ABSTRACT

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

INTRODUCTION

The sexual reproductive phase from gamete development to seedling emergence is one of the most critical stages in the lifecycle of plants (Harper 1977). The success of an individual in passing through this phase is greatly dependent on the maternal environment (Roach & Wulff 1987; Donohue 2009). Effects of the maternal environment on seed set, maturation, predation, germination and seedling survival are all well documented (e.g. Meunier et al. 2007; De Frenne et al. 2009, 2010b). With respect to seed traits, however, seed nutrient concentration is often overlooked, although for multiple reasons it deserves as much attention as, for example, seed size (Kitajima 2002): the seed nutrient concentration has important implications for several processes within the sexual reproductive phase, including interactions with nitrogen-limited seed predators (Mattson 1980; Jolivet & Bernasconi 2006; De Menezes et al. 2010), efficiency of seed dispersal (Boulay et al. 2006; Delatte & Chauberie 2008) and seedling performance (Parrish & Bazzaz 1985; Stock et al. 1990; Naegle et al. 2005). In addition, global warming is likely to affect plant reproduction in many ways (Hedhly et al. 2009), but current knowledge about the effects of global warming on the number, mass and nutrient concentration of seeds in natural settings is still very limited (Hovenden et al. 2008; De Frenne et al. 2009, 2010b; Verheyen et al. 2009).

One approach to assess the impact of the maternal environment on plant performance in general and on seed

Keywords
Collection date; latitude; nutrient stoichiometry; seed nitrogen; seed predation; seed provisioning; sexual reproduction; wood anemone.

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RESEARCH PAPER

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

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

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

Here, we investigate latitudinal variation in seed nutrient concentrations of a widespread understorey forest herb in Europe. Anemone nemorosa L. (Ranunculaceae) may be considered a model species for slow-colonising perennial understorey forest herbs (De Frenne et al. 2010b). Given its wide distribution (Hultén & Fries 1986) and high degree of phenotypic plasticity (Shirreffs 1985; De Frenne et al. 2010a), A. nemorosa is potentially an excellent species to study variation in seed nutrients. Given the presence of a gradient in seed nutrient concentration, it also offers the possibility to examine effects of growing degree-hours >5 °C (cf. growth rate hypothesis) and soil characteristics (cf. geochemical hypothesis) relative to latitude itself. Therefore, two main research questions were formulated. (i) Is there a latitudinal gradient in seed nutrient concentration and stoichiometry of A. nemorosa? (ii) What is the relative importance of growing degree-hours, soil characteristics and latitude on seed nutrient concentration and stoichiometry? We further consider possible causes, other than temperature and soil characteristics, and discuss consequences of the possible presence/absence of a latitudinal gradient in seed nutrients.

MATERIALS AND METHODS

Study species

Anemone nemorosa L. (wood anemone) is a widespread perennial herb, mainly occurring in the understorey of temperate deciduous forests (Shirreffs 1985). The species occurs from northern Spain to northern Scandinavia, and from Ireland to western Russia (Hultén & Fries 1986). Shoots emerge in early spring and flowering starts a few weeks later. Flowers (one per shoot) are typically white, hermaphrodite, largely self-incompatible and pollinated by insects, although selfing does occur (Müller et al. 2000). Each ramet produces on average 18.1 ± 0.6 (±SE) achenes (referred to as ‘seeds’ hereafter), with a mature seed mass of 1.96 ± 0.04 mg (De Frenne et al. 2010b). Seeds are gravity-, ant- and slug-dispersed (Delatte & Chabrerie 2008; Türke et al. 2010), show epicotyl morpho-physiological dormancy (Mondoni et al. 2008) and germination rates ranging from 5% to 100% across its distribution range (Eriksson 1995; Mondoni et al. 2008; De Frenne et al. 2010b). Vegetative growth through rhizomes is very slow, and it is assumed that seeds are the most important dispersal organ accounting for population persistence and spread (Brunet & Von Oheimb 1998).

Seed collection

In 2009, two A. nemorosa populations were sampled in each of eight regions along a 1900-km latitudinal gradient, spanning 14° (we sampled only one population in northern Sweden due to rarity in that region) (Fig. 1; Table S1). The difference in mean annual temperature between the southernmost and northernmost populations was 6.5 °C (based on 1961–1990; FAO 2005; Table S1). All populations were large (>10,000 ramets), situated in deciduous forest (mixed forests in northern Sweden and Estonia) with 40–95% canopy cover and were >1 km apart in each region.

Sampling occurred on three to seven seed collection dates: because of nutrient relocation between mother plant and embryo and endosperm, the variation in growing season length and large phenological differences with latitude, the correct estimation of seed maturity is extremely important. It is likely that the seed collection date strongly affects seed nutrient concentrations (cf. Zerche & Ewald 2005) and was therefore incorporated into the sampling design. Usually, seed ecologists harvest seeds at only one point in time, although it is known that the collection date can have a clear influence on, for example, germination and dormancy (Hay & Probert 1995; Baskin & Baskin 1998; Handley & Davy 2005). Seeds were therefore collected every fifth day, from the date when
the first seeds formed (27–39 days post-anthesis) to when most seeds had already been shed (53–60 days post-anthesis; Table S1). This resulted in 79 samples from 15 population · 3–7 collection date combinations (Fig. 2). At every collection date, we sampled and pooled the seeds of 15–20 randomly chosen ramets per population.

Environmental variables

We collected five 4-cm deep soil cores from below the litter layer in each population in 2008 (France, Belgium, northeast Germany, south Sweden, north Sweden) or 2009 (northwest Germany, central Sweden, Estonia). Soil samples from each population were pooled, air-dried to constant mass and passed through a 2-mm sieve. All samples were analysed for pH (determined from a solution of 10 g soil and 25 ml 0.01 m CaCl₂ using a standard glass electrode). Furthermore, 5 g dry soil were extracted in 100 ml ammonium lactate solution [9.01 ml lactic acid (88%) + 18.75 ml acetic acid (99%) + 7.75 ml NH₄-acetate diluted to 1 l] according to the modified method of Egner et al. (1960) and analysed for Ca, Mg and K using atomic absorption spectrometry (SpectAA-220; Varian, Santa Clara, CA, USA) and for P in a spectrophotometer (Cary 50; Varian) according to the colorimetric method of Schel (1936), with molybdenum vanadate as colour reagent. Finally, the percentages of C and N were analysed with an element analyser. Growing degree-hours >5 °C were used for the accumulated temperature sum until seed collection date (Meunier et al. 2007). Growing degree-hours between 1 January and the collection date were calculated according to Lindsey & Newman (1956) using data obtained from weather stations close to the sampled populations (in all cases <30 km). Since the growing degree-hours and seed collection dates were closely correlated in each region (Fig. 2), and since the amount of degree-hours is the ecologically more relevant variable, we only included this variable in the data analyses. Latitude and growing degree-hours were not correlated when considering all seed collections (r = −0.157, n = 79, P = 0.168) or only the last seed collection (r = 0.171, n = 15, P = 0.543).

Seed traits

The seeds were air-dried to constant mass for ca. 1 week. We determined mean seed mass by weighing 50 seeds (to the nearest 0.1 mg) per seed sample and dividing the obtained mass by 50. For each seed sample, three Petri dishes were lined with moist filter paper and 20–50 randomly chosen seeds were distributed in each dish (the number of seeds within each dish depended on seed availability) within 15 days of collection for all seed samples. Based on earlier studies (Mondoni et al. 2008), seeds were given 8 weeks of warm stratification (simulating summer: 23 °C) before they were placed in cool stratification (simulating autumn: 10 °C) in temperature-controlled growth chambers. The number of germinated seeds (seeds with emerged radicle) was recorded weekly for at least 8 weeks (from the start of cool stratification) until germination had ceased in all dishes. The sets of dishes were randomly rotated after each weekly recording and distilled water was supplied as necessary.
A subsample of seeds not used for the germination trials was oven-dried to constant mass (50 °C for 72 h), analysed for C and N (element analyser; %) and, subsequent to wet acid digestion with HNO₃ and HClO₄, analysed for P (colorimetrically as above), K, Ca and Mg (atomic absorption spectrometry as above) (P and cations in ppm or µg g⁻¹ seed dry mass). C:N and N:P ratios were calculated from these results as C/N and N/P × 10,000. Since we found a clear latitudinal gradient in seed N (see Results), we also did a selective extraction of alkaloids on the two seed lots with extreme total N concentration (i.e., the northernmost and southernmost seed provenances) in a second step. Plants may resist herbivory using physical and/or chemical defence mechanisms (e.g., spines, hairs, physical toughness or secondary compounds), most of which are said to increase towards the equator (Schemske et al. 2009). Also, increasing concentrations of secondary compounds are related to better defence mechanisms (Bennett & Wallsgrove 1994; Chen 2008). Therefore, we aimed to check whether the higher N concentrations in seeds of our southernmost populations are related to increased chemical seed defence. The most widespread secondary compounds involved in chemical plant defence containing N and occurring in the Ranunculaceae are alkaloids (Larcher 2003). Therefore, we used a microwave technique on the southernmost and northernmost A. nemorosa seeds, and subsequently screened for alkaloids using thin layer chromatography (TLC) with the Dragendorff’s reagent for visualisation.

Data analysis

To explore the relative importance of growing degree-hours (which includes the seed collection date effect due to the strong correlation within regions; see Fig. 2), soil variables and latitude on seed traits, mixed-effect model analyses were performed considering either all collection dates or only the last collection date per population (i.e., the most mature seeds). Region was included in the models as a random factor to account for possible spatial autocorrelation of the populations within a region. Population was included as a random factor in models in which repeated measurements within one population were analysed (i.e., different collection dates). To examine the proportion of variance explained by the grouping structure in mixed-effect models (i.e., by random factors ‘region’ and ‘population’), the intra-class correlation was calculated according to Hox (2002). The results showed that between 0% and 69% (when considering all seed collections) or between 1% and 93% (only final seed collection) of the variance in seed traits of A. nemorosa was caused by the grouping structure.

Since some soil variables were highly correlated, we first performed a principal components analysis (PCA) with VariationMax rotation on the soil variables (pH, C, N, P, K, Ca, Mg), extracted the first two PCA axes (comprising 49.1% and 31.4% of variability in soil variables, respectively) and used these axes for analysis. The first PCA axis was positively correlated with plant available soil nutrients (P, K, Ca, Mg; r > 0.607) and pH (r = 0.852), whereas the second PCA axis was positively correlated with total soil C and N (r > 0.980; i.e., organic matter axis). The variance inflation factors of the predictor variables (Quinn & Keough 2002). To avoid over-fitting, maximal models were simplified by subsequently excluding predictors with P > 0.1; only the final reduced models are presented in the results. To fulfill normality and homoscedasticity assumptions, germination percentage and seed P concentration were first arcsine square-root and log₁₀-transformed, respectively. All other data were untransformed. All data were on the population × collection date level and analysed in SPSS (version 15.0, IBM Corp., Somers, NY, USA).

RESULTS

Seed N and N:P ratio were significantly negatively related to latitude, whereas the seed C:N ratio increased with increasing latitude (Table 1; Fig. 3). Seed Ca was negatively related to latitude only when all seed collection dates were considered (Table 1a). Across all seed collection dates, mean seed N and seed N:P ratio (±SE) decreased from 3.33 ± 0.22% and 8.4 ± 0.5 in northern France to 2.52 ± 0.07% and 6.5 ± 0.2 in northern Sweden, respectively (Fig. 3a and e). Seed C:N ratio increased from 15.4 ± 0.8 in the southernmost populations to 20.1 ± 0.6 in the northernmost population (Fig. 3c). When considering only the last collection date in every population (i.e., the most mature seeds), the strength of the relationships with latitude increased (Table 1; Fig. 3; comparison of parameter estimates and t-values). Across all collection dates, seed Ca decreased from 5245 ppm (±450) in northern France to 3502 ppm (±607) in northern Sweden (Tables 1a and 1c). The other seed nutrient concentrations showed no significant latitudinal cline. Soil conditions consistently affected seed C (positive effect of soil PCA 2) and seed N:P ratio (negative effect of soil PCA 1), while positive effects on germination and negative effects on seed Ca were apparent only when all or only the last collection date was considered, respectively (effects of both soil PCA 1 and 2; Table 1). In the TLC analysis, no alkaloids were found in any of the seed lots with extreme total N concentration (i.e., northernmost and southernmost seed provenances).

Furthermore, all seed traits of Anemone nemorosa were affected by the seed collection date (effects of growing degree-hours in Table 1a). For example, the earliest collected seeds with the lowest degree-hours failed to germinate (0% for all except the two German regions; data not shown) whereas seeds collected later showed germination rates up to 94%. Overall, Table 2 shows that germination percentages were positively affected by seed mass, seed C and N as well as seed N:P ratio but negatively affected by seed K, Ca and Mg concentrations. Seed mass and seed C showed a threshold effect and a clear correlation with germination percentage. This pattern was independent of the climatic zone where the seeds were collected (Fig. 4).

DISCUSSION

Seeds of Anemone nemorosa collected along a 1900-km long latitudinal gradient from northern France to northern Sweden showed a significant latitudinal cline in seed N and Ca concentrations and in seed C:N and N:P ratios. Seed nutrients displayed variations in concentration depending on the seed collection date, but the latitudinal clines were still significant when considering only the most mature seeds (final collection date; except for seed Ca).
Table 1. Effects of temperature (growing degree-hours), soil variables (two soil PCA axes) and latitude on seed traits of Anemone nemorosa along a latitudinal gradient. In (a) all seed collection dates are considered, in (b) only the last seed collection (i.e., most mature seeds) of each population. Soil PCA1 was positively correlated with plant available soil nutrients (P, K, Ca, Mg; r > 0.607) and pH (r = 0.852), whereas soil PCA2 was positively correlated with total soil C and N (r > 0.988); i.e., organic matter axis.

(a) all seed collection dates

<table>
<thead>
<tr>
<th>seed trait</th>
<th>predictor</th>
<th>par. est.</th>
<th>df</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>seed mass (mg)</td>
<td>Degree-hours</td>
<td>4.20E-4</td>
<td>69.2</td>
<td>9.06*</td>
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<tr>
<td></td>
<td>Degree-hours</td>
<td>1.90E-4</td>
<td>62.0</td>
<td>12.65*</td>
</tr>
<tr>
<td></td>
<td>Soil PCA2</td>
<td>1.34E-1</td>
<td>13.3</td>
<td>2.60**</td>
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<tr>
<td></td>
<td>Soil PCA1</td>
<td>1.40E-1</td>
<td>11.5</td>
<td>2.36**</td>
</tr>
<tr>
<td></td>
<td>Latitude</td>
<td>-2.95E-2</td>
<td>11.3</td>
<td>-2.05***</td>
</tr>
<tr>
<td>seed C (%)</td>
<td>Degree-hours</td>
<td>1.83E-3</td>
<td>64.3</td>
<td>17.63*</td>
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<tr>
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<td>Soil PCA2</td>
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<td>15.4</td>
<td>2.47**</td>
</tr>
<tr>
<td>seed N (%)</td>
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<td>-7.93E-2</td>
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<td>-3.52†</td>
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<td>seed P (ppm)</td>
<td>Degree-hours</td>
<td>8.10E-5</td>
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</tr>
<tr>
<td>seed K (ppm)</td>
<td>Degree-hours</td>
<td>-1.17</td>
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<td>-6.90*</td>
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<td>seed Ca (ppm)</td>
<td>Degree-hours</td>
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<td></td>
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<td>seed Mg (ppm)</td>
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<td>seed C:N</td>
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</tr>
<tr>
<td>seed N:P</td>
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<td>Latitude</td>
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<td>-1.87***</td>
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</table>

(b) final seed collection date

<table>
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<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree-hours</td>
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<td>2.55**</td>
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</tr>
<tr>
<td>Latitude</td>
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<td>4.99†</td>
</tr>
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</table>

*p < 0.001; **p < 0.05; ***p < 0.1; †p < 0.01; df calculated with Satterthwaite approximation.

Parameter estimate.

None of the studied predictors was significant.

Latitudinal gradient in seed nutrients of Anemone nemorosa

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

Possible causes and consequences of the latitudinal gradient in seed nutrients

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

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

Alternatively, the higher seed N concentration in southern plants may result from increased seed provisioning due to increased soil N availability or a potential shift in reproductive allocation. Indeed, although we found no effect of total soil N (soil PCA 2) on seed N, total soil N is not a very good measure of plant available N. Mineralisation rates in northern boreal forests are much slower than in temperate forests mainly due to low temperatures and recalcitrant organic matter (Coutéaux et al. 2001; Robinson 2002). Further, Holland et al. (2005) clearly show a northward decline in N deposition input across Europe (from ~20 kgN ha⁻¹ year⁻¹ in northern France to nearly zero in northern Sweden). This
A latitudinal gradient in seed nutrients

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Fig. 3. Nitrogen concentration (N), carbon:nitrogen (C:N) and nitrogen:phosphorus (N:P) ratio of seeds of Anemone nemorosa along a latitudinal gradient from northern France to northern Sweden. In (a), (c) and (e) all seed collections are considered. In (b), (d) and (f) only the last seed collection date (i.e., the most mature seeds) of each population is included. Significance values are given in Table 1: ‘region’ and ‘population’ were included in the models as random factors to account for possible autocorrelation of the populations within one region and the repeated measurements within one population, respectively.

potentially increased N availability for growth and reproduction of A. nemorosa in the south may result in more available resources for seed provisioning with N-rich compounds (i.e., mostly proteins). Second, northern peripheral populations of clonal plants tend to increase the ratio of investment in asexual reproduction over investment in sexual reproduction (Mooney & Billings 1961; Houle & Babeux 1994; Dorken & Eckert 2001). This suggests that lower provisioning of seeds but greater investment in clonal reproduction may be an adaptive strategy in northern A. nemorosa populations. To conclude, two possible mechanisms may explain why the seed N concentration decreases to the north: potentially lower soil N availability and greater investment in clonal reproduction. Hence, further (experimental) research seems worthwhile to explicitly identify the environmental factors driving the latitudinal clines in seed nutrient concentrations and to investigate how global change (e.g., increasing temperatures may alter soil N availability through changes in decomposition) affects the seed nutrient concentrations of this forest herb. Finally, differences in the inter-population genetic ability to store N in seeds may also account for the observed differences, but this can only be investigated using genetic analyses or common garden experiments.

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

Seed collection date effects

Our results also highlight the importance of the seed collection date for ecologists studying plant sexual reproduction. Harvesting A. nemorosa seeds at an earlier date biased seed mass and germination towards lower values (Hay & Probert).
CONCLUSIONS

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

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article:

Table S1. Characterisation of the eight study regions, anthesis, seed collection dates (in days following anthesis) and mean seed trait values (±SE) across all collection dates of 15 *Anenome nemorosa* populations along a latitudinal gradient in 2009.

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