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Research

The importance of biotic filtering on boreal conifer recruitment at alpine treeline

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Treeline, the ecotone where forest transitions to alpine or tundra ecosystems, is considered the thermal limit to tree growth and survival. Despite temperature increases across mountainous areas and high latitudes globally, there has been no ubiquitous change in treeline position. The process of range expansion must initially depend on increased recruitment at, or beyond current range limits and recruitment limitations have been hypothesized as a mechanism for the variable response of treeline position to climate warming. We conducted a unique series of observational and experimental studies to quantify early-life stage constraints, from seed production to seedling establishment, on black spruce *Picea mariana* and tamarack *Larix laricina* recruitment at a model alpine treeline in Newfoundland, Canada. We found recruitment at treeline to be simultaneously seed and establishment limited. The treeline population produced fewer seeds than the forest population and black spruce seeds produced at treeline were less viable. Tamarack was more seed limited than black spruce where seed viability was low regardless of altitudinal position. Post-dispersal seed predation greatly constrained recruitment across the altitudinal gradient; however, black spruce seeds experienced the lowest levels of invertebrate seed predation on the lichen mat at treeline. If seeds were not consumed, individuals at treeline were establishment limited where germination and seedling establishment was both less abundant and delayed on lichen substrate. Our study highlights the need for multiple factors to align temporally for significant recruitment at treeline to occur.

Keywords: altitudinal treeline, *Larix laricina*, *Picea mariana*, range expansion, recruitment limitations, seed predation

Introduction

Trees are sessile organisms, fixed in space; therefore, any landscape-scale changes in geographic distribution are dependent on recruitment via the production or dispersal of viable seeds and subsequent seedling establishment and survival at, or beyond range limits (Nathan and Muller-Landau 2000, Briceño et al. 2015, Johnson et al. 2017). Globally, the distributional limit of trees, where forests transition to alpine or tundra ecosystems and herein termed the treeline ecotone, is ascribed as the thermal limit to

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tree growth, survival and reproduction (Körner 1998). The alleviation of thermal constraints, driven by human-induced climate change, is predicted to have widespread effects on species distributions (Chen et al. 2012) and treelines are expected to advance beyond their current climatic tolerances to greater elevations and latitudes (Malcolm et al. 2002). Despite average temperatures increasing across mountainous areas and high latitudes around the world (IPCC 2014, Pepin et al. 2015), there has been no ubiquitous change in the geographical position of global treelines (Harsch et al. 2009), suggesting that non-climatic factors, including dispersal limitations and biotic interactions, operating at local to regional scales may constrain recruitment more than direct temperature constraints (Brown and Vellend 2014, Jameson et al. 2015, Kroiss and Hille Ris Lambers 2015, Kambo and Danby 2017).

The transition from seed production to seedling establishment has long been recognized as an important bottleneck to recruitment (Harper 1977), as individuals are both abundant and highly vulnerable at early-life stages (Chambers and MacMahon 1994, Nathan and Muller-Landau 2000, Clark et al. 2013). The reproduction hypothesis states that the functional position of treeline is dictated by the failure for trees to successfully recruit beyond range limits (Körner 1998). Recruitment limitations, and the fate of seeds, must be examined across multiple stages from seeds attached to parent plants, pre-dispersal processes, to seedling establishment and survival, post-dispersal processes (Clark et al. 1999, 2013, Nathan and Muller-Landau 2000). Seed limitations, defined as the failure for a sufficient number of viable seeds to arrive at uncolonized sites, can occur due to either low seed production and/or failure to disperse (Clark et al. 2013). While recruitment at treeline cannot occur without the production and dispersal of seed, simply the arrival of seed at treeline does not guarantee recruitment will occur (Nathan and Muller-Landau 2000, Clark et al. 2013). Establishment limitations occur when environmental conditions at uncolonized sites limit seedling establishment regardless of seed arrival (Nathan and Muller-Landau 2000, Clark et al. 2013). Seed and establishment limitations are not mutually exclusive and can be thought as being inversely related, where solely seed limited populations are on the opposite end of a gradient from solely establishment limited ones (Clark et al. 2007).

Individuals at treeline have been found to produce fewer seeds relative to individuals within range limits (for example see Sirois 2000). While it is predicted that fecundity will increase with the alleviation of climatic constraints (Case and Taper 2000, Roland et al. 2014), increased production of seed does not necessarily lessen seed limitations as evidence suggests patterns in seed production do not consistently match patterns in seed viability (Sirois 2000, Roland et al. 2014, Brown et al. 2019). Pollen limitations driven by low genetic diversity and/or low abundance in parent tree populations can produce empty, non-viable seeds (Kroiss and Hille Ris Lambers 2015, Brown et al. 2019), while viable seeds produced may be removed from the population through pre-dispersal predation (Jameson et al. 2015, Kambo and Danby 2017). Treeline populations composed of krummholz individuals (i.e. stunted, clonal trees) likely produce lower viability seed due to decreased genetic diversity (Viktora et al. 2011); however, these populations might experience lower levels of pre-dispersal seed predation due to decreased stand densities (Gärtner et al. 2011). If pre-dispersal limitations are sufficiently strong, seed-mediated treeline advance will be dependent on the dispersal of viable seed from within-range populations and subsequent post-dispersal processes (Chambers and MacMahon 1994, Clark et al. 1999).

Post-dispersal processes may have additive effects on seed limitations, further constraining recruitment at treeline. Post-dispersal seed predation has long been considered an important process in regulating plant demographics (Hulme 1998). While the strength of post-dispersal seed predation has been found to decrease with increasing altitude (Hargreaves et al. 2019), experimental evidence suggests that predation at temperate and boreal treelines can significantly limit recruitment (Munier et al. 2010, Wheeler et al. 2011, Brown and Vellend 2014, Kambo and Danby 2017). Substrate can greatly influence germination success of tree species and unsuitable substrate may impede forest range expansion (LePage et al. 2000, Charron and Greene 2002, Dufour-Tremblay and Boudreau 2011, Wheeler et al. 2011, Brown et al. 2015). Moreover, high abundance of allelopathic shrub species, particularly ericaceous shrubs, at alpine treelines have been found to constrain tree recruitment (Bråthen et al. 2010, Dufour-Tremblay et al. 2012b). Allelopathic compounds are leached from leaves following rainfall or spring snow melt and are absorbed into seeds during imbibition, a crucial first stage of germination (Chiapusio et al. 1997). Substrate composition, vascular plant community composition, and the magnitude of post-dispersal seed predation likely have interactive effects. Given these complex factors, it is not surprising that recruitment at treeline has been compared to a very challenging hurdle race (Holtmeier 2009); to successfully recruit, a seed must first make it through a series of biotic filters and arrive where local environmental conditions meet the requirements for germination and establishment.

Despite the recognized importance of biotic interactions as drivers of species' geographic distributions (Hille Ris Lambers et al. 2013, Hargreaves et al. 2014) and the understanding that early-life stages can disproportionately influence the abundance and distribution of species (Harper 1977), there remains uncertainty surrounding the role of biotic interactions in recruitment limitations at tree species' distributional limits. Given the intrinsically variable nature of early-life stages (e.g. seed production and mast years), the identity and strength of biotic filters will likely vary both spatially and temporally (Clark et al. 1999). Adding further complexity, climate change may indirectly alter the identity and strength of biotic filters by altering species in which early-life stages interact (Hille Ris Lambers et al. 2013). A critical first question to ask is, do biotic interactions control recruitment at current range limits (Hille Ris Lambers et al. 2013)? If yes, these interactions will likely play

a role under alternative temporal and spatial scenarios (Hille Ris Lambers et al. 2013). Experimental and observational studies across environmental gradients that transition from within range to at, or beyond, range limits are the first steps to examining the direction and magnitude of biotic interactions at range limits and, thus, how biotic interactions may alter geographic response to climate change (Hille Ris Lambers et al. 2013, Hargreaves et al. 2014).

Individuals may be removed from a population at any life stage as a cohort of potentially germinable seeds transitions from parent plants to established seedlings. The relative importance of seed and establishment limitations on recruitment are often examined through seed addition experiments; however, seed addition experiments are limited in the ability to examine processes that drive recruitment limitations (Clark et al. 2007). As many processes can result in seed mortality, ideally seed addition experiments should be paired with studies that examine specific mechanisms (Clark et al. 2007). To quantify the magnitude of each potential biotic filter on black spruce *Picea mariana* and tamarack *Larix laricina* recruitment at alpine treeline, we conducted an unique series of observational and experimental studies along an ecologically representative altitudinal gradient in central Newfoundland, Canada (Fig. 1). Specifically, we examined whether predicted biotic filters drive: 1) seed limitations, and 2) establishment limitations, and how the magnitude of these limitations differ between forest and treeline populations. Compared to forest populations, we expected the treeline population to: 1) produce fewer viable seeds, 2) experience lower, yet still constraining, levels of post-dispersal seed predation and 3) have lower seedling emergence and establishment, driven by competitive interactions with lichen substrate and ericaceous shrubs.

Methods

The study took place on a south-west facing slope on the Baie Verte Peninsula in central Newfoundland, Canada (49°35.2′N, 56°13.7′W; Fig. 2). This ecoregion has the most continental climate of the island, with relatively warm summers and cold winters (Damman 1983), experiencing a mean annual temperature of approximately 3°C (2007–2017; Middle Arm weather station, Environment Canada). Precipitation mirrors island wide averages, with approximately 1200 mm total annual precipitation and about 350 cm falling as snow (2007–2017; Middle Arm weather station, Environment Canada). The central Newfoundland ecoregion is the most distinctly boreal assemblage on the island (Damman 1983). With increasing elevation, the study slope's vegetation transitions from a closed canopy black spruce forest to an ericaceous heath with isolated patches of stunted black spruce and tamarack. The study slope is ecologically representative of the broader geographic region, where altitudinal treelines, across the island of Newfoundland, occur at relatively low altitudes (rarely exceeding 350 m a.s.l.) and areas at, or above treeline support krummholz and alpine heaths (MacPherson 1995).

We established two study sites: the forest site at low elevation within the study species range (~130 m a.s.l.) and the treeline site at the study species range limits (~240 m a.s.l.; Fig. 2). The forest site understory has sparse vascular plant cover (predominately *Cornus canadensis* and *Clintonia borealis*) and is dominated by feathermoss (predominately *Pleurozium schreberi* with *Hylocomium splendens* also present). The treeline site is composed of distinct krummholz islands of black spruce interspersed in open areas dominated by ericaceaous shrubs (predominately *Kalmia angustifolia*,

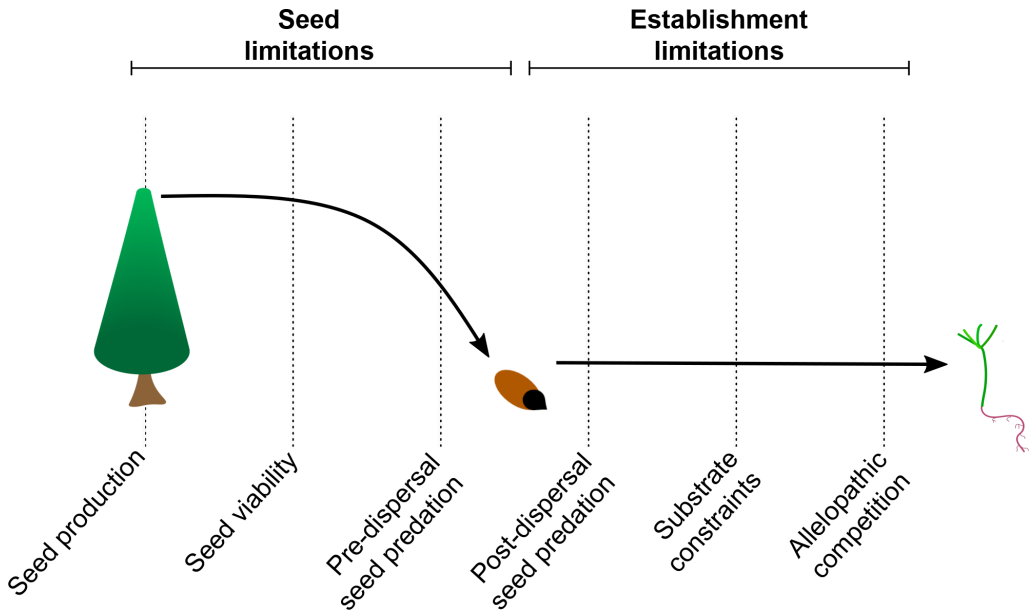


Figure 1. Predicted biotic filters to recruitment that were examined through a series of observational and experimental studies along an altitudinal gradient in central Newfoundland, Canada.

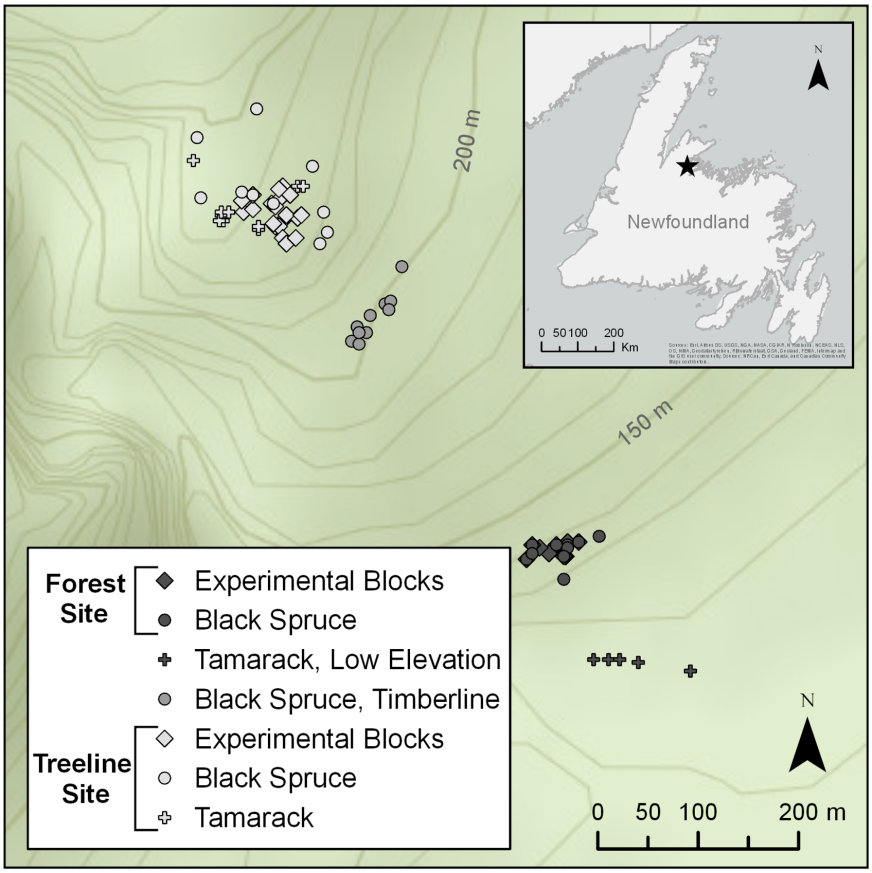


Figure 2. A map of the south-west facing study slope in King's Point, NL. The experimental blocks mark the location of both the field germination and post-dispersal seed predation experiment study blocks, while the black spruce and tamarack symbols denote the location of individuals where cones were harvested for the seed production, viability and pre-dispersal predation observational study. The star on the inset map marks the location of King's Point, NL.

followed by *Rhododendron groenlandicum* and *Vaccinium angustifolium*). Caribou lichens (predominately *Cladonia stellaris*) dominate the treeline understory, while isolated patches of mosses (predominately *Pleurozium schreberi*) occur at the base of black spruce and tamarack individuals. A subset of biotic filters were additionally examined at timberline, defined as the uppermost limit to the forest population where trees are still in arborescent form but are less dense than closed canopy forest (~200 m a.s.l.). The understory community at timberline represents a mid-point between the forest and treeline sites, where ericaceous shrubs occur at intermediate abundances and isolated patches of caribou lichens are interspersed between the feathermoss substrate.

Pre-dispersal seed production and viability

Maturing black spruce and tamarack cones were harvested at the end of the 2017 growing season (19–21 September). Cones were collected from low and high elevation, in the general proximity of the established forest and treeline study sites; and in addition, black spruce cones were also collected at timberline. At the treeline site, we harvested all the black spruce cones within 10 tree krummholz islands (aggregations of stunted and deformed, shrub-form trees; Harsch and Bader 2011) and harvested a subset of cones from 10 tamarack individuals. At the forest site, where black spruce individuals were too tall to harvest cones directly, we collected individual cones and cone-bearing twigs recently clipped by red squirrels *Tamiasciurus hudsonicus*, which were abundant on the forest floor, from directly under the canopies of 10 individuals. No cones collected from the forest floor showed signs of red squirrel predation. As no tamarack occurred directly within the forest site, low elevation tamarack cones were collected from five individuals growing in an open, wet meadow approximately 75 m away. At timberline, we harvested cones from 10 black spruce individuals; tamarack was absent from this location. Across all sites, individuals from which cones were harvested were chosen at random. Cones were collected into paper bags, brought back to the laboratory, and stored at 20°C until seed extraction.

In the laboratory, seed extraction from cones followed established standard protocols (black spruce protocol modified from Safford 1974, Leadem et al. 1997, and Green unpubl.; tamarack protocol modified from Pauley 1965, Rudolf 1974). Cones were soaked in deionized water for approximately 24 h and let dry at room temperature for

24–72 h. Black spruce cones were then dried at 60°C for 16 h, while tamarack cones were dried at 50°C for 8 h. Following drying, cones were tumbled in a sieve shaker for 10 min to separate extracted seed from woody cone material. The extraction cycle was repeated three times for black spruce and twice for tamarack. Extracted seeds were grouped by individual.

Extracted seeds were counted and seed production was calculated as the number of seeds extracted divided by the number of cones harvested per individual. To determine the viability of extracted seed, we conducted a 28-d laboratory germination trial where 25 seeds harvested from each individual were placed on moist filter paper in a 9 cm Petri dish and watered with deionized water every second day. If fewer than 25 seeds were extracted from an individual, we used all the seeds that were extracted. Seeds experienced 16 h of light per day (6400 K full-spectrum, T5 lamp with omnimax reflector; Jump Start, Hydrofarm, Petaluma, CA, USA), which mimics sunlight duration at peak growing season in the study area, at room temperature (~20°C). Seeds were considered germinated if the length of their radicle was at least four times that of the seed coat (Leadem et al. 1997) and seed viability was calculated as the total number of germinants divided by the number of seeds. To quantify predispersal damage, 25 seeds from each individual were visually inspected under a dissecting microscope for holes in the seed coat and longitudinally sectioned to assess embryo condition (Supplementary material Appendix 2 Fig. A1). When fewer than 25 seeds were extracted, we visually inspected the seeds that failed to germinate in the seed viability germination trial.

Field germination experiment

To examine the relative importance of seed and establishment limitations in situ, we established a seed addition experiment (October 2016), following the basic framework of the Global Treeline Range Expansion Experiment (2 species, +/0 seed addition, +/0 substrate scarification; G-TREE; Brown et al. 2013) at both the forest and treeline site. Within each site, experimental plots (0.5 × 0.5 m) were organised in blocks of six and established in areas of similar slope (n = 180). To assess substrate suitability (i.e. establishment limitations), we scarified half of the experimental plots, removing all surface plant litter and living surface cover (mosses and lichens). To test whether seed availability is limiting, we sowed approximately 250 black spruce seeds or 200 tamarack seeds to plots assigned seedling treatments (seed origin, storage and viability detailed in Supplementary material Appendix 1). Plots were surveyed monthly for emergent seedlings from May to September 2017. For complete field germination experiment methods see Supplementary material Appendix 1.

Due to minimal emergence across the field experiment, with only 12 seedlings observed (five black spruce and seven tamarack; all in plots with scarified substrate), we were unable to analyze emergence and seedling establishment. However, incidental observations of ant activity and seed predation during seedling emergence surveys led to our formation of further hypotheses explaining biotic constraints on treeline range expansion at the study site, described below.

Post-dispersal seed predation experiment

Driven by the low emergence in the field germination trial, we examined the role of post-dispersal seed predation in limiting recruitment by establishing a post-dispersal seed predation experiment (experimental design adapted from Côté et al. 2005). Treatments were installed at both the forest and treeline site and replicated at each of the established field germination experiment blocks described above (+/0 cage, +/0 scarified, 2 species; n = 240). Cylindrical cages, where the top was pinched and secured shut (galvanized hardware 0.64 cm mesh cloth, 15 cm diameter × 30 cm height), were used to exclude birds and small mammals. Cage bottoms were buried 5 cm and secured in the ground with 10 cm long staples. Seed cups were constructed out of 5 cm diameter PVC pipe cut into 4 cm sections. Two layers of 1 mm mesh tulle was glued to the bottom of each section to create a cup that was permeable to water but prevented the loss of seeds. Two seed cups, one with black spruce seeds and one with tamarack seeds, were installed within each cage to quantify invertebrate seed predation. Two uncaged seed cups were placed in close proximity (between 25 and 50 cm) to quantify total seed predation (small mammal, birds and invertebrate predation). To test how substrate composition influences post-dispersal seed predation, we installed seed cups and cages on both undisturbed and scarified substrates. Seed cups were filled with the local substrate; undisturbed treatments were filled with feathermoss at the forest site and caribou lichen at the treeline site, and scarified treatments were filled with local organic soil. Five seeds were placed in each seed cup, black spruce or tamarack respectively, and left in situ for approximately one month (13 August–20 September 2017). At the end of the experimentation period, the entire contents of each seed cup were collected, brought to the lab, and examined for remaining seeds. Predation odds was calculated using the formula: 1 − (number of seeds relocated in the laboratory/5) (Côté et al. 2005).

Substrate suitability laboratory trial

To further investigate substrate suitability, the field germination experiment was paired with a laboratory germination trial that examined germination ability and seedling establishment of black spruce and tamarack across substrates collected from the forest and treeline sites. A laboratory germination trial allowed us to compare substrate suitability across substrate types while controlling for factors that co-vary along the elevational gradient.

Feathermoss, predominantly *Pleurozium schreberi*, was collected from the forest site and fruticose caribou lichen (predominantly *Cladonia stellaris*) was collected from the treeline site. We transplanted substrate into aluminum trays (20.3 × 9.8 × 6.3 cm), modified to have drainage holes, on top of a thin layer of dampened commercial peat. Control

trays were completely filled with peat, which mimics the substrate, organic soil, in the scarified plots of the field germination experiment. We randomly sowed 50 seeds of either black spruce or tamarack per tray (n = 27 per species). Seeds experienced the same light and temperature conditions described for germination trials, above, and were sprayed with water every second day. The arrangement of trays was changed every two to three days, to avoid any spatial biases.

Following the first germination event, trays were surveyed for germinants every three days for 28 d. As with the petri dish germination trials, seeds were considered germinated when their radicle was four times the length of the seed coat (Leadem et al. 1997). Germination index was calculated as the number of germinants observed divided by the product of the number of seeds sown and the proportion of seed that was viable, which standardizes the number of germinants observed by seed viability and allows for comparisons across species.

Kalmia-biotic community laboratory trial

Kalmia angustifolia, an ericaceous shrub, has long been understood to interfere with black spruce establishment and growth; although the exact mechanism(s) of interference is still debated, there is evidence suggesting allelopathic effects on seedling establishment (Mallik 2003). We hypothesized that the high abundance of *Kalmia* at the treeline site may have contributed to the low emergence observed from the initial field germination experiment.

Allelopathic competition by *Kalmia* is difficult to quantify in the field due to multiple, interacting competitive effects between shrubs and seedlings (Mallik 2008). To examine potential allelopathic effects of *Kalmia* on black spruce and tamarack germination, we conducted a laboratory germination trial using *Kalmia* and soil leachates. Mature, living *Kalmia* leaves were collected from several individuals across the treeline site, and organic soil was collected from three distinct locations from each of the forest and treeline sites, at the end of the 2017 growing season (21 September) and transported to the lab. Organic soil samples were homogenized at the site level with large roots and living plant material removed, and frozen until used. To produce leachate for the experiment, 5 g of *Kalmia* leaves, 10 g of treeline site organic soil and 10 g of forest site organic soil were each mixed with 250 ml deionized water and allowed to sit for 24 h, four hours of which the slurries were agitated at low speed using an orbital shaker. Slurries were filtered through Whattman no. 1 filter paper and were kept refrigerated at 6°C (leachate protocol adapted from Mallik 1987).

The effect of leachates on black spruce and tamarack germination was determined in a 28-d laboratory germination trial under the laboratory conditions used in all previous trials, described above. Each petri dish contained 25 seeds on filter paper moistened with one of the three leachates or deionized water, used as a control (n = 10 dishes per treatment). Each dish was moistened every second day with the assigned treatment. Petri dishes were surveyed every two days and number of germinants were recorded. At the end of the trial period, germinants were dried for at 60°C for 48 h and weighed.

Statistical analyses

All statistical analyses were performed using R ver. 3.3.1 (R Core Team) via RStudio ver. 1.1.149 (RStudio Team 2016). We used the 'glmmTMB' package for beta distributed and Poisson distributed generalized linear mixed models (Brooks et al. 2017).

We used generalized linear models to analyze the response of 1) black spruce and tamarack seed production (Poisson distribution for count data), 2) black spruce and tamarack seed viability (binomial distribution) and 3) black spruce and tamarack pre-dispersal damage (binomial distribution) between elevational sites.

To assess site, cage and substrate effects on post-dispersal black spruce and tamarack seed predation in our field experiment, we used generalized linear mixed models with a beta distribution and block included as a random effect. Beta distributions are appropriate when proportional data fail to meet the assumption of independence (Ospina and Ferrari 2010). Here, we assume that if one deployed seed was consumed it was more likely another seed deployed in the same seed cup would be consumed, and thus each seed could not be assumed to have an independent response to our treatments.

To assess substrate suitability for black spruce and tamarack emergence in our laboratory trial, we used generalized linear models to analyze how 1) time to first black spruce and tamarack germination event (normal distribution) and 2) black spruce and tamarack germination odds (binomial distribution for proportion data) varied between substrates (lichen, moss and peat). Likewise, we used generalized linear models to analyze how 1) time to first black spruce and tamarack germination event (normal distribution) and 2) black spruce and tamarack germination odds (binomial distribution for proportion data) responded to the leachate treatments. We assessed model fit for all models using residual diagnostics (Zuur et al. 2009).

Results

Pre-dispersal seed production and viability

Individuals of both species produced fewer seeds per cone at the treeline site compared to individuals at the forest site; black spruce individuals produced on average 4.5 times fewer seeds per cone, while tamarack individuals produced on average 7 times fewer seeds per cone at treeline compared to within the forest (Fig. 3, Table 1). Black spruce seed production (i.e. number of seeds per cone) did not vary between individuals at the forest site and those at timberline, both producing on average roughly 39 seeds per cone, although the seeds

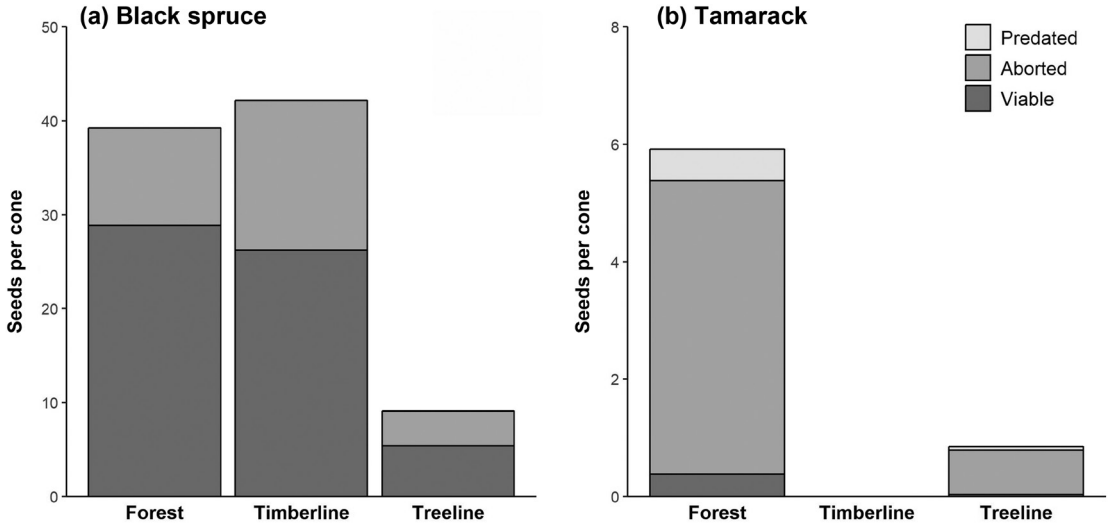


Figure 3. Mean number of seeds produced per cone across sites across the altitudinal range limits of (a) black spruce (n = 30) and (b) tamarack (n = 25). No tamarack individuals occurred at timberline. The number of viable seeds per cone was calculated using seed viability determined in the lab germination trial. The number of aborted seeds and pre-dispersal predation per cone was calculated from the proportions determined through visual inspection. Please note y-axes are of different scale.

produced at the forest site were more viable (73.2 ± 9.25% compared to 64.0 ± 24.4% respectively; Fig. 3). Across the elevation gradient, black spruce seed viability decreased with increasing elevation, where seeds produced at treeline were the least viable (50.5 ± 22.8%; Fig. 3). No evidence of black spruce pre-dispersal seed predation was observed at the forest site, and was rarely observed at the treeline site (0.48 ± 0.007%), and non-viable black spruce seeds were, effectively, solely caused by embryo abortion regardless of site. Tamarack seed viability was low across both forest and treeline sites, where on average only around 1 out of every 20 seeds produced germinated (7.20 ± 3.44% viable at the forest site and 4.19 ± 1.34% viable at treeline; Fig. 3). The majority of non-viable tamarack seeds had aborted embryos (83.2 ± 5.99% aborted at the forest site and 94.7 ± 2.17% aborted at treeline; Fig. 3), although low levels of pre-dispersal seed predation did occur (10.7 ± 3.79% seeds consumed at forest site and 5.27 ± 2.17% seeds consumed at treeline; Fig. 3).

Table 1. Summary of results from generalized linear models on black spruce and tamarack seed production, seed viability and pre-dispersal seed damage (degrees of freedom (df) = 29 for black spruce and df = 24 for tamarack). Black spruce and tamarack cones were harvested along an elevational gradient at the forest site and the treeline site, in addition black spruce cones were harvested from timberline (n = 10, except tamarack at forest site where n = 5). The sites were used as predictor variables and the intercept represents the forest site. Seed production models assume a Poisson distribution and the seed viability and seed damage models assume a binomial distribution. Values in bold indicate a significance difference ($\alpha \leq 0.05$).

Parameter	Estimate	SE	z-value	p-value
Seed production				
Black spruce				
Intercept	39.4	3.50	11.5	**< 0.0001**
Timberline	0.139	4.95	0.028	0.978
Treeline	−30.8	4.95	−6.22	**< 0.0001**
Tamarack				
Intercept	5.81	0.820	7.09	**< 0.0001**
Timberline	–	–	–	–
Treeline	−4.99	1.00	−4.97	**0.0002**
Seed viability				
Black spruce				
Intercept	1.01	0.143	7.04	**< 0.0001**
Timberline	−0.429	0.194	−2.21	**0.027**
Treeline	−0.927	0.194	−4.77	**< 0.0001**
Tamarack				
Intercept	−2.56	0.346	−7.39	**< 0.0001**
Timberline	–	–	–	–
Treeline	−0.277	0.475	−0.583	0.560

Post-dispersal seed predation experiment

Site, cage treatment and scarification treatment all had significant effects on the predation pressure (the proportion of seeds removed from seed cups) of black spruce seeds (Fig. 4, Table 2). Overall, the cage treatment had the largest effect, where the proportion of removed (presumed consumed) black spruce seeds was consistently lower under cages. Independent of whether they were within the forest site or at treeline, all uncaged seeds were effectively consumed (i.e. missing and presumed predated upon). Site had a weak effect on black spruce seed predation, where marginally fewer seeds were consumed at treeline. In general, scarification had a negative effect on black spruce seed predation, although there were significant interactions between site and scarification, and cage and scarification. Scarification at treeline switched the sign of the model estimate from positive to negative, meaning that black spruce seeds on scarified substrate at treeline were more likely to be consumed, but black spruce seeds on scarified substrate in the forest site were less likely to be consumed. Similarly,

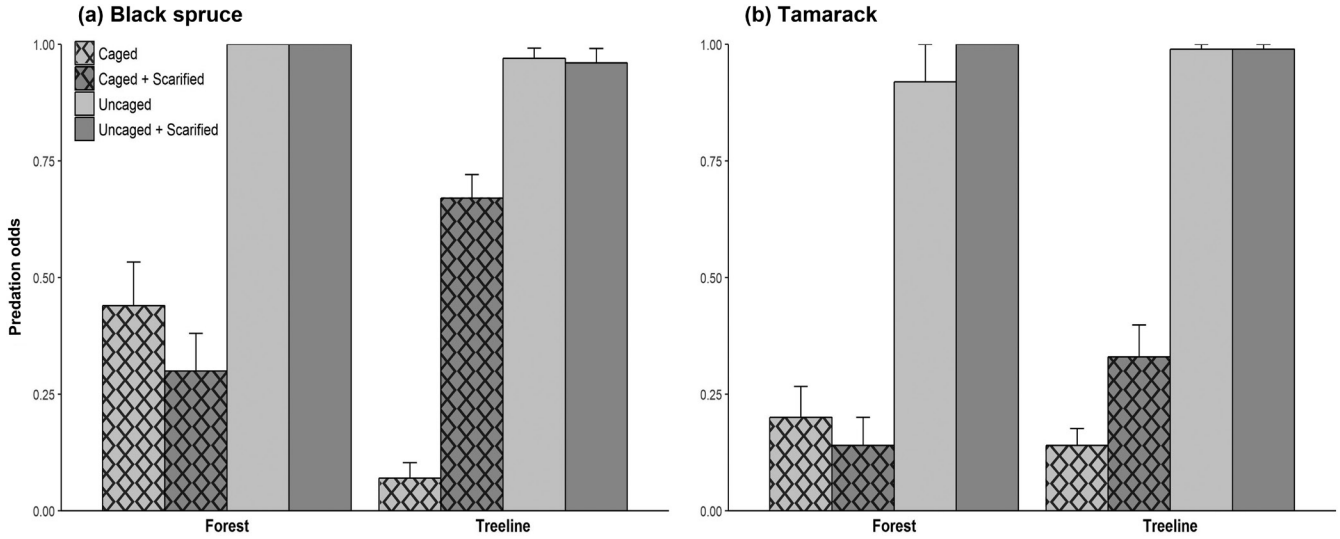


Figure 4. Odds of (a) black spruce and (b) tamarack post-dispersal seed predation (mean ± SE) under cages (cross-hatching) and control seed cups filled with local or scarified substrate at both the forest (n = 40) and treeline (n = 80). Where a predation odd of zero means all seeds sown were recovered and, conversely, a predation odd of one means no seeds were recovered (i.e. all seeds were presumably predated).

the interaction between cage and scarification switched the sign of the model estimate to negative, meaning that a lower proportion of caged black spruce seeds on unscarified substrate were consumed than those on scarified substrate. This effect is likely driven by the treeline site, where on average 7.0 ± 14.9% of caged black spruce seeds on unscarified substrate were removed compared to 67.0 ± 22.7% of caged black spruce seeds on scarified substrate. In contrast to black spruce, the proportion of consumed tamarack seeds solely responded to the cage treatment, where tamarack seeds under cages were significantly less likely to be consumed; all tamarack seeds not protected by a cage were effectively consumed (Fig. 4, Table 2).

Substrate suitability laboratory trial

Seedling emergence on lichen was both delayed and less abundant than on moss or control substrate (Fig. 5, Supplementary material Appendix 2 Table A1). Lichen had the greatest significant effect on time until first germination event for both black spruce and tamarack, where it took over two times longer for black spruce and nearly two times longer for tamarack radicle emergence on lichen than on the control substrate ($t = 8.36$, $p < 0.001$ and $t = 4.75$, $p = 0.0001$ respectively). Moss also slowed the time it took for the first germination event for both black spruce and tamarack relative to the control substrate but lesser so than lichen ($t = 2.55$,

Table 2. Summary of results from the generalized linear mixed models of predation pressure on black spruce and tamarack seeds in the post-dispersal seed predation experiment (df = 119). Experimental sites were located at two elevational sites, the forest site (n = 80) and at treeline (n = 40). Both models assume binomial distribution and include block as a random effect. Values in bold indicate a significance difference ($\alpha \leq 0.05$).

Parameter	Estimate	SE	z-value	p-value
Black spruce				
Intercept	−3.09	0.456	−6.79	**< 0.0001**
Caged	4.05	0.576	7.08	**< 0.0001**
Scarified	1.14	0.459	2.49	**0.0127**
Treeline	1.04	0.439	2.36	**0.0184**
Caged:Scarified	−1.78	0.452	−3.94	**< 0.0001**
Scarified:Treeline	−1.69	0.504	−3.36	**0.0008**
Caged:Treeline	−0.117	0.481	−0.243	0.808
Tamarack				
Intercept	−2.54	0.372	−6.83	**< 0.0001**
Caged	4.37	0.526	8.31	**< 0.0001**
Scarified	0.237	0.405	0.585	0.559
Treeline	0.205	0.378	0.543	0.587
Caged:Scarified	−0.421	0.413	−1.02	0.309
Scarified:Treeline	−0.413	0.436	−0.947	0.344
Caged:Treeline	−0.301	0.436	−0.690	0.490

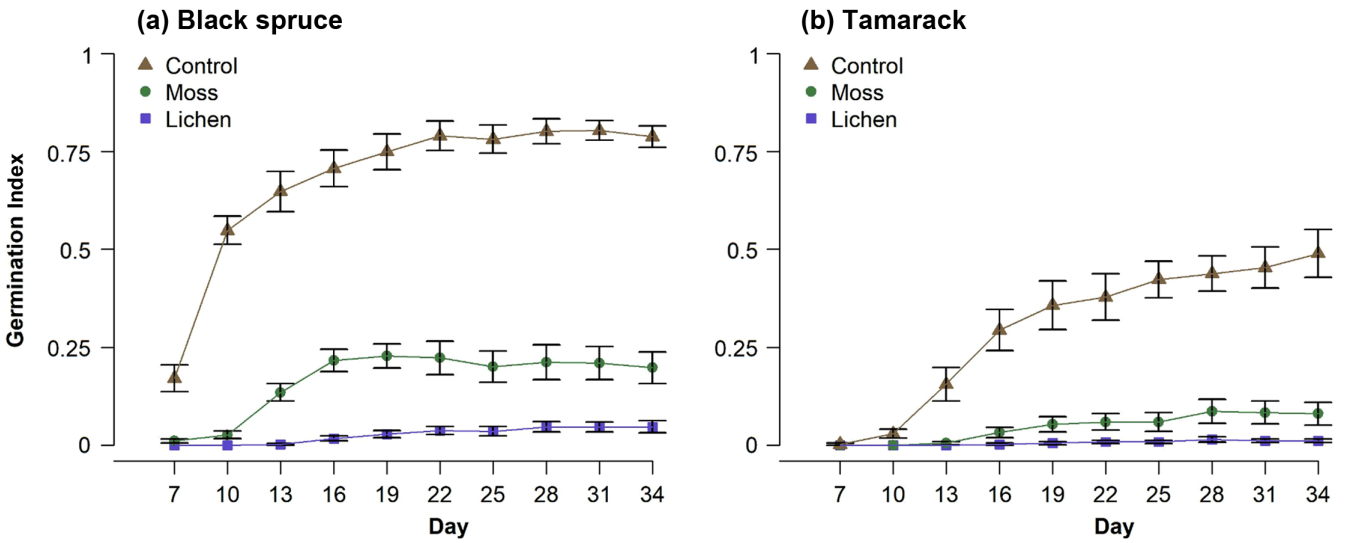


Figure 5. Germination index, calculated as the number of germinates observed divided by the product of the number of seeds sown and seed viability, (mean ± SE) of (a) black spruce (n = 27) and (b) tamarack (n = 27) across the three substrate types (control-commercial peat, feathermoss and caribou lichen) over the duration of the laboratory substrate germination trial.

$p = 0.018$ and $t = 3.15$, $p = 0.005$ respectively). Similarly, lichen had the greatest effect on seedling emergence where the proportion of emerged seedlings on lichen was very low; on average only 6.32 ± 3.99% of black spruce and 1.8 ± 1.91% of tamarack seeds germinated compared to 82.4 ± 9.15% of black spruce and 52.5 ± 15.8% of tamarack seeds germinated on control substrate ($z = -18.3$, $p < 0.0001$ and $z = -9.62$, $p < 0.001$ respectively). Moss also reduced the proportion of emerged black spruce and tamarack seedlings but had less of an effect than lichen, where on average 25.3 ± 10.3% of black spruce seeds germinated and 9.00 ± 9.36% of tamarack seeds germinated ($z = -15.8$, $p < 0.0001$ and $z = -11.0$, $p < 0.0001$).

Kalmia-biotic community laboratory trial

Leachate treatments had minimal to no detectable effects on the timing and odds of black spruce and tamarack germination (Supplementary material Appendix 2 Table A2). The timing of the first black spruce germination event was the same across all treatments and leachate treatments had no effect on black spruce germination odds ($p > 0.05$). The *Kalmia* leachate significantly delayed the date of the first tamarack germination event ($t = 3.00$, $p = 0.005$), although the magnitude of this effect was minimal (delay = 1 d). The organic treeline soil leachate had a marginally significant effect on date of first tamarack germination event ($t = 2.40$, $p = 0.022$) but, similarly, the magnitude of this effect was minimal (delay < 1 d) and likely not biologically significant. The organic forest soil leachate had a significant, positive effect on tamarack germination odds ($z = 2.74$, $p = 0.0062$), although the strength of this effect was small as the odds of germination increased by approximately 1.1 times, and therefore is likely not biologically significant.

Discussion

Through a series of sequential observational and experimental studies, we demonstrate that recruitment at a model alpine treeline on the island of Newfoundland is simultaneously seed and establishment limited (Fig. 6). Recruitment is firstly seed limited, where production of viable black spruce and tamarack seed was lower at treeline compared to the forest population. When viable seed is available and dispersed, recruitment both within range and at treeline is greatly constrained by post-dispersal seed predation, where effectively all black spruce and tamarack seeds were consumed, a constraint that may be overcome by masting. Yet, even if a larger proportion of seeds escape predation during a mast year, we found recruitment at treeline will likely not occur because the dominant, lichen substrate poses a significant barrier to germination and seedling establishment. While bare ground greatly increased germination and seedling establishment, suggesting that a pulse of recruitment may follow a disturbance event, we show that seeds dispersed to bare ground are more likely to be consumed before they can establish. The mismatch between conditions that promote germination and seedling establishment and decrease invertebrate post-dispersal seed predation at this treeline presents an interesting conflict, where disturbance that removes the lichen mat reduces establishment limitations while simultaneously increasing seed limitations. We conclude that recruitment at this model treeline, and thus changes in geographical position, is greatly constrained by a series of biotic filters that will likely override any positive effects associated with the alleviation of climate constraints. Here, we discuss the implications of pre- and post-dispersal processes and their cumulative constraints; contextualizing our findings with those from other North American coniferous treelines.

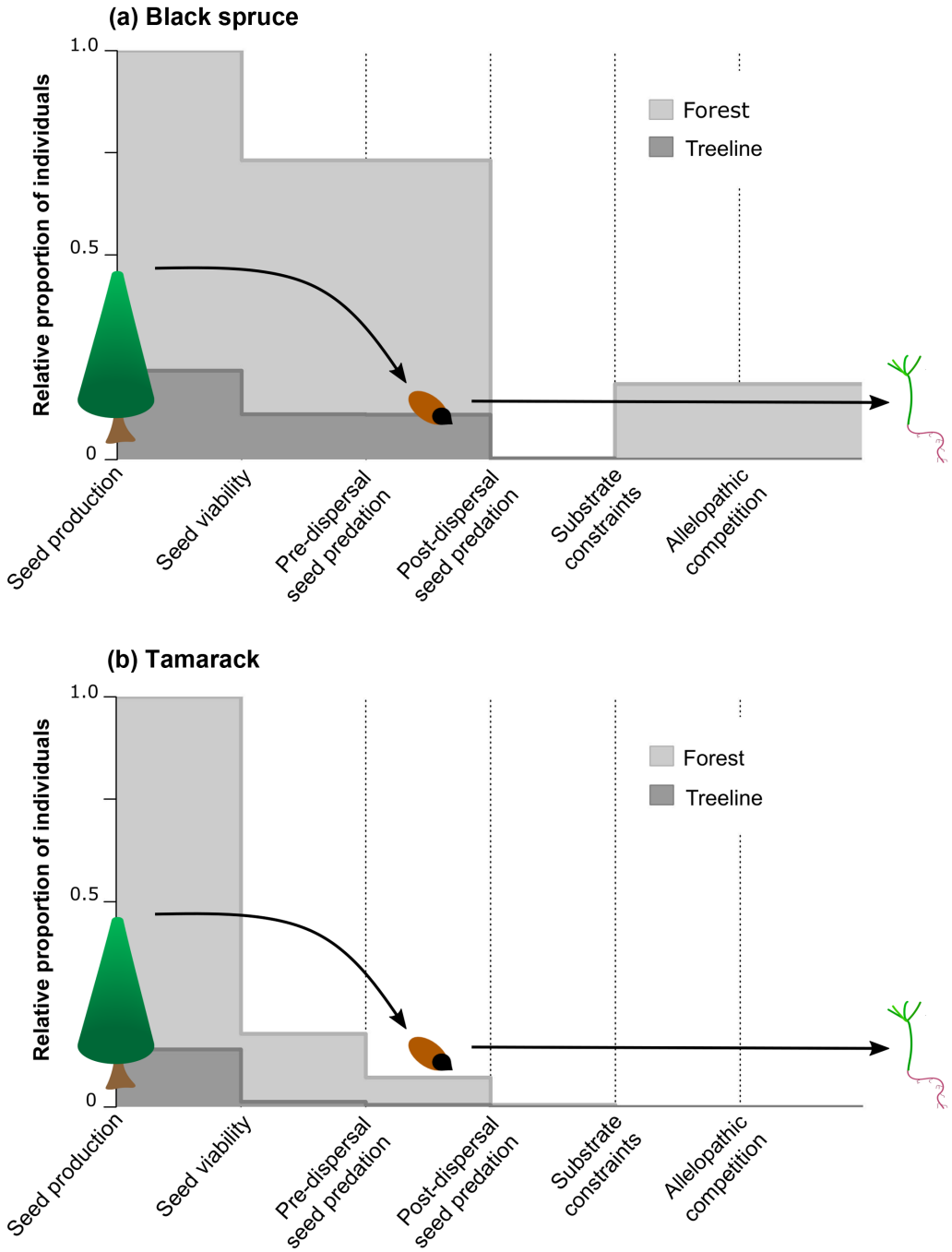


Figure 6. Flow diagram illustrating the relative proportion of (a) black spruce and (b) tamarack individuals at each predicted biotic filter. Seed production by the forest population was set to 1.0, while seed production by treeline population represents the proportion of seeds produced by the treeline populations compared to the forest populations. The relative proportion of individuals were calculated sequentially across each biotic filter, for both the forest and treeline populations, using the mean results from our observational and experimental studies. All black spruce individuals at the forest population were lost to post-dispersal seed predation, so flow diagram assumes that biotic filter is overcome and proportion of individuals lost to substrate constraints is calculated from the number of viable seeds.

Pre-dispersal processes: seed limitations at treeline

At the most fundamental level, seed production is controlled by climate, and studies have attributed unfavourable climatic conditions at treeline to decreased seed production and viability (Sirois 2000, Meunier et al. 2007, Krebs et al. 2012, Roland et al. 2014, Brown et al. 2019 but see Kroiss and Hille Ris Lambers 2015). Similar to other coniferous range edge populations, we found that production of viable black spruce and tamarack seed was lower at treeline, suggesting

that recruitment at range limits is currently seed limited (Sirois 2000, Meunier et al. 2007, Viglas et al. 2013, Jameson et al. 2015, Brown et al. 2019). Seed production at treeline is expected to increase with the alleviation of climatic constraints as longer growing seasons will likely result in increased resource accumulation and, in turn, increased resource allocation to reproduction (Krebs et al. 2012). We anticipate that landscape-level seed production at treeline will remain seed limited in the short term, despite expected increases in seed production, because treeline populations have lower stand densities where there are fewer reproductive individuals. Stand densification will need to occur for landscape-level seed production at treeline to equal that within range limits (Kroiss and Hille Ris Lambers 2015).

As expected, black spruce seed produced by the treeline population was less viable than seed produced from the within range, forest population. Pollen limitations, via failure to fertilize or self-pollination, are major drivers of low seed viability (Sorensen 1969, Owens and Blake 1985, Owens et al. 2005) and have been found to operate at range edge populations (Elliott 1979, Sirois 2000). Black spruce is anemophilous and successful wind pollination has been linked to conspecific density and plant height (Friedman and Barrett 2009), characteristics that both decline at treeline. No seeds were completely absent of an embryo, suggesting that black spruce individuals at treeline were successfully fertilized. Our findings align with previous research on eastern North American alpine treelines (Jameson et al. 2015), where reproductive capacity of boreal conifers was hypothesized to not be pollen limited as the distances between forest and treeline populations were within pollen dispersal distances (as quantified by O'Connell et al. 2007). Similarly, the distance between our forest and treeline populations (~290 m) were well within the pollen dispersal distance (250–3000 m; O'Connell et al. 2007). Unlike Jameson et al. (2015), however, we found effectively no signs of pre-dispersal black spruce seed predation and found that non-viable seeds were driven by embryo abortion. While we cannot determine the causal mechanism for embryo abortion, we speculate that an increase in non-viable, aborted seeds at treeline is driven by increased self-fertilization. Clonal reproduction of black spruce is common at treeline (Payette and Gagnon 1979, Viktora et al. 2011) and with this increasing genetic interrelatedness of individuals the likelihood of self-fertilization increases (Mimura and Aitken 2007). Alternatively, decreased seed viability at the treeline population may be driven by, or partially driven by, altitudinal climatic constraints; for example, late spring frost can lead to embryo abortion (Caron and Powell 1989).

Tamarack seed viability was low across both populations, with the majority of seeds produced per cone being non-viable. Like black spruce, and in alignment with Jameson et al. (2015), no tamarack seeds were completely void of an embryo meaning that failure to fertilize is not driving low seed viability across the altitudinal gradient. However, in contrast to findings along another eastern North American alpine treeline (Jameson et al. 2015), only a small proportion of non-viable tamarack seeds were caused by pre-dispersal seed predation with most non-viable seeds containing aborted embryos. Low seed viability is common among *Larix* spp. and is often driven by a high rate of embryo abortion (Farmer and Reinholt 1986, Lewandowski et al. 1991). In line with previous studies, most of the seeds produced by both populations contained aborted embryos. Genetic diversity of tamarack stands have been found to be lower than other boreal conifers and self-fertilization has been identified as a leading cause of aborted embryos (Knowles et al. 1987). The proportion of non-viable seeds did not vary between populations suggesting that both populations experience similar rates of self-fertilization. Genetic diversity increases, and thus self-fertilization decreases, with stand density (Knowles et al. 1987), and the density of tamarack individuals is low across the altitudinal gradient.

Our results, in alignment with previous studies, demonstrate that recruitment at treeline is currently seed limited or, at the minimum, is seed limited some of the time. It is important to note the highly variable nature of seed production, where inter-annual variation in climate and intrinsic periodicity (i.e. masting) drive temporal variability (Sirois 2000, Gallego Zamorano et al. 2018). While relatively little is known about black spruce and tamarack masting in Newfoundland, it is commonly recognized that black spruce and tamarack have years of increased seed production (an estimated 2–6 yr and 3–6 yr for black spruce and tamarack respectively; Johnston 1990, Sirois 2000) that may overcome seed limitations observed by this study. Alternatively, dispersal from source populations at lower elevations may lessen the magnitude of, or overcome, seed limitations at treeline (Johnson et al. 2017). We did not quantify seed rain but we speculate that dispersal may alleviate black spruce seed limitations. While seed rain is, understandably, related to seed production (Kambo and Danby 2017; > 300 m as estimated by Piotti et al. 2009), suggesting that dispersal from lower elevations can augment viable seed produced at treeline. In contrast, dispersal from the low elevation tamarack population is unlikely to alleviate tamarack seed limitations at treeline as the low elevation population is beyond the effective dispersal zone of tamarack seed (60 m as estimated by Johnston 1975) and the majority of seeds produced by the low elevation population were non-viable. More attention is needed to evaluate the details of this filter, such as: population level cone production, dispersal ability and temporal variability in viable seed production.

Post-dispersal processes: cumulative constraints on establishment

Despite experimentally overcoming seed limitations in the field germination experiment, effectively none of the seeds added survived to the seedling stage, indicating that establishment limitations are of greater relative importance than seed limitations in constraining recruitment (Clark et al. 2007). Through complementary empirical tests, we demonstrate

that multiple post-dispersal processes constrain recruitment in conflicting ways. There is an interesting mismatch between substrate that promotes seed retention and substrate that is suitable for seed germination and seedling establishment at treeline. The complex, three-dimensional characteristic of the lichen mat results in decreased invertebrate black spruce seed predation but is a significant barrier to germination and seedling establishment. Therefore, disturbance that removes the lichen mat simultaneously decreases establishment limitations and increases seed limitations. The opposing direction of these filters on black spruce recruitment at the treeline site supports the argument that multiple factors need to align temporally for geographical range shifts to occur (Kroiss and Hille Ris Lambers 2015, Kambo and Danby 2017). Therefore, treeline advance will likely be dependent on episodic periods of seedling establishment, when conditions align to overcome the multiple filters to recruitment (Harsch et al. 2009, Kambo and Danby 2017).

Our post-dispersal seed predation experiment results highlight that boreal conifer recruitment across this altitudinal gradient is greatly constrained by seed predation. We found higher levels of post-dispersal seed predation than previous studies (Côté et al. 2003, 2005, Munier et al. 2010, Wheeler et al. 2011), as effectively all seeds of both black spruce and tamarack that were not protected from vertebrates were consumed regardless of site or substrate. The large magnitude of seed consumption is likely why there was effectively no seedling emergence observed in the initial field germination trial. It is important to note that predation pressure will likely differ between seasons, as food availability changes (Côté et al. 2003). Here, seeds were disseminated in the fall for the field germination trial, while the post-dispersal seed predation experiment quantified summer seed predation only. However, Côté et al. (2003) found increased predation pressure on black spruce seeds in the winter than the summer, suggesting that post-dispersal seed predation is likely to be a dominant constraint on regeneration following natural seed dispersal in the fall. Masting is a commonly recognized mechanism for overcoming seed predator limitations, where regionally high years of seed production lowers the percentage of seeds lost to seed predators (Kelly and Sork 2002) and we argue that masting is likely required to overcome post-dispersal seed predation constraints and for successful recruitment at treeline to occur.

Most post-dispersal seed predation studies do not differentiate between the relative importance of different guilds of seed consumers and the importance of invertebrates as post-dispersal seed predators has likely been underestimated (Hulme 1998). Here, we assume that any seeds removed from seed cups that were protected by cages, which excluded vertebrate seed predators, were consumed by invertebrate seed predators (Côté et al. 2005). We found that black spruce seed consumption by invertebrates was dependent on both site and scarification treatment, where post-dispersal invertebrate predation pressure was lowest on undisturbed substrate, lichen, at treeline and the greatest on disturbed substrate at treeline. Although Côté et al. (2005) did not study an altitudinal gradient, they similarly observed lowest levels of invertebrate predation on lichen – spruce forest and the greatest levels in a non-vegetated, recently burned site, which our substrate disturbance treatment mimics. We speculate that the complex three-dimensional physical structure characteristic of fruticose lichens (e.g. *Cladonia* spp.) decreases the probability of an invertebrate encountering a seed, as opposed to the relatively two-dimensional structure of bare ground. Based on this argument, we expected a similar trend for the forest population, as undisturbed moss substrate is more structurally complex than disturbed ground; however, we found invertebrate seed consumption was greater on undisturbed *Pleurozium* moss substrate than on the scarified, disturbed bare ground. While relatively more complex in structure compared to bare soil, *Pleurozium* moss is much denser compared to *Cladonia* lichen substrates, and seeds were observed to stay on the surface (Wheeler et al. 2011, unpubl.), likely increasing the likelihood of a seed being encountered by a predator. In contrast with black spruce, invertebrate predation of tamarack seeds did not differ between substrate treatments at treeline. Tamarack seeds are larger than black spruce, and our field observations indicate they did not penetrate the lichen mat, preventing its beneficial protection.

If treeline is establishment limited, seed predation will likely play a minor role in constraining recruitment because most seeds will be unable to germinate and become established regardless of seed predation; in other words, seeds that are lost to predation are doomed anyway (Hulme 1998, Clark et al. 2007). In agreement with numerous other studies, our substrate suitability lab trial clearly demonstrates that substrate composition has an effect on establishment success (LePage et al. 2000, Charron and Greene 2002, Dufour-Tremblay and Boudreau 2011, Wheeler et al. 2011, Brown and Vellend 2014). Suitability of substrate is a function of its chemical and physical characteristics, with particular importance ascribed to: substrate temperature, moisture availability and penetrability (Leadem et al. 1997). Previous work has found conifer establishment is reduced on lichen substrates (Charron and Greene 2002, Dufour-Tremblay and Boudreau 2011, Wheeler et al. 2011) and as expected, we found that lichen both delayed and reduced the abundance of black spruce and tamarack establishment. Black spruce and tamarack germination is dependent on consistent soil moisture (Holtmeier 2009) and the poor water-retention capacity of lichen mats may explain low rates of establishment (Allen 1929, Charron and Greene 2002). Moreover, the porous nature of a thick lichen mat could prevent seedling radicles from reaching the soil, or alternatively if seeds fall deep into the lichen mat, emerged seedling may be unable to penetrate the upper layers of the substrate (Allen 1929, Asplund and Wardle 2016). The decreased establishment success on *Cladonia* lichen compared to *Pleurozium* moss suggests that recruitment at treeline, where lichen substrate dominates, is more establishment limited than the forest.

Seedling establishment was much greater on our control peat substrate, suggesting that recruitment at treeline may be dependent on disturbance that exposes bare soil and decreases establishment limitations. Bare soil has long been recognized as the ideal substrate for black spruce and tamarack germination and establishment (Johnston 1990, Viereck and Johnston 1990) and seeding experiments that have simulated disturbances (Munier et al. 2010, Wheeler et al. 2011) or have examined naturally occurring disturbances (Dufour-Tremblay and Boudreau 2011) have found increased emergence on disturbed substrate compared to intact alpine substrate.

Disturbances can occur across variety of scales from cryoturbation (Sullivan and Sveinbjörnsson 2010), to caribou trampling (Dufour-Tremblay and Boudreau 2011) and bear digs (Wheeler et al. 2011), to fires (Brown and Johnstone 2012, Brown et al. 2015). However, in harsh environments such as alpine treeline, the relative importance of facilitative interactions can outweigh that of competitive interactions (Choler et al. 2001, Callaway 2007, Bronstein 2009). Wheeler et al. (2011) found black spruce emergence to be greatest on simulated disturbed ground, however *Pleurozium* moss substrate was found to enhance seedling establishment and survival at treeline and suggesting that the physical structure of *Pleurozium* may ameliorate the challenging alpine environment. Moreover, despite widespread evidence that lichen substrates are unsuitable for conifer establishment, Dufour-Tremblay et al. (2012a) observed disproportionate numbers of tamarack seedlings established on lichens at treeline, suggesting that lichens may lessen establishment limitations, potentially via microsite amelioration (e.g. increased levels of humidity; Dufour-Tremblay et al. 2012a). While seedling establishment in the field germination experiment was extremely low, all seedlings that were observed occurred in scarified plots, we speculate that facilitative effects at this alpine treeline do not outweigh the competitive effects observed in the substrate suitability lab trial.

Ericaceous shrubs, in particular *Kalmia angustifolia*, are highly abundant at the treeline population (unpubl.) but, contrary to predictions, we found *Kalmia* and soil leachates to have no detectable effect on black spruce and tamarack germination. Leachates from other ericaceous shrubs, *Empetrum nigrum* and *Vaccinium uliginosum*, have been found to decrease black spruce and to a lesser extent tamarack germination (Dufour-Tremblay et al. 2012a). *Empetrum nigrum* and several *Vaccinium* species occur at our treeline population, although the lack of response to the high site soil leachate suggests that they occur at low enough abundance that their negative impacts are negligible. It is important to note that allelopathy is difficult to quantify in natural conditions due to multiple co-occurring plant–plant interactions (Mallik 2008) and, while we used similar methodology to previous studies, experimental leachates likely differ from concentrations experienced in situ. *Kalmia* has long been recognized to inhibit black spruce regeneration (Mallik 1987) and following disturbance black spruce forests stands in Newfoundland have failed to regenerate, undergoing a state change to *Kalmia* heath (Mallik 1995). Our results suggest that *Kalmia* does not competitively exclude the seedling emergence life stage but the high *Kalmia* abundance at the treeline population may have additive effects on establishment limitations, constraining seedling growth and survival at slightly later post-emergence life stages (Mallik 1987, Bradley et al. 1997, Wallstedt et al. 2002, Zeng and Mallik 2006).

Conclusions

Our study highlights the complex nature of tree species' range limits and, thus, the challenge in predicting how treelines will respond to changing climatic conditions. Using a model alpine treeline in central Newfoundland, we conclude that recruitment at treeline can be simultaneously seed and establishment limited and that changes to geographic treeline position will likely be dependent on the temporal alignment of multiple factors. The mismatch we observed between substrate that decreases post-dispersal seed predation and that increases seedling establishment demonstrates that variables affecting recruitment can be interwoven and contradictory; we argue that future studies need to assess interactions between biotic filters to understand their impact on recruitment. Our study enhances previous work that highlights the importance of local to regional scale, non-climatic constraints in shaping geographical distribution of treeline and reinforces the necessity to better understand the generality of non-climatic constraints in prediction of global treeline response to a warming climate.

Data availability statement

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.9p8cz8wcg> (Crofts and Brown 2020).

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Author contributions – ALC and CDB conceived of the ideas and designed methodology. ALC conducted field sampling, performed laboratory analyses and analyzed the data. Both authors contributed critically to the drafts and gave final approval for publication.

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Supplementary material (available online as Appendix ecog-04899 at <www.ecography.org/appendix/ecog-04899>). Appendix 1–2.

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