The response of forest plant regeneration to temperature variation along a latitudinal gradient

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• *Background and Aims* The response of forest herb regeneration from seed to temperature variations across latitudes was experimentally assessed in order to forecast the likely response of understorey community dynamics to climate warming.
• *Methods* Seeds of two characteristic forest plants (*Anemone nemorosa* and *Milium effusum*) were collected in natural populations along a latitudinal gradient from northern France to northern Sweden and exposed to three temperature regimes in growth chambers (first experiment). To test the importance of local adaptation, reciprocal transplants were also made of adult individuals that originated from the same populations in three common gardens located in southern, central and northern sites along the same gradient, and the resulting seeds were germinated (second experiment). Seedling establishment was quantified by measuring the timing and percentage of seedling emergence, and seedling biomass in both experiments.
• *Key Results* Spring warming increased emergence rates and seedling growth in the early-flowering forb *A. nemorosa*. Seedlings of the summer-flowering grass *M. effusum* originating from northern populations responded more strongly in terms of biomass growth to temperature than southern populations. The above-ground biomass of the seedlings of both species decreased with increasing latitude of origin, irrespective of whether seeds were collected from natural populations or from the common gardens. The emergence percentage decreased with increasing home-away distance in seeds from the transplant experiment, suggesting that the maternal plants were locally adapted.
• *Conclusions* Decreasing seedling emergence and growth were found from the centre to the northern edge of the distribution range for both species. Stronger responses to temperature variation in seedling growth of the grass *M. effusum* in the north may offer a way to cope with environmental change. The results further suggest that climate warming might differentially affect seedling establishment of understorey plants across their distribution range and thus alter future understorey plant dynamics.

Key words: *Anemone nemorosa*, climate change, common garden, growth chambers, latitudinal gradient, local adaptation, *Milium effusum*, plant regeneration, range edges, recruitment, seedling establishment, temperature.

INTRODUCTION

Early life-history stages are among the most critical phases in the life cycle of plants and important determinants of population dynamics. Assessing changes in the regeneration from seed in response to temperature variation is therefore key to forecast the likely response of plant communities to climate warming (Adler and HilleRisLambers, 2008; Hedhly *et al.*, 2009; Shevtsova *et al.*, 2009; Klady *et al.*, 2011; Saatkamp *et al.*, 2011; Walck *et al.*, 2011). Temperature may affect seed set as well as germination rates and timing through direct effects on the mother plants and seeds (Hedhly *et al.*,

2009), and warming may also affect the performance of the resulting seedlings. To maximize reproductive success, plants avoid exposure to unfavourable environmental conditions by shifting seedling emergence both spatially and temporally (Probert, 2000; Fenner and Thompson, 2005). The timing of seed germination and seedling emergence, as well as the seedling emergence rate are important estimates to assess population dynamics and persistence. Our understanding of the responses of early life-stages to climatic change factors, however, is still limited (Hovenden *et al.*, 2008; De Frenne *et al.*, 2009; HilleRisLambers *et al.*, 2009).

The response of plant regeneration to temperature variation likely differs across the distribution range of species. Alpine or northern subarctic populations are subject to higher local environmental variability, for example in terms of microclimatic conditions, than populations of the same species growing in a temperate climate or lowland region (Armbruster *et al.*, 2007; Scherrer and Körner, 2010; Graae *et al.*, 2012). Accordingly, adult plant individuals in northern populations are generally more plastic in response to environmental heterogeneity than southern populations (Ghalambor *et al.*, 2006; Lacey *et al.*, 2010). Furthermore, vegetative and reproductive fitness, as well as population densities, generally decrease from the centre to the edge of the distribution range in many plant species (e.g. Jump and Woodward, 2003; for a recent review, see Sexton *et al.*, 2009). An intraspecific shift from sexual to vegetative reproduction towards the north has also been found (Mooney and Billings, 1961; Houle and Babeux, 1994; Dorken and Eckert, 2001). Hence, forecasting plant-community responses to climate change requires understanding variation in early life-history stages in response to temperatures across the distribution range.

Plants with different phenologies and life forms generally respond differently to temperature variation (Walker *et al.*, 2006; Sherry *et al.*, 2007; De Frenne *et al.*, 2009, 2011a, b; Elmensdorf *et al.*, 2012). Sherry *et al.* (2007), for instance, showed that plants flowering before the summer heat peak in a North American tallgrass prairie tended to advance their phenology in response to warming, whereas later-flowering plants delayed their phenology. De Frenne *et al.* (2011a) also suggested that climate warming may have a stronger influence on spring-flowering understorey plants than on summer-flowering plants. It has also been shown that grasses generally respond more strongly than forbs to resource alterations such as nitrogen enrichment and temperature increases (Dormann and Woodin 2002; Walker *et al.*, 2006; De Schrijver *et al.*, 2011; Klady *et al.*, 2011).

In spite of their importance for forest biodiversity and ecosystem functioning (Gilliam, 2007), there have been few experimental tests of the effects of climate change on herbaceous understorey plants. Svenning and Skov (2006) showed that the bioclimatic envelope of many herbaceous understorey plants might shift dramatically towards northern latitudes in Europe in the near future. Whether the future distribution of understorey plants will ever match their predicted bioclimatic envelope is doubtful, with potentially serious consequences for the long-term persistence of these species. Investigating the response of understorey plants to rising temperatures is thus highly relevant.

In this study, we assessed the regeneration from seed of two forest understorey plant species along a 1900- and a 2300-km-long latitudinal gradient. The study species were the spring-flowering geophyte *Anemone nemorosa* and the early summer-flowering grass *Milium effusum*. Divergent responses of these species are to be expected due to their contrasting phenology (early vs. later-flowering) and life forms (forb vs. grass) (De Frenne *et al.*, 2011a).

First, seeds of both species were wild-collected from plants growing along the latitudinal gradient and placed in growth chambers under three spring-temperature scenarios. By raising progeny in different spring-warming scenarios, the response of seedling establishment to spring temperature can be examined. Not only is spring a very important period for understorey plant growth and recruitment, but the IPCC (2007) also predicted that early-season warming in northern Europe will be greater than summer and autumn warming (relative increase of $+4{\cdot}3\,°C$ in December–February and $+3{\cdot}1\,°C$ in March–May vs. $+2{\cdot}7\,°C$ in June–August and $+2{\cdot}9\,°C$ in September–November by 2080–2099 compared with 1980–1999). Because seeds from this first experiment were wild-collected and directional selection may have favoured plant ecotypes adapted to local environmental conditions (Leimu and Fischer, 2008), we also performed a common-garden experiment. Common-garden experiments offer the opportunity to relocate ecotypes from a colder region into a warmer region with a longer growing season and can be used to study local adaptation and growth responses to temperature variation across a species' range (Haggerty and Galloway, 2011; Offord, 2011). Therefore, in a second experiment, we reciprocally transplanted adult individuals that originated from the same populations as in the first experiment in three common gardens (i.e. similar maternal environment) located into a southern, central and northern site along the same gradient (De Frenne *et al.*, 2011a) and used the seeds produced in the common gardens. Seedling establishment was quantified by measuring the rate and percentage of seedling emergence, and seedling biomass in both experiments.

Specifically, we tested the following hypotheses: (*a*) emergence percentages and seedling growth are lower in plants from northern peripheral origin than from southern origin near the core of the distribution range; (*b*) seedling establishment of understorey plants with contrasting phenology and life form is differentially affected by temperature along a latitudinal transect; (*c*) seedling establishment is locally adapted, i.e. seedlings perform better when the mother plant has been transplanted closer to the home site.

MATERIALS AND METHODS

Study species

Two widespread European understorey plants were selected for this study: *Anemone nemorosa* (Ranunculaceae) is a spring-flowering geophyte while *Milium effusum* (Poaceae) is an early summer-flowering hemicryptophytic grass. The northern distribution edge of *A. nemorosa* is situated around 67 °N whereas *M. effusum* is distributed as far as 71 °N. Their southern range limits are around 38 – 40 °N (Hultén and Fries, 1986). The flowers of *A. nemorosa* are hermaphroditic and pollinated by insects, and selfing sometimes occurs. The species also propagates clonally with rhizomes. *Milium effusum* is

wind pollinated (Tyler 2002), and also develops short stolons for re-sprouting. Regeneration from seed is the most important means of population persistence and spread for both species (Brunet and von Oheimb, 1998; Tyler, 2002).

Latitudinal gradient and in situ *seed collection (first experiment)*

In situ seeds were collected in 2008 in seven (*A. nemorosa*) and eight (*M. effusum*) regions located along an approx. 1900-km (*A. nemorosa*) to 2300-km (*M. effusum*) latitudinal gradient from northern France via Belgium and Germany to northern Sweden (Fig. 1). Mean air temperatures between March and July 2008 were 12·8 °C in the southernmost (Amiens) and 6·6 °C and 2·7 °C in the northernmost (Umeå and Abisko) populations of *A. nemorosa* and *M. effusum*, respectively. More detailed temperature and growing degree-hour data along the latitudinal gradient are provided in De Frenne *et al.* (2011b). We randomly selected two populations of each species in each region, resulting in a total of 14 *A. nemorosa* and 16 *M. effusum* populations. Within each

FIG. 1. Map of northwestern Europe showing the latitudinal gradient along which seeds were sampled *in situ* in natural *Anemone nemorosa* and *Milium effusum* populations (both circles and squares; excluding Abisko for *A. nemorosa*) and the common gardens (squares) where *ex situ* seeds were collected. Two populations per region were sampled which resulted in 14 *A. nemorosa* and 16 *M. effusum* populations.

region, the populations were selected within an area of approx. 20×20 km^2. All populations occurred in deciduous forests with no major recent disturbances in terms of management, grazing, etc. Within each population, the seeds of 15 randomly chosen individuals were collected at the time of seed maturity and pooled. In both species, a random subsample of 50 seeds was weighted per seed lot, and the mean seed mass was determined as the 50-seed mass divided by 50. The region- and species-specific seed collection dates are shown in the Appendix.

Ex situ *seed collection (second experiment)*

Adult individuals (i.e. rhizome fragments in *A. nemorosa* and whole root clumps in *M. effusum*) were collected in each of the natural populations along the latitudinal gradient between May and August 2008 and reciprocally transplanted in three common-garden sites near Gontrode (Belgium), Alnarp (southern Sweden) and Umeå (northern Sweden) in September 2008 (Fig. 1). Hence, the maternal plants for the *ex situ* experiment originated from the same populations as used for the *in situ* study. The adult individuals were transplanted in 1·5-L pots (four replicate pots per population and per species in each common garden) filled with standard potting soil. The potting soil consisted of a 2 : 1 volume mixture of peat and coconut fibres that was assumed to resemble litter-rich forest soil and had the following characteristics: pH-KCl of 5·5; ammonium acetate-EDTA extractable P of 0·35 mg kg^{-1} dry soil and KCl extractable NO_3- of 2·68 mg kg^{-1} dry soil. Moreover, a $15-8-11-2·5$ N : P : K : Mg fertilizer containing trace elements (Osmocote Exact Lo.Start 16–18M; Scotts International BV, Geldermalsen, The Netherlands) was added at an application rate of 6 g fertilizer L^{-1} potting soil at the beginning of the experiment. The same pots, potting soil, fertilizer and experimental design were used in the three common gardens. The pots were then placed in a randomized design in forest ecosystems characteristic of the region where the common garden was established (in terms of tree-species composition and over-storey canopy cover). More details on the design of the common-garden sites and detailed microclimatic data are provided in De Frenne *et al.* (2011a).

The *ex situ* seeds were then collected from the transplanted mother plants in 2009 in each of the three common-garden sites at the time of seed maturity; again, the exact collection dates were site-dependent (Appendix), seeds were pooled per population $\times$ common garden combination and seed mass was determined as described above. *Anemone nemorosa* had to be omitted from the *ex situ* analyses because individuals produced no or only few seeds in the common gardens in 2009 (of 56 individuals in each common garden, only eight produced seeds in Belgium, one in southern Sweden and four in northern Sweden).

Growth-chamber experiments: seed sowing and measurements

The seeds were germinated in temperature- and light-controlled growth chambers. The *in situ* and *ex situ* seeds were handled in exactly the same way. Seeds of *A. nemorosa* were stored in moistened white sand at room temperature

between seed collection and the start of the experiment (for approx. 12 weeks) to avoid desiccation and viability loss (Ali *et al.*, 2007). Distilled water was added when needed to the sand-seed mixture. Seeds of *M. effusum* were stored at room temperature until sowing; short-term desiccation poses no threat to the viability of its seeds (Thompson, 1980). On 1 Sepember 2008 (*in situ* seeds of both species) and 1 September 2009 (*ex situ* seeds of *M. effusum*), the seeds were sown in containers filled with potting soil (same soil–fertilizer mixture as used in the common gardens). The containers were subdivided into 28 cells of 17 cm^3 each. In each cell, ten seeds were sown. Each population (2008) and population × common garden combination (2009) was randomly assigned to a cell (seven replicates per population and per treatment). The containers were then placed in growth chambers (Sanyo MLR-351 Versatile Environmental Test Chamber; Osaka, Japan) and exposed to the same seasonal cycle in both years (starting on 1 September): (*a*) autumn simulation at 10 °C for 8 weeks (continuous light); (*b*) winter simulation at 2 °C for 11 weeks (8 h light, 16 h darkness); (*c*) early spring simulation at 10 °C for 2 weeks (8 h light, 16 h darkness).

In the *in situ* experiment, the containers were then (after the 2 weeks at 10 °C mentioned above) randomly assigned to three different growth chambers for 6 weeks representing three possible spring scenarios across the species' range: cool (10 °C), intermediate (15 °C) or warm (20 °C) with 12 h light and 12 h darkness in all cases. In the *ex situ* experiment, the containers were placed in a growth chamber with an intermediate 15 °C spring scenario (12 h light, 12 h darkness) for 6 weeks. The number of growing degree-hours >5 °C at the end of the experiment (following Lindsey and Newman, 1956) calculated for the three scenarios amounted to 6960, 12240 and 17520 °C-hours, respectively. These values are within the range of growing degree-hours in the species' natural habitats between northern France and northern Sweden (seed maturity of *A. nemorosa* and *M. effusum* occurs at approx. 8000–12 000 °C-hours and approx. 12 000–18 000 °C-hours, respectively; De Frenne *et al.*, 2011*b*). Thus, provenances were grown in environments similar to their natural habitats. Photosynthesis photon flux density in the centre of the growth chambers was always set at 112 μmol m^{-2} s^{-1} during light and at 0 μmol m^{-2} s^{-1} during darkness. In both experiments, the containers were rotated weekly within each growth chamber and watered as necessary.

The number of seedlings (visible emerged shoot) was recorded weekly from the start until the end of the experiment. On 10–12 March 2009 (*in situ* seeds) and 11–12 March 2010 (*ex situ* seeds) the final emergence percentage (calculated as the number of seedlings at the end of the experiment divided by the original seed input) was determined and their above-ground biomass was harvested. All biomass samples were dried for 48 h at 60 °C and weighed. Subsequently, the above-ground biomass per cell was divided by the number of seedlings to determine the biomass per seedling. Additionally, in the case of *A. nemorosa*, above-ground and below-ground biomass was separated for one randomly chosen seedling per cell and dried as above to determine below-ground biomass and root : shoot ratio (in the case of *M. effusum*, it was impossible to determine below-ground biomass due to difficulties of

separating fine roots and potting soil). Finally, the mean emergence time (MET) was calculated as (adapted from Deines *et al.*, 2007; Milbau *et al.*, 2009):

$$\text{MET} = \sum_1^i (n_i t_i)/N$$

where n_i is the number of emerged seedlings within consecutive time intervals, t_i is the time between the beginning of the experiment and the end of a time interval (in weeks), and N is the final number of emerged seedlings.

Data analysis

The effects of the latitude of origin and temperature treatment (*in situ*) or of the latitude of origin and common-garden site (*ex situ*) on the seedling traits (i.e. emergence percentage, mean emergence time, and biomass and root : shoot ratio) were analysed with linear mixed-effect models, using the *lme*-function of the *nlme*-library in R 2·13·0 (R Development Core Team, 2011). To account for potential maternal effects of seed mass on seedling establishment (e.g. Moles and Westoby, 2002), seed mass data were included in the models as covariate. 'Region' and 'population' nested within 'region' were added to the models as random-effect terms to account for potential autocorrelation of populations from the same region and replicates within populations. The effects of the latitudinal home-away distance [i.e. the absolute value of the latitudinal difference between the latitude of origin (L_O) and the latitude of the common-garden transplant site (L_{CG}); i.e. $\Delta Latitude = |L_O - L_{CG}|$] were analysed with a similar mixed-effect model. Emergence percentage data were arcsine square-root transformed, and above-ground biomass data of *M. effusum* and below-ground biomass and root : shoot ratio data of *A. nemorosa* were log$_{10}$-transformed prior to the analyses.

RESULTS

In the *in situ* experiment, both the emergence percentage (significant only for *M. effusum*) and above-ground seedling biomass (for both species) decreased with increasing latitude of seed origin (Table 1 and Fig. 2) indicating lower seed quality and seedling performance for individuals of northern origin. Rising spring temperatures consistently increased the above-ground seedling biomass of both species, the below-ground biomass and root : shoot ratio of *A. nemorosa*, and decreased and (marginally) increased the emergence time in *A. nemorosa* and *M. effusum*, respectively (Table 1). Temperature did not consistently affect emergence percentages in *A. nemorosa* (Table 1 and Fig. 2A). Significant interaction terms were present for *M. effusum*, indicating a differential response to temperature depending on the latitude of seed origin (Table 1). More specifically, the above-ground seedling biomass increased more with increasing temperature in *M. effusum* seedlings from northern than from southern origin (Fig. 2F). No such pattern could be seen in *A. nemorosa*. Seed mass significantly increased the biomass of *A. nemorosa* seedlings and the emergence time of *M. effusum* seedlings (Table 1).

TABLE 1. *The effects of the latitude of seed origin and spring temperature treatment on seedling traits of (A)* Anemone nemorosa *and (B)* Milium effusum *in the* in situ *experiment*

	Latitude		Temperature		Latitude × temperature		Seed mass	
	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value
(A) *A. nemorosa*								
Emergence (%)	5	0·91 n.s.	267	↕7·26**	267	0·55 n.s.	6	2·10 n.s.
Emergence time	5	0·02 n.s.	200	↓5·38**	200	2·25 n.s.	6	0·54 n.s.
Above-ground biomass	5	↓25·48**	200	↑3·93*	200	0·12 n.s.	6	↑7·95*
Below-ground biomass	5	4·05 n.s.	196	↑15·98***	196	1·80 n.s.	6	↑4·89 (*)
Root : shoot ratio	5	↑5·15 (*)	199	↑21·45***	199	0·80 n.s.	6	0·01 n.s.
(B) *M. effusum*								
Emergence (%)	6	↓23·29**	334	1·05 n.s.	334	3·46*	7	2·74 n.s.
Emergence time	6	0·70 n.s.	328	↑2·70 (*)	328	1·74 n.s.	7	↑18·85**
Above-ground biomass	6	↓7·23*	324	↑34·98***	324	28·02***	7	1·35 n.s.

Seed mass was included as a covariate in each of the models.

Results from linear mixed-effect models with 'region' and 'population' nested within 'region' as random effects. n.s., $P > 0.1$; (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. d.f., Denominator degrees of freedom. Directions of the effect are reported if $P < 0.1$: ↓ and ↑ indicate a negative and positive effect, respectively. In the case of the temperature scenarios, ↕ specifies mixed effects (i.e. a negative or positive effect for the 15 °C or 20 °C scenario compared with the 10 °C scenario).

In the *ex situ* experiment, the biomass of *M. effusum* seedlings decreased significantly with the latitude of seed origin in each of the three common gardens; similar to the results of the *in situ* experiment, above-ground seedling biomass significantly decreased from 52 ± 10 mg in the southern populations to 12 ± 2 mg in the northernmost populations (Table 2A and Fig. 3). Conversely, the common-garden transplant site of the mother plant significantly affected seed quality and seedling performance in terms of emergence and above-ground biomass of *M. effusum*. Across all provenances, the above-ground biomass in seedlings where the mother plant was grown in the southern transplant sites was lower than the biomass in seedlings where the mother plant was grown in the northern sites (Table 2A and Fig. 3C, F, I). The significant interaction between common-garden site and latitude of origin for the emergence percentage and timing again indicates differential behaviour in each transplant site depending on the latitude of origin (Table 2A). The emergence percentage was significantly higher for the seeds produced by the mother plants that were transplanted closer to the home site (i.e. with lower Δ*Latitude*), suggesting local adaptation (Table 2B and Fig. 3A, D, G). Conversely, the above-ground seedling biomass increased with increasing Δ*Latitude* (Table 2B). Seed mass positively affected the emergence percentage of *M. effusum* in the second experiment (Table 2).

DISCUSSION

By applying a combination of raising progeny in different spring-warming scenarios and using seeds collected from adult individuals that were reciprocally transplanted in three common gardens, we were able to assess the effects of the maternal environment as well as direct temperature effects on the seedling establishment of the characteristic understorey plants *Anemone nemorosa* and *Milium effusum* along a latitudinal gradient. We found decreasing emergence percentages and seedling growth from the centre to the northern edge of the distribution range for both species. Furthermore, elevated spring temperatures had positive effects on the growth of the seedlings which suggests that spring warming might enhance seedling growth and, in turn, improve seedling survival and plant regeneration. Biomass of *M. effusum* seedlings from northern origin increased more with increasing spring temperature than in those from southern origin. *Milium effusum* populations close to the range edge thus seemed to respond more strongly to variation in temperature. Finally, the emergence percentage decreased with increasing home-away distance in seeds from the transplant experiment, confirming our hypothesis and suggesting that the maternal plants were best adapted to their home environment.

The emergence percentage and seedling biomass of the study plants decreased in peripheral populations with colder climate compared with southern populations situated within the core of the distribution range. This is consistent with the findings obtained for many other plants (e.g. Jump and Woodward, 2003; Sexton *et al.*, 2009) and might be one of the causes for the current range limits observed in these species. Reduced emergence percentages and seedling growth in northern peripheral populations may reflect that northern plants tend to invest more in clonal reproduction (e.g. Dorken and Eckert, 2001) and possibly put less effort in, for instance, seed production. This is partly supported by our finding of an increasing root : shoot ratio towards the north in *A. nemorosa*. Since the decrease in seedling performance was generally unrelated to seed-mass differences, possibly a latitudinal decline in seed nutrient concentrations forms the basis for the reduced seedling growth for northern provenances. It has previously been found that the seed nitrogen concentration in *A. nemorosa* more than doubled between plants growing at 63·8°N and plants growing at 49·8°N (De Frenne *et al.*, 2011c) which may account for the decreasing performance of northern seedlings, irrespective of seed mass effects. Hence, further research could aim at disentangling whether decreasing sexual reproductive fitness from the centre to the edge of the distribution range of plant species (Dorken and Eckert,
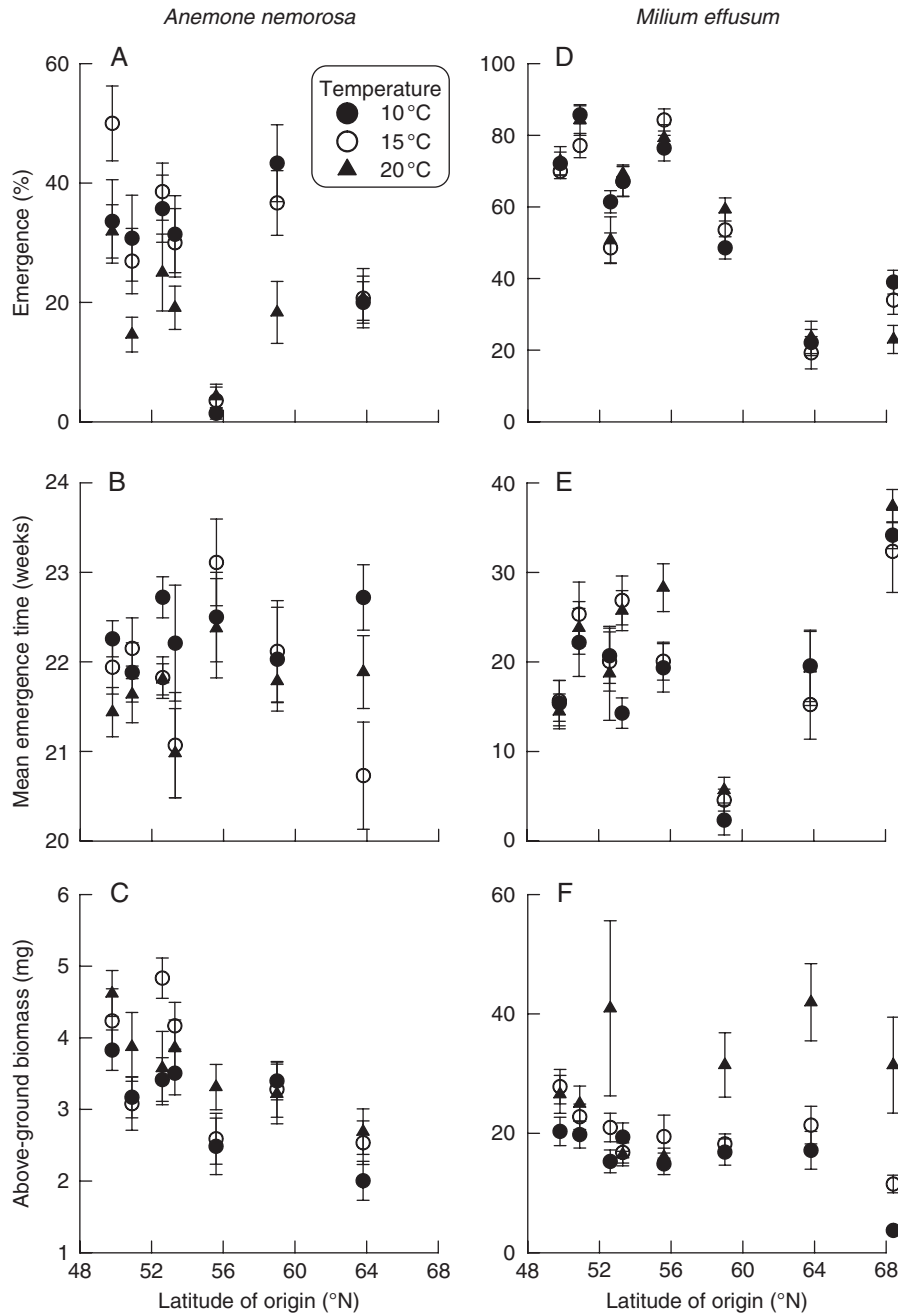
FIG. 2. Relationships between seedling traits and latitude of origin of seeds collected in natural populations of the forest herbs *Anemone nemorosa* (left; A–C) and *Milium effusum* (right; D–F); the seeds were exposed to three different spring-temperature treatments (10 °C, 15 °C, 20 °C, as indicated). The data were averaged across the two populations within a region (only for this figure, not for statistical analyses). Bars denote s.e.

2001) might be caused by differential seed provisioning strategies within species. We believe that intraspecific differences in dormancy are most likely not biasing the recruitment success of these study species in the context of the present study. First, *A. nemorosa* seeds from different provenances germinate very consistently at cooler temperatures following warm stratification (Mondoni *et al.*, 2008). Secondly, *M. effusum* seeds collected in exactly the same populations and year, and sown under field conditions in forests along a latitudinal gradient still showed lower

seedling emergence with increasing latitude of seed origin 3 years after sowing (De Frenne *et al.*, 2011a) which suggests that the pattern of northwards decreasing seedling emergence in the growth chambers likely reflects patterns observed in the field.

Apart from the seedling emergence percentage, the timing of seedling emergence is also a key factor in the potential reproductive success of plants, especially in terms of the predicted changes in phenology across the globe. Although we found no significant linear latitudinal relationship in

TABLE 2. *Effects of (A) the latitude of origin of the mother plant, common-garden transplant site and their interaction and (B) the latitudinal home-away distance (i.e. the absolute value of the latitudinal difference between the latitude of origin and the latitude of the common-garden transplant site; ΔLatitude), common-garden transplant site and their interaction on seedling traits of* ex situ *seeds of* Milium effusum *collected in three common-garden transplant sites located along a latitudinal gradient*

(A)

	Latitude		Common garden		Latitude × common garden		Seed mass	
	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value
Emergence (%)	5	0·40 n.s.	151	↓15·41***	151	11·77***	151	↑24·65***
Emergence time	5	0·57 n.s.	147	↓10·76***	147	12·07***	147	0·01 n.s.
Abovegr. biomass	5	↓15·01*	144	↑10·36***	144	2·02 n.s.	144	0·77 n.s.

(B)

	ΔLatitude		Common garden		*ΔLatitude* × common garden		Seed mass	
	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value
Emergence (%)	150	↓53·72***	150	↓4·92***	150	1·35 n.s.	150	↑8·32**
Emergence time	146	0·58 n.s.	146	↓14·11***	146	6·48**	146	0·71 n.s.
Abovegr. biomass	143	↑6·35*	143	↑5·79**	143	0·72 n.s.	143	0·57 n.s.

Seed mass was included as a covariate in each of the models.

Results from linear mixed-effect models with 'region' and 'population' nested within 'region' as random effects. n.s., $P > 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. d.f., Denominator degrees of freedom. The direction of the effects is reported if $P < 0.1$: ↓ and ↑ indicate a negative and positive effect, respectively. In the case of the effect of the common gardens, ↓ and ↑ indicate a northward decrease and increase, respectively.

emergence time in the first, i.e. *in situ*, experiment, the northernmost *M. effusum* seedlings still emerged approx. 20 weeks after the southernmost seedlings. Thompson (1980) already suggested that *M. effusum* seeds may germinate in autumn in southern populations, whereas seeds stay dormant during autumn and winter in northern Europe. Hence, delayed seedling emergence in *M. effusum* until the next spring seems to be an adaptive strategy in northern populations to avoid the harsh winter conditions, whereas growing conditions in the south are favourable enough to build-up a growth advantage before the winter. Secondly, although spring warming shortened the emergence time of *A. nemorosa* and thus has the potential to lengthen the growing season and positively affect the regeneration success, all seedlings emerged after the winter treatment and showed limited geographical variation. An expansion of the length of the first growing season of *A. nemorosa* seedlings might provide them with an important survival advantage (see also Milbau *et al.*, 2009).

The emergence percentage significantly decreased with increasing distance between the latitude of origin and the transplant site (Δ*Latitude*), which suggests that the maternal plants were best adapted to their home environment. This may have been related to the lower adult plant performance and seed size when the maternal plants were transplanted farther from their home site (see also De Frenne *et al.*, 2011*a*). Populations of widespread plant species generally display adaptation to the local site conditions due to divergent natural selection (Leimu and Fischer, 2008). The transgenerational local adaptation observed here cannot be attributed to soil properties or competitive interactions because all mother plants were grown principally without competition in exactly the same soil in the three common gardens. Rather, this pattern must be due to adaptation to other local factors,

including climate, photoperiod or canopy cover (De Frenne *et al.*, 2011*a*). Yet, the seedling biomass was generally the highest for the southern provenances, irrespective of the transplant site of the mother plant, and thus the maternal environmental effect seemed to disappear during the development of the progeny (Teng *et al.*, 2009).

In conclusion, we showed that both the maternal environment as well as the temperature directly experienced by the offspring differentially affected the seedlings across the distribution range of the two study species. Spring warming had a positive effect on the growth of understorey seedlings. The larger biomass growth response of seedlings of the grass *M. effusum* originating from northern populations might enable these populations to cope with more extreme microclimatic heterogeneity in the northern subarctic, than in temperate regions (e.g. Armbruster *et al.*, 2007; Lacey *et al.*, 2010; Graae *et al.*, 2012) and possibly with the climatic changes projected for the coming decades (Hof *et al.*, 2011; Walck *et al.*, 2011). Other studies investigating seed production, germination and/or seedling establishment of plants were inconclusive, showing both positive (Milbau *et al.*, 2009; Klady *et al.*, 2011) and negative (Hovenden *et al.*, 2008; Shevtsova *et al.*, 2009) effects of rising temperatures on sexual plant reproduction. Of course, the impact of changing temperatures on germination and seedling establishment also depends on other factors including water and nutrient availability, herbivory and competition (Ali *et al.*, 2007; Shevtsova *et al.*, 2009; reviewed by Walck *et al.*, 2011). We demonstrated that the question of whether rising temperatures will favour or constrain plant regeneration in the future is not solely a species-dependent issue. Seedling establishment may also show wide intraspecific variation across broad geographical areas. Hence, these phenomena should be incorporated into predictions of
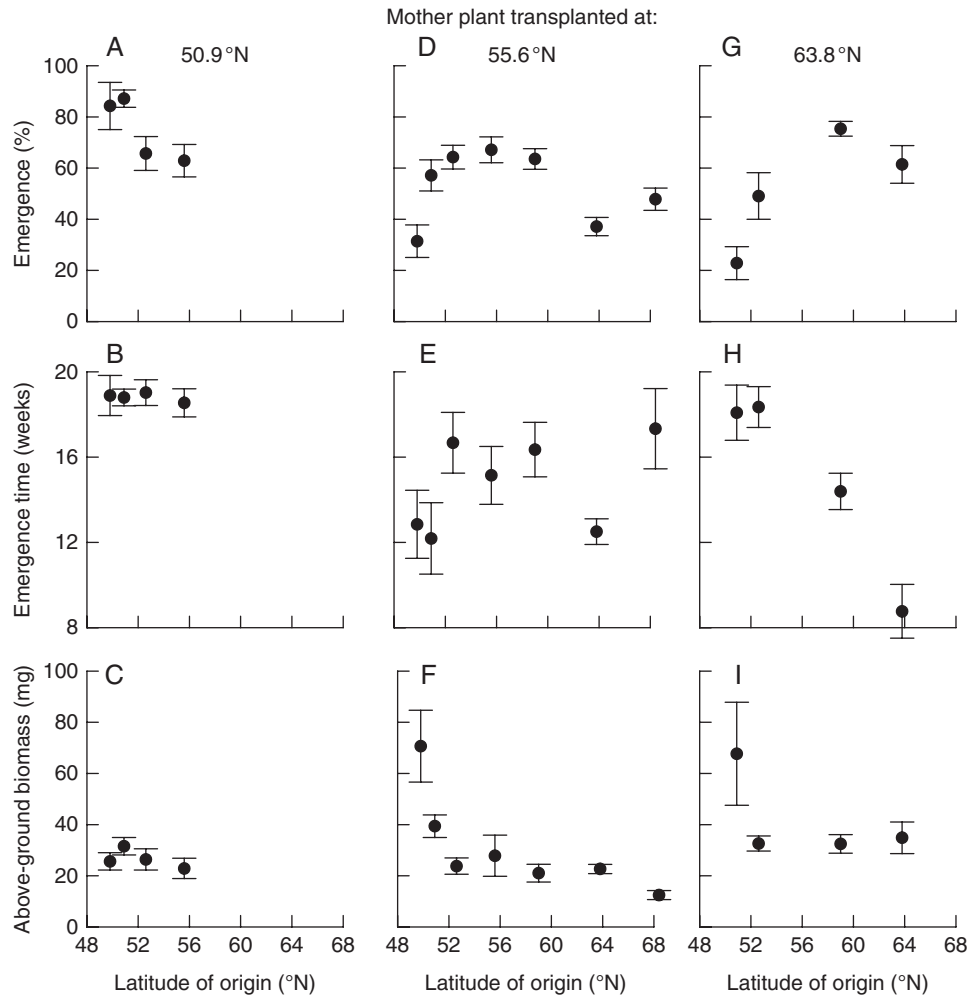
Mother plant transplanted at:

FIG. 3. Seedling traits of the understorey forest plant *Milium effusum* along a latitudinal gradient: (A–C) *ex situ* collected seeds from the southernmost common garden (Belgium, 50·9°N); (D–F) *ex situ* collected seeds from the central common garden (southern Sweden, 55·5°N); (G–I) *ex situ* collected seeds from the northernmost common garden (northern Sweden, 63·8°N). The data were averaged across the two populations within a region (only for this figure; not for statistical analyses). Bars represent s.e.

future population dynamics across species' ranges. Our results further indicate that climate warming might affect the seedling establishment and regeneration success of plants across their distribution range and thus differentially change understorey plant dynamics across Europe in the near future.

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APPENDIX

Seed collection dates (first, i.e. *in situ* experiment: 2008; second, i.e. *ex situ* experiment: 2009; in Julian days) along the latitudinal gradient.

Region	City	Latitude (°N)	Longitude (°E)	Seed collection dates *in situ*		Seed collection dates *ex situ* M. effusum[†]
				A. nemorosa	*M. effusum*	
France	Amiens	49·8	2·1	140	157	
Belgium	Gontrode	50·9	3·8	130	157	162
NE Germany	Potsdam	52·6	13·0	136 and 137	162 and 164	
NW Germany	Bremen	53·3	9·2	142	165	
S Sweden	Alnarp	55·6	13·3	141	170 and 171	173
C Sweden	Stockholm	59·0	17·5	157	198	
N Sweden	Umeå	63·8	20·0	176	187 and 198	195
N Sweden	Abisko	68·4	18·8	–*	235 and 239	

* *A. nemorosa* has no natural occurrences near Abisko.
[†] *A. nemorosa* had to be omitted from the *ex situ* analyses because individuals produced only few seeds in the common gardens in 2009 (see text).