed provisioning (De Frenne *et al.*, 2011). This could imply that it may not be unlikely that *G. urbanum* and *M. effusum* seed bank patterns would at least partly be genetically embedded through genetic control over germinable seed production. If the seed bank patterns would persist due to genetic adaptation under climate change, then the low numbers of banked seeds may not fulfil their critical buffering role within the changing population dynamics (Kalisz & McPeck, 1992; Toräng *et al.*, 2010). The lack of ample infusion of new individuals from the seed bank might limit their chances to adopt a demographical regime in equilibrium with any new climatic regime (Toräng *et al.*, 2010), potentially jeopardizing the long-term persistence and survival of such populations. As seed bank patterns show clear interspecific variation in their response to climate, dependent upon the interaction of decreasing germinable seed inputs towards northern latitudes and their seed longevity, not all seed banking plant species may be as well equipped to buffer climatic changes. As the first study linking seed bank density and climatic conditions on this scale, further research should focus on quantifying climatic variation in internal seed bank processes such as seed survival rates or *in situ* seed losses through germination. Then, we can fully appreciate how climate imposes the observed seed bank patterns.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1 Geographical distribution of the number of sampled populations in all four forest herb species along the latitudinal gradient covering six European countries.

Table S2 Effects of latitude, growing degree days (GDD10; 10 year average, 2001–2011) and the temperature range on population size (PC1) of the four herbaceous forest species along the latitudinal gradient, established using Generalized Estimating Equations.

BIOSKETCH

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