Feathermoss seedbeds facilitate black spruce seedling recruitment in the forest–tundra ecotone (Labrador, Canada)

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As climate warms, conifers are expected to expand their ranges into alpine tundra where ecological factors such as seedbed availability, and post-dispersal seed and seedling predation may control local recruitment. Seedbed composition may influence microhabitat, nutrients, physical structure, and predation level and, therefore, affect the success of conifer recruitment, thereby providing the template for future expansion. In the boreal forest, seedbed–seedling competition dominates such that seedbed removal increases black spruce recruitment. In the harsher climate of the Mealy Mountains boreal forest–tundra ecotone (Labrador, Canada) the Stress gradient hypothesis (SGH) predicts that facilitation may dominate seedbed–seedling interactions. This study investigated potential mechanisms of seedbed facilitation (tempera-ture, water, nutrients, physical protection) in three conifer seedbeds (*Pleurozium schreberi, Cladonia spp.*, bare soil) and examined whether seed predation and/or seedling herbivory varied among seedbeds over three years. Seed emergence was low overall (<10% on all treatments), but highest on *Pleurozium* (6.3%), followed by bare ground (4.6%) and *Cladonia* (0.3%). Facilitation was observed between *Pleurozium* and black spruce as seedling height increase (31%) and survival (55%) were highest; herbivory, seed predation and overwinter mortality were lowest compared to both *Cladonia* and bare ground seedbeds. Unlike in the closed canopy boreal forest, seedlings recruited poorly on bare soil as seedling height increase and survival were 20.5% and 26%. Temperature and water availability were similar across seedbeds, while nutri-ent availability was higher on *Pleurozium*. The physical structure of *Pleurozium* likely protects first to third-year seedlings from temperature extremes and predators. As climate warms and seed availability increases, *Pleurozium* may facilitate black spruce recruitment and treeline expansion.

Climate is often considered the primary driver of altitudi-nal treeline position across landscape and continental scales (Holtmeier and Broll 2005); however, mountain treelines often show less advance than predicted based on the magni-tude of local climate change (Harsch et al. 2009) suggesting that local biotic and environmental factors likely affect the spatial variability of seedling survival and thus tree recruit-ment. Differential environmental conditions required for seed germination and seedling recruitment have been repre-sented by the 'sieve' model where microhabitats form 'safe' and 'unsafe' locations for germination and growth (Harper 1977). The limitation of safe microhabitats can be the pri-mary factor controlling plant recruitment across a variety of habitats (Eriksson and Erhlén 1992, Zobel et al. 2000). Microhabitat may facilitate recruitment directly through physical features of the microhabitat, or indirectly through biotic interactions such as plant–plant facilitation. Facilita-tive plant interactions may be most important for defining safe microhabitats in climatically stressful environments as suggested by the Stress gradient hypothesis (SGH; Bertness and Callaway 1994). The SGH predicts that the benefits of growing in proximity to neighboring vegetation outweigh

the effects of competition and inhibition in environmentally stressful habitats (Brooker et al. 2008) and that facilitative effects are more important than competition and inhibition during the early seedling stage in high-stress environments (Lortie and Callaway 2006).

The altitudinal forest–tundra ecotone is a climatically stressed habitat for trees as a result of low temperatures, short growing seasons due to snow burial, wind and water stress, and seed limitation (Stevens and Fox 1991, Malanson et al. 2007, Weiser and Tausz 2007). Microhabitat is thought to play a key role in seedling establishment, acting as a template for future seedling distribution (Germino et al. 2002, Smith et al. 2003, Malanson et al. 2007, Batllori et al. 2009). In the forest–tundra ecotone, seedlings that germinate in sub-optimal microhabitats usually die as a result of limited carbon uptake and root establishment, reduced mycorrhizal infection or water stress (Smith et al. 2003). Thus, optimal microhabitats form critical recruit-ment patches for seedlings.

Seedbed, the groundcover at the site of seedfall, strongly influences recruitment in boreal conifer species during critical stages such as emergence and early establishment

(Duchesneau and Morin 1999, LePage et al. 2000). In the boreal forest of North America and Eurasia, moss and lichen seedbeds structure the microhabitat, influence soil temperature, moisture and nutrients (Bonan and Shugart 1989, Deluca et al. 2002, Stark and Hyvarinen 2003) and may play an important role in alpine conifer treeline dynamics (Holtmeier and Broll 2005, Cairns et al. 2007). Seedbed suitability for conifer establishment is often a function of the chemical and physical characteristics of the substrate, such that in altitudinal treeline environments, low alpine soil temperatures may be one of the primary factors limiting treeline elevation (Smith et al. 2003). Optimal microhabitat limitation (Germino et al. 2002, Maher and Germino 2006, Malanson et al. 2007), soil temperature, moisture and penetrability (Smith et al. 2003, Butler et al. 2004, Holtmeier and Broll 2005, Malanson et al. 2007) have been discussed as potential impediments of conifer expansion into the alpine tundra, but the role of microclimatic conditions within vegetated seedbeds in successful recruitment of conifer seedlings has rarely been studied at the treeline.

Most studies examining black spruce *Picea mariana* recruitment and seedbed interactions within forested ecosystems have demonstrated that exposed mineral soil is an optimal seedbed for black spruce establishment relative to moss or lichen groundcovers (Prévost 1997, Charron and Greene 2002, Hébert et al. 2006). On a seedbed of exposed mineral soil, black spruce seedlings may have reduced competition for water, nutrients and light, and less allelopathic, ectomycorrhizal, thermal and overgrowth interference (Bonan and Shugart 1989, Hornberg et al. 1997, Hébert et al. 2006). However, the SGH suggests that facilitative biotic interactions among plants may play a more important role in black spruce recruitment in the more climatically stressed forest–tundra ecotone.

Facilitative interactions among plant species growing at treeline are generally studied at the level of higher-canopy vegetation such as adult trees (Germino et al. 2002, Smith et al. 2003, Maher and Germino 2006, Batllori et al. 2009) and among species of similar sizes such as seedlings and herbaceous layer species (Maher et al. 2005, Ball et al. 1997). None, however, discuss the potential facilitative effects of seedbeds on conifers in the treeline ecotone. In harsh environments, the seedbed could act as a facilitator of seedling establishment, growth and survival through its sheltering effects, and positive influences on soil moisture, temperature and nutrients. Seedbed can also facilitate seedling establishment and recruitment by inhibiting herbivory and granivory (Côté et al. 2005). Post-dispersal seed predation may act as a biological limitation of recruitment at the treeline (Castro et al. 1999) and, since the impacts of herbivores can be amplified by environmental stress, predators may also play a key role in seedling survival and recruitment in the treeline ecotone (Cairns and Moen 2004).

Like many arctic and sub-arctic ecosystems, temperatures in Labrador (Canada) are expected to warm over the coming decades; predictions for Labrador indicate a warming of 2°C by 2090 (Jacobs et al. 2007). As a consequence, upslope tree expansion into alpine tundra habitats has been predicted in the Mealy Mountains National Park Reserve (Munier et al. 2010). Understanding the importance of seedbed composition in promoting/inhibiting the establishment and success of tree seedlings at the forest–tundra ecotone will aid in predicting the dynamics of upslope expansion of forests into alpine environments and the subsequent threat to the biodiversity and genetic structure of increasingly isolated and shrinking alpine habitats (Malanson et al. 2007, Munier et al. 2010).

Since the SGH predicts facilitation as an important interaction among plants in stressful environments, we examined the potential facilitative effects of vegetated seedbeds on black spruce recruitment in the climatically stressed alpine treeline ecotone in the Mealy Mountains, Labrador (Canada). The seedbeds examined in this study are the feathermoss *Pleurozium schreberi,* the fruticose lichens *Cladonia stellaris* and *C. arbuscula*, and exposed mineral soil. *Pleurozium* seedbeds are densely packed, with a relatively unbroken surface, while *Cladonia* form a more fractured surface; both seedbeds grow to heights between 5 and 10 cm. In the forest–tundra ecotone of the Mealy Mountains, *Pleurozium* and *Cladonia* groundcovers are non-continuous and patchy in distribution. In the forest–tundra ecotone, exposed mineral soil is generated by freeze-thaw action and pits dug by foraging black bears *Ursus americanus*. Exposed soil is a relatively rare microhabitat in the forest–tundra ecotone (5–11% of seedbed composition across ecotone) compared to feathermoss (20–36%) and lichen seedbeds (17–29%; Munier et al. 2010).

The objectives of this forest–tundra ecotone study were to: 1) determine whether vegetated seedbeds (*Pleurozium schreberi* and *Cladonia* spp.) facilitate black spruce germination, growth and survival by comparing recruitment among vegetated seedbeds and exposed mineral soil; 2) quantify how abiotic factors varied among seedbeds and how this variation might suggest mechanisms promoting germination, growth and survival differences among the three seedbed types; and 3) compare seedling vulnerability to slug herbivory between seedbed types (slugs are a major seedling herbivore at the study site; Munier et al. 2010). We predict that moss and lichen seedbeds will be more optimal seedling recruitment sites than bare soil, with greater seed germination, higher seedling growth and lower overall predation in this transition zone.

Methods

Study site and experimental design

The Mealy Mountains (Labrador, Canada) study area falls within the boundaries of the Mealy Mountains/Akamiuapishkᵘ National Park Reserve (53°36'6"N, 158°49'0"W). The experimental plots for this study were established above the discontinuous boundary of open canopy forest in the forest–tundra transition zone at approximately 600 m a.s.l. The dominant conifer is black spruce with white spruce *Picea glauca*, balsam fir *Abies balsamea* and eastern larch *Larix laricina* present at lower abundance (R. Jameson pers. comm.). The shrub layer is dominated by arctic dwarf birch *Betula glandulosa*, bilberry *Vaccinium uliginosum* and black crowberry *Empetrum nigrum* (Cranston 2009). The groundcover is dominated by the feathermosses *Pleurozium*

schreberi and *Hylocomium splendens* in shaded, partially shaded, and suitably moist open areas (e.g. areas of higher snow capture peripheral to shrub stands), by *Sphagnum* in wetter areas and by the lichens *Cladonia stellaris* and *C. arbuscula*, in open, non-shaded areas. Summers are cool (13.2°C at 570 m a.s.l., July average), winters cold (–15.4°C at 570 m a.s.l., January average) and yearly precipitation averages between 2000–3000 mm (approximately 50% of which falls as snow). Elevation influences temperature at a lapse rate of approximately –0.7°C per 100 m. The permafrost layer is discontinuous, and annual mean soil temperatures (1 m depth) range from –0.1°C at 570 m a.s.l. to –0.7°C in the alpine tundra (Jacobs et al. 2007). The soil types in the study area are primarily loamy sand (Sutton 2008).

A total of sixty 25 × 25 cm experimental plots were split between two adjacent sites within 25 m, where *Cladonia* or *Pleurozium* covers were greater than 75% (by visual estimation). No naturally occurring black spruce seedlings were found in the study site (Cranston 2009). After the locations for the experimental plots were selected and marked, 24 plots (12 of each lichen and moss-dominated) were haphazardly selected and all surface vegetation was removed within a 45 × 45 cm area (mimicking the approximate size of natural black bear digs observed at the site) leaving the bare mineral soil uncovered and disturbed. More experimental bear dig plots were established as higher transplant mortality was expected due to desiccation.

Slug herbivory had been observed in previous studies in this area (Munier et al. 2010) and therefore a slug control treatment was implemented at half the sites. Organic, nutrient-neutral slug pellets (slug and snail bait (0.76% ferric phosphate)) was spread in small quantities (less than 5 g) on and surrounding slug-controlled plots every four days and following each rainfall event during the first two summers. The experimental plot treatments were thus bare soil with (+) pesticide, bare soil without (–) pesticide, *Cladonia* +/– and *Pleurozium*+/–. Slope, orientation, vegetation type, soil temperature and moisture were similar in both parts of the study area.

Abiotic comparison of seedbeds

Abiotic variables (soil temperature, moisture, nutrient content) were collected in 20–24 of the seedling plots. Soil moisture and temperature were measured between 8–10 cm below the seedbed surface and compared during the height of the growing season. Measurements were collected during July 2008 using a probe for temperature (°C) and a probe for moisture (% water content) but neither was taken within 24 h of a precipitation event. The data were collected over the course of six 1–2 day intervals in July 2008 on eight haphazardly selected plots of each seedbed type over a two-week period.

Soil nutrient availability was measured using 20 ion-exchange Plant Root Simulator (tm) probe sets (eight probes per set). The probes' anion and cation exchange resin membranes mimic the surface of a plant root and when buried, the probe assess nutrient supply rates by continuously adsorbing charged ions from the soil. The PRS probe sets were buried approximately 10 cm below the seedbed layer in the established treatment plots for five weeks (24 June – 02 Aug 2008), then analyzed for total N, NO_3^-, NH_4^+, Ca, Mg, K, P, and Fe at the Western AG laboratories (<www.westernag.ca>). Ten probe sets were planted in each vegetated seedbed type; simulated bear dig/ bare soil treatment was not included in this study due to limited PRS probe availability.

Seed emergence

On each of 10 randomly-selected *Cladonia,* 10 *Pleurozium* and 14 bear dig plots, thirty black spruce seeds (viability >95%) collected within100 km of the study site near Goose Bay, Labrador, Canada (50 m a.s.l.) were sown and marked on 23 June 2008 for a total of 1020 seeds. Seeds were monitored daily and emergence was quantified for each seedbed type until 20 July 2008, then again on 13 Sept 2008.

Planted seedling growth and survival

Black spruce seeds collected within 100 km of the study site near Goose Bay (Labrador, Canada) were planted in peat pellets without additional fertilizer in June 2007 in temperature-controlled greenhouses of the Provincial Nursery in Goose Bay. Seedlings were grown on raised platforms to prevent contamination by foreign mycorrhizae and were watered daily. They were grown for 15 days until early July 2007 when seedlings (n = 300) were transported to the Mealy Mountains field site. Five seedlings were planted in each of the 60 study plots on 5 July 2007 and monitored for growth over two seasons, and survival over three seasons (2007–2009). Differences in height of planted seedlings among groundcover treatments were measured using calipers (±1 mm) six times (once every three days) in summer 2007, and 18 Sept 2007, and three times (25 June, 20 July and 18 Sept 2008) and again 27 June 2009.

Primary sources of seedling mortality

Herbivory
During the 2007 and 2008 growing seasons, herbivory damage on seedlings was estimated visually when growth measurements were taken. Damage from herbivory was categorized based on the percentage of seedling removal: 1 = <25%, 2 = 25–50%, 3 = 50–75%, 4 = >75%, 5 = removed. Partial leaf tissue removal was assigned to slugs as their distinctive rasping feeding behavior removes a portion of the needles over a longer period of time (Moss and Hermanutz 2009).

Overwinter mortality
Seedlings that were dead at the first survey of the second growing season were attributed to over-winter mortality.

Desiccation
During the 2007 and 2008 growing seasons, desiccation was identified as the cause of mortality when a seedling's stem and needles were brown and dry, with no living tissue observed.

Relative seed predation

Relative seed predation across seedbed treatments was examined in 2008 using seed cards onto which 10 black spruce seeds were lightly glued using spray adhesive to 5 × 5 cm

medium-grade sandpaper squares (Marino et al. 2005). Seed predation was examined in eight randomly selected plots from each seedbed type, for a total of 24 plots; the same plots were used for each repetition of the experiment. The seed cards were secured to the ground with 5 cm roofing nails and were collected and replaced on a 5-day interval, for a total of four replacements. Every disturbed card (i.e. > 1 seed removed) was counted as a predation event since the likelihood of individual seed removal increased if a predator encountered the card. The data were pooled for analysis.

Data analyses

Data analyses were carried out using SAS ver. 9.1, Minitab ver. 13 and R 2.6.0. Temperature and moisture were analyzed using general linear models (ANOVAs) where temperature and water availability were the response variables, and seedbed and date were the explanatory variables. Nutrient supply totals were analyzed using a principal components analysis (PCA) after processing the data by Euclidean normalization. A Poisson regression model was used for seed emergence, with count of emerged seeds as the response variable and seedbed as the explanatory variable. Seedling growth was analyzed using a weighted-means ANOVA, in which the response variable was the percent height increase from the initial planted height, and explanatory variables were seedbed and pesticide treatment. Percent height increase for each seedling was weighted by plot survivorship so growth on treatments with fewer surviving seedlings did not have an undue weight. Seed predation, seedling herbivory, over-winter mortality and desiccation were all analyzed using logistic regression models, in which the response variable was the odds of the event (predation, mortality), and explanatory variables were seedbed and in the case of predation and herbivory, pesticide treatment. Exposed soil/simulated dig plots with no pesticide were chosen as the reference treatment for all logistic regression models (all results for other treatments are expressed as odds ratios relative to this treatment) because mineral soil is considered to be the optimal seedbed in the boreal forest and thus any significant differences of event likelihood on the other treatments can be attributed to the presence of an intact seedbed and/or slug pesticide. In both Poisson and logistic regression, incidence and odds ratios show the treatment differences.

Results

Soil temperature, moisture and nutrients

During the 2008 growing season, soil temperatures were not significantly different across seedbeds (DF = 2, F = 0.10, p = 0.904). July 2008 soil temperatures were lower at the beginning of the sampling period (mean = 11.8°C, SE = 0.23), peaked mid-season (mean = 13.0°C, SE = 0.17), and declined at the end of the sampling period (mean = 11.2°C, SE = 0.11). The average soil temperature was 12.3°C on all treatments through the sampling period. Average percent soil moisture ranged from 23% on bare ground digs, to ~26% on *Cladonia* and *Pleurozium* seedbeds, and was not significantly different between treatments at each individual sampling period.

The first two principal component axes of the PCA explained 71% of the variation in soil nutrient supply rates. PC axis 1 reflected calcium, magnesium and iron concentrations, (component loading –0.495), while PC axis 2 reflected positive iron and negative sulphur (0.607 and –0.569) loadings. The distribution of *Cladonia* and *Pleurozium* samples on the biplot indicates a stronger positive relation between soil nutrients and *Pleurozium*, and a negative relation to *Cladonia*, suggesting the lichen seedbed is more nutrient-deficient for all nutrients except boron when compared to *Pleurozium*. Soil nutrient supply rates were highly variable across *Pleurozium* seedbeds.

Seed emergence

Emergence of black spruce was very low across all treatments (total n emergents = 36/1020 = 3.5%), but differed significantly across seedbeds over the 2008 growing season (DF = 2, χ^2 = 221.19, p ≤ 0.0001). Black spruce emergence was highest on *Pleurozium* (n emergent = 19, 6.3%), followed by bare soil (n emergent = 16, 5.3%) and was lowest on *Cladonia* (n emergent = 1, 0.3%). Seed emergence was earliest on bare soil treatments (12 July – 13 Sept), followed by *Pleurozium* (13 July – 13 Sept), with latest emergence on *Cladonia* (single seed emerged on 17 July). Incidence of black spruce emergence was almost equally likely on bare soil and *Pleurozium* (incidence ratio = 1.19, CI = 0.97–1.44), while emergence likelihood was significantly lower on *Cladonia* (incidence ratio = 0.063, CI = 0.033–0.12).

Seedling growth

For each of the three measurement periods, seedling growth, as indicated by survival-weighted percent height increase, was greatest on *Pleurozium*+ relative to bare soil– treatment (Fig. 1A) and the order of seedling growth by treatment was the same (highest to lowest: *Pleurozium*+, bare soil+, *Pleurozium*–, *Cladonia*+, *Cladonia*–, bare soil–; the reference treatment; Fig. 1A). After the first growing season (Sept 2007), seedling growth differed significantly across seedbed (DF = 2, F = 6.27, p = 0.002) and pesticide (DF = 1, F = 13.16, p = 0.0001) treatments. In the second measurement period (June 2008), seedling growth differed significantly across seedbed (DF = 2, F = 9.89, p ≤ 0.0001), but not between pesticide treatments (DF = 1, F = 2.69, p = 0.103), which was expected since pesticide was not added to the treatment plots between August 2007 and June 2008 (i.e. overwinter). After two full growing seasons (Sept 2008), seedling growth differed significantly across seedbed (DF = 2, F = 8.13, p = 0.001) but not across pesticide treatment (DF = 1, F = 2.69, p = 0.104). After the first growing season, pesticide had no effect on seedling growth. When measurements for Sept 2008 were pooled across seedbeds, survival-weighted growth is significantly higher on *Pleurozium* (30.9%) than on *Cladonia* (21.7%) or bare soil (20.5%; Fig. 1B).

Seed predation

Black spruce seeds were significantly less vulnerable to predation on *Pleurozium* plots relative to *Cladonia* and bare soil.
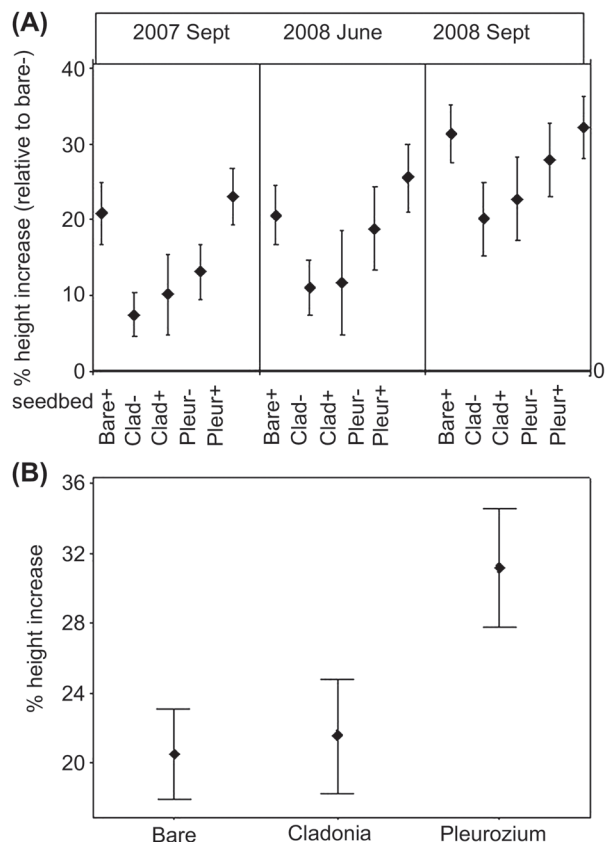
Figure 1. (A) Seedling weighted percent height increases (with 95% CI) of seedlings on bare soil+ (Bare+), *Cladonia*+/– (Clad+/–) and *Pleurozium*+/– (Pleur+/–) relative to seedlings on bare soil-treatments (values greater than y = 0 indicate greater relative % height increase); points indicate average percent height increase weighted by survival on each treatment type during each growing period, relative to average percent height increase on bare soil-during each period and (B) Seedling weighted percent height increases (with 95% CI) pooled across bare soil+/– (Bare), *Cladonia*+/– (Clad–) and *Pleurozium*+/– (Pleur–) pesticide treatments after two growing seasons (Sept 2008).

During the 2008 growing season, the odds of seed predation were significantly different across seedbed (DF = 2, χ^2 = 31.62, p < 0.0001) and pesticide treatments (DF = 1, χ^2 = 17.01, p ≤ 0.0001) with no significant interaction effects (DF = 2, χ^2 = 2.14, p = 0.3438). The probability of a seed predation event was highest on bare soil + (83%), followed by *Cladonia*+ (75%), *Cladonia*– and bare soil– (69%), and was lowest on *Pleurozium*+ (62%) and *Pleurozium*– (50%). During the 2008 growth season seed predation was more likely to occur on pesticide treatments than those without pesticide (OR = 2.27, less likely to occur on *Pleurozium* (OR = 0.45) and equally likely to occur on *Cladonia* (OR = 1) relative to bare soil (odds = 2.2; Table 1).

Seedling survival and mortality factors

Of the 300 seedlings initially planted across all six treatment types, 206 seedlings (68.7%) survived the 2007 growing season, 118 (39.3%) survived 2008, and 99 (33%) survived to June 2009 (Fig. 2). The proportion of total seedlings surviving was highest on *Pleurozium*+ (49%) and lowest on

Table 1. Odds ratios of relative seed predation likelihood by seedbed and pesticide treatment (+ with – without; 2008). Odds of seed predation occurring on reference treatment bare soil – pesticide = 2.2; ratios > 1 = higher predation likelihood, ratio < 1 = lower predation likelihood. CI = confidence intervals.

	Odds ratios	OR CI	
Cladonia– versus bare soil–	1	0.688	1.453
Pleurozium– versus bare soil–	0.45	0.317	0.652
+ versus –	2.27	1.362	3.791
Cladonia+ versus *Cladonia*–	1.36	0.411	4.529
Pleurozium+ versus *Pleurozium*–	1.75	1.049	5.749

the bare soil (18%). Factors causing mortality in the first growing season included herbivory (26%), drowning (2%) and unknown causes (3%; Fig. 3). Mortality factors in the second year included overwinter mortality (15%), probable desiccation (12%), and overgrowth by moss (1%; Fig. 3). Overwinter mortality was the only cause of seedling loss in 2009, since the study period only extended to June 2009.

Seedling herbivory by slugs

Because the majority (>90%) of seedling herbivory was attributed to native slugs (*Deroceras laeve*), analyses were designed to evaluate differences in slug herbivory across treatments. Seedlings exhibited vulnerability to slug predation only during the first growing season; therefore only herbivory data from 2007 were analyzed. During the 2007 growing season, herbivory mortality was significantly different across seedbed (DF = 2, χ^2 = 12.31, p = 0.0021). Pesticide treatment (DF = 1, χ^2 = 1.21, p = 0.2707) and interaction effects (DF = 2, χ^2 = 0.67, p = 0.7158) were non-significant. Herbivory mortality was highest on bare soil + (35%), followed by bare soil– (31.7%), *Pleurozium*+ (20%), *Cladonia*+ (17.8%), *Cladonia*– (15.6%) and was lowest on *Pleurozium*– (11.1%). Slug herbivory was almost equally likely to occur on pesticide-treated plots than those without pesticide (OR = 1.166) and slug herbivory was less likely to occur on *Pleurozium* (OR = 0.27) and *Cladonia* plots (OR = 0.4) relative to bare soil (odds = 0.46; Table 2A).
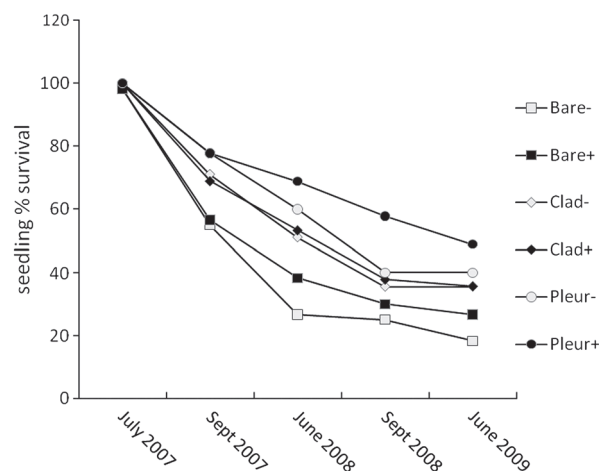

Figure 2. Survivorship curves showing percent survival of black spruce seedlings on bare soil+/–, *Cladonia*+/– and *Pleurozium*+/– treatments (2007–2009).
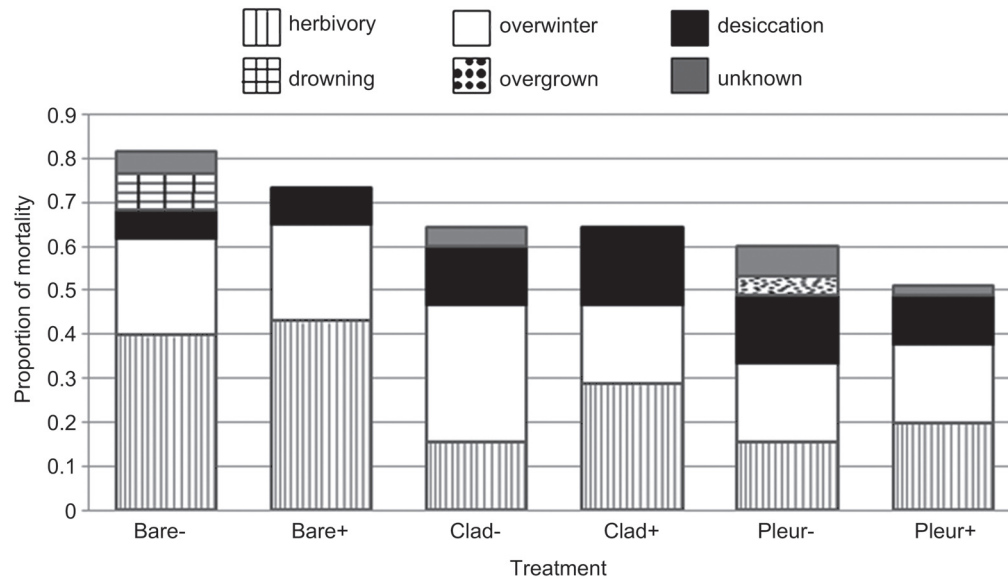
Figure 3. Total percent mortality and proportion of contributing mortality factors for black spruce seedlings across all treatments (2007–2009).

Overwinter mortality and desiccation

Overwinter mortality was the primary cause of new mortality in 2008 (4–22%) and the only measured cause of mortality in 2009. In 2008, overwinter mortality was significantly different across seedbeds (DF = 2, χ^2 = 8.07, p = 0.0176) as it was highest on *Cladonia* (18.9%), followed by bare soil (18.3%), and was lowest on *Pleurozium* (6.7%). While the odds of overwinter mortality are approximately equal on bare soil (odds = 0.225) and *Cladonia* (OR = 1.027) they are significantly lower on *Pleurozium* (OR = 0.313; Table 2B). In 2009, overwinter mortality remained highest on *Cladonia* (21.1%), followed by bare soil (20.9%), and was lowest on *Pleurozium* (12.2%), but did not differ significantly across seedbeds (DF = 2, χ^2 = 3.38, p = 0.1841). While the odds of overwinter mortality are approximately equal on the bare soil reference treatment (odds = 0.26) and *Cladonia* (OR = 1.01) they are lower on *Pleurozium*, though not significantly (OR = 0.53; Table 2C). In 2007 and 2008, overall probable desiccation mortality was highest on *Cladonia* (15%), followed by *Pleurozium* (13%) and bare soil (8%), but did not differ significantly across seedbeds (DF = 2, χ^2 = 331, p = 0.1912). The odds of probable desiccation mortality were approximately twice as high on *Cladonia* (OR = 2.18) and *Pleurozium* (OR = 1.81) relative to bare soil (odds = 0.08); these differences were not significant (Table 2D).

Discussion

At treeline, *Pleurozium* appears to facilitate the establishment and growth of black spruce seedlings during the early life history stages. Relative to the other substrates tested, *Pleurozium* seedbeds showed a higher incidence of black spruce seed emergence, significantly greater seedling height increase after two growing seasons and had significantly lower odds of first-winter mortality, seed predation, and slug herbivory relative to bare soil. Also, there was no clear pattern of improved success on either *Cladonia* versus bare soil relative to each other; black spruce emergence was lower on *Cladonia* relative to bare soil but the odds of slug herbivory were lower on bare soil.

These results differ from the pattern of black spruce recruitment observed by the majority of studies conducted in the boreal forest, in which bare soil represents the optimal seedbed for black spruce recruitment and *Pleurozium* and *Cladonia* seedbeds are relatively poorer seedbeds (Prévost 1997, Charron and Greene 2002, Hébert et al. 2006). Only a single study in the boreal forest reported higher rates of seedling growth on *Pleurozium* relative to bare soil, *Sphagnum* and other moss seedbeds (Lavoie et al. 2006, 2007). In this study, highest yearly height and above-ground biomass increase were observed in black spruce seedlings planted on

Table 2. Odds ratios and confidence intervals (CI) of seedling mortality likelihood by seedbed relative to mortality on bare soil reference treatment (2007–2008). Odds ratios > 1 = higher mortality likelihood, ratio < 1 = lower mortality likelihood.

	Cause of mortality	Mortality odds on bare soil/(CI)		Odds ratio (OR), *Cladonia* relative to bare soil/(CI)		Odds ratio (OR), *Pleurozium* relative to bare soil/(CI)	
A	Slug herbivory (2007)	0.46	(0.27, 0.80)	0.40	(0.15, 1.06)	0.27	(0.09, 0.79)
B	Overwinter death (2007)	0.23	(0.14, 0.36)	1.03	(0.51, 2.07)	0.31	(0.12, 0.81)
C	Overwinter death (2007–2009)	0.26	(0.17, 0.41)	1.01	(0.52, 1.98)	0.53	(0.24, 1.14)
D	Desiccation (2008)	0.08	(0.04, 0.16)	2.18	(0.89, 5.31)	1.81	(0.73, 4.61)

Pleurozium and *Pleurozium*-derived seedbeds in lowland boreal forests, with relatively less growth on bare soil, likely as a result of flooding (Lavoie et al. 2006, 2007). We suggest that under the environmentally stressful conditions in the treeline ecotone, *Pleurozium* seedbeds enhance black spruce seedling growth, survival and recruitment, shifting the interaction balance from potential competition to facilitation.

The odds of first winter mortality were much higher on bare soil and *Cladonia* relative to *Pleurozium*, suggesting that moss seedbeds protect first-year seedlings from the low temperature extremes that likely occur on bare soil during cold periods when insulating snow cover is absent; at the altitude of this study, daily minimum temperatures reach freezing in all months (Jacobs and Chan pers. comm. 2010). Soil temperatures under moss and lichen seedbeds may be colder than, or more highly insulated and warmer than exposed soil, depending on the season (Bonan and Shugart 1989, Colombo et al. 2003, den Herder et al. 2003). In our study, there were no soil temperature differences among seedbed during the height of the growing season in early to mid-July 2008, which had the highest average temperatures and greatest number of degree days relative to any other month for 2008 (Jacobs and Chan pers. comm. 2010). Seedbed temperatures may be more variable between substrate types during the fall, when removal of the insulating seedbed layer can cause extremes in soil temperatures, especially during frost periods before snow cover is established (Colombo et al. 2003, den Herder et al. 2003). The thermal control regulated by seedbed insulating properties may facilitate conifer growth and survival because cold soils are associated with limitations in both photosynthesis and root growth in conifer seedlings (Smith et al. 2003). *Cladonia* have high reflectivity of solar radiation (Bonan and Shugart 1989), so lichen seedbeds are colder than the darker *Pleurozium* patches which would absorb more solar radiation, especially in the spring after the snow cover has retreated and before the development of the peripheral shrub canopy.

Soil water availability and temperature appear to be the primary factors limiting black spruce seed germination in many studies (Power 2005, Lavoie et al. 2006). Temperature did not differentially limit germination in the Mealy Mountains study area, since all seedbed temperatures during the 2008 growing season exceeded the 10°C minimum germination temperature for spruce (Black and Bliss 1980). *Pleurozium* and *Cladonia* seedbeds are generally wetter than exposed soils (Bonan and Shugart 1989, Suzuki 2007), but can also desiccate during the boreal summer (Hörnberg et al. 1997). In our study, seeds and seedlings likely did not experience significant moisture deficits in the 2007 or 2008 growing seasons. Moist summers have characterized the Mealy Mountains forest–tundra ecotone during the past 8 years of climate monitoring (Jacobs pers. comm.).

Nutrient availability is another factor that may account for the differences in the overall growth and survival of seedlings in our study. In the boreal forest, relatively nutrient-rich bare soil is usually associated with post-fire habitats, where vegetative seedbeds have burned and previously-immobilized nutrients become available (Hart and Chen 2006). Since fire is not a disturbance factor in the Mealy Mountains forest–tundra ecotone (Trindade et al. 2011), bare soil is not associated with this post-burn nutrient mobilization, which may explain the poor performance of black spruce seeds and seedlings on

this seedbed. With regard to the vegetated seedbeds, lichen groundcovers are generally associated with nutrient-poor soils (Bonan and Shugart 1989) and in our study *Cladonia* seedbeds were moderately nutrient-deficient relative to *Pleurozium* seedbeds. Calcium, which is required for cell wall and membrane integrity and in building tolerances to stressful abiotic conditions such as cold and drought (Fink 1991, DeHayes et al. 1999), was in higher supply in *Pleurozium* seedbeds. This enrichment of *Pleurozium* may be a consequence of litter leachates from dwarf birch growing in close proximity to the moss seedbeds (DeHayes et al. 1999), and not directly due to the moss. Dwarf birch shrubs were not shown to facilitate the growth or survival of black spruce seedlings in the Mealy Mountains treeline ecotone (Cranston 2009).

Bare soil is thought to be an optimal germination site in boreal habitats because developing roots are better able to penetrate bare mineral soil relative to vegetated seedbeds (Butler et al. 2004, Malanson et al. 2007). In our study, *Cladonia* seedbeds inhibited emergence, possibly because seeds tended to fall deeper into the labyrinthine lichen mats and, as a consequence, emerging black spruce may have been unable to penetrate the upper seedbed surface during the growing season. While Power (2005) demonstrated increased black spruce seed emergence after fire in lichen fractures, the fissures in the mats may have provided a suitable microhabitat not present in the wetter, intact lichen mats of the Mealy Mountains. On the denser surface of *Pleurozium* and bare soil, seeds tended to remain on or near the surface and thus emerged with greater success.

The physical structure of seedbeds and associated vegetation may also facilitate seedlings by sheltering them from low temperatures and photoinhibition (photosynthetic reduction caused by overexposure to high-intensity light; Germino and Smith 1999). This problem would not be expected in the boreal forest due to the presence of a full or partial canopy protecting seedlings from sky exposure. Full sky exposure increases photoinhibition, which reduces photosynthetic carbon gain and growth, and has been linked to high mortality rates in some conifer species (Smith et al. 2003, Maher and Germino 2006). Sheltering plants reduce sky exposure, which alters the temperature regime (warmer night and cooler day temperatures) and increases photosynthetic carbon gain, root growth, mycorrhizal infection and survival (Smith et al. 2003, Maher and Germino 2006). Seedlings planted in bare soil have no vegetation growing around them to provide shelter and are almost completely exposed to the sky relative to those planted in *Pleurozium* and *Cladonia* plots that have some direct shelter provided by the seedbed growing up around them. Since seedling growth was greatest and odds of survival highest on the *Pleurozium* groundcover, this suggests that the physical structure of this seedbed may directly reduce sky exposure and regulate the temperature regime of the microhabitat, facilitating the recruitment of black spruce. However, further research is needed to explicitly quantify sky exposure and determine whether it directly affects black spruce seedling survival.

Seed and seedling herbivory

Not only did black spruce establish and grow better in plots with *Pleurozium* groundcover but their seedlings and seeds

were less likely to be eaten by herbivores and seed predators. Herbivory by the slug *D. laeve* was the major cause of seedling mortality during the first growing season, accounting for more than 90% of all herbivory. Moreover, herbivory was most prevalent in bare soil plots suggesting that vegetated seedbeds shelter seedlings from slug predators. These results are consistent with Côté et al.'s (2005) study of black spruce seedling herbivory in the boreal forest and Munier et al.'s (2010) findings of slug herbivory across an elevational gradient in the Mealy Mountains. Because slug pesticides containing ferric phosphate have been used effectively to reduce *D. reticulatum* herbivory in agricultural field trials (Speiser and Kisler 2002), our result that seedling herbivory attributed to *D. laeve* was almost equally likely to occur on plots scattered with and without organic slug pesticide was unexpected. This study suggests ferric phosphate pellets, as applied in our study, are ineffective as a control against *D. laeve* herbivory of black spruce seedlings.

Post-dispersal predation of black spruce seed was also lowest on vegetated plots, particularly on *Pleurozium* plots. Côté et al. (2005) found a similar result in boreal forest habitat, where predation of post-dispersal black spruce seed was lowest in *Pleurozium*–spruce forests, followed by *Cladonia*–spruce forest and was highest in non-vegetated burned sites. The likelihood of seed predation was highest, in our study, on pesticide-treated plots suggesting either that the pesticide is ineffective against seed predation by slugs, or that other granivores, such as carabid beetles (Moss and Hermanutz 2009) are feeding on black spruce seed. Although our study did not attempt to isolate the causes of post-dispersal seed predation, other than that by slugs, our experimental seed predation results are, nonetheless, consistent with our other results suggesting that a *Pleurozium* seedbed facilitates black spruce establishment at treeline.

Implications of seedbed facilitation for black spruce recruitment and expansion

The ability of black spruce to germinate, establish and survive three growing seasons above the treeline in the Mealy Mountains forest–tundra ecotone was facilitated by seedbeds vegetated by *Pleurozium* relative to seedbeds vegetated by either lichen or especially to bare soil. This result differs from what was found in the boreal forest where black spruce generally established best on bare soils (Prévost 1997, Charron and Greene 2002, Hébert et al. 2006) and thus is consistent with the predictions of the Stress gradient hypothesis, that facilitative interactions outweigh competition as the most ecologically important plant–plant interaction in environmentally stressful habitats (Brooker et al. 2008). Moreover, our results are consistent with Munier et al.'s (2010) findings that planted black spruce seed and seedlings can establish successfully at or above treeline. In early years, seedlings develop within the sheltering vegetated seedbed; once they grow above the seedbed, they may face different microclimatic conditions. Further research is needed to explicitly quantify seedling growth and survival in the microhabitat above the seedbed. The potential for *Pleurozium* facilitation of seed emergence and early establishment above the standing treeline exists, if viable seeds are being produced within or transported to the forest–tundra boundary. Lack of viable seed is an important factor limiting plant establishment in alpine habitats and the forest–tundra ecotone (Lindgren et al. 2007). Currently in the Mealy Mountains, black spruce seed production at the treeline is a bottleneck to establishment (R. Jameson pers. comm. 2010). However, if climates warm, as predicted, it is expected that the probability of viable seed reaching treeline sites will increase.

Moss and lichen seedbeds represent a significant proportion of high-latitude plant diversity and biomass and are important for habitat structure and nutrient cycling in these ecosystems. In the Mealy Mountains forest–tundra ecotone, mosses compose on average between 20 to 36% of the groundcover, while lichen seedbeds account for an average of 17 to 29%; shrubs, vascular plants, and other groundcovers account for the remaining area (Munier et al. 2010). However, there are few studies that examine the impact of shifting climate regimes on these seedbed communities (Nilsson and Wardle 2005, Jägerbrand et al. 2006). A shift towards a more facilitative seedbed, as a consequence of warming climates, could increase the recruitment of black spruce in the treeline ecotone and promote tree expansion upslope, by providing optimal microhabitat for emergence, growth and survival.

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