Increased seedling establishment via enemy release at the upper elevational range limit of sugar maple

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Abstract. The enemy release hypothesis is frequently invoked to explain invasion by non-native species, but studies focusing on the influence of enemies on natural plant range expansion due to climate change remain scarce. We combined multiple approaches to study the influence of plant–enemy interactions on the upper elevational range limit of sugar maple (*Acer saccharum*) in southeastern Québec, Canada, where a previous study had demonstrated intense seed predation just beyond the range limit. Consistent with the hypothesis of release from natural enemies at the range limit, data from both natural patterns of regeneration and from seed and seedling transplant experiments showed higher seedling densities at the range edge than in the core of the species' distribution. A growth chamber experiment manipulating soil origin and temperature indicated that this so-called "happy edge" was not likely caused by temperature (i.e., the possibility that climate warming has made high elevation temperatures optimal for sugar maple) or by abiotic soil factors that vary along the elevational gradient. Finally, an insect-herbivore-exclusion experiment showed that insect herbivory was a major cause of seedling mortality in the core of sugar maple's distribution, whereas seedlings transplanted at or beyond the range edge experienced minimal herbivory (i.e., enemy release). Insect herbivory did not completely explain the high levels of seedling mortality in the core of the species' distribution, suggesting that seedlings at or beyond the range edge may also experience release from pathogens. In sum, while some effects of enemies are magnified beyond range edges (e.g., seed predation), others are dampened at and beyond the range edge (e.g., insect herbivory), such that understanding the net outcome of different biotic interactions within, at and beyond the edge of distribution is critical to predicting species' responses to global change.

Key words: *biotic interactions; climate change; enemy release; range expansion; sugar maple.*

Introduction

Global climate change is expected to result in widespread geographic shifts of species distributions (Parmesan 2006). Studies have indeed documented poleward and upward elevational expansions of species' ranges in response to environmental changes in recent decades (Walther et al. 2005, Beckage et al. 2008, Feeley et al. 2011, Zhu et al. 2012, Delzon et al. 2013, Boisvert-Marsh et al. 2014, Urli et al. 2014). However, the magnitude and even the direction of range shifts are not always as predicted based on a warming climate (Crimmins et al. 2011, Foster and d'Amato 2015). For example, many studies have shown that suitable climatic conditions for a given species have shifted much faster than species range limits (Davis 1989, Beckage et al. 2008, Bertrand et al. 2011, Zhu et al. 2012, Corlett and Westcott 2013, Delzon et al. 2013, Savage and Vellend 2015). Such time lags might be due to purely demographic processes

(limited dispersal distances and long life spans), or to non-climatic factors that deterministically slow migration. Ultimately, predicting species responses to climate change requires understanding not only the direct influence of climatic variables on range limits, but also the way by which non-climatic factors (e.g., soil properties or biotic interactions) vary along putatively climatic gradients (e.g., latitude or elevation) and how those factors also influence various demographic processes (Lafleur et al. 2010, Blois et al. 2013, HilleRisLambers et al. 2013, Brown and Vellend 2014).

Temperature or temperature-related variables (e.g., growing season length) are widely recognized as important determinants of cold- or leading-edge plant geographic range limits, especially at broad spatial scales (Hewitt 2000, Hampe and Petit 2005, Walther et al. 2005). Cold and extreme climatic events can reduce survival or prevent completion of the life cycle via their influence on flowering, fruit ripening, or seed set (Pigott and Huntley 1981, Gaston 2003, Morin et al. 2007). However, non-climatic variables can also play an important role in determining range limits. For example, soil properties,

such as nutrient availability or pH, beyond the current range limit may facilitate or hamper species range expansion, if they are more or less favorable than in the extant range, respectively (Lafleur et al. 2010).

Climate change is also expected to create novel combinations of co-occurring species (Bertrand et al. 2011), and the potential for range expansion will be influenced by various biotic interactions (Davis and Shaw 2001, Aitken et al. 2008, Boulangeat et al. 2012, Meier et al. 2012, HilleRisLambers et al. 2013). If leading-edge populations encounter relatively strong negative interactions (e.g., seed predation or competition) or relatively weak positive interactions (e.g., absence of mycorrhizal mutualists), range expansion will be slower than that predicted based on models assuming constancy of biotic interactions across space (van der Putten et al. 2010). Likewise, weak negative or strong positive interactions can accelerate range expansion. For example, colonizing non-native species as well as native species at naturally low densities may experience "enemy release" (Callaway et al. 2004, van der Putten et al. 2010), as predicted by the Janzen-Connell hypothesis (Janzen 1970, Connell 1971). Some empirical case studies have found that leading-edge plant populations experience reduced negative impacts of herbivores or pathogens (Engelkes et al. 2008, Van Grunsven et al. 2010, Lakeman-Fraser and Ewers 2013), while others find no such effect (e.g., Skou et al. 2011). However, we cannot draw generalizations at present given that so few empirical studies have tested how non-climatic factors influence the leading edge of natural species ranges (HilleRisLambers et al. 2013, Brown and Vellend 2014).

For trees (and for plants in general), range expansion requires viable seed-producing populations at or near the current range edge, dispersal beyond the range edge, successful establishment, and subsequent population growth (Hewitt 2000, Hampe and Petit 2005, Aitken et al. 2008, Thuiller et al. 2008, Meier et al. 2012). Seedling establishment, involving germination, emergence, and the initial years of seedling survival, is especially sensitive to both physical factors (e.g., temperature, moisture, and nutrients) and biotic factors (e.g., litter depth, seed and seedling predators, and pathogens) (Clark et al. 1998). Seedling establishment is thus thought to be more sensitive to climate change than is adult tree growth and survival, thus constituting a potential bottleneck to population persistence and establishment at range edges and beyond (Walck et al. 2011). Early life stages thus represent an especially important focus of studies of plant range limits and potential expansion.

Here we present several observational and experimental studies aimed at testing the hypothesis that enemy release leads to increased seedling establishment at the upper elevational range limit of a dominant tree species in eastern North America, sugar maple (*Acer saccharum*). We also report results concerning the influence of temperature and soil on regeneration along the same elevational gradient. At our montane field site in southern Québec, Canada (Parc national du Mont Mégantic), sugar maple is the dominant species at low elevations (~700 m above sea level), but is absent above 850 m, up to the maximum elevation of ~1100 m, where boreal species such as balsam fir (*Abies balsamea*) dominate. Using experiments involving transplantation of soil and seeds within and beyond sugar maple's upper elevational range edge, as well as exclusion of granivores, Brown and Vellend (2014) showed that (1) high seed predation strongly reduced seedling establishment beyond the current range edge (>850 m above sea level) and (2) seedling establishment was lower in soil from the high-elevation boreal forest than in soil from within sugar maple's range, when both were grown within the range. Interestingly, seedling establishment over one year (regardless of soil) was higher in sites right at the species range edge (~800 m) than at sites within the range (~700 m). This led us to hypothesize that edge populations were experiencing enemy release, perhaps from insect herbivores, which were observed in abundance at low elevations. Previous experiments were neither able to assess the influence of soil at the colder temperatures found at high elevation (due to >90% seed predation), nor to disentangle climate effects from other unmanipulated factors that covary with elevation.

Here we combine multiple approaches to test different predictions of the enemy release hypothesis: (1) Using (a) natural patterns of abundance, regeneration, and seed availability (b) data for one additional year on the seedlings established in the soil-transplant experiment of Brown and Vellend (2014), and (c) a new seedling transplant experiment, we tested whether seedling establishment was indeed greater at the range edge than in the center of the range. (2) Using an insect-herbivore-exclusion experiment, we tested explicitly for enemy release at the range edge. We predicted the greatest leaf damage and the greatest influence of herbivory on seedling survival within the species range where it is most abundant. (3) Using a growth chamber experiment, we tested the influence of soil origin (within the range, at the range edge, or beyond the range edge) and temperature regime (low vs. high elevation) on seedling survival and growth. This growth chamber experiment also provided a very preliminary test of the possibility of release from the negative effects of soil pathogens at and beyond the range edge, with the prediction that seedling survival should be lowest on soils from within the species range (Table 1).

Materials and Methods

Study site

We conducted this study in Parc national du Mont Mégantic (45°26′51″ N, 71°06′52″ W), located in southeastern Québec, Canada (Appendix S1: Fig. S1a, b). Vegetation is typical of the northern Appalachian Mountains (Marcotte and Grantner 1974, Savage and Vellend 2015), with an elevational vegetation zonation

TABLE 1. Overview of the different key questions and predictions tested in this paper with their associated study or experiment and data sets.

Study/experiment	Key question (for this paper)	Happy-edge prediction	Data
(1) Natural patterns of abundance	How do the densities of adults, seedlings, and seeds vary with elevation?	Seedling density is maximal at the range edge, despite declining adult and seed densities.	Plot/trap-based observations on adults (2012), seedlings (2012–2014) and seeds (2013, 2014).
(2) Soil transplant experiment	How does seedling survival vary with elevation?	Seedling survival is maximal at the range edge.	Follow-up of seedlings planted by Brown and Vellend (2014); here only interested in elevation (not soil).
(3) Herbivore exclusion experiment	How does the effect of insect herbivory on seedlings vary with elevation?	Negative effects are greatest at low elevation.	Seedlings planted at different elevations and protected (or not) from herbivores.
(4) Growth chamber experiment	How do climate and soil source influence seeds/seedlings?	Higher performance on soil from the range edge (possibly due to pathogen release).	Plant seeds on soils from different elevations in warm and cold growth chambers.

from temperate forest dominated by sugar maple (*Acer saccharum* Marshall) at low elevation to boreal forest dominated by balsam fir (*Abies balsamea* (L.) Mill.) and spruce (*Picea* spp.) at high elevation (elevational range 430–1105 m above sea level; SEPAQ 2010). The climate in this region is characterized by cold winters with abundant snowfall, and wet and warm summers (SEPAQ 2010). During 2013–2014 at low (599 m) and high (1089 m) elevations, mean annual temperatures in the park were 3.9°C and 1.2°C, respectively (weather stations IQUBECNO2 and IQUBECNO3, Personal Weather Station Network; *available online*).[6] Combined with data from iButtons (Maxim Integrated, San Jose, California, USA) collected at our specific study plots between July 2012 and June 2013 (Appendix S2), this corresponds to an adiabatic lapse rate of ~0.6°C/100 m. At higher elevations, the soil tends to be more acidic, especially in the boreal forest zone, where decomposition is relatively slow and the soil is shallow (Marcotte and Grantner 1974). Our study had four main components (see Table 1), as follows.

Natural patterns of adult tree, seedling, and seed densities

In order to quantify natural variation in the abundance of mature trees and seedlings, we surveyed 14 plots of 400 m^2 (20 × 20 m), distributed evenly along two transects (referred to as the north and south transects) spanning the elevational range of sugar maple on Mont St-Joseph (see Appendix S1: Fig. S1d). In July–August 2012, we identified and counted all trees >1.4 m in height and ≥10 cm diameter at breast height (DBH; 1.37 m from ground) in each plot. We report only the results for sugar maple here. In July and August 2012, 2013, and 2014, sugar maple seedlings were counted in 20 1 m^2 subplots spaced systematically along two perpendicular transects

[6]www.wunderground.com/weatherstation/overview.asp

intersecting the center of each 400-m^2 plot (10 subplots per transect, spaced ~2 m apart). In 2013 and 2014 (but not 2012), seedling ages were also determined by counting terminal bud scars (Graignic et al. 2013).

Following Brown and Vellend (2014), for most analyses, we distinguished three elevational zones along these transects (see Appendix S1: Fig. S1c,d): (1) "within" sugar maple's distribution where the species represents >80% of adult trees (697–752 m and 716–783 m on the north and south transects, respectively), (2) at the "edge" of sugar maple's distribution where relative density averages <50% (783–813 m and 800–845 m for the north and south transects, respectively), and (3) "beyond" sugar maple's elevational limit (>851 m and >893 m for the two transects).

In order to estimate seed availability, we monitored 80 seed traps positioned along four transects located between the north and south transects and between ~710 to 800 m in 2011 and 2012, and 57 traps along the north and south transects (plus two transects between them) between ~710 m to 840 m in 2013. Each trap consisted of a 45 × 30 × 20 cm plastic box that was fixed to the soil and covered with mesh that was sunken slightly downward into the box to collect seeds.

Seedling establishment in the soil transplant experiment 2011–2013

Brown and Vellend (2014) conducted a soil reciprocal transplant experiment to test the effects of soil provenance on seedling establishment at different elevations (and therefore climatic conditions). In October 2011, seeds were sown at six sites: three positions with respect to sugar maple range (Within, Edge, and Beyond) along each of two elevational transects (north and south; Appendix S1: Fig. S1d). Ten seeds were sown in each experimental unit (2-L peat pots, filled with soil from different elevations) in October 2011 (a total of 800 seeds at Within and Edge

sites, 600 at Beyond sites) and seedlings were thinned to two per pot in June 2012. The number of seedlings was recorded in 2012 (reported in Brown and Vellend 2014) and in 2013 (reported here). Here we analyze only seedling survival for Within and Edge sites, because seedling establishment beyond the range was very low in Brown and Vellend (2014). While there was a significant tendency for reduced survival on Beyond soils in 2012 (Brown and Vellend 2014) and in 2013 (results not shown), the effect was very weak, and more importantly there was no elevation × soil source interaction. Therefore, we do not consider the soil treatment as a factor in our analyses. In addition, after two years of growth, we expect roots to grow beyond our 2-L peat pots, and thus to experience similar soil conditions, irrespective of the soil that had been placed in the 2-L pot originally.

Seedling transplant experiment with herbivore exclusion

Using sugar maple seedlings, we conducted a transplant experiment with herbivore exclusion treatments in order to test for enemy release at and Beyond the species' upper elevational range limit. On 7–9 October 2013, two one-year-old sugar maple seedlings were carefully excavated (allowing part of the rhizosphere soil to adhere to roots) from locations across the elevational gradient (720–830 m). Seedlings were stored in dampened plastic bags at 3°C before transplantation. On 10–12 October 2013, 720 seedlings were planted at six sites: Within, Edge, and Beyond along two elevational transects (north and south; Appendix S1: Fig. S1d). This experiment was initially designed to also include a watering treatment (to alleviate summer moisture stress), although given relatively high rainfall, the weekly water addition treatment had no influence on soil water content so it was not incorporated into the analyses (see Appendix S3).

During May and early June 2014 (immediately following snowmelt), we applied herbivore exclusion treatments, consisting of three levels of protection (assigned at random to seedlings): complete exclusion of herbivores, denoted as H; partial exclusion, (H); and no exclusion or control, C. Complete exclusion consisted of a cube-shaped tent (30 × 30 × 20 cm) constructed with fabric designed specifically to protect plants from insects (PRO17, AgroFabric, Atlanta, Georgia, USA), and held in place with four wire stakes. The bottom of the cube had a small opening and draw string to close around the base of the seedling. PRO17 AgroFabric is permeable to water and permits passage of 95% light. Using HOBO sensors (HOBO Pro v2 logger, Onset, Cape Cod, Massachusetts, USA), we detected no difference in temperature or relative humidity surrounding seedlings with or without protection (paired t test, $t = 0.14$, $P = 0.84$ and $t = -0.193$, $P = 0.89$ for temperature and relative humidity respectively, $n = 8$) during preliminary tests. Partial leaf protection (restricting but not completely limiting access to insects) consisted of the same tent, except with holes (~110 cm^2) cut into two opposite faces of the tent.

At each site, 12 seedlings were planted in each of 10 blocks to account for environmental heterogeneity. In each block, three seedlings were assigned to the complete exclusion treatment, three to the partial exclusion treatment, and three to six to the control treatment (the latter depending on how many seedlings survived the winter). Seedlings that died prior to the assignment of treatments were not included in the experiment. Seedling survival and the presence of foliar damage (FD) caused by insects or diseases were monitored throughout the growing season, approximately once every two weeks. At three time points (once in June, July, and August), we distinguished the following types of foliar damage: holes, tatter, and other (see photos in Appendix S4). For sugar maple seedlings, holes are considered indicative of damage from caterpillars and beetles, while leaf tatter is mostly caused by thrips. The "other" category typically included subtle brown or white flecks. Brown flecks with physical damage (not evident early in the growing season) could be skeletonized or mined leaves, indicative of damage from slugs, caterpillars and flies. Fungi, air pollution, or disease could cause some brown flecks without physical damage. White flecks are likely caused by leafhoppers but some could also be a symptom of plant nutrient deficiency (Gardescu 2003; Marc T. J. Johnson, *personal communication*).

Growth chamber experiment: soil × temperature

To test the effects of temperature regime and soil provenance (biotic and abiotic effects combined) on seedling establishment and growth, we sowed sugar maple seeds in field-collected soils in growth chambers. In autumn 2012, 10 soil samples were collected to 10 cm depth, after removing the litter layer, from each of the six field sites used in the soil transplant experiment. Soil samples were stored at 2°C until the beginning of the growth chamber experiment. The 10 soil samples from each site were combined to produce a single soil sample per site (hereafter "soil source"). Soil was sieved to remove roots and large (>3 cm) rocks and debris, and 20, 11 cm diameter, ~1-L, plastic pots were filled with soil from each of the six sites (120 pots total). Each pot was sown with five germinated sugar maple seeds. Seeds were collected from Within and Edge zones in autumn 2011 to be used in the soil-transplant experiment (Brown and Vellend 2014) and the growth chamber experiment. As Brown and Vellend (2014) showed no effect of seed source on seedling recruitment and only a marginal effect on seedling growth, for the growth chamber experiment, we drew seeds randomly from the pooled seed collection, rather than including seed source as an additional treatment. Prior to planting, seeds were stratified for eight weeks at 2°C on moist peat moss to stimulate germination. Only germinated seeds were planted in the experiment to ensure the presence of seedlings in each pot.

Five pots from each soil source were randomly assigned to each of four growth chambers. Two temperature

treatments were simulated, with two replicate chambers per treatment: "warm" and "cold" chambers corresponded to low- and high-elevation temperature regimes in the study area, respectively. This translated to a 2°C temperature difference (~300 m elevation difference) between treatments (Appendix S2). Temperatures for the warm treatment were determined based on ground-level air temperatures monitored in 2012 using iButtons at the Within sites used in the soil transplant experiment. For the first four weeks of the experiment, chambers were maintained at mean May daytime and nighttime temperatures (warm 8.1°/13.6°C, cold 6.1°/11.6°C night/day), followed by four weeks of mean June temperatures (warm 11.6°/17.1°C, cold 9.6°/15.1°C) and then mean July temperatures (warm 14.8°/20.3°C, cold 12.8°/18.3°C). Chambers were exposed to 15 h of light per day (640 μmol·m^{-2}·s^{-1}) throughout the experiment, corresponding to mean daylight hours during the simulated period of May–July in the study area. Pots were watered three times per week, and seedlings were thinned to a maximum of two seedlings per pot once true leaves emerged.

We monitored seedling survival and height throughout the duration of the experiment (January–May 2013). The experiment ended once seedling leaves began to senesce, at which time seedlings were clipped at the soil surface, air dried, and weighed to determine aboveground dry biomass.

Statistical analyses

With observational data, we used (1) generalized linear mixed-effects models (GLMMs) to analyze the response of adult tree density to elevation with transect as a random effect, (2) GLMMs to analyze the response of seedling density to elevation and year with transect and plot (nested within transect) as random effects, (3) GLMMs to analyze the response of seedling density to elevation and seedling age (1-, 2-, 3-, 4-, and >4-yr-old seedlings) in 2013 and 2014 with transect as a random effect, and (4) generalized linear models (GLMs) to analyze the response of number of seeds collected per seed trap to elevation and year (2011 and 2013, no seeds were found in the seed traps in 2012). These models were fit with a Poisson or a negative binomial distribution of residuals depending on the dispersion of the data. A model was considered overdispersed if the ratio of residual deviance to residual degrees of freedom was >1, in which case, a negative binomial distribution of residuals was assumed (Zuur et al. 2009). GLMs with a quadratic term and a log link function were used to estimate the elevation at which maximum density occurred, elev$_{opt}$ (see Appendix S5 for details).

For the soil-transplant experiment, we used GLMMs to model the number of seedlings per block as a function of elevational zone and year, with transect and block (nested in transect) as random effects. These models assumed a negative binomial distribution of residuals.

For the herbivore exclusion experiment, we used GLMMs with a negative binomial distribution of residuals to analyze the response of (1) seedling survival, (2) occurrence of seedlings with foliar damage, and (3) occurrence of seedlings with each type of foliar damage (holes, tatter, and others) to time, elevational zone, and herbivory treatment with transect and experimental unit (nested in transect) as random effects. For the growth chamber experiment, we used GLMMs to analyze the response of seedling survival (binomial distribution) at the end of the experiment and linear mixed-effects models to analyze the response of seedling height and biomass to soil source (Within, edge, and Beyond) and chamber temperature (warm vs. cold) with pot (nested in chamber and transect) as a random effects.

In all analyses, standard procedures for model diagnostics were conducted. All analyses were conducted using R version 3.0.1 (R Development Core Team 2012) using the lme4 package for GLMMs (Bates et al. 2015) and multcomp for post hoc tests (Hothorn et al. 2016).

RESULTS

Natural patterns of adult tree, seedling, and seed densities

Adult tree density decreased markedly with elevation (Fig. 1a, Appendix S6: Table S1a), and was at its maximum at relatively low elevations (elev$_{opt}$ = 733 m and 750 m for north and south transects, respectively). Natural seedling density was best predicted by elevation, year, and the elevation × year interaction (Appendix S6: Table S1b). Seedling density was strongly influenced by elevation in all years ($t = -5.1$, $P < 0.0001$, Fig. 1b–d), and also showed inter-annual variation, being lower in 2013 than in 2012 ($t = -5.35$, $P < 0.0001$, Fig. 1b, c) but then higher in 2014 ($t = 4.19$, $P < 0.0001$, Fig. 1d). The interaction between elevation and year was highly significant (2013 $t = 4.37$, $P = 0.001$ and 2014 $Z = -4.45$, $P < 0.0001$; Fig. 1b–d), with the position of maximum density occurring at relatively high elevations in 2012 (e.g., ~800 m for south transect, Fig. 1b) and in 2013 (>769 m for both transects, Fig. 1c) and at lower elevation in 2014 (Fig. 1d).

In the analyses incorporating seedling age (2013 and 2014 only), results varied between years. In 2013, seedling density was best predicted by a GLMM that included elevation and seedling age as fixed factors (Appendix S6: Table S1c). In 2014, seedling density was best predicted by a GLMM that included elevation, seedling age, and the elevation × age interaction as fixed factors (Appendix S6: Table S1d). Interestingly, the mortality rate of seedlings from 2012 cohort between 2013 and 2014 was higher at Within (81%) than at Edge (58%; see Appendix S7).

The number of seeds per seed trap decreased with elevation in 2011 and 2013 ($Z = 5.59$, $P < 0.0001$; Appendix S6: Table S1e), with an absence of seeds beyond 810 m (Fig. 2). Seed availability was high in both 2011 and 2013,
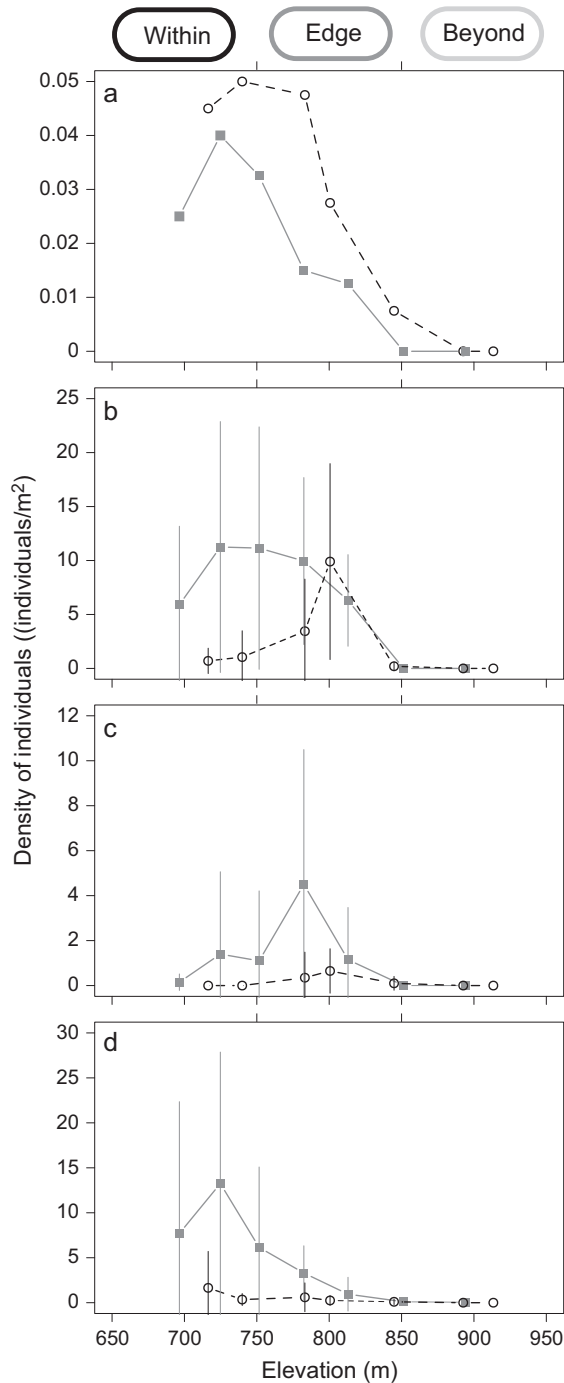
FIG. 1. (a) Adult tree and (b–d) seedling densities along the elevational gradient. Seedling densities are shown separately for (b) 2012, (c) 2013, and (d) 2014 along the north (gray squares and lines) and south (open black circles and dashed lines) transects. Symbols and vertical lines represent the mean and standard deviation of density of individuals in 20 1-m² plots.

whereas no seeds were found in seed traps in 2012. The decline in seed density with elevation was relatively weak in 2011 compared to 2013 (elevation × year, $Z = -6.25$, $P < 0.0001$; Appendix S6: Table S1e), such that seed

density in Edge sites was much higher in 2011 (19 ± 8 seeds [mean ± SD]) than 2013 (5 ± 6 seeds), with little difference between years at Within sites (25 ± 10 and 20 ± 11 seeds in 2011 and 2013, respectively, Fig. 2).

Seedling establishment in the soil transplant experiment 2011–2013

The number of seedlings per block from the soil reciprocal transplant experiment was best predicted by a GLMM including zone, year, and the zone × year interaction (Appendix S6: Table S2a). Mortality led to fewer seedlings in 2013 than in 2012 ($t = -4.96$, $P < 0.0001$, Fig. 3a), and seedling establishment was markedly higher at Edge than at Within sites ($t = 9.97$, $P < 0.0001$, Fig. 3a).

Seedling transplant experiment with herbivore exclusion

Focusing first on differences among elevational zones, a GLMM showed that the number of seedlings varied significantly among zones (Appendix S6: Table S2b), with survival higher at Edge ($z = 4.66$, $P < 0.0001$) and Beyond ($z = 6.14$, $P < 0.0001$) than Within (Fig. 3b).

Incorporating experimental treatments, seedling survival was best predicted by a GLMM that included time, zone, herbivory treatment, and the three two-way interactions (Appendix S6: Table S3a), while the probability of occurrence of foliar damage (FD) was best predicted by a GLMM that included time, zone, herbivory treatment, and two two-way interactions (Appendix S6: Table S3e). Not surprisingly, across all elevational zones and herbivory treatments, seedling survival decreased and occurrence of foliar damage increased as the growing season progressed ($z = -10.69$, $P < 0.0001$, Fig. 4a–c and $z = 15.50$, $P < 0.0001$, Fig. 4d–f). Seedlings with herbivores completely excluded showed higher survival and lower occurrence of foliar damage than those in the partial exclusion treatment ($z = 3.15$, $P = 0.001$ for
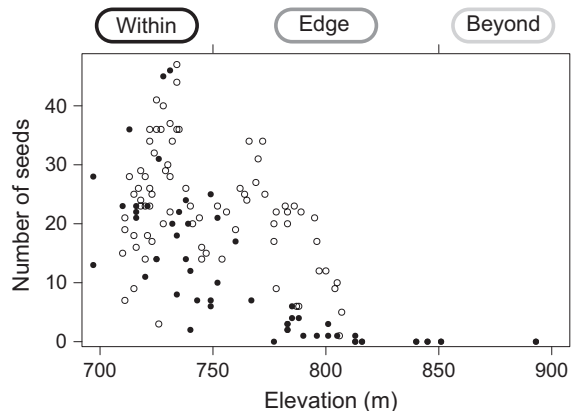

FIG. 2. Number of seeds per seed trap along the elevation gradient in 2011 (open circles) and 2013 (solid circles). These seeds produced the seedlings of the 2012 and 2014 cohorts, respectively.
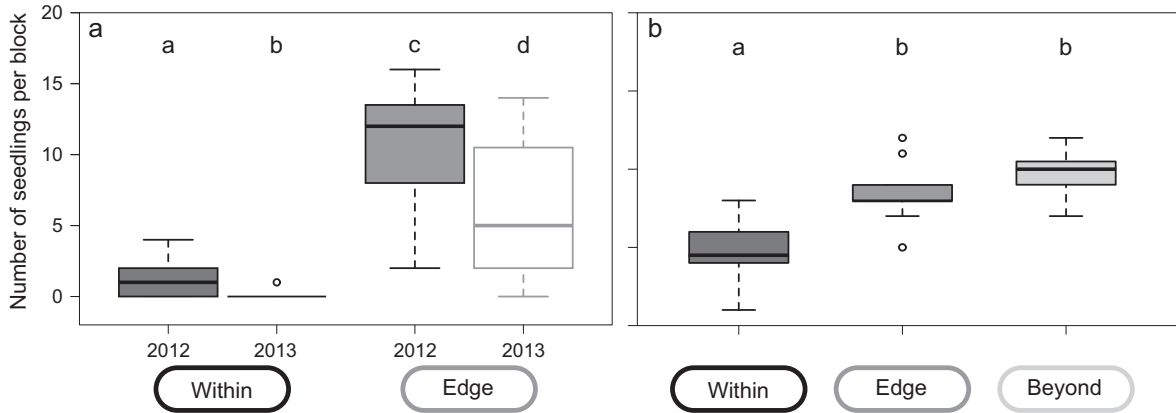
Fig. 3. The number of seedlings per block (a) between elevational zones in 2012 and 2013 in the soil transplant experiment and (b) between elevational zones during the last week of the seedling transplant experiment (week 40 in 2014). Different lowercase letters indicate significant differences ($P < 0.05$; comparisons within panels).
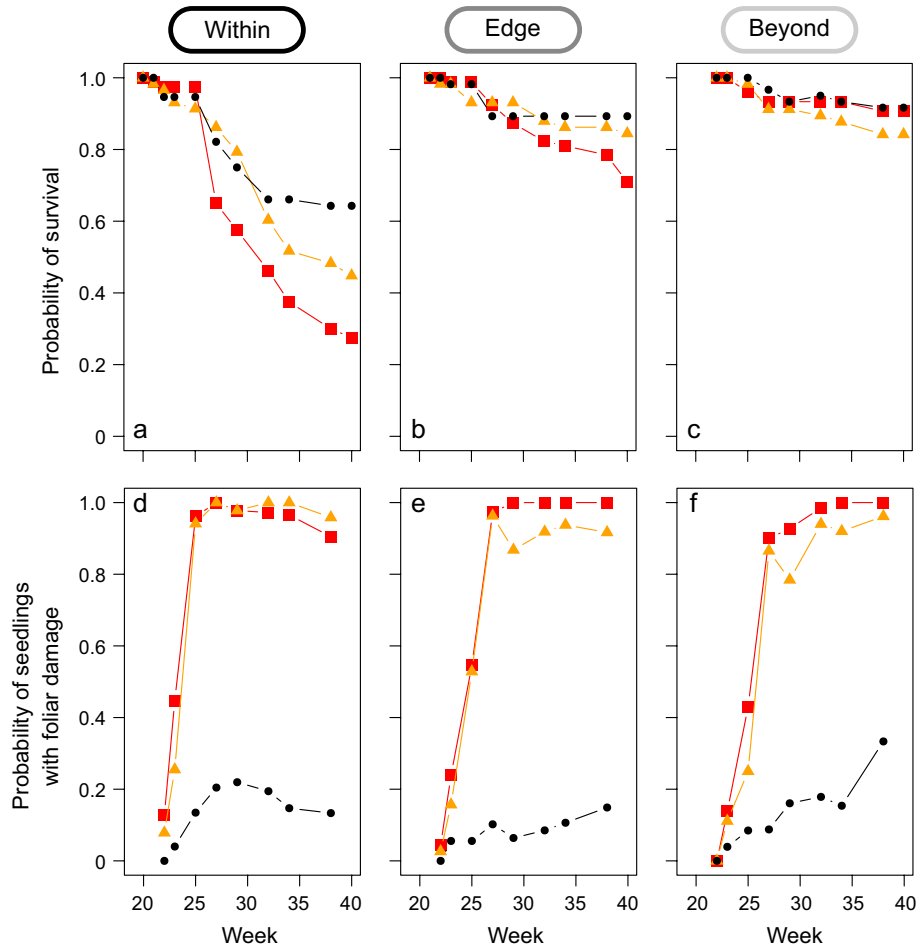

Fig. 4. Probability of (a–c) seedling survival and (d–f) foliar damage during the growing season of 2014, between 13 May (week 20) and 2 October (week 40), in the three elevational zones and for the three herbivory treatments: complete herbivore exclusion (black dots, H), partial exclusion (orange triangles, (H)) and the control treatment (red squares, C). Treatments were implemented immediately following snowmelt: on week 20 at Within, week 21 at Edge and week 23 at Beyond. These probabilities were calculated from raw data. [Color figure can be viewed at wileyonlinelibrary.com]

survival and $z = 3.29$, $P = 0.001$ for FD) and in the control treatment ($z = 3.86$, $P = 0.0001$ for survival and $z = 1.99$, $P = 0.047$ for FD). For all forms of foliar damage (holes, tatter, or other), most damage occurred between June and July and remained stable in August (Appendix S6: Table S4). As indicated above, seedling survival was significantly lower at Within than at Edge and Beyond ($z = 3.00$, $P = 0.003$ and $z = 2.63$, $P = 0.0001$, respectively Fig. 4a–c). In addition, the occurrence of foliar damage was significantly higher at Within than at Edge and Beyond ($z = -4.12$, $P < 0.0001$ and $z = -5.95$, $P < 0.0001$, respectively Fig. 4d–f). Moreover, the time at which 95% of seedlings (without protection) experienced at least some foliar damage occurred earlier during the growing season at Within (week 25) than at Edge (week 27) and Beyond (week 32; Fig. 4d–f).

As predicted by the enemy-release hypothesis, the effect of the herbivore exclusion on seedling survival and occurrence of foliar damage was more pronounced at Within than at Edge and Beyond (significant zone × herbivory interaction, Appendix S6: Table S3a, e). Indeed, at the end of the growing season at Within, seedling survival in the complete herbivore exclusion treatment (~65%) was twice that observed in the control treatment (<30%; Fig. 4a; $z = 4.3$, $P < 0.0001$; Appendix S6: Table S3b) whereas at Edge and Beyond, survival was >75% regardless of herbivory treatment (Fig. 4b, c, Appendix S6: Table S3c, d). We observed the same trends for each type of foliar damage (Fig. 5, Appendix S6: Table S4), most strikingly for leaf holes (Fig. 5). Most foliar damage consisted of holes or other types of damage, with 100% of seedlings in the control treatment presenting these types of foliar damage at Within by the end of the growing season (Fig. 5).

Growth chamber experiment: soil × temperature

Seedling survival in the growth chamber was lower on soil from Within the range than on soil from the range Edge or Beyond (best model included soil source, Appendix S6: Table S5a, Table 2). We detected no

differences in seedling growth metrics (seedling height and aboveground dry biomass) among soil sources. Temperature had an effect on height and aboveground dry biomass (but not on seedling survival), with the height and biomass of seedlings in warm chambers being higher than those in cold chambers (best predicted model included temperature; Appendix S6: Table S5b, c).

DISCUSSION

Enemy release at the range edge

Several lines of evidence presented here are consistent with the hypothesis of enemy release at the upper elevational range limit of sugar maple. Despite declines with elevation in both mature tree abundance and seed availability, maximal seedling densities were found near the range edge, rather than at lower elevations, in 2012 and 2013. Exceedingly few seeds were produced above 775 m elevation in 2013, such that the observation of a steady decline in seedling density with elevation in 2014, due almost entirely to 1-year-old seedlings, is not inconsistent with increased seedling survival at the range edge. Increased seedling survival at the range edge was clear from seed sowing experiment initiated in 2011, as well as the seedling transplants initiated in 2013.

It is possible that a "happy edge" could be due to climate warming having shifted the species' temperature optimum up to its range edge, but two results suggest that this is unlikely: (1) the growth of surviving seedlings was *greater* under low-elevation rather than high-elevation temperature regimes in growth chamber experiments and (2) the growth of surviving seedlings in the field did not vary among elevational zones (soil transplant experiment; Brown and Vellend 2014). The latter result also makes it unlikely that abiotic factors (e.g., soil nutrients, some of which are at slightly higher concentrations at Edge relative to Within; Brown and Vellend 2014) play a major role here as sugar maple has a broad ecological amplitude allowing its establishment in both boreal and hardwood forests (Barras and Kellman 1998). It is
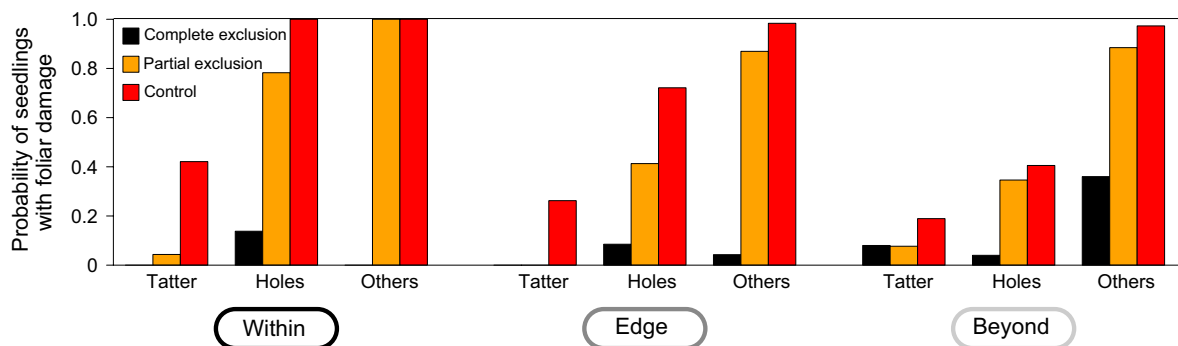

FIG. 5. Probability of seedlings with tattered leaves, holes, and other types of foliar damage at the end of the growing season of 2014 in the three elevational zones and for the three herbivory treatment levels: complete herbivore exclusion (black bars), partial exclusion (orange bars), and the control treatment (red bars). These probabilities were calculated from raw data. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. Responses of the probability of seedling survival, seedling height, and aboveground dry biomass to soil source and temperature in the growth chamber experiment.

Soil source and chamber temperature†,‡	Probability of survival	Height (cm)	Aboveground dry biomass (g)
Within			
Cold	0.250 ± 0.173	7.280 ± 0.761	0.102 ± 0.030
Warm	0.350 ± 0.173	8.029 ± 1.420	0.119 ± 0.037
Edge			
Cold	0.475 ± 0.150	7.000 ± 1.710	0.092 ± 0.032
Warm	0.450 ± 0.289	7.606 ± 1.235	0.102 ± 0.038
Beyond			
Cold	0.625 ± 0.050	7.420 ± 1.467	0.096 ± 0.029
Warm	0.400 ± 0.216	8.125 ± 1.385	0.117 ± 0.023

Notes: "Warm" growth chambers were set to mimic the day–night temperature cycle as measured at the Within field sites, updated every four weeks to mimic seasonal change starting in May. "Cold" chambers were maintained 2°C colder than warm chambers, with otherwise identical cycling and seasonal changes (details in *Materials and methods*). Values are mean ± SD.
†The probability of survival is significantly lower on soil from Within than beyond the range, there is no significant difference of probability of survival on soil from Within and at the edge of the range, and at the edge and Beyond the range.
‡Temperature had a significant effect on height and aboveground dry biomass: seedlings growing in the warmer chambers had higher height and biomass.

important to note that we cannot definitively exclude a possible influence of climate and other abiotic factors (e.g., on survival rather than growth). However, for sugar maple seedlings, pathogens and herbivores are major causes of mortality in general (Gardescu 2003, Cleavitt et al. 2014), and release from such mortality agents at higher elevations represent likely contributors to regeneration success.

Our herbivore exclusion experiment showed that insect herbivory was a major cause of seedling mortality within the core of sugar maple's elevational range, with more than 50% of seedling mortality caused by insect herbivores. In turn, seedlings growing at or beyond the range edge are, to some degree, released from herbivore pressure. Sugar maple seedlings are prone to attack by many different insects (Gardescu 2003, Cleavitt et al. 2014) and, at our site, the species most frequently encountered was *Phyllobius oblongus* (L.) (the European snout weevil, likely to fall in the "holes" category in Fig. 5), which is known as an important herbivore on sugar maple (Coyle et al. 2012). Our data and qualitative field observations allow us to speculate that the underlying mechanisms might also involve a phenological mismatch between herbivores and seedlings at higher elevations: there was a greater time lag between fully expanded leaves (phenological stage 5; Appendix S8) and 95% of seedlings showing foliar damage at higher elevations (3 weeks at Within plots, 4 weeks at Edge, and 7 weeks at Beyond).

However, insect herbivores do not provide a complete explanation for the happy edge because, even with protection from aboveground herbivores, survival was higher at Edge than Within. This points to the possibility of additional release at the range edge from the effects of microbial pathogens (above- or belowground) or belowground herbivores. Reduced seedling survival on soil from Within in the growth chamber experiment is

consistent with this hypothesis, although we are unable to pinpoint which soil properties drove this result. Root damage via soil enemies was also hinted at by the frequent observation of leaf wilting on seedlings at low elevations, where water does not appear to be limiting (i.e., wilting was not likely caused by abiotic stress). Soil moisture was in fact higher in Within sites (see Appendix S3: Fig. S1), and oomycetes, which thrive in moist soils, are well-known to cause such wilting symptoms (Agrios 2005). Some cases of density-dependent tree seedling recruitment, consistent with the Janzen-Connell hypothesis, have been found to be caused by oomycetes like *Pythium* spp. or *Phytophtora* spp. (e.g., Bell et al. 2006). The role of soil pathogens across the elevational gradient is clearly an important avenue for future research.

One of our results, reduced seedling survival on Within soils in the growth chamber experiment, is difficult to reconcile with the finding of the soil-transplant experiment of Brown and Vellend (2014) of *increased* seedling establishment on Within soils (relative to Edge and Beyond soils) for seeds planted at the Within field site. These two experiments used the same seed source. We can speculate that the effect of enemies was magnified relative to the effect of other factors (e.g., moisture retention) in the growth chamber experiment, although the only general conclusion we can draw is that the combined effect of different soil properties (e.g., nutrients, moisture, mutualists, and enemies) is likely contingent on any number of factors that differed between the field and the growth chambers (e.g., light or above-ground species interactions).

Implications for understanding range limits in a changing climate

While natural enemies have frequently been invoked to explain the success of nonnative species (Callaway et al. 2004, Cincotta et al. 2009, Maron et al. 2014) and in

explaining patterns of density-dependent seedling establishment within particular sites (Klironomos 2002, HilleRisLambers et al. 2013, Fricke et al. 2014), their relationship with species range limits and potential range expansion has only rarely been studied. A few studies have found reduced herbivore or pathogen pressure in leading-edge populations (Engelkes et al. 2008, Van Grunsven et al. 2010, Lakeman-Fraser and Ewers 2013), although this result is not universal (e.g., Skou et al. 2011). Combined with the results of Brown and Vellend (2014), our results have some important implications for understanding potential responses of species distributions to climate warming, as described in the following paragraphs.

Within the core of its elevational range, sugar maple is the dominant species, but current regeneration is limited by high insect herbivory and possibly other natural enemies. Climate warming may make such stands prone to invasion by more warm-adapted species whose recruits experience relatively low enemy pressure. At its upper elevational range limit, sugar maple grows in the hardwood-boreal-forest ecotone, where release from enemy pressure and increasingly suitable climatic conditions might lead to (or may have already led to) increased abundance. Some earlier studies support this. In an ecologically similar site in the Green Mountains of Vermont, Beckage et al. (2008) observed increases between 1964 and 2004 in sugar maple basal area of 60% and 115% in sites at 732 m and 792 m elevation, respectively. Similarly, Goldblum and Rigg (2002) observed sugar maple seedlings in the absence of adults in boreal forest stands located adjacent to maple-dominated deciduous forest. Using somewhat coarse community composition data at our field site in 1970 and 2012, Savage and Vellend (2015) observed a slow convergence of vegetation composition at high elevation toward that found at low elevations. In short, sugar maple may well be slowly invading the boreal forest.

Things get especially interesting beyond sugar maple's current upper elevational range limit. Brown and Vellend (2014), via a granivore exclusion experiment, found that most seeds sown just ~100 m beyond the range limit were consumed by seed predators, leading to very low regeneration from seed. However, some seeds do indeed escape predation, and once beyond the initial seed-to-seedling transition, survival was actually higher at Beyond than at Within or Edge and growth did not vary with elevation (Brown and Vellend 2014). Seed predation thus appears likely to slow upward elevational range expansion, but for seeds that escape predation, strong seedling survival and growth may speed up range expansion. If indeed edge populations are on the rise, seed dispersal beyond the range edge would also rise, thereby increasing the probability that some seeds make it past the predation barrier. The ultimate cause of higher seed predation beyond the range edge is unknown, but if it involves the very low availability of large tree seeds itself, predator satiation might occur eventually, thus relaxing this constraint on range expansion. This is speculative and will require further study before strong conclusions can be

made, but regardless of the details, the results emphasize the major importance of biotic interactions of various kinds in determining the potential for range expansion under climate change. From an applied perspective, if one is interested in establishing tree species beyond their current range limits for economic reasons, a major topic of current debate (Aitken et al. 2008, Dumroese et al. 2015), the common forestry practice of planting saplings instead of seeds (Nyland 2007) would overcome what appears to be the major barrier to sugar maple establishment beyond its upper elevational range limit.

In sum, our study provides one of the few compelling case studies demonstrating release from natural enemies at a leading range edge, as well as clear evidence more generally that biotic interactions play a central role in determining how species distributions will respond to climate change. The net outcome of different biotic interactions can change drastically between sites at the range edge itself and sites just beyond the range edge, making geographically explicit and detailed studies of such interactions critical for anticipating species responses to global change.

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