

Effects of enhanced nitrogen inputs and climate warming on a forest understorey plant assessed by transplant experiments along a latitudinal gradient

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Received: 27 November 2013 / Accepted: 10 April 2014 / Published online: 27 April 2014
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Abstract Global warming and enhanced nitrogen (N) inputs are two key global-change drivers affecting temperate forest ecosystems simultaneously. Interactive effects of multiple drivers might cause species responses to differ from those in single-factor experiments; therefore, there is an urgent need for more multi-factor studies. Here, we assessed the growth and reproductive performance of multiple populations of a widespread grass of deciduous forests (*Milium effusum*) sampled along a latitudinal gradient and

subjected to experimental manipulations of temperature and nitrogen availability. Common garden transplant experiments along the latitudinal gradient were used to manipulate temperatures and combined with experimental N addition to assess intraspecific responses of the study species to global-change drivers as well as to determine local adaptation. The total biomass, number of seeds and seedling emergence time of *M. effusum* increased when transplanted in the southern common garden. Apart from effects on the seed mass, the species did not respond to N addition alone. Yet, interactive effects between warming and N addition were found: N addition led to increased biomass growth but only in the northern common garden. Significant home-site advantages were apparent, most likely because of increased mycorrhizal colonization of roots of local transplants. We show that multiple global-change drivers may alter

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Communicated by Christian Rixen.

Electronic supplementary material The online version of this article (doi:10.1007/s11258-014-0341-z) contains supplementary material, which is available to authorized users.

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dynamics in understorey communities of temperate forests. Our study reinforces the need to increase our understanding of plant responses to future environmental changes by expanding the multi-factor research framework.

Keywords Climate change · Common garden experiment · Forest understorey · Latitude · Local adaptation · Mycorrhiza · Nitrogen deposition · Deciduous forest

Introduction

Global environmental change is severely altering forest composition and dynamics worldwide, thereby threatening their diversity. Two major drivers of biodiversity change in forests are (i) airborne pollution and the resulting acidification and eutrophication (e.g. Gilliam 2006; Dirnböck et al. 2013) and (ii) climate warming (Bertrand et al. 2011; De Frenne et al. 2011, 2013b). The burning of fossil fuels that is accompanied by production of NO_x as well as the intensification of agricultural systems substantially increased atmospheric N deposition levels in temperate forests to up to 50 times pre-industrial levels (Galloway et al. 2004). In N-limited forests, short-term effects of enhanced N inputs comprise increased plant productivity originating from stimulated soil N mineralization, either directly through fertilization which may increase microbial activity or indirectly through altered soil organic matter quality (e.g. lower soil C:N ratio) (Aber et al. 1998; Ma et al. 2011). In the long run, increased N availability may lead to altered competitive interactions and increased pests and pathogen attacks, ultimately resulting in reduced plant species richness and biodiversity losses in some sites

(Gilliam 2006; Bobbink et al. 2010; De Schrijver et al. 2011). In addition, increased temperatures caused by rising atmospheric greenhouse gas concentrations may affect plant phenology, performance and distribution directly (Lovejoy and Hannah 2005) as well as indirectly through changes in competitive interactions and N availability (Rustad et al. 2001) and through altered nutrient uptake capacities (Bassirrad 2000).

An important gap in our understanding of the effects of global change on natural ecosystems is the simultaneous influence of multiple drivers, potentially resulting in unexpected interactive effects (Rustad 2008; Dieleman et al. 2012). The concurrent changes in temperatures and N inputs in ecosystems, for example, may trigger non-additive effects on some processes such as soil N mineralization. In this regard, Ma et al. (2011) found an antagonistic effect of combined warming and N addition on mineralization rates with higher mineralization under separate warming and increased N inputs than under combined treatments. Other studies show that soil N availability and leaching in high-elevation spruce and fir forests will increase in response to warming under N-saturated conditions (Garten 2000), whereas Turner and Henry (2009) demonstrated that warming increased plant N uptake in an old field and, hence, may partially mitigate soil N losses resulting from increased N deposition. Therefore, single-factor experiments may not realistically model future plant responses to global change as a whole (Templer and Reinmann 2011). Hence, more integrative studies including multiple factors are needed to support future biodiversity conservation strategies. A step towards a unified multidisciplinary approach is to superimpose experiments across gradients, since this allows researchers to better assess the likely response of plants to climate change across a broader range of environments (Dunne et al. 2004; Rustad 2008; De Frenne et al. 2013a). Within the existing multi-factor experiments, forests and forest-floor plants represent a particularly under-represented ecosystem and species group, respectively, while forest understorey plants are key for temperate forest biodiversity and ecosystem dynamics in general. For instance, forest-floor plants can significantly influence tree regeneration or nutrient cycling (Nilsson and Wardle 2005; Gilliam 2007).

Here, we combine experimental temperature and nitrogen treatments with the sampling of multiple populations (both seeds and adult plant material) of a

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widespread Holarctic grass of deciduous forests, *Milium effusum*, along a latitudinal gradient. Transplant experiments in two common garden sites along the latitudinal gradient were combined with experimental nitrogen addition. This experimental design allowed us to determine the effects of changed temperatures and nitrogen availability on growth and reproduction of understorey plant populations and at the same time assess long-term adaptation to the local environment. We specifically assessed whether (i) the species responded to warming and nitrogen addition, and whether interactive effects were found, (ii) populations from different latitudes of origin responded differently, and (iii) plants transplanted near their home site performed better than those transplanted further away. Since N is commonly a growth-limiting factor in temperate forests and temperature generally increases the rate of biological processes, the single effects of N addition and warming may improve the growth and reproduction of the species. Consequently, we hypothesize that the combined effects of N and warming are stronger than the sum of the single effects.

Materials and methods

Study species

Milium effusum L. (Poaceae) is an early summer flowering hemicytrophitic grass and is widely distributed in European ancient deciduous forests. The European northern distribution edge of *M. effusum* is situated around 71°N. The southern range limit is around 38–40°N (Hultén and Fries 1986). The inflorescences of *M. effusum* of up to 1.5 meter high are wind-pollinated (Tyler 2002) and usually produce between 100 and 300 caryopses (further referred to as *seeds*) per year and per shoot (further referred to as *individual*). The species also reaches maximum vegetative growth rates of 20 cm year⁻¹ (Brunet and von Oheimb 1998) by means of short stolons. Seeds are mainly gravity-dispersed, but myrmecochory, epizoochory and endozoochory occur as well (Graae 2002; Heinken and Raudnitschka 2002; von Oheimb et al. 2005; Delatte and Chabrier 2008), making the species a relatively fast colonizer compared to other forest understorey species (Brunet et al. 2012).

Experimental treatments

Source material was sampled from two populations in each of eight regions located along an approximately 2,300 km latitudinal gradient from northern France to northern Sweden in 2008, and transplanted into common gardens near Gontrode (Belgium) and Alnarp (southern Sweden; Fig. 1). This resulted in a total of 16 source populations. Both seeds and adult individuals (further referred to as *seeds* or *adults*) were used as source material. All source populations (that is, two sites per region) were located in deciduous forests that were unaffected by recent major disturbances (e.g. clear-cuts, heavy grazing) and that were at least 1 km apart in each region. Within each population, the seeds of 15 randomly chosen individuals were collected at the time of seed maturity, pooled and 40 seeds per population were then sown into 40 ml pots containing standard potting soil to avoid seed desiccation. At the same time, whole root lumps of adults were collected and stored in containers with soil from the collection sites. In September 2008, after all populations had been sampled along the gradient, the seeds and adults were transferred into 1.5 l-pots containing standard potting soil (40 seeds or one *M. effusum* root lump per pot) and transplanted into the two common gardens in a randomized block design [$n = 4$ replicates per species, population and establishment method (seeds or adult material)]. This led to a total sample size of 256 pots (2 common gardens \times 8 regions \times 2 populations \times 4 replicates \times 2 establishment methods). The common gardens were located in sites similar to deciduous forests of that specific latitude in terms of tree species composition and overstorey canopy cover: (i) in Belgium, the pots were placed in a mixed *Fraxinus excelsior*, *Quercus robur* and *Fagus sylvatica* forest with 89.9 % canopy cover; (ii) in southern Sweden, the common garden was situated in a mixed *Quercus robur* and *Fagus sylvatica* forest with 85.0 % canopy cover. However, the forest where the Swedish common garden site was installed was harvested in August 2011; therefore, the pots were moved to a neighbouring forest with 65 % canopy cover ca. 500 m away. Because of the experimental set-up, the populations were subjected to diverse transplantation directions and distances: the populations from Stockholm, Umeå and Abisko were only transplanted towards the south; populations from Bremen and Potsdam were transplanted in both directions, whereas

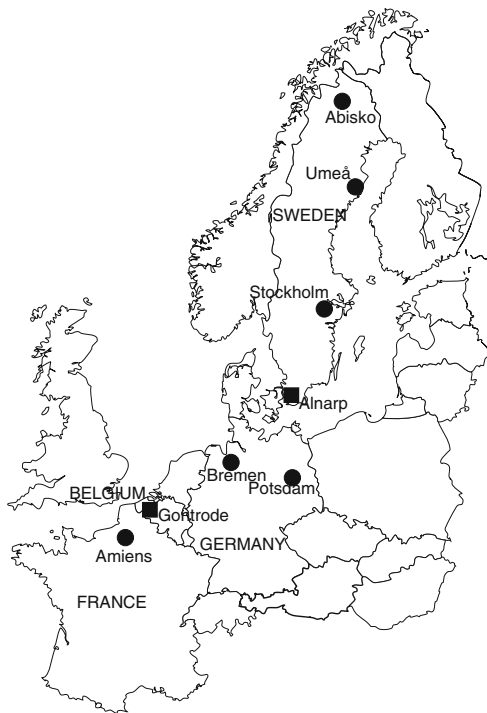


Fig. 1 The eight regions (circles and squares) where the source populations of *Milium effusum* were sampled along a 2,300 km latitudinal gradient from northern France to northern Sweden. The two common garden locations used in this study (Gontrode and Alnarp) are indicated with squares

the populations from Amiens were only transplanted northwards (Fig. 1). The aboveground biomass of *M. effusum* was already harvested in 2010 to investigate the effects of warming by means of transplantation (De Frenne et al. 2011). In the present study, however, we also incorporated another global-change driver, i.e. elevated inputs of N, and can therefore also assess interactive effects. The latitude of origin of the sampled populations was significantly correlated with the air temperature (annual mean climatic data for 1981–2010 from FetchClimate, Microsoft Research, <http://research.microsoft.com/fetchclimate>; $r = -0.984$, $p < 0.001$), precipitation ($r = -0.785$, $p = 0.021$), growing-season photoperiod (on 1 July, calculated according to Forsythe et al. 1995; $r = 0.958$, $p < 0.001$), and total sum of dry and wet oxidized and reduced N deposition values (EMEP database, <http://www.emep.int>, data for the reference year 2000; $r = -0.905$, $p = 0.002$) at each site. More details on the design of the common garden sites are provided in De Frenne et al. (2011).

In September 2011, the nitrogen addition started. A total equivalent of $62.5 \text{ kg N ha}^{-1}$ was applied to half of the replicates in both common gardens spread across three occasions ($12.5 \text{ kg N ha}^{-1}$ in Sept. 2011, 25 kg N ha^{-1} in Jan. 2012 and 25 kg N ha^{-1} in Apr. 2012). Nitrogen was added as ammonium nitrate fertilizer, dissolved in 50 ml distilled water per pot and injected in the soil by means of an injection syringe to avoid the evaporation of ammonium and leaf tissue damage. At these occasions, the controls received the same amount of distilled water.

Data loggers were installed in each common garden to monitor the air temperature (at 20 cm above the soil surface), the temperature of the litter layer (at the soil surface: 0 cm) and the soil temperature (at 5 cm below the soil surface). In Alnarp, microclimate Em50 data loggers (Decagon Inc., Pullman, WA, USA) and in Gontrode, Type T miniature Thermocouples (TC Direct, Nederweert, NL) were used to log data at two-hour and twenty-second intervals, respectively. The air, surface and soil temperature during the experiment (1 October 2011 till the collection dates of *M. effusum*) was 2.3, 3.9 and 6.3 °C higher in Belgium than in the Swedish common garden (Supporting Information Table S1). These effects were particularly evident in the accumulated heat sum: air, surface and soil growing degree-hours above 5 °C between 1 February and the collection date were 16.357, 11.001 and 6.049 °C h in the Swedish common garden and 21.873, 16.385 and 16.116 °C h in the Belgian common garden, respectively.

Plant measurements

At the moment of peak biomass during the growing season of 2012 (as indicated by natural seed dispersal, monitored around every 3 days), we harvested all biomass per pot and measured several growth and reproductive traits: the number of adult and fruiting individuals, vegetative and generative height, the aboveground vegetative and reproductive biomass, the total number of seeds per flowering individual, mean seed mass, specific leaf area (SLA) as well as germination percentage and mean emergence time (the latter two from germination trials). These traits are related to plant fitness, competitive ability (e.g. biomass and plant height) and colonization capacity (e.g. seed quantity and germination). Vegetative height was measured as the maximal height of the

foliage and the generative height as the maximal height of the inflorescence above the soil surface of the largest individual in each pot. All harvested biomass was oven-dried at 50 °C for 3 days. The total above-ground biomass in each pot was calculated as the sum of aboveground vegetative and reproductive tissue. Seeds were collected separately per pot. All seeds and a random subsample of 50 seeds (50-seed mass) were weighed per pot, after which the total number of seeds per pot was estimated as the total seed mass divided by the mean seed mass (i.e. 50-seed mass divided by 50). The total number of seeds per flowering individual was then calculated by dividing the total number of seeds by the number of inflorescences per pot. SLA was determined on one randomly selected leaf per pot (flat dried between dry sheets of paper for 3 days at 50 °C) as leaf area measured using the Li-Cor Portable Area Meter LI-3000) divided by dry leaf mass.

Subsequently, germination trials were conducted to assess the effects of the experimental treatments on seed germination and mean emergence time of seedlings. For each seed sample collected per pot, one Petri dish was lined with moist filter paper, and 5–50 randomly chosen seeds were distributed in each dish (the number of seeds within each dish depended on seed availability) within one month after collection. Seeds were given 10 weeks of warm stratification (room temperature) followed by cold stratification in three 4-week blocks: 10 °C (autumn), 2 °C (winter), 10 °C (spring) and finally warm stratification (20 °C; summer) until germination had ceased in all dishes (De Frenne et al. 2012). The number of germinated seeds (i.e. with an emerged radicle) was recorded weekly and distilled water was added as necessary. Germination percentages were then calculated as the final number of emerged seedlings divided by the total number of seeds in the dish at the start of the trial. Additionally, the mean emergence time (MET) for each seed sample was calculated as (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 start of the experiment and the end of a time interval (in days) and N is the final number of emerged seedlings. Following Milbau et al. (2009), samples

with very poor germination (<5 %) were excluded from the analyses for the calculation of MET.

Mycorrhizal colonization of roots

Since plants were transplanted into exactly the same pots (with perforated bottom) and potting soil in both common gardens, differences in plant traits among common gardens cannot be attributed to abiotic soil characteristics. Because the pots were dug into the soil so that the top edge of the pots was level with the soil surface, this may have facilitated contact with mycorrhizae. Therefore, to assess the degree to which belowground biotic interactions varied along the latitudinal gradient, roots of sown *M. effusum* plants (to avoid the effect of a soil inoculum in the adult transplants) in the Belgian common garden were sampled at the time of seed maturity, and kept at –18 °C until investigation for mycorrhizal abundance. This was done by means of the Grid Line Intersection Method, a procedure based on the presence or absence of colonization at each intersection of root and gridline under a microscope (Giovannetti and Mosse 1980). After clearing the plant roots by boiling for 1 min in a 10 % KOH solution, rinsing with tap water and staining the mycorrhizal structures with an ink-vinegar solution (Vierheilig et al. 1998), the roots were placed above a grid and then counted under a dissecting microscope at 40× magnification (McGonigle et al. 1990).

Data analyses

First, to examine the effects of (i) the common garden transplant site, (ii) nitrogen addition and (iii) latitude of origin of each population on plant growth and reproductive performance in the two common gardens, linear mixed-effect models were performed by means of the *lmer*-function of the *lme4*-library in R 2.15.2 (R Core Team 2012) with maximum likelihood estimation. *Population* nested within *region* were the random-effect terms to address the autocorrelation and hierarchy of the populations within regions and replicates per population, respectively. Transplanted adults and sown seeds were analysed separately. Model assessment followed Zuur et al. (2009) using likelihood ratio tests (χ^2 test statistic) of nested models and dropping non-significant terms. More specifically,

Table 1 The effects of the common garden transplant site (CG), nitrogen addition (N), the latitude of origin (Lat), the home-away distance (Δ Lat) and their interactions on growth and reproductive traits of *Milium effusum*

	CG	N	Lat	CG \times N	CG \times Lat	N \times Lat	Δ Lat	CG \times Δ Lat	N \times Δ Lat
<i>Transplanted adults</i>									
No. individual stems ^b	↓3.5 ^(*)	ns	ns	ns	4.5*	6.1*	↓6.6*	ns	5.3*
% Fruiting individuals ^a	↑9.5***	ns	↑10.3**	ns	ns	ns	ns	ns	ns
Vegetative height ^b	↓17.8***	ns	↑8.3**	ns	5.6*	2.9 ^(*)	↓7.9***	ns	ns
Generative height	ns	ns	↑4.0*	ns	5.3*	ns	↓8.8**	ns	ns
Vegetative biomass ^b	↑2.9 ^(*)	ns	↑3.0 ^(*)	ns	15.7***	5.7 ^(*)	↓13.0***	3.1 ^(*)	5.6*
Reproductive biomass ^a	ns	ns	↑4.2*	ns	6.5*	ns	↓7.1**	ns	ns
Total biomass ^b	↑3.8 ^(*)	ns	↑3.7 ^(*)	ns	16.3***	5.1*	↓13.8***	3.3 ^(*)	5.1*
Leaf area ^b	ns	ns	ns	ns	3.8 ^(*)	7.5**	ns	3.2 ^(*)	6.6*
Specific leaf area ^a	↑35.0***	ns	↑4.0*	ns	16.4***	ns	↑8.7**	17.5***	3.7 ^(*)
Mean seed mass ^a	↓30.1***	ns	ns	ns	2.7 ^(*)	3.8 ^(*)	ns	ns	ns
Total no. seeds/ind ^a	↑17.8***	ns	ns	ns	9.9**	ns	ns	3.1 ^(*)	ns
Germination % ^c	↓7.3**	ns	ns	ns	5.8*	ns	ns	16.0***	ns
Mean emergence time	↑7.0**	ns	↑8.6**	ns	4.3*	ns	ns	6.3*	ns
<i>Sown seeds</i>									
No. individual stems ^a	↓22.5***	ns	ns	4.4*	ns	ns	↓2.8 ^(*)	ns	3.9*
% Fruiting individuals ^a	↑14.5***	ns	ns	ns	ns	ns	ns	ns	ns
Vegetative height	ns	ns	ns	ns	6.5*	ns	↓6.8**	ns	5.1*
Generative height	↑13.8***	ns	ns	ns	ns	ns	ns	ns	ns
Vegetative biomass ^b	ns	ns	ns	8.0**	5.8*	ns	↓9.3**	ns	4.0*
Reproductive biomass ^a	↑11.9***	ns	ns	ns	ns	ns	ns	ns	ns
Total biomass ^b	↑3.4 ^(*)	ns	ns	7.7**	6.2*	ns	↓9.6**	ns	4.3*
Leaf area ^b	ns	ns	ns	ns	6.9**	ns	ns	7.5**	ns
Specific leaf area ^a	ns	ns	ns	ns	ns	ns	↑3.6 ^(*)	ns	ns
Mean seed mass ^a	ns	↑2.9 ^(*)	ns	4.3*	4.2*	ns	ns	4.4*	ns
Total no. seeds/ind ^a	ns	ns	ns	ns	ns	ns	ns	ns	ns
Germination % ^c	ns	ns	ns	ns	ns	11.7***	ns	ns	11.1***
Mean emergence time	↑5.5*	ns	ns	ns	5.6*	ns	ns	4.0*	ns

χ^2 values and significances from likelihood ratio tests of mixed-effect models. The direction of the effect is given with an arrow: ↑ corresponds to an increase in the trait value to the south (CG), with addition of nitrogen (N), with decreasing latitude of origin (Lat) or with increasing home-away distance (Δ Lat), while ↓ corresponds to a decrease in the trait values. ns: $p > 0.1$, (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

^a Log₁₀ transformed

^b Sqrt-transformed

^c Arcsine sqrt-transformed

in order to test the significance of interaction effects, we started with a full model (including the three predictor variables and all two-way interactions) and always compared this model with a model that dropped one interaction term. In order to test the significance of main effects, null models (excluding all predictors and interactions) were repeatedly compared with a model including one predictor. To meet the

assumptions of the statistical tests, normality was checked by means of a histogram, QQ-plot and Shapiro-Wilcoxon test and the data were log₁₀, square-root or arcsine-square-root transformed if necessary (Zuur et al. 2009).

Second, to investigate whether a home-site advantage was present in all populations we examined the effects of the home-away distance quantified as the

absolute value of the latitudinal difference between the latitude of origin and the latitude of the common garden transplant site [Δ Latitude] (De Frenne et al. 2011) on plant growth and reproductive performance using similar models as described above. Additionally, we examined these effects for the reciprocally transplanted populations only, i.e. populations from Gontrode, Belgium and Alnarp, southern Sweden, using similar models as described above.

Third, to investigate whether local adaptation may have affected responses of the study species to N addition, we analysed the effects of N addition on plant growth and reproductive performance of the locally transplanted populations only. That is, Belgian populations in the Belgian common garden and populations from Alnarp in the Swedish common garden were considered for this analysis. Again, mixed-effect models were used, after which the results could be compared with those of the first analyses.

Fourth, a similar mixed-effect modelling procedure was followed to analyse whether there was different mycorrhizal colonization of *M. effusum* roots in the Belgian common garden in local vs. non-local transplants.

Results

For both the transplanted adults and sown seeds, the transplant site explained most differences in growth and reproductive traits of *M. effusum* (Table 1). For instance, across all populations of transplanted adults, plants grew significantly taller, produced less biomass and had leaves with a lower SLA in the Swedish than in the Belgian common garden. Seeds from plants grown in the Belgian common garden germinated less and slower than seeds produced in the Swedish common garden.

Overall, nitrogen addition explained very little of the differences in growth or reproductive performance (Table 1). Only one marginally significant direct N response was found: individuals resulting from sown seeds of *M. effusum* produced heavier seeds in response to N addition. Other effects of N addition were detected when including only locally transplanted populations. That is, N addition increased vegetative ($\chi^2 = 3.6$; $p = 0.076$) and total ($\chi^2 = 3.4$; $p = 0.084$) biomass of *M. effusum* but no other significant effects were found. It is important to note

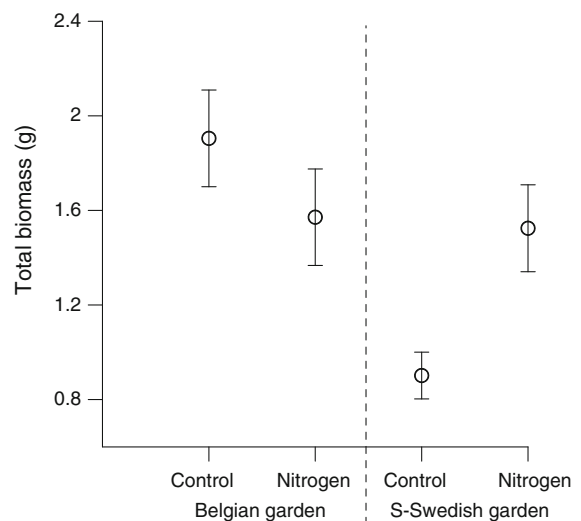


Fig. 2 The effects of nitrogen addition on total biomass production of *Miliun effusum* collected along a latitudinal gradient (seeds as source material) and sown in the common garden in Belgium and southern Sweden. Error bars denote SE

that the only significant N responses of *M. effusum* were found for sown seeds and not for adult transplants.

Significant common garden \times nitrogen interactions were found for several plant traits of *M. effusum*, i.e. for the number of individuals, vegetative and total biomass and mean seed mass, denoting divergent responses of plants to the nitrogen treatment in the common gardens. For instance, total biomass as well as number of individuals significantly increased with N addition in the Swedish common garden ($p = 0.0029$), while this trend was not seen in the Belgian common garden (Fig. 2).

The latitude of origin also significantly affected growth and reproduction (Table 1). For instance, across both common gardens, transplanted adults from northern origin had less fruiting individuals than populations from southern origin. Significant common garden \times latitude interactions were found for most of the analysed traits in *M. effusum*, denoting a differential effect of the transplantation site in populations from different latitudes of origin, i.e. the effect was larger when plants were transplanted farther away from home (Table 1; Fig. 3). For example, in the northern common garden, individuals grew taller and produced leaves with a lower SLA, but these effects were more pronounced for northern than for southern populations (Fig. 3a, b).

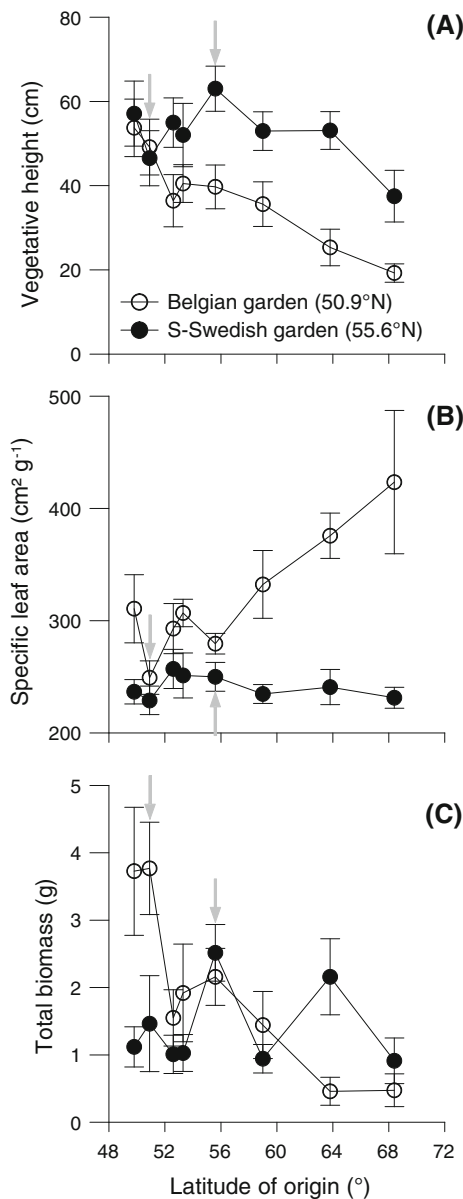


Fig. 3 The effects of the common garden transplant site and the latitude of origin on the vegetative height (a), specific leaf area (b) and total aboveground biomass (c) of *Milium effusum* adults (mean \pm SE) collected along a latitudinal gradient. The mean across the two populations from each region is given in this graph. The grey arrows indicate the home transplants

Several indications of local adaptation of the sampled populations were found. First, a generally negative effect of the home-away distance on several plant traits was found when including all provenances, implying that plant performance generally decreased

Table 2 The effects of the common garden transplant site (CG), nitrogen addition (N) and the home-away distance (Δ Lat) on growth and reproductive traits of reciprocal transplants of *Milium effusum* considering only the populations from Belgium (Gontrode) and southern Sweden (Alnarp)

	CG	N	Δ Lat
<i>Transplanted adults</i>			
No. individual stems	$\downarrow 6.5^*$	ns	$\downarrow 6.5^*$
% Fruiting individuals ^b	$\uparrow 5.4^*$	ns	$\uparrow 3.4^{(*)}$
Vegetative height	$\uparrow 3.3^{(*)}$	ns	$\downarrow 4.6^*$
Generative height ^a	$\uparrow 12.6^{***}$	ns	$\downarrow 5.7^*$
Vegetative biomass	ns	ns	$\downarrow 6.0^*$
Reproductive biomass ^a	ns	ns	ns
Total biomass ^b	$\uparrow 3.4^{(*)}$	ns	$\downarrow 6.0^*$
Leaf area	ns	ns	ns
Specific leaf area	$\uparrow 4.4^*$	ns	ns
Mean seed mass ^a	$\downarrow 10.9^{**}$	ns	$\downarrow 6.0^*$
Total no. seeds/ind ^a	$\uparrow 14.0^{***}$	ns	$\uparrow 2.8^{(*)}$
Germination % ^c	ns	ns	ns
Mean emergence time	$\uparrow 4.3^*$	ns	ns
<i>Sown seeds</i>			
No. individual stems ^a	$\downarrow 24.8^{***}$	ns	ns
% Fruiting individuals ^a	$\uparrow 8.5^{**}$	ns	ns
Vegetative height	$\uparrow 3.3^{(*)}$	ns	ns
Generative height	$\uparrow 12.6^{**}$	ns	$\downarrow 4.6^*$
Vegetative biomass	ns	ns	$\downarrow 8.5^{**}$
Reproductive biomass ^a	$\uparrow 11.8^{***}$	ns	$\downarrow 3.6^{(*)}$
Total biomass ^b	ns	ns	$\downarrow 8.6^{**}$
Leaf area ^b	ns	ns	$\uparrow 4.8^*$
Specific leaf area	ns	$\uparrow 4.5^*$	ns
Mean seed mass ^a	$\uparrow 9.4^{**}$	ns	ns
Total no. seeds/ind ^b	$\downarrow 2.9^{(*)}$	ns	ns
Germination % ^c	ns	ns	ns
Mean emergence time	$\uparrow 7.2^{**}$	ns	ns

χ^2 values and significances from likelihood ratio tests of mixed-effect models. The direction of the effect is given with an arrow: \uparrow corresponds to an increase in the trait value to the south (CG), with addition of nitrogen (N) or with increasing home-away distance (Δ Lat), while \downarrow corresponds to a decrease in the trait values. ns $p > 0.1$; (*) $p < 0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

^a Log₁₀ transformed

^b Sqrt-transformed

^c Arcsine sqrt-transformed

when plants were transplanted farther away from their 'home site' (Table 1, Δ Lat-effects). The positive effect of Δ Lat on SLA complemented by the absence

of Δ Lat-effects on leaf area implies that the increase in SLA originated from a decrease in leaf dry matter. Thus, *M. effusum* produced thinner leaves when transplanted further from its home site. Second, a negative effect of the home-away distance on several plant traits was found for the reciprocally transplanted populations (Table 2, Δ Lat): e.g. vegetative and generative height, mean seed mass and biomass production decreased significantly with increasing home-away distance. This implies that the Belgian and southern Swedish populations showed a decreased plant performance when transplanted away from their 'home site' into the other common garden. Generally, similar effects of the home-away distance were found in both common gardens. In some cases, however, significant common garden \times home-away distance as well as nitrogen \times home-away distance interactions (Table 1) was found.

When assessing the roots of *M. effusum* plants from the Belgian common garden for mycorrhizal colonization, we found that roots of local plants (mean 32.75 % \pm SE 10.56) were colonized more by mycorrhizae than non-local populations (20.76 % \pm 2.67) ($\chi^2 = 2.82, p = 0.093$). Nitrogen addition had no effect on mycorrhizal colonization ($\chi^2 = 0.57, p = 0.450$).

Discussion

Increasing temperatures via transplantation into a southern common garden reduced the size, but increased SLA and biomass production of adult *M. effusum* plants compared to transplantation into the northern common garden. Higher temperatures in the southern common garden may have led to an increased biomass productivity of *M. effusum*, indicating a positive growth effect on this forest plant in a future warming climate. Instead of the temperature difference between the common gardens, however, also the difference in light availability may drive the latitudinal trade-off between plant size and SLA in *M. effusum*. Higher light availabilities exist in the northern common garden, originating from a lower canopy cover as well as a longer photoperiod in the growing season. Since *M. effusum* grows and flowers after tree canopy flush, we assume that plants in the Belgian common garden invested more energy in thinner leaves with a larger leaf area (high SLA) to use the less available light more efficiently (Poorter et al. 2009), whereas plants in the Swedish common garden

invested more in taller plant statures. This trade-off demonstrates the capacity of *M. effusum* to adapt to changing environmental conditions. Larger effects of the transplant site were found on the height and SLA of individuals from northern populations than on individuals from more southern populations, suggesting a differential impact within the distribution range of this species. The latter may enable northern *M. effusum* populations to cope with more extreme microclimatic heterogeneity than in temperate regions and also indicates that they might acclimate better to environmental change in the future (De Frenne et al. 2012). This is, for instance, also relevant when selecting seed provenances (northern vs. southern) of plant species to use in restoration projects in the face of climate change. Higher SLA in plants from different biomes has been linked with elevated growth rates due to their contribution to photosynthesis regulation (Cornelissen et al. 1996; Reich et al. 1997), which can explain the increased biomass production in the southern common garden. On the other hand, transplanting *M. effusum* in the southern common garden resulted in a lower sexual reproductive performance of *M. effusum*: seed germination, for instance, was lower when mother plants were grown in the Belgian common garden. Besides southerly increasing temperatures and decreasing photoperiod in the growing season, our latitudinal gradient also coincides with a N deposition gradient, with higher deposition rates towards the south. Therefore, increased biomass production could also be attributed to the larger southerly background N deposition rates. While each empirical approach to infer warming effects on plants has specific advantages and drawbacks, covariation of environmental factors other than temperature comprises a disadvantage of gradient studies that complicates disentangling temperature effects from these other drivers. This highlights the importance of complementary integrated approaches to infer warming effects on plants (Rustad 2008; De Frenne et al. 2013a).

Nitrogen addition led to higher biomass and more individuals in Sweden, whereas no effects were seen in the Belgian common garden. This interaction could be explained by the fact that the species may only utilize the extra N when light is not a growth-limiting factor and that *M. effusum*—as a typical example of understorey grasses and tall herbs which survive under closed canopies—needs canopy gaps to reproduce and grow well. Similarly, Verheyen et al. (2012) suggested light limitation to be responsible for the apparent resistance of

understorey communities in European deciduous forests to species losses following decades of N eutrophication. They suggest chronic N deposition in closed-canopy forests should be regarded to as the building up of a ‘N time bomb’. This apparent resistance may decline if forest canopies are opened up again so that light becomes a less limiting resource (Verheyen et al. 2012; De Frenne et al. 2013b). Since background N deposition levels are lower in the Swedish than in the Belgian common garden, another explanation could be that *M. effusum* in Sweden may still benefit from the additional N (reflected by a higher biomass production in response to N addition), whereas plants in Belgium may already have been at their maximal uptake, responding no more to extra N. Cumulative N inputs from wet and dry atmospheric deposition in the Belgian and Swedish common garden over the experimental period amounted to 81.7 kg N ha⁻¹ (deposition of 20.4 kg N ha⁻¹ year⁻¹) and 50.5 kg N ha⁻¹ (deposition: 12.6 kg N ha⁻¹ year⁻¹), respectively. Hence, differential atmospheric N deposition potentially influenced experimental N addition responses (Hedwall et al. 2013). Additionally, none of the N responses for *M. effusum* were found for transplanted adults, but only for seeds. Transplanted adult individuals (root lumps) serve as efficient underground N storage organs for the perennial species (Suzuki and Stuefer 1999). Hence, transplanted adults might not take advantage of the additional N in short-term experiments due to their larger belowground storage organs, explaining the stronger effects of our N addition on sown seeds.

No other clear evidence was found that increased N addition affects growth or reproductive performance of the species studied. Although early reproductive stages may be positively influenced (heavier seeds) in *M. effusum* due to a higher seed N provisioning, no effects on later reproductive stages were found (germination percentage or emergence time). We hypothesize that N responses might be largely absent due to several reasons: (i) the study species shows no responses to increased N deposition, which is, however, contradictory to earlier short- and long-term studies (Turnau et al. 1992; Thimonier et al. 1994; Falkengren-Grerup et al. 2000), (ii) the duration of N addition (1 year) might have been too short to account for any short- (fertilizing) or long-term (acidifying) effects of N addition (Clark and Tilman 2008); (iii) responses to N addition might be limited by background N deposition (Hedwall et al. 2013) which

could have been stored in the underground storage organs of the plants (see above); (iv) the presence of local adaptation might have confounded experimental results such that plants transplanted further away from their home site exhibited lower fitness. When only the locally transplanted populations were considered, N addition led to an increased biomass production. These results suggest that the home-site advantage may have partly affected the N response.

Even though warming induced plastic responses, local adaptation was apparent. Plant performance decreased with increasing home-away distance (De Frenne et al. 2011, 2012). In other words, individuals that were transplanted closer to the home site performed better than the other populations at that site or they performed better than when transplanted further away. The SLA specifically is determined by factors such as irradiance and nutrient availability (Poorter et al. 2009), and thinner leaves do not necessarily imply better plant performance. Therefore, it is unclear whether thinner leaves in plants transplanted further away from their home site imply higher or lower fitness. Mycorrhizal colonization of *M. effusum* roots was higher in local plants than in non-local plants. This finding suggests that the higher plant performance found in *M. effusum* populations replanted at their home site along the latitudinal gradient is due to higher incidence of mycorrhizal fungi. The symbiosis between host plant and mycorrhizal fungus implies that the plant receives more mineral nutrients due to an extended surface area with mycorrhizal hyphae (up to 25 % of the nitrogen demand can be met; Johnson et al. 1997) as well as a potentially increased growth, resistance to stress and general plant fitness (Harrison 1997). This adaptation to the local mycorrhizae may have confounded responses of the different *M. effusum* populations to transplantation and N addition. The findings of common garden × home-away distance interactions as well as N addition × home-away distance interactions corroborate this hypothesis since plants responded differentially to the transplant site or N addition when transplanted closer or further from their home site. For instance, the effect of N addition on total biomass was larger for *M. effusum* individuals transplanted farther from their home site, possibly because they could not rely on N supply from their local mycorrhizae. Usually, grass species also have endophytic fungi that are vertically transferred from parent plant to offspring. Hence, individuals from different geographic origins may

exhibit contrasting performance due to different endophytes. However, we only implemented a quantitative approach to assess root colonization by fungi.

In sum, superimposing experiments across gradients can be useful to better assess the future response of species to global change across a broader range of environments (Dunne et al. 2004; Rustad 2008; De Frenne et al. 2013a). Here, we combined common garden transplant experiments and N addition in plants sampled along a wide latitudinal gradient. *Milium effusum* clearly showed growth and reproductive responses to the transplant site, whereas the effects of N addition on the species were less evident. Nitrogen addition led to very limited plant responses, suggesting that plants were either not influenced by increased N deposition, or that they were non-responsive to the treatment due to its short duration, possible cumulative N deposition legacies or the presence of local adaptation along the gradient. Nevertheless, our results indicated a potential positive growth response to increased N deposition, but only under high-light conditions. Significant home-site advantages were found, most likely related to a higher incidence of mycorrhizal fungi in local populations of *M. effusum*. Our gradient study illustrates the existence of complex interactive effects between global-change drivers and accentuates the need for multi-factor as well as long-term experiments for predicting future forest understorey dynamics.

Acknowledgments We thank the Research Foundation—Flanders (FWO) for funding the scientific research network FLEUR (<http://www.fleur.ugent.be>). Support for this work was provided by the Kempe Foundation, Petra and Karl Erik Hedborg Foundation, an EU Transnational Access Program ATANS Grant (Fp6 506004), FWO post-doctoral fellowships (to PDF and EDLP) and the Special Research Fund of Ghent University (to RG). We are also grateful to Anna Shevtsova, Bente Graae, Thilo Heinken, Sharon Stanton, An De Schrijver, Luc Willems, Greet De Bruyn, Rob Dhondt, Emma Holmström and Eva Sorgeloos and the staff at the Abisko Scientific Research Station for discussions, accommodation, facilities and assistance with the field work.

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