**The effect of biotic interactions on boreal conifer recruitment at alpine treeline in central Newfoundland.**

by

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**Abstract**

Treeline, the ecotone between forest and alpine or tundra ecosystems, is perceived as the thermal limit to tree growth, reproduction, and survival and in a warming world, we expect treelines to shift to higher elevation and latitudes. Despite increases in temperatures, there has been no ubiquitous change in treeline position. Shifts in treeline position will be dependent on increased recruitment, the production or dispersal of viable seed followed by germination and seedling establishment and survival, at treeline. To examine how biotic interactions constrain or facilitate black spruce and tamarack recruitment at alpine treeline, we conducted a series of observational and experimental studies along an altitudinal gradient in central Newfoundland, Canada. We found treeline population to be simultaneously seed and establishment limited, however if seedlings become established we found seedling survival to be high. Our results highlight the need for multiple factors to align temporally for recruitment to occur.

**Keywords:** altitudinal treeline, range expansion, biotic interactions, recruitment limitations, seed viability, seed predation, substrate suitability, facilitation, *Picea mariana*, *Larix laricina*

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**Chapter 1: Introduction and thesis overview**

**1. 1 Introduction**

   Understanding drivers of species' abundance and distributions has been a central

theme throughout the history of biogeographical studies and has current implications for

modern ecological issues involving impacts of global climate change. Species'

distributions are the spatial representation of the combination of abiotic and biotic

conditions that support a specie's growth, survival, and reproduction (Harper, 1977).

While biotic conditions have long been recognized to influence species' range limits (e.g.,

Connell, 1961), historically there has been a focus on abiotic conditions, particularly

temperature and precipitation, as determinants of species' distributions, especially

species' uppermost altitudinal and latitudinal limits (e.g., Dana, 1953; Dobzhansky, 1950;

MacArthur, 1972). This focus on abiotic drivers is not unfounded, as species

physiological tolerance to abiotic conditions unequivocally influence species distributions

(Sexton *et al.*, 2009). However, the focus on abiotic drivers has resulted in limited

empirical knowledge on the relative importance of biotic factors in driving species' range

limits and we are currently limited in our ability to generalize across species, interactions,

and conditions in which biotic interactions drive range limits (Hille Ris Lambers *et al.*,

2013).

   Climate change is predicted to have widespread effects on species' distributions, with

populations expected to shift upslope and/or to higher latitudes (Chen *et al.*, 2012).

However, observed variability in the magnitude and direction of species' responses to

changing climatic conditions suggests that biotic factors are driving, or interacting with

abiotic conditions to drive species' range limits (Hille Ris Lambers *et al.*, 2013). In response, biotic factors, in particular interactions, have been increasingly recognized as determinants of species distributions at broad spatial scales (Lewis *et al.*, 2017). Competitors, consumers, mutualists, and facilitators will influence a species' ability to grow, survive, and reproduce and therefore, intuitively, affect a species' abundance and distribution (Wiens, 2011). However, there is currently a paucity of empirical evidence on when biotic interactions control species' range limits and in turn, how these biotic interactions will mediate species' response to climate change (reviewed by Hille Ris Lambers *et al.*, 2013). Studies of climate change effects on species distributions will allow for the increased understanding of the relative importance of abiotic and biotic drivers on determining species' range limits (Sexton *et al.*, 2009).

Treeline, the ecotone between forest and tundra or alpine ecosystems, represents the uppermost latitudinal or altitudinal conditions that support tree growth, survival, and reproduction (Körner, 1998). Despite the term treeline, there is no 'line' that represents forest range limits; treeline is the zonal transition marked by a decline in tree density from closed canopy forest to tree species limit (Smith *et al.*, 2003). The geographical position of treeline is thought to be primarily controlled by thermal conditions, as evidenced by global scale correlations between seasonal mean temperature and treeline position (Körner & Paulsen, 2004; Körner, 2012; Paulsen & Körner, 2014). Therefore, with the alleviation of thermal constraints, treeline position is expected to shift upslope and to greater latitudes. Particular focus has been placed on predicting the response of trees; not only is the geographical limit of forests the most conspicuous transition in vegetation

structure but forests cover approximately half of the Earth's terrestrial surface (Aitken *et al.*, 2008).

The process of tree range expansion is initially dependent on successful recruitment at, or beyond, the treeline ecotone. Early-life stages have long been recognized to disproportionally affect recruitment, as early-life stages are both most abundant and most vulnerable (Harper, 1977). Therefore, seed survival, germination, and seedling establishment and survival can have long standing effects on species abundance and distribution (Harper, 1977; Chambers & MacMahon, 1994; Clark *et al.*, 2013) The reproduction limitation hypothesis poses that the position of treeline is governed by the failure of trees to successfully recruit beyond range limits (Körner, 1998).

Fundamentally, climate controls plant recruitment; warm summers promote pollen and seed cone initiation (Owens & Blake, 1985), minimum heat sums are required for proper embryo development (Sirois *et al.,* 1999), seasonal cues drive germination and seedling emergence (Walck *et al*, 2011), and seasonal frosts can result in death across all early-life stages (Zasada, 1971). However, despite atmospheric temperatures increasing worldwide, there has been no ubiquitous change in global treeline position (Harsch *et al.*, 2009). This variability in treeline response suggests that non-climatic factors, including biotic interactions, may override or modulate the effects of temperature on treeline position, inhibiting or slowing treeline response to changing climatic conditions. Multiple biotic interactions can modulate the effects of atmospheric warming on recruitment either directly (i.e., consumption by seed predators; Brown & Vellend, 2014; Jameson *et al.*, 2015) or indirectly (i.e., amelioration of environmental conditions by facilitators; Wheeler

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*et al.*, 2011). Despite the recognized importance of early-life stages and biotic interactions in governing species distributions, there is uncertainty about how biotic drivers interact with early-life stages to shape current and future species distributions.

Recruitment at treeline is generally considered to be seed limited (e.g., Sirois, 2000; Meunier *et al.*, 2007; Brown *et al.*, 2018). Climate change is predicted to increase seed production (Krebs *et al.*, 2012); however, the production and/or dispersal of seed to treeline does not guarantee recruitment will occur. Recruitment limitations can result from numerous abiotic and biotic factors and that occur across multiple life stages, from seeds attached to parent plants to germination, seedling establishment and survival (Clark *et al.*, 1999, 2013; Nathan & Muller-Landau, 2000). Consequently, recruitment limitations, and the fate of seeds, need to be assessed at both pre-dispersal and post-dispersal stages (Nathan & Muller-Landau, 2000; Clark *et al.*, 2013). Patterns of seed production do not consistently align with patterns of seed viability and pre-dispersal processes, such as pollen limitations and pre-dispersal seed predation, may keep treeline populations seed limited (Sirois, 2000; Jameson *et al.*, 2015; Kroiss *et al.*, 2015; Kambo & Danby, 2017; Brown *et al.*, 2018). Subsequent post-dispersal processes, including predation, competition, and herbivory, may further constrain recruitment where, despite the arrival of viable seed to treeline, seedlings fail to establish (Clark *et al.*, 1999; Bråthen *et al.*, 2010; Munier *et al.*, 2010; Wheeler *et al.*, 2011; Dufour-Tremblay *et al.*, 2012; Kambo & Danby, 2017).

Successful germination does not guarantee seedling establishment and survival, as abiotic requirements at one life stage may not be advantageous at another (Cranston &

Hermanutz, 2013). Due to the small stature of seedlings, microsite conditions are likely to have a greater influence on recruitment than local climatic conditions (Resler, 2006; Körner, 2016; Renard et al., 2016). Established individuals at treeline have been found to alter microsite conditions (e.g., increasing snow accumulation, providing protection from wind and herbivory, and reducing radiative extremes; reviewed by Holtmeier, 2009). Conifer seedlings at treeline have been found to be positively associated with tree islands (Alftine & Malanson, 2004; Renard et al., 2016) and individual krummholz (Batllori *et al.*, 2009), suggesting that established individuals at treeline alter microclimatic conditions in a way that facilitates seedling establishment and survival. Facilitation, the interaction of one species altering the environment in a way that enhances the growth, survival, or reproduction of a neighbouring species (Bronstein, 2009), is an important factor at range limits and facilitative interactions between established individuals and seedlings has been proposed as a potential mechanism for treeline advance (Holtmeier & Broll, 2007; Presas *et al.*, 2009; Cranston & Hermanutz, 2013; Renard *et al.*, 2016).

**1.2 Thesis rationale**

The province of Newfoundland and Labrador, to date, is 1.5°C warmer than historical average (1968-1990) and is projected to continue to warm across the island of Newfoundland, with temperature changes most pronounced during the winter (+2-4°C by mid-21st century) and to a lesser extent in the summer (+1°C by mid-21st century; Finnis, 2013; Finnis & Daraio, 2018). Temperature changes of maritime areas are expected to occur slowly due to the moderating effects of the ocean. The mean annual temperature of central Newfoundland is approximately 3°C (2007-2017; Middle Arm weather station,

Environment Canada), implying that increases in mean winter temperatures will result in more precipitation falling as rain than snow. The number of frost days are projected to decrease dramatically by the mid-21st century, with the island experiencing 10 to 15 fewer frost events a year, with autumn and spring becoming more like summer than winter (Finnis, 2013; Finnis & Daraio, 2018). A shortened winter will result in an increased growing season, with 200 to 400 additional growing degree days projected for the island (Finnis, 2013; Finnis & Daraio, 2018). The impacts of climate change on precipitation across the province are expected to be minimal, where frequency and intensity of drought and/or dry spells are projected to remain constant and small increases in mean winter and spring precipitation are projected (Finnis, 2013; Finnis & Daraio, 2018). The projected changes in Newfoundland's climatic conditions support the global prediction that forest range limits are expected to shift upslope, and to greater latitudes.

Evidence suggests that non-climatic factors, including biotic interactions, likely slow or inhibit the ability for forest distribution to respond to broad scale climatic change (Harsch *et al.*, 2009). The effect of biotic interactions (e.g., competition, consumption, facilitation) on species' ability to track changing climate controls are difficult to predict, as there is lack of empirical evidence on how biotic interactions control current range limits and in turn, how the magnitude and direction of these biotic drivers may be modified in a warmer world (Hille Ris Lambers *et al.*, 2013). In order to accurately predict treeline advance to climate change, we must first increase our understanding on the direction and magnitude biotic drivers have on reproduction, growth, and survival at current range limits (Hille Ris Lambers *et al.*, 2013; Hargreaves *et al.*, 2014).

While asexual reproduction is common at treeline (Payette & Gagnon, 1979; Viktora *et al.*, 2011), significant changes in treeline position will depend on successful recruitment at, or beyond current range limits. Despite the recognized importance early-life stages have on the abundance and distribution of species (Harper, 1977), there is uncertainty about how biotic drivers affect seed production and germination, and subsequent seedling survival at range limits. Observational studies alone cannot parse the multiple, interacting factors involved with range expansion, thus there is a critical need for manipulative experiments to understand the response of species' distributions to a warming world (Hille Ris Lambers *et al.*, 2013). Here, we present findings from a series of complementary observational and experimental studies examining how predicted biotic drivers' affect black spruce (*Picea mariana*) and tamarack (*Larix laricina*) recruitment at altitudinal treeline in Newfoundland. The studies presented in this thesis offer a novel examination of recruitment at treeline, examining the effect of several biotic interactions across a series of early life stages. Our findings complement previous studies examining the importance of ecological processes in shaping the geographical position of treelines and fill a geographical knowledge gap in treeline research. To the best of our knowledge, we are the first to experimentally examine whether biotic interactions will affect altitudinal treeline advance on the island of Newfoundland, filling a geographical gap in treeline research.

## 1.3 Study site

The study took place on a south-west facing slope on the Baie Verte Peninsula in central Newfoundland, Canada (49º35.2' N, 56º13.7' W; Figure 1.1). The central

Newfoundland 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; 49°41' N, 56°06'W; 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; 49°41' N, 56°06'W; Middle Arm weather station, Environment Canada).

The growing season spans mid-May through the end of September (Damman, 1983),

accumulating approximately 1200 growing degree days (2007-2017; 49°41' N, 56°06'W;

Middle Arm weather station, Environment Canada; GDD based on 5°C threshold,

MacPherson & MacPherson 1981). To date, the island of Newfoundland has warmed

approximately 1.5°C (Finnis, 2013; Finnis & Daraio, 2018). Due to the regulating effects

of the ocean, projected temperature changes are minimal (+2-4°C by mid-21st century);

however, total GDD is expected to increase by 30 to 40% (Finnis, 2013; Finnis & Daraio,

2018).
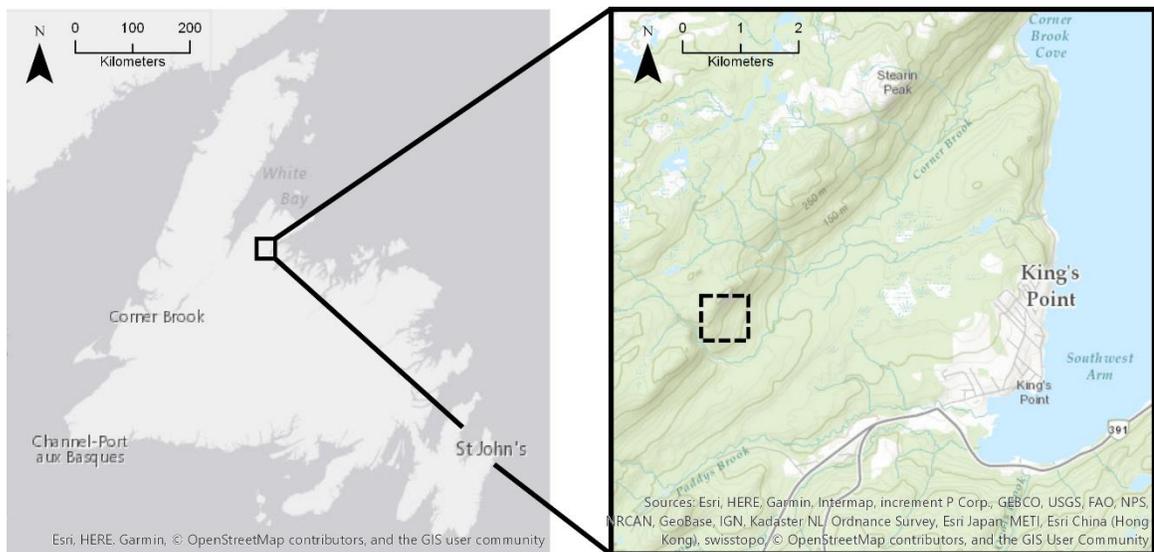
**Figure 1.1** Map of the study site on the Baie Verte Peninsula of Newfoundland, Canada. The dashed box outlines the altitudinal gradient in which the studies were conducted.

The central Newfoundland ecoregion is the most distinctly boreal assemblage on the island (Damman, 1983). Predominantly black spruce (*Picea mariana* [Mill.] B.S.P.) stands are common in the northcentral subregion (i.e., the Baie Verte Peninsula) and arctic-alpine flora is restricted to areas of increased topographic relief (Damman, 1983). Altitudinal treeline, across the island of Newfoundland, occurs at low altitudes, rarely exceeding 350 m a.s.l., and areas at, or above treeline support krummholz (locally referred to as tuckamore), heathlands, and bogs (MacPherson, 1995).

With increasing elevation, the study site's vegetation transitions from a closed canopy black spruce dominant forest to an ericaceous heath with isolated patches of stunted black spruce and scattered tamarack (*Larix laricina* [Du Roi] K. Koch) (Figure 1.2a). We designated two study sites across the altitudinal gradient, the forest site (~130 m; Figure 1.2b) and the treeline site (~240 m; Figure 1.2c). The forest site is characterized as a black spruce- feathermoss forest type, where the canopy is predominately composed

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of black spruce with a few balsam fir (*Abies balsamea* [L.] Mill.) and the feathermoss

substrate is predominantly *Pleurozium schreberi* and at lower abundance *Hylocomium*

*splendens* and *Ptillium crista-castrensis*. The forest transitions to ericaceous heath at

approximately 200 m a.s.l., the uppermost elevation of erect, tree-form black spruce

individuals. The treeline site is characterized as a *Kalmia* heath (Meades, 1983), where

ericaceaus dwarf shrub cover is dominated by *Kalmia angustifolia*, with *Rhododendron*

*groenlandicum* and *Vaccinium angustifolium* being the next two most abundant shrubs,

and groundcover vegetation is composed of fruticose, caribou lichens predominately

*Cladonia stellaris*. Isolated patches of stunted, krummholz black spruce and scattered

tamarack individuals occur across the treeline site.
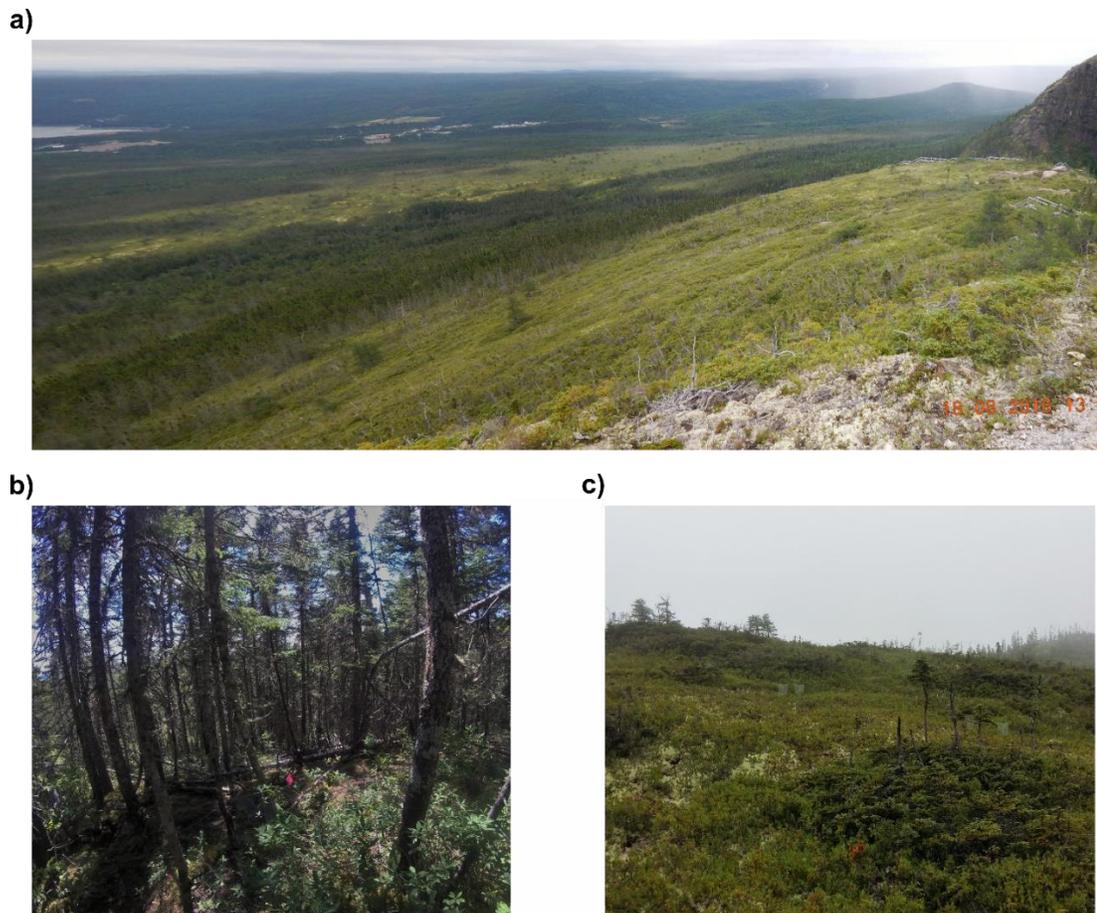
**Figure 1.2** Photographs of a) the south-west facing altitudinal gradient (49º35.2' N, 56º13.7' W), b) the forest site characterized as black spruce- feathermoss forest type, and c) the treeline site characterized as *Kalmia* heath.

## 1.4 Study species

### 1.4.1 *Black Spruce*

Black spruce is the dominant tree species at the study sites and stands are common throughout the northcentral subregion of the central Newfoundland ecoregion (Damman, 1983). The species is widely distributed across the North American boreal forest, inhabiting a broad range of environmental conditions, with populations often forming latitudinal  and alpine treeline (Timoney *et al.*, 1992). Mature individuals, within range limits, exhibit tree form and reach average heights of 12 to 20 m (Viereck & Johnston,

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1990), while at treeline, black spruce individuals are often stunted and deformed exhibiting a semiprostrate, shrub form (Viereck & Johnston, 1990).

Sexual reproduction follows two year cycles, with reproductive buds developing in the growing season of the first year, a period of winter dormancy, pollination and fertilization occurring in the spring and subsequent maturation of cones and fertilized embryos by the fall of the second year. Black spruce seed cones are semi-serotinous where high temperatures experienced during fires open cones and increase seed release. Absent of fire, cones remain partially closed and seeds are gradually released throughout the year. Seeds are primarily wind dispersed; although despite the small size and presence of wings, seeds are rarely dispersed greater than 80 m from the stand (Viereck & Johnston, 1990). Vegetative reproduction, by layering, is common at treeline and is an important mode of reproduction during periods of harsh environmental conditions that impede sexual reproduction (Viereck & Johnston, 1990; Holtmeier & Broll, 2010).

### 1.4.2 *Tamarack*

Tamarack is absent from the forest site but occurs alongside black spruce, at lower densities, at the treeline site. Tamarack has a wide distribution, spanning the North American boreal forest from Newfoundland and Labrador to central Alaska. At latitudinal and alpine treeline, tamarack is often found in association with black spruce (Payette, 1993). While deformed, shrub-like growth forms can occur, tamarack individuals at treeline, despite being stunted, usually occur in tree form (Payette, 1993).

Similar to black spruce, sexual reproduction of tamarack follows two year cycles from reproductive bud differentiating to seed production. Small cones are borne

throughout the crown and small, winged seeds are released in the fall upon cone ripening (Johnston, 1990). Tamarack seeds are larger than black spruce seeds (550,000 -710,000 seeds/kg and 890,000 seeds/kg respectively; Johnston, 1990; Viereck & Johnston, 1990). Few seeds are dispersed greater than twice the height of the individual (Johnston, 1990). While vegetative reproduction, through layering, can occur, it is considered much less important than regeneration by seed (Payette *et al.,* 1982).

## 1.5 Thesis objectives

To identify how biotic interactions may constrain or facilitate black spruce and tamarack recruitment at altitudinal treeline, we conducted a series of complementary observational and experimental studies in both field and laboratory settings. We examined the effects of predicted biotic interactions across two early-life stages: 1) seed production and germination (Chapter 2) and 2) seedling survival (Chapter 3).

For recruitment to occur, there are several filters that seeds must surpass, starting with the production of viable seed by parent plants to the arrival and retention of seed in microsites that allow for germination and seedling establishment (Clark *et al*., 2013; Chambers & MacMahon, 1994). Given the complex nature of recruitment, it is not surprising that recruitment at treeline has been compared to a very difficult hurdle race (Holtmeier, 2009). Chapter 2 aims to quantify a suite of recruitment limitations, from seed production to germination. To do so, we ask a sequence of research questions, addressing predicted life stage specific hurdles to conifer seed production, germination, and seedling emergence. Specifically, we ask how i) seed limitations and ii) establishment limitations vary across altitudinal species range limits.

Successful seedling germination and establishment does not guarantee seedling survival; in Chapter 3, we examine whether established black spruce and tamarack individuals at treeline facilitate the survival of seedlings. Facilitation by tree islands has been identified as a potential route for range expansion of boreal conifers (Alftine & Malanson, 2004; Renard et al., 2016). Due to the small stature of seedlings, microsite conditions drive establishment and survival (Resler, 2006). In Chapter 3, we aim to i) quantify how microclimatic conditions change with distance from established black spruce and tamarack at altitudinal treeline and ii) determine how established individuals and microclimate conditions interact to affect seedling survival at treeline.

The studies presented in this thesis will increase our understanding of the mechanisms governing recruitment at treeline in Newfoundland and, therefore, will provide insight into how Newfoundland's altitudinal treelines will respond to changing climatic conditions. This in turn, will help advance the understanding of the role of biotic interactions play in driving current treeline position more broadly and help generalize how and when biotic interactions will modulate species' response to climate change.

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## Co-Authorship Statement

All chapters in this thesis were co-authored with Dr. Carissa Brown. I was the principal contributor across all aspecets, including project proposal, experimental design, implimentation of field methods, analysis of data, and the preperation of the manuscripts. Dr. Brown supported the project across all aspects and contributed significantly to experimental design, analysis of data, and preperation of the manuscrips.

# Chapter 2: Biotic filtering of boreal conifer recruitment at alpine treeline

## Abstract

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. Recruitment limitations have been hypothesized as a mechanism for the variable response of treeline position to climate warming. We conducted a 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 an altitudinal treeline ecotone in central Newfoundland, Canada. We found recruitment at treeline to be simultaneously seed and establishment limited. The treeline populations produced fewer seeds than the forest populations and black spruce seeds produced by the treeline population were of lower viability. 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 are not consumed, individuals at treeline are establishment limited because 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.

## 2.1 Introduction

As sessile organisms, trees are fixed in space; therefore, any 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 & Muller-Landau, 2000; Briceño *et al.*, 2015; Johnson *et al.*, 2017). Dispersal ability, abiotic conditions, and biotic interactions may each constrain recruitment, where recruitment limitations can occur through multiple processes and across multiple life stages (Clark *et al.*, 1999). Globally, the distributional limit of trees where forests transition to alpine or tundra ecosystems, herein termed the treeline ecotone, is ascribed as the thermal limit to tree growth, survival, and reproduction (Körner, 1998). Human-induced environmental change and the alleviation of thermal constraints 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 & Vellend, 2014; Jameson *et al.*, 2015; Kroiss *et al.*, 2015; Kambo & 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 & MacMahon, 1994; Nathan & 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 (Nathan & Muller-Landau, 2000; Clark *et al.*, 2013). Seed limitations, defined as the failure for a sufficient number of seeds to arrive at uncolonized sites, can occur due to either low seed production and/or poor dispersal ability (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 & Muller-Landau, 2000; Clark *et al.*, 2013). Establishment limitations occur when environmental conditions at uncolonized sites limit seedling establishment regardless of seed arrival (Nathan & 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 (Muller-Landau *et al.,* 2002; Clark *et al.*, 2007).

Individuals at treeline have been found to produce fewer seeds relative to individuals within range limits, and it is predicted that fecundity will increase with the alleviation of climatic constraints (Case & Taper, 2000; Krebs *et al.*, 2014; Roland *et al.*,

2014). However, 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.,* 2018). Pollen limitations driven by low genetic diversity and/or low abundance in parent tree populations can produce empty, non-viable seeds (Kroiss *et al.*, 2015; Brown *et al.,* 2018), while viable seeds produced may be removed from the population through pre-dispersal predation (Jameson *et al.*, 2015; Kambo & 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 & MacMahon, 1994; Clark *et al.*, 1999), discussed below.

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 (e.g., Hulme, 1998) and experimental evidence suggests that predation at temperate and boreal treelines can be significantly limiting (Munier *et al.*, 2010; Wheeler *et al.*, 2011; Brown & Vellend, 2014; Kambo & Danby, 2017). Substrate, the groundcover on which a seed falls, can greatly influence germination success of tree species and unsuitable substrate may impede forest range expansion (LePage *et al.*, 2000; Charron & Greene, 2002; Dufour Tremblay

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& 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 the 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 in the literature (Hille Ris Lambers *et al.*, 2013) 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 mitigating tree species responses to climate change at their distributional limit. To accurately predict whether climate induced treeline advance will occur, we must first understand how biotic interactions control recruitment at current range limits. 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 magnitude and direction of biotic interactions at range limits and in turn 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 (Figure 2.1). 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 interaction on black spruce (*Picea mariana* [Mill.] B.S.P.) and tamarack (*Larix laricina* [Du Roi] K. Koch) recruitment at alpine treeline, we conducted a series of observational and experimental studies along an altitudinal gradient. Specifically, we examined whether predicted biotic interactions 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: (i) produce fewer seeds of lower viability, (ii) experience greater levels of post-dispersal seed predation, in particular by vertebrate seed predators, and (iii) have less germination and seedling establishment, driven by competitive interactions with lichen substrate and ericaceous shrubs.
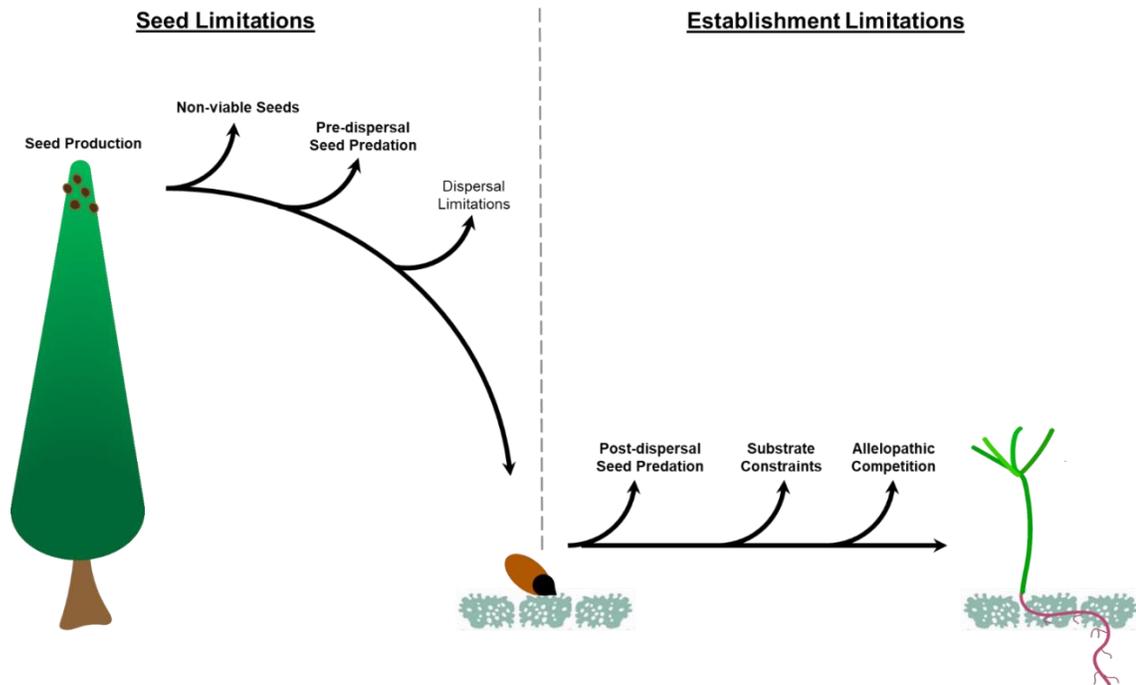
**Figure 2.1** Predicted biotic filters to recruitment at altitudinal treeline. We conducted a series of observational and experimental studies to examine the bolded biotic filters.

## 2.2 Methods

The study took place on a south-west facing slope on the Baie Verte Penisula in central Newfoundland, Canada (49º35.2' N, 56º13.7' W). 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. We established two study sites, the forest site at low elevation within the study species range (~130 m) and the treeline site at the study species range limits (~240 m). The forest site understory is composed of sparse vascular plant cover (predominately *Cornus canadensis* and *Clintonia borealis*) and is dominated by feathermoss (predominately *Pleurozium schreberi* and lesser so *Hylocomium splendens*). The treeline site is composed of distinct islands of stunted black spruce interspersed in open areas dominated by woody, ericaceaous shrubs (predominately *Kalmia angustifolia* and lesser so *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.

### 2.2.1 *Pre-dispersal seed production and viability*

Maturing black spruce and tamarack cones were harvested at the end of the 2017 growing season (Sept 19-21st). Cones were collected from low and high elevation, in the general proximity of the established forest and treeline study sites. Black spruce cones were also collected at timberline, the uppermost limit of the forest population, defined as the area where trees are still in arborescent form but are less dense than closed canopy forest. At the treeline site, we harvested all the black spruce cones within 10 tree islands (aggregations of stunted and deformed, shrub-form trees; Harsch & Bader, 2011) and harvested on average 48 cones each 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. No cones collected from the forest floor showed signs of red squirrel predation. Low elevation tamarack cones were collected from five individuals growing in an open, wet meadow approximately 75 m away from the forest site. At timberline, we harvested cones from 10 black spruce individuals; tamarack was absent from this location. 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, 1997; and Green, pers. comm.; tamarack protocol modified from Pauley, 1965; Rudolf, 1974). Cones were soaked in deionized water for approximately 24 hours and let dry at room temperature for 24-72 hours. Black spruce cones were then dried at 60°C for 16 hours, while tamarack cones were dried at 50°C for 8 hours. Following drying, cones were tumbled in a sieve shaker for 10 minutes 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 per individual as the number of seeds extracted divided by the number of cones harvested. To determine the viability of extracted seed, we conducted a 28-day 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 hours of light per day (6400 K full-spectrum, T5 lamp with omni-max 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 pre-dispersal 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 (Figure 2.2). When fewer than 25 seeds were extracted, we visually inspected the seeds that failed to germinate in the seed viability germination trial.
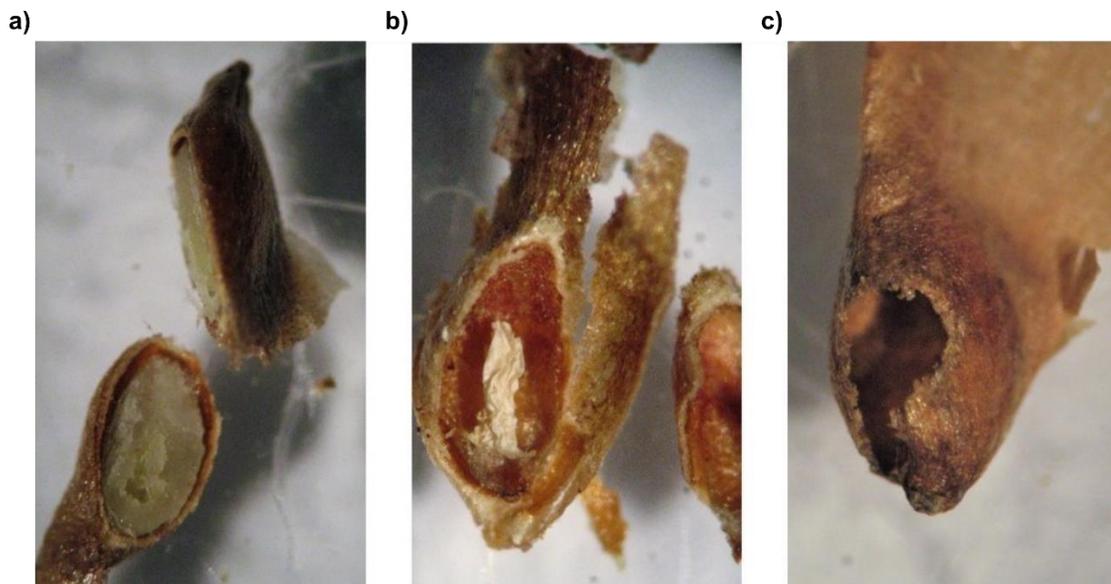


**Figure 2.2** Examples of pre-dispersal damage observed for tamarack seeds: a) no damage, b) aborted embryo, c) pre-dispersal insect predation.

### 2.2.2 *Field germination experiment*

Experimental plots were established in October 2016; seed availability and substrate structure were manipulated following the basic framework of the Global Treeline Range Expansion Experiment (G-TREE; Brown *et al.*, 2013) (2 species, +/0 seed

addition, +/0 substrate scarification) at both forest and treeline sites. Within each site (treeline and forest), plots (0.5 x 0.5 m) were organized into blocks of six, and blocks were systematically placed in areas of similar slope. At the treeline site, where facilitation is predicted to play an important role (Choler *et al*., 2001, Bruno *et al*., 2003), blocks were established in close proximity to and on the leeward side of tree islands, and paired blocks were established in open areas, presumably void of tree island facilitation (Figure 2.3). Each of the treatments was replicated 10 times for a total of 180 plots.
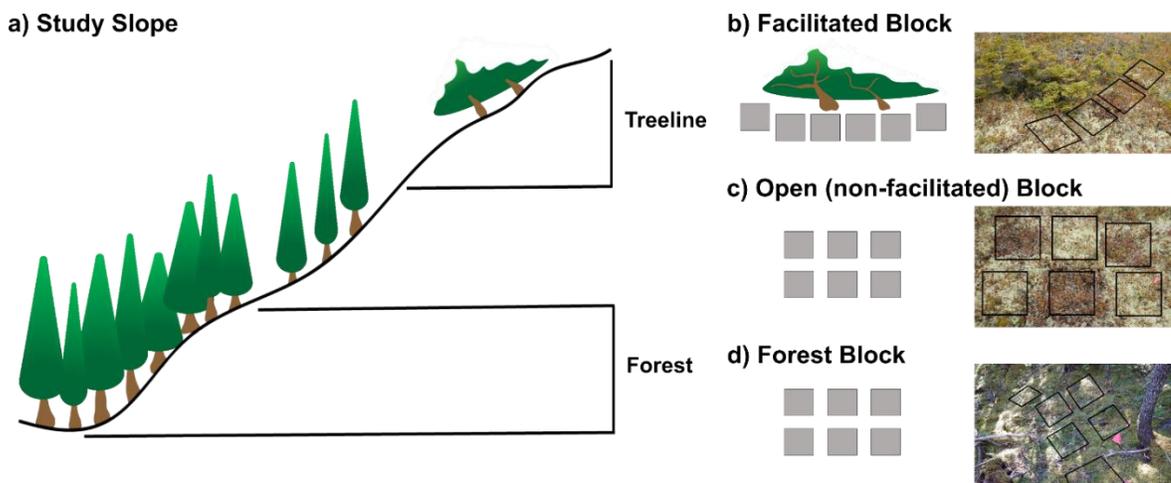


**Figure 2.3** Schematic diagram of the field germination experiment. a) Two sites, forest and treeline, were established along the study slope. Within each site, plots (0.5 m²) were organized into blocks of six, one plot per treatment (2 species, +/0 seed addition, +/0 substrate scarification). At the treeline site, b) facilitated blocks (established on the leeward side of tree islands, presumed facilitators; n=10) were paired with c) open blocks (established in open areas, presumably void of facilitation; n=10) and at the forest sites, d) forest blocks were established (n=10). Black boxes were overlaid on photos of each block type to outline plots visible in each picture.

To test whether seed availability is limiting, we experimentally sowed approximately 250 (0.376±0.0001 g) *P. mariana* seeds or exactly 200 *L. laricina* seeds in plots assigned the seeding treatments. All seeds were obtained from the Department of Fisheries, Forestry and Agrifoods' Wooddale Provincial Tree Nursery, where they were

stored between -8 to -10°C. *Picea mariana* seed (>95% viable) was collected from the

Springdale provenance, approximately 12 km southwest of the study site, in 1988 and *L.*

*laricina* seed (74% viable) was collected from the Wooddale Tree Nursery lot, whose

parent population was composed of individuals from across the island, in 2015. Seed

viability was determined in a 28-day laboratory germination trial following the procedure

described above, where 50 seeds were placed on moist filter paper in each 9 cm Petri

dish.

To assess substrate suitability under field conditions, we scarified half of the

experimental plots using a hand cultivator. This process removed all surface plant litter

and living surface cover of mosses and lichens, while leaving vascular plants intact. To

determine biomass removed, scarified substrate from a subset of plots (n=15 per site) was

dried at 60°C for two days, and weighed.

Plots were surveyed monthly for emergent seedlings from May to September

2017. Due to minimal emergence across the field experiment, with only 12 germinants (5

black spruce and 7 tamarack; all in plots with scarified substrate) observed, 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.

**2.2.3 *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 (+/0 cage, +/0 scarified, 2 species; n=10). Cylindrical cages, where the top was pinched and secured shut (galvanized hardware 0.64 cm mesh cloth, 15 cm diameter x 30 cm height; Figure 2.4), 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; Figure 2.4). 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 (Aug 13th to Sept 20th, 2017). At the end of the experimentation period, the entire contents of each seed cup were collected and seeds were removed from the substrate by hand in the lab. Predation pressure was calculated using the formula (1 – (number of seeds relocated in the laboratory/ 5)) (Côté *et al.*, 2005).
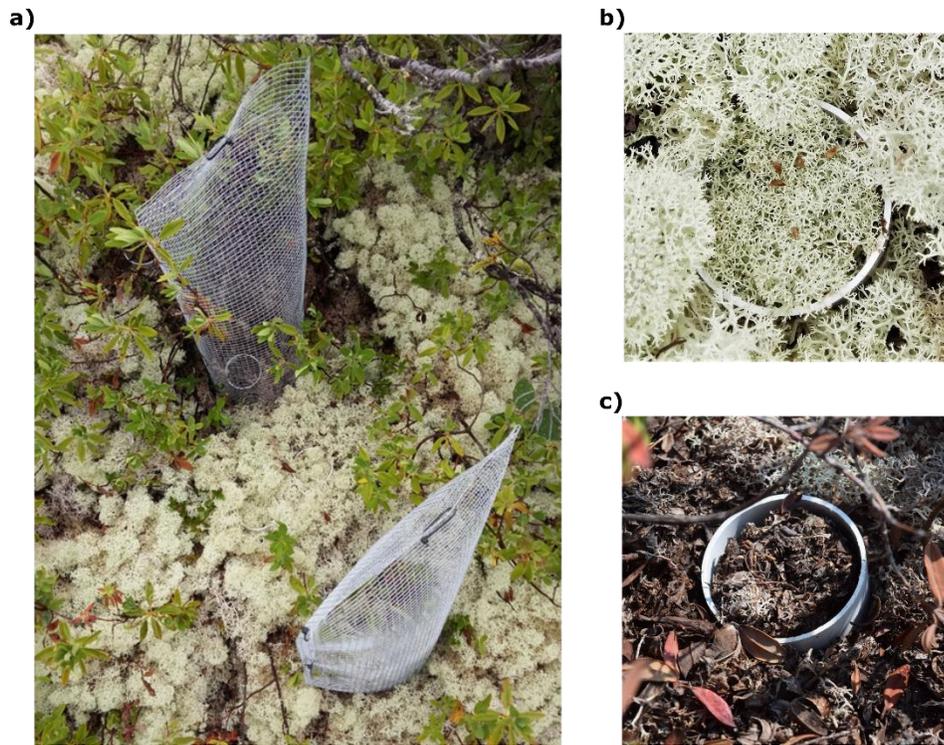
**Figure 2.4** Post-dispersal seed predation experimental design, a) two seed cups, one with black spruce seeds and one with tamarack seeds, were placed inside each seed predator exclosure cage and two seed cups were placed in close proximity to, but outside predator exclosure cages. Seed cups and cages were installed on both b) intact substrate and c) scarified substrate. Treatments were installed at both the forest and treeline site (+/0 cage, +/0 scarified, 2 species; n=10); the treeline site is pictured here.

### 2.2.4 *Substrate suitability laboratory trial*

To further investigate substrate suitability, the original G-TREE seeding experiment was paired with a laboratory germination trial that examined germination ability, establishment, and survival of black spruce and tamarack across substrates collected from the low and high elevation sites of the seeding experiment. A laboratory germination trial allowed us to compare substrate suitability across substrate types while controlling for factors that co-vary along the elevational gradient. Moreover, it allowed for more intensive monitoring of germinant establishment than in the field.

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 x 9.8 x 6.3 cm), modified to have drainage holes, on top of a thin layer of dampened commercial peat (Figure 2.5). Control trays were completely filled with peat (Figure 2.5), which mimics substrate in the scarified plots and is a commonly used substrate in siliviculture. We randomly sowed 50 seeds of either black spruce or tamarack per tray (n=27 per species). Seeds experienced 16 hours of light per day (6400 K full-spectrum T5 lamp with omni-max reflector; Jump Start, Hydrofarm, Petaluma, CA, USA), mimicking sunlight duration at peak growing season, room temperature (~20⁰C), and were sprayed with water every day for the first 28 days. 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 days. 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). Seedlings were considered alive if they had at least one green needle (Renard *et al*., 2016).

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.

**Figure 2.5** The three substrates used in the laboratory substrate germination experiment, from right to left: moss (predominately *P. schreberi*), lichen (predominately *C. stellaris*), and control (commercial peat). Each tray was sown with either 50 black spruce or tamarack seeds (n=27 per species) and following the first germination event, trays were surveyed for germinates every 3 days for 28 days.

### 2.2.5 *Kalmia-biotic community laboratory trial*

*Kalmia angustifolia* 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 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 (Sept 21st) and transported to the

lab. Organic soil samples were homogenized at 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 hours, 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).

Germination capacity of black spruce and tamarack under the leachates was

determined in a 28-day 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; Figure 2.6). 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

hours and weighed.
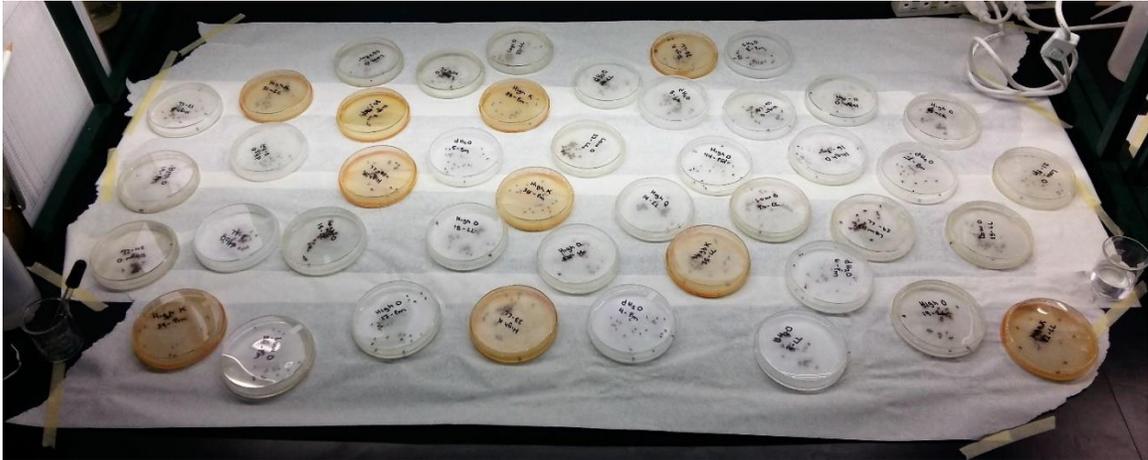
**Figure 2.6** The *Kalmia*-biotic community germination trial. Each petri dish contained either 25 black spruce or tamarack seeds on moistened filter paper, one of the three leachates or deionized water used as a control (n=10 per treatment). Petri dishes were moistened every second day with the assigned treatment. The petri dishes were the same across all treatments; however, the *Kalmia* leaf leachate stained the filter paper yellow and the petri dishes that appear yellow were those that were watered with *Kalmia* leaf leachate.

### 2.2.6 *Statistical analyses*

All statistical analyses were performed using R version 3.3.1 (R Core Team, 2016) via RStudio version 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 (i) black spruce and tamarack seed production (Poisson distribution for count data), (ii) black spruce and tamarack seed viability (binomial distribution), and (iii) black spruce and tamarack pre-dispersal damage (binomial distribution) between elevational sites. Seed viability and pre-dispersal damage models assumed binomial distribution as the response variables are proportional data (e.g., number of seeds germinated divided by the total number of seeds sown; Zuur *et al*., 2009).

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 & 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 (i) time to first black spruce and tamarack germination event (normal distribution) and (ii) 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 (i) time to first black spruce and tamarack germination event (normal distribution) and (ii) 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).

## 2.3 Results

### 2.3.1 *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 (Figure 2.7; Table 2.1).

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 produced at the forest site had greater viability

($73.2 \pm 9.25\%$ viable compared to $64.0 \pm 24.4\%$ viable respectively; Figure 2.7). Across

the elevation gradient, black spruce seed viability decreased with increasing elevation,

where seeds produced at treeline were the least viable ($50.5 \pm 22.8\%$; Figure 2.7). No

evidence of pre-dispersal seed predation was observed and non-viable seeds were 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 ($7.20 \pm 3.44\%$ viable at the forest site and $4.19 \pm 1.34\%$ viable at

treeline; Figure 2.7). The majority of non-viable tamarack seeds had aborted embryos

($83.2 \pm 5.99\%$ aborted at the forest site and $94.7 \pm 2.17\%$ aborted at treeline), although

low levels of pre-dispersal seed predation did occur ($10.7 \pm 3.79\%$ seeds consumed at

forest site and $5.27 \pm 2.17\%$ seeds consumed at treeline; Figure 2.7).

**Table 2.1** 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 | 39.3538 | 3.4984 | 11.249 | **<0.0001** |
| | Timberline | 0.1387 | 4.9476 | 0.028 | 0.9780 |
| | Treeline | -30.7869 | 4.9476 | -6.223 | **<0.0001** |
| *Tamarack* | | | | | |
| | Intercept | 5.8104 | 0.8201 | 7.085 | **<0.0001** |
| | Timberline | - | - | - | - |
| | Treeline | -4.9915 | 1.0044 | -4.97 | **0.00026** |
| **Seed Viability** | | | | | |
| *Black Spruce* | | | | | |
| | Intercept | 1.0048 | 0.1428 | 7.037 | **<0.0001** |
| | Timberline | -0.4294 | 0.1943 | -2.21 | **0.0271** |
| | Treeline | -0.9268 | 0.1942 | -4.771 | **<0.0001** |
| *Tamarack* | | | | | |
| | Intercept | -2.5564 | 0.346 | -7.388 | **<0.0001** |
| | Timberline | - | - | - | - |
| | Treeline | -0.2768 | 0.475 | -0.583 | 0.5600 |
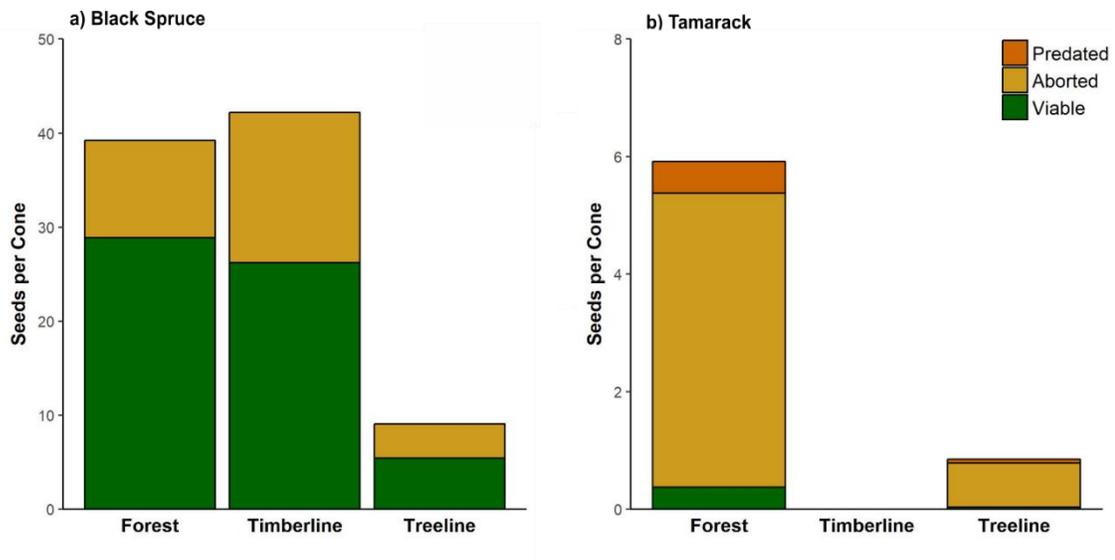
**Figure 2.7** Mean number of seeds produced per cone across sites across the altitudinal range limits of a) black spruce and b) tamarack. No tamarack individuals occurred at timberline. The number of viable seeds per cone was calculated using seed viability determined in the lab germination trial. The 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.

### 2.3.2 *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 (Figure 2.8; Table 2.2). 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

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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, 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 (Figure

2.8; Table 2.2).

**Table 2.2** 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. Experimental sites were located at two elevational sites, the forest site and at treeline. 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 | -3.091 | 0.4555 | -6.785 | **<0.0001** |
| Caged | 4.050 | 0.5755 | 7.037 | **<0.0001** |
| Scarified | 1.144 | 0.4590 | 2.492 | **0.0127** |
| Treeline | 1.036 | 0.4392 | 2.358 | **0.0184** |
| Caged:Scarified | -1.782 | 0.4518 | -3.943 | **<0.0001** |
| Scarified:Treeline | -1.692 | 0.5035 | -3.361 | **0.0008** |
| Caged:Treeline | -0.117 | 0.4810 | -0.243 | 0.8084 |
| *Tamarack* | | | | |
| Intercept | -2.5395 | 0.3719 | -6.829 | **<0.0001** |
| Caged | 4.3667 | 0.5257 | 8.307 | **<0.0001** |
| Scarified | 0.2369 | 0.4049 | 0.585 | 0.559 |
| Treeline | 0.2052 | 0.3777 | 0.543 | 0.587 |
| Caged:Scarified | -0.4205 | 0.4132 | -1.018 | 0.309 |
| Scarified:Treeline | -0.4129 | 0.4359 | -0.947 | 0.344 |
| Caged:Treeline | -0.3007 | 0.4361 | -0.690 | 0.490 |
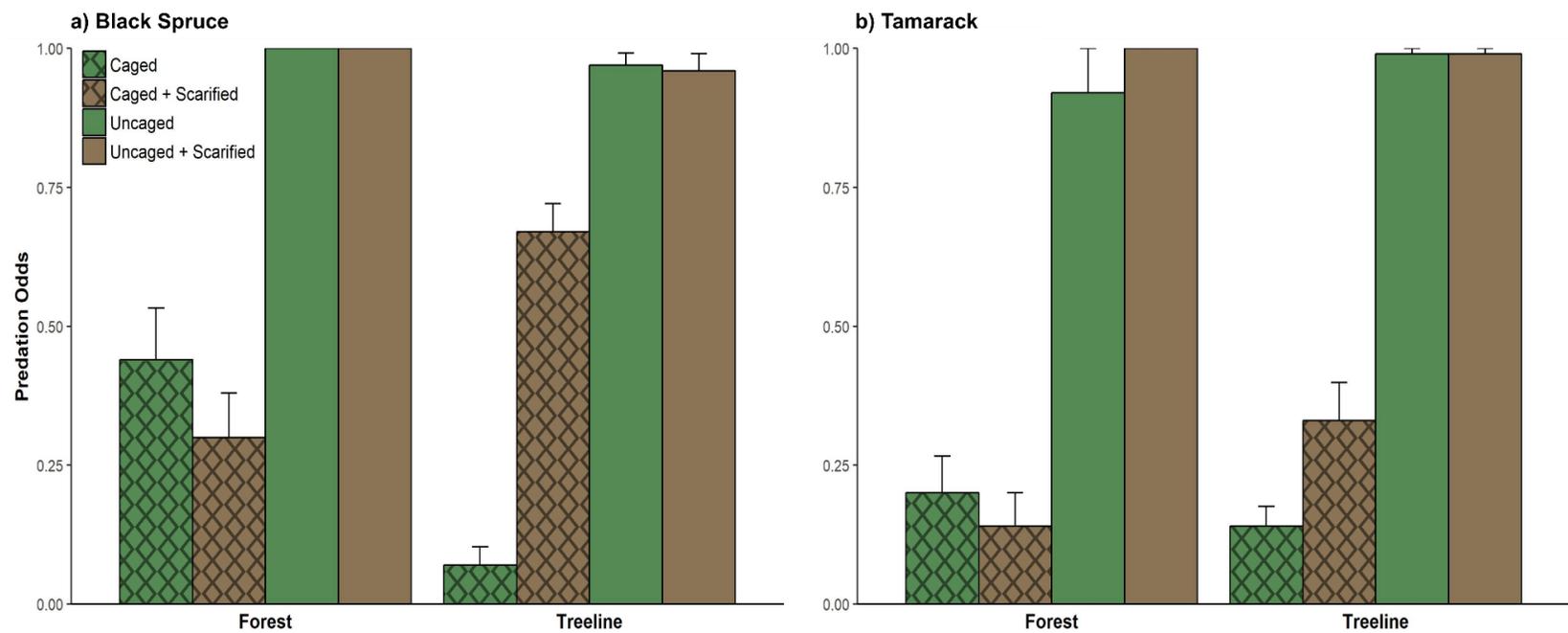
**Figure 2.8** Odds of post-dispersal seed predation (mean ± SE) in cages (cross-hatching) and control plots across substrate treatment and elevation.

### 2.3.3 *Substrate suitability laboratory trial*

Germination on lichen was both delayed and less abundant than on moss or control substrates (Figure 2.9; Table 2.3). 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 to emerge on lichen than on the control substrate. Moss also slowed the time it took for the first germination event for both black spruce and tamarack relative to the control substrate, but had a smaller effect than lichen. Similarly, lichen had the greatest effect on the odds of germination where the proportion of germinated black spruce seeds on lichen was very low; on average only 6.32% ± 3.99% of black spruce seeds germinated and 1.8% ± 1.91% of tamarack seeds germinated (compared to 82.4% ± 9.15% and 52.5% ± 15.8% germinated seeds on control respectively). Moss also significantly reduced black spruce and tamarack germination odds but had less of an effect than lichen, where on average 25.3% ± 10.3% of black seeds germinated and 9.00% ± 9.36% of tamarack seeds germinated.

**Table 2.3** Summary of results from generalized linear models of black spruce and tamarack date of 1$^{st}$ germination event and germination odds from the substrate germination trial (df=26). Moss was collected from the forest site, lichen was collected from the treeline site, and peat was used as a control. Substrates were used as predictor variables and the intercept represents the control substrate. The date of 1$^{st}$ germination models assume normal distribution and the test statistic is a t-value, while the germination odds models assume binomial distribution and the test statistics is the z-value. Values in bold indicate a significance difference ($\alpha \leq 0.05$).

| Parameter | Estimate | SE | Test Statistic | p-Value |
|---|---|---|---|---|
| **Date of 1$^{st}$ Germination** | | | | |
| *Black Spruce* | | | | |
| Intercept | 7.000 | 0.831 | 8.426 | **<0.0001** |
| Moss | 3.000 | 1.175 | 2.553 | **0.0178** |
| Lichen | 10.125 | 1.211 | 8.360 | **<0.0001** |
| *Tamarack* | | | | |
| Intercept | 11.333 | 1.342 | 8.445 | **<0.0001** |
| Moss | 6.167 | 1.956 | 3.152 | **0.0053** |
| Lichen | 10.667 | 2.246 | 4.750 | **0.0001** |
| **Germination Odds** | | | | |
| *Black Spruce* | | | | |
| Intercept | 1.543 | 0.125 | 12.370 | **<0.0001** |
| Moss | -2.627 | 0.166 | -15.840 | **<0.0001** |
| Lichen | -4.239 | 0.232 | -18.300 | **<0.0001** |
| *Tamarack* | | | | |
| Intercept | 0.102 | 0.110 | 0.931 | 0.3520 |
| Moss | -2.415 | 0.221 | -10.945 | **<0.0001** |
| Lichen | -4.100 | 0.426 | -9.618 | **<0.0001** |
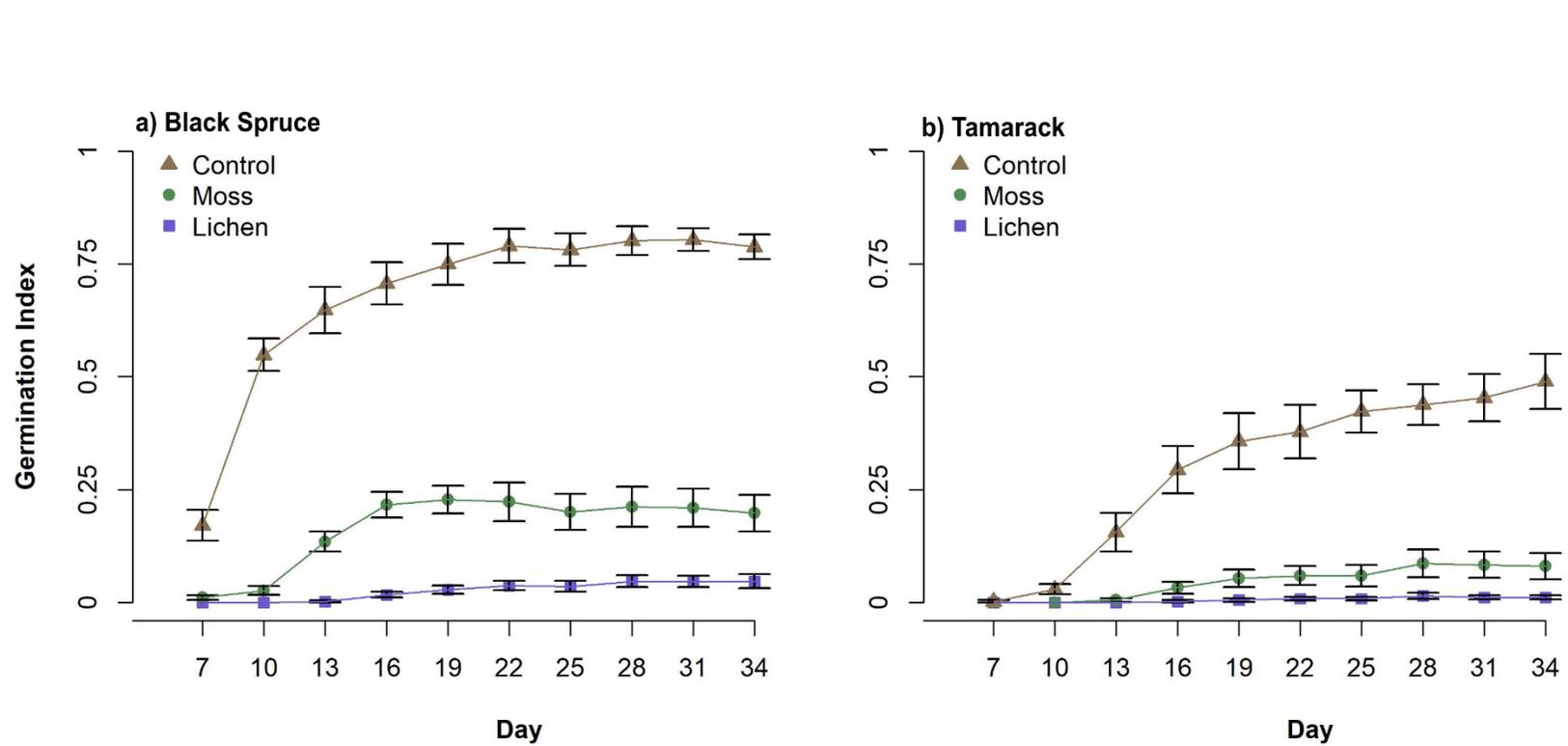
**Figure 2.9** Germination index, calculated as the number of germinates observed divided by the product of the number of seeds sown and seed viability, (mean ± SE) of a) black spruce and b) tamarack across the three substrate types (control- commercial peat, feathermoss, and caribou lichen) over the duration of the laboratory substrate germination trial.

### 2.3.4 *Kalmia-biotic community laboratory trial*

Leachate treatments had minimal to no detectable effects on the timing and odds of black spruce and tamarack germination (Table 2.4). 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. The *Kalmia* leachate significantly delayed the date of the first tamarack germination event, although the magnitude of this effect was minimal (delay = 1 day). The organic treeline soil leachate had a marginally significant effect on date of first tamarack germination event, but as residuals deviated slightly this effect is likely not statistically meaningful. The organic forest soil leachate had a significant, positive effect on tamarack germination odds, 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.

**Table 2.4** Summary of results from generalized linear models of black spruce and tamarack date of $1^{st}$ germination event and germination odds from the leachate germination trial (df=40). Leachates were made using organic soil collected from the forest and treeline sites, fresh *Kalmia* leaves from the treeline site, and deionized water was used as a control. Leachates were used as predictor variables and the intercept represents the deionized water. The date of $1^{st}$ germination model assume normal distribution and the test statistic is a t-value, while the germination odds models assume binomial distribution and the test statistics is the z-value. The date of $1^{st}$ germination model failed to meet model assumptions and should be interpreted with caution. Values in bold indicate a significance difference ($\alpha \leq 0.05$).

| Parameter | Estimate | SE | Test Statistic | p-Value |
|---|---|---|---|---|
| **Date of $1^{st}$ Germination** | | | | |
| *Tamarack* | | | | |
| Intercept | 7.000 | 0.2357 | 29.700 | **<0.0001** |
| Organic soil$_F$ | 0.600 | 0.3333 | 1.800 | 0.0803 |
| Organic soil$_T$ | 0.800 | 0.3333 | 2.400 | **0.0217** |
| *Kalmia* leaves$_T$ | 1.000 | 0.3333 | 3.000 | **0.0049** |
| **Germination Odds** | | | | |
| *Black Spruce* | | | | |
| Intercept | 4.119 | 0.5040 | 8.172 | **<0.0001** |
| Organic soil$_F$ | 0.701 | 0.8707 | 0.805 | 0.4210 |
| Organic soil$_T$ | 1.398 | 1.1216 | 1.247 | 0.2120 |
| *Kalmia* leaves$_T$ | 0.701 | 0.8707 | 0.805 | 0.4210 |
| *Tamarack* | | | | |
| Intercept | 0.925 | 0.1403 | 6.593 | **<0.0001** |
| Organic soil$_F$ | 0.592 | 0.2163 | 2.736 | **0.0062** |
| Organic soil$_T$ | 0.020 | 0.1988 | 0.099 | 0.9208 |
| *Kalmia* leaves$_T$ | 0.250 | 0.2046 | 1.222 | 0.2217 |

## 2.4 Discussion

Recruitment of black spruce and tamarack at alpine treeline is greatly constrained by biotic filters occurring across multiple life stages, as seeds transition from parent plants to established seedlings (Figure 2.10). Here, we provide empirical evidence that both tree species produced few seeds per cone at treeline and that those seeds produced were less viable, suggesting that recruitment at treeline is primarily seed limited. If such severe seed limitations were overcome, we have shown that subsequent establishment

limitations strongly inhibit recruitment. First, seeds experience intense predation pressure, as we found that effectively all seeds were consumed by post-dispersal seed predators. Surviving seeds must then germinate and persist on the substrate they have dispersed to, yet we have empirically shown that the odds are against them. Lichen, the substrate on which more seeds escaped predation at the treeline population, was also the substrate on which germination was both delayed and less abundant.

## Black Spruce

### a) Forest



### b) Treeline
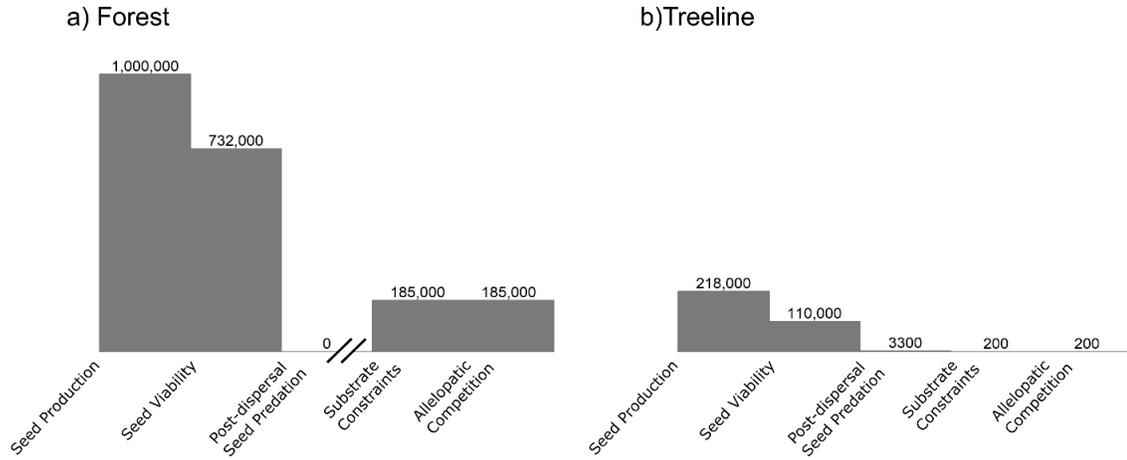


## Tamarack
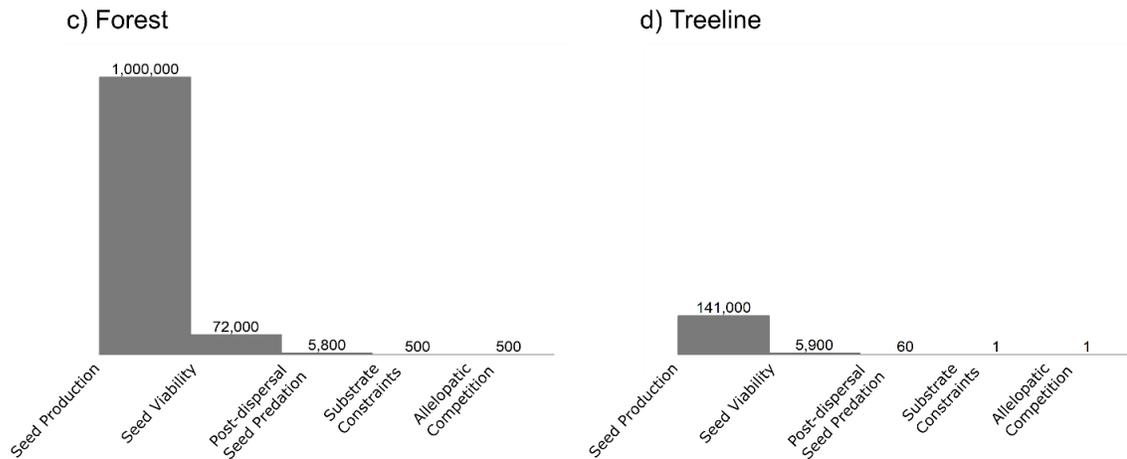
### c) Forest



### d) Treeline



**Figure 2.10** Flow diagram illustrating the proportion of individuals lost for every 1,000,000 seeds produced by the forest population at each measured biotic filter for black spruce a) forest population and b) treeline population and tamarack c) forest population and d) treeline population. Number of seeds produced at treeline compared to the forest represents the proportion of seeds produced by the treeline populations compared to the forest populations, as determined by the observational seed production study. 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.

**2.4.1 *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., 2018  but see Kroiss & Hille Ris Lambers, 2015). We found that production of viable black spruce and tamarack seed was lower at treeline; meaning, recruitment at range limits is currently seed limited, similar to other coniferous range edge populations (e.g., Sirois, 2000; Meunier *et al.*, 2007; Viglas *et al.*, 2013; Jameson *et al.*, 2015; Brown *et al.*, 2018). 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 of lower stand densities at treeline 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 & Hillrislambers, 2015).

As expected, black spruce seed produced by the treeline population was of lower viability 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 & 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 & 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 (~290m) were well within the pollen dispersal distance (250-3000m; 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 & Gagnon, 1979; Viktora *et al.*, 2011) and with this increasing genetic interrelatedness of individuals the likelihood of self-fertilization (Mimura & Aitken, 2007). However, 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 & 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.

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However, in contrast to findings along another eastern North American alpine treeline (Jameson *et al.*, 2015), a small proportion of non-viable tamarack seeds were caused by pre-dispersal seed predation with the most non-viable seeds containing aborted embryos. Low seed viability is common among *Larix spp.* and is driven by a high rate of embryo abortion (Farmer & 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 with stand density, therefore self-fertilization decreases with stand density (Knowles *et al.*, 1987), and the density of tamarack individuals is low across the altitudinal gradient.

Our results suggest that recruitment at treeline is currently seed limited; however, 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 & Danby, 2017), our black spruce timberline is within the effective dispersal zone of spruce seeds (79 m as estimated by Johnston & Smith, 1983; >300m 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

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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.

Moreover, black spruce and tamarack undergo can mast events, years of increased seed

production, (Johnston, 1990; Sirois, 2000), and seed limitations at treeline might be

overcome during mast years.

### 2.4.2 *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 that results in decreased

invertebrate black spruce seed predation 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 treeline highlights how

multiple factors need to align temporally for geographical range shifts to occur (Kroiss *et

al.*, 2015; Kambo & Danby, 2017). 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 & Danby, 2017).

Our post-dispersal seed predation experiment results highlight that boreal conifer recruitment, across the 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 over the winter 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 & Sork, 2002). Both black spruce and tamarack experience masting (Johnston, 1990; Sirois, 2000) and we argue that masting is 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). To the best of our knowledge, only one other study has investigated post-dispersal seed predation by invertebrates in North America's boreal forest; in this study Côté *et al.* (2005) attributed seed consumption to generalist boreal ants (*Myrmica* spp. and *Formica* spp.) and the ground beetle, *Pterostichus adstrictus*. 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), 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 (e.g., LePage *et al.*, 2000; Charron & Greene, 2002; Dufour Tremblay & Boudreau, 2011; Wheeler *et al.*, 2011; Brown & 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 & Greene, 2002; Dufour Tremblay & 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 & 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 & 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.

Establishment was much greater on our control peat substrate, suggesting that recruitment at treeline may be dependent on disturbance that exposes bare soil, decreasing establishment limitations. Bare soil has long been recognized as the ideal substrate for black spruce and tamarack germination and establishment (Viereck & Johnston, 1990; 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 & Boudreau, 2011) have found increased emergence on disturbed substrate compared to intact alpine substrate. Disturbances can occur across variety of scales from cryoturbation (Sullivan & Sveinbjörnsson, 2010), to caribou trampling (Dufour Tremblay & Boudreau, 2011) and bear digs (Wheeler *et al.*, 2011), to fires (Brown & 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.* (2012) 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.,* 2012). While seedling establishment associated with the field germination experiment was extremely low, all seedlings that were observed occurred in scarified plots, we speculate that facilitative effects in the field may 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 (unpublished data) 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.*, 2012b). *Empetrum nigrum* and several *Vaccinium* species occur at our treeline population although the lack of response to the high soil leachate suggests that they occur at low enough abundance that their negative impacts are negligible. However, 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 (e.g., 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 competitive exclusion by *Kalmia* does not occur at the seedling emergence 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 & Mallik, 2006).

### 2.4.3 *Conclusions*

We demonstrate, through a series of observational and experimental studies, that recruitment at treeline, and thus changes in geographical position of treeline, is constrained by a series of biotic filters that likely will override any positive effects associated with the alleviation of climatic constraints. Recruitment is firstly seed limited, where production of black spruce and tamarack seed was lower at treeline compared to the forest population. Black spruce seed viability decreased with increasing elevation and recruitment at treeline is likely dependent on dispersal of viable seed from individuals at lower elevations. Tamarack was more seed limited with very few viable seeds being produced, regardless of altitudinal position. 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. Recruitment both within range and at treeline are 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, recruitment at treeline will likely not occur because lichen substrate poses as a significant barrier to germination and seedling establishment. While bare ground greatly increased

germination and seedling establishment, suggesting that a pulse of recruitment will follow a disturbance event, any seed that disperses to bare ground will likely be eaten before it can establish. The mismatch between conditions that promote germination and seedling establishment and decrease invertebrate post-dispersal seed predation at treeline presents an interesting conflict, where disturbance that removes the lichen mat reduces establishment limitations while simultaneously increasing seed limitations. Despite the high abundance of ericaceous shrubs at treeline, we detected no allelopathic effects on black spruce and tamarack initial seedling emergence. Competitive exclusion through allelopathy may occur at later life stages than assessed here, and future research should examine whether *Kalmia* heath at treeline negatively affects seedling establishment, growth, and survival. We conclude that recruitment at alpine treeline in central Newfoundland is simultaneously seed and establishment limited and that changes to geographic treeline position are dependent on the temporal alignment of multiple factors. Our study compliments 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 global treeline response to a warming climate.

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**Chapter 3: Can established individuals at treeline facilitate seedling survival?**

**Abstract**

Facilitation, the interaction of one species altering the environment in a way that enhances the fitness of a neighbouring species, has long been considered to be an important factor at range limits. Due to their small stature, seedlings can experience microsite conditions and facilitation by established conifers at treeline, and this has been identified as a potential route for range expansion of boreal trees. To quantify how microclimatic conditions change with distance away from established conifers and how microclimate and established conifers interact to promote seedling survival, we transplanted black spruce and tamarack seedlings along transects leading away from black spruce tree islands and tamarack individuals, both presumed facilitators, and in open areas. Island treeline advance is equally dependent on increased recruitment and decreased dieback; we paired the transplant experiment with an observational study to quantify black spruce tree island growth constraints. Despite changes in microclimatic conditions with distance away from presumed facilitators and between transect types, there were no trends in black spruce and tamarack seedling survival. Seedling survival was high across summer and winter (>85%) indicating this life stage is not a bottleneck to recruitment. The symmetrical pattern in height across black spruce tree islands suggests that dieback is not limiting and tree islands are expanding.

**Keywords**: altitudinal treeline, range expansion, biotic interactions, tree islands, facilitation, seedling transplants, *Picea mariana*, *Larix laricina*

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**3.1 Introduction**

Evidence is mounting that species are not consistently responding to climate change at the rate or in the direction predicted by climate factors alone (Hille Ris Lambers *et al.*, 2013). Treeline, the ecotone characterized by the transition between forest and alpine or arctic ecosystems, is perceived as the thermal limit to tree survival, growth, and reproduction and therefore is expected to shift upslope and poleward in response to atmospheric warming. However, due to the small-stature of seedlings, it is not atmospheric conditions but microsite conditions, controlled in part by surrounding vegetation and topography, that are critical drivers of seedling survival and thus a species' distribution (Resler, 2006; Körner, 2016; Renard *et al.*, 2016). The discrepancy between the alleviation of global-scale thermal constraints and small-scale microsite conditions may explain why there has been no ubiquitous trend in treeline response to atmospheric warming to date (Harsch *et al.*, 2009).

Treeline response to climate change is dependent on the treeline form, i.e., the spatial pattern of established individuals at treeline (Harsch *et al.*, 2009; Harsch & Bader, 2011). Island treelines consist of patches of trees or krummholz, which are deformed, multi-stemmed trees, occurring beyond the continuous forest limit and interspersed in a matrix of alpine or tundra vegetation (as defined by Harsch & Bader, 2011). Krummholz are a common response to exposed environments and have been reported extensively at alpine and arctic treelines, particularly at sites described as wind exposed (Harsch & Bader, 2011). The formation and persistence of tree islands has long been attributed to positive feedback driven by facilitative interactions that modulate the negative effects of

exposure (e.g., Marr, 1977; Holtmeier, 1982), where dieback is greater on the windward side of tree islands and growth and recruitment is greater on the leeward side (Bekker *et al.*, 2009; Harsch & Bader, 2011; Renard *et al.*, 2016).

Facilitation is defined as an interaction where an established individual ameliorates the environment in a way that increases the fitness of a neighbouring individual (Bronstein, 2009). For plants, facilitative and competitive interactions are inexorably linked as all plants require the same basic resources (Brooker & Callaghan, 1998). The balance between facilitative and inhibitive effects are often context dependent and conditional (Bronstein, 2009). For example, the relative strength of facilitation and competition is predicted to vary inversely along environmental stress gradients, with positive interactions typically outweighing negative interactions in harsh environments, such as alpine ecosystems (Callaway, 2007). Despite findings that support the role facilitation plays in forming stable plant communities, little research has focused on the role facilitation has on species' responses to global change impacts (reviewed by Brooker, 2006), which is surprising given that facilitative interactions have been found to increase the occurrence of individuals at their species' range limits and has been proposed as a mechanism for range expansion (Choler *et al.*, 2001; Germino *et al.*, 2002; Bruno *et al.*, 2003).

Conifer seedlings at treeline have been found to have non-random spatial association, suggesting that amelioration of the environment at microscales will increase seedling survival (Resler *et al.*, 2005; Malanson *et al.*, 2007; Kambo & Danby, 2018). There is evidence that the occurrence of conifer seedlings at treeline is positively affected

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by: tree islands (Alftine & Malanson, 2004; Renard *et al.*, 2016), individual krummholz (Batllori *et al.*, 2009), topographic features (Resler *et al.*, 2005; Resler, 2006), herb cover (Germino *et al.*, 2002; Maher & Germino, 2006), and moss (Wheeler *et al.*, 2011). These suitable microsites, or 'safe sites', that facilitate recruitment are often attributed to taller neighbours that ameliorate the harsh environment by reducing the effects of multiple stressors, in particular creating shelter from damaging winds and radiative extremes (reviewed by Holtmeier, 2009). Exposure to high winds and radiative extremes have cascading effects, altering soil temperature and moisture, snow regime, and soil nutrient availability, potentially resulting in seedling desiccation, freezing damage, and photodamage (reviewed by Holtmeier, 2009). The compounding effects of multiple abiotic stressors support the prediction that the upslope treeline expansion will depend on the availability of 'safe sites' (Holtmeier, 2009; Cranston & Hermanutz, 2013).

Feedback between individual plants and their environment at micro-scales can drive patterns in vegetation composition at larger scales (Alftine & Malanson, 2004). There is a critical need to better understand how microsite modifications by established trees, or groups of trees, will affect treeline response to a warming world (Harsch & Bader, 2011). The mechanisms of how established trees, or tree islands, modify the environment will likely be structure- and species- dependent (Holtmeier, 2009; Holtmeier & Broll, 2010). To quantify how established conifers feedback to affect seedling survival at treeline, we conducted a seedling transplant experiment where black spruce (*Picea mariana* [Mill.] B.S.P.) and tamarack (*Larix laricina* [Du Roi] K. Koch) seedlings were transplanted along transects leading away from the leeward side of presumed facilitators

(i.e., black spruce tree islands and tamarack) and in open areas, presumably void of facilitation. We predicted that microsite conditions would change with distance from established conifers, and that microsite conditions experienced on the leeward side of presumed facilitators would positively feedback to promote transplant seedling survival. We further examined if tree island height varied with distance from windward side, working under the hypotheses that if height increases with distance from windward side, then tree islands' growth is constrained by wind exposure. If positive effects of seedling survival are accompanied by equal negative dieback, tree islands, and thus treeline, will remain static (Harsch & Bader, 2011).

## 3.2 Methods

Our study was conducted along a south-west facing slope on the Baie Verte Peninsula in central Newfoundland, Canada (49⁰35.2' N, 56⁰13.7' W). The climate in the area is boreal with a strong maritime influence, experiencing a mean annual air temperature of 3°C and on average 1200 mm of annual precipitation, of which about 350 cm accumulates as snow (2007-2017; Middle Arm weather station, Environment Canada). With increasing elevation, the study slope's vegetation transitions from a closed-canopy black spruce dominated forest to alpine treeline, an ericaceous heath with scattered tamarack and isolated islands of krummholz black spruce. At treeline, all black spruce individuals occurred as krummholz islands, while all tamarack individuals occurred as erect, upright individuals; herein, black spruce will be referred to as tree islands and tamarack as individuals. For a comprehensive study site description, see Chapter 1.

### 3.2.1 *Experimental design*

To assess the potential role of tree islands as facilitators at the treeline site, we systematically established short (~2 m) transects extending downslope on the leeward side of established conifers (black spruce tree islands and individual tamarack; presumed facilitators) and in open patches (presumably void of facilitation). Predominant wind direction was determined by observing tree growth and flagging. To examine how survival varies with distance from established individuals, we transplanted one black spruce and one tamarack seedling at five positions along each transect: i) base of established individual (within tree island), ii) under tree island canopy, iii) canopy edge, iv) transition to treeless patch, and v) treeless patch (Figure 3.1). Transects extended from both established black spruce (n=10) and tamaracks (n=5) to examine inter- and intraspecific effects. We established 120 cm transects in open patches (n=10) parallel to the slope and transplanted one black spruce and one tamarack at five equally spaced positions, mimicking the tree island transects. Distance to the nearest upslope facilitator was measured for each open transect. To contextualize seedling survival at range edges compared to survival within the current range, the procedure for establishing open patch transects was replicated at the forest site (n=10).
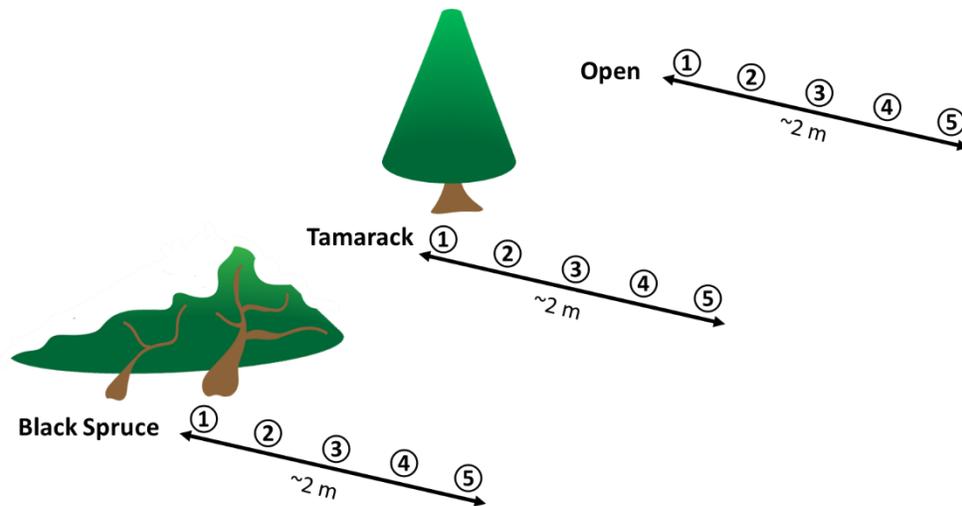
**Figure 3.1** Schematic diagram seedling transplant experimental design, illustrating one block. Approximately 2 m long transects extended downslope, on the leeward side of black spruce tree islands (n=10) and tamarack individuals (n=5), as well as in open areas presumably void of facilitation (n=10). One black spruce and one tamarack seedling was transplanted at 5 positions along the facilitated transects: ① at the base of individuals, ② under the conifer canopy, ③ canopy edge, ④ transition to treeless patch, and ⑤ in open, treeless patch; and every 30 cm along open transects.

Seedlings for the field experiment were grown in the laboratory from seed acquired from black spruce and tamarack populations in central Newfoundland (see Chapter 2 for seed source details). Seedlings were propagated in celled forestry trays (67 3.5 cm diameter by 9 cm deep cells per tray) in potting soil, experiencing 16 hours of light per day (6400 K full-spectrum T5 lamp with omni-max reflector; Jump Start, Hydrofarm, Petaluma, CA, USA) at room temperature (~20°C) for 127 days. In the week prior to transplanting, seedlings were exposed to ambient temperature and solar radiation outside of the laboratory in an effort to reduce transplantation stress. To control for differences between individuals, we measured seedling height and number of buds before transplanting (Renard *et al.,* 2015). At the beginning of the 2017 growing season (June

13th and 16th, 2017), the four month old seedlings were transplanted to the experimental sites. At the treeline site, where there is a thick *Cladonia* sp. mat, we planted seedlings flush with the soil underneath the lichen mat, to mimic where establishment would likely occur from naturally dispersed seed (Asplund & Wardle, 2016; Deines *et al*., 2007). It is important to note that the effect of transplant stress on seedling performance is confounded with the effect of microsite conditions on seedling performance. To further minimize transplant stress, we watered all transplants on June 16th, 2017 (Grau *et al*., 2012; Castanha *et al*., 2013). Seedlings were surveyed monthly from July to September 2017 and again in June 2018. During each survey, we measured seedling survival (alive/dead) and qualitatively ranked the health of living seedlings as good, fair, or poor (Figure 3.2). Seedlings were considered alive if they had at least one green needle (Renard *et al*., 2015).
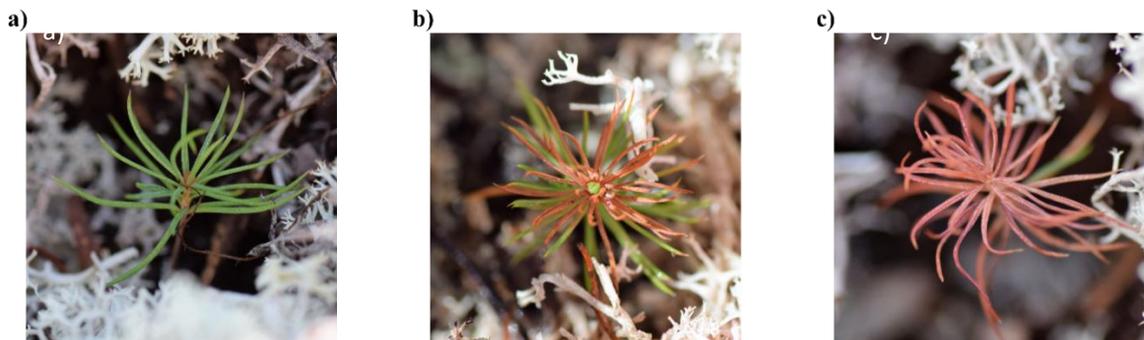


**Figure 3.2** Black spruce seedlings qualitatively ranked as: a) good, b) fair, and c) poor (note the single green needle).

**3.2.2 *Biotic measurements***

To quantify the biotic environment experienced by seedlings, we visually estimated the percent cover of all vascular plants, bryophytes, and lichens (to species), and litter in a 25 x 25 cm quadrat surrounding each transplanted seedling. Total and

species-specific canopy cover were estimated using a spherical densitometer. All percent cover estimations were rounded to the nearest 5%.

To quantify the structure (height and area) of the black spruce tree islands, we measured the dimensions of the 10 black spruce tree islands that were associated with seedling transplant transects, as well as an additional 7 tree islands (n=17). Area of the tree islands was approximated using the formula for the area of an ellipse ($A = \pi ab$; where $a$ is the length of the tree circle perpendicular to the slope and $b$ is the length parallel to the slope; Albertsen *et al.*, 2014). Height of the tallest vertical stem was measured every metre along the length of tree island parallel to the slope. Only black spruce tree islands were analyzed, as tamarack did not form clonal clusters in our study area.

### 3.2.3 *Abiotic, microclimatic measurements*

To assess soil temperature experienced by seedlings, we buried soil temperature data loggers (Maxim Integrated, iButton Thermochron, DS1921G-F5#) along a subset of transects at the treeline site (n=5 open transects, n=5 black spruce transects, and n=2 tamarack transects). Temperature was recorded every 4 hours for the duration of the experiment (June 13th, 2017 to June 4th, 2018). As protection from temperature extremes is a commonly ascribed benefit facilitated plant-plant interactions (Brooker, 2006), we calculated the average minimum and maximum soil temperatures for each season (summer: June 13th – Sept 21st, 2017; winter: Sept 22nd, 2017 to June 4th, 2018) by taking the mean of the daily minimum and maximum temperatures recorded.

Soil moisture measurements were taken monthly over the course of the growing

season at each of the five positions along the experimental transects. Soil moisture was

measured at 5 cm depth using a water moisture sensor (volumetric soil water content;

Procheck, Decagon Devices, Pullman, WA, USA), except for the July survey where soil

cores collected from ~5 cm were brought back to the lab for moisture assessment. We

dried 5 g of wet soil for 48 hours and 60°C and July soil moisture was calculated by

dividing dry weight by wet weight. We measured soil pH in the laboratory by combining

5 g of soil with 10 mL of deionized water, stirred, and allowed to come to equilibrium for

30 minutes prior to measuring pH with pH tracer pocketester (LaMotte Company,

Chestertown, MD, USA).

We measured average surface light intensity at each position along each transect

over a 15 second period. At the treeline site, light intensity measurements were taken with

a quantum sensor (Li-Cor Inc., Lincoln, NE, USA) at i) the lichen surface level,

underneath any upright vegetation, and ii) approximately 1 m, above any vegetation. At

the forest site where we could not measure above vegetation light intensity, we took three

light intensity measurements in a canopy gap. Measurements were taken on clear days

(July 14th and 16th, 2017), effectively void of cloud cover, over a 4 hour (treeline site) and

2 hour (forest site) time period encompassing solar noon. To increase the generality of the

light measurements, we used the unobstructed light intensity measurements, taken above

any vegetation, to standardize the surface light intensity measurements. Light availability,

a standardized light metric, was calculated as one minus the difference between average

above vegetation light intensity and average surface light intensity divided by the above

vegetation light intensity. We assume that light availability remains constant over varying light conditions experienced over the summer growing season.

Wind speed measurements (EA-3010 handheld anemometer; La Crosse Technology, La Crescent, MN, USA) were taken at each position along the transects during peak growing season (July 14th-15th, 2017). To account for high variability between wind speed measurements, we simultaneously measured wind speed at substrate surface level and directly above at 2 m and calculated wind interception as the difference between wind speed at 2 m and wind speed at surface level, divided by wind speed at 2 m. As for light availability, we assumed that wind interception, a standardized wind metric, remains constant across varying wind conditions.

To capture the pulse of soil nutrients available to alpine plants following spring thaw (Edwards *et al.*, 2007; Zinger *et al.*, 2011), we deployed separate cation and anion exchange membranes (Plant Root Simulator (PRS®) probes; Western Ag Innovations Inc., Saskatoon, SK, Canada) along a subset of transects at the treeline site (n=5 open transects, n=5 black spruce transects, and n=2 tamarack transects). Probes were buried at three positions along each transect: the beginning, middle, and end. To account for soil heterogeneity (Johnson *et al.*, 2010; Johnson *et al.*, 2011; Das Gupta *et al.*, 2015), three probes of each type were used at each sampling location and averaged for data analysis. Probes were left *in situ* over the winter (Oct 22nd, 2017 to June 4th, 2018; burial length=255 days) and were collected the following spring. Western Ag Innovations Inc. conducted nutrient supply rate analyses ($NO_3^-$, $NH_4^+$, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd).

**3.2.4** *Statistical analysis*

All statistical analyses were performed using R version 3.3.1 (R Core Team, 2016) via RStudio version 1.1.149 (RStudio Team, 2016). To explore biotic trends at the treeline site, we performed nonmetric multi-dimensional scaling (nMDS) with a Bray-Curtis dissimilarity index using the treeline site plant community abundance data and the 'vegan' package version 2.4-4 (Oksanen *et al*., 2013). We ran the analysis using one to six dimensions and selected a three-dimensional model as it minimized both stress and dimensions (McCune and Grace, 2002). We performed two interpretative aids on the nMDS: 1) to interpret whether the biotic communities differed among transect treatment and along transects, we drew convex hulls that enclosed all sites within each transect treatment (black spruce, tamarack, or open) and fit distance to established individual to the ordination, and 2) to aid in the visualization of seedling survival at the treeline site, we coloured each site to correspond with the qualitative seedling health ranking made at the end of the growing season (September 2017 survey) and at the end of the winter (June 2018 survey).

To examine how microclimatic conditions vary with distance from facilitator and between facilitator types, we ran linear mixed models predicting variation within biologically important abiotic factors as a function of distance (*Dist*), facilitator identity (*Fa*), and their interaction (Equation 1; using the 'lm4' package; Bates *et al*., 2015). To account for spatial dependence along transects, transect (*T*), nested within block (*Blk*), was included as a random factor (Keitt *et al.*, 2002; Equation 1).  We ran residual diagnostics to test all models met assumptions.

$$Abiotic\ Factor \sim Dist\ + Fa + Dist * Fa + (1|Blk{:}T) \qquad [1]$$

Analysis of covariance (ANCOVA), with type III sums of squares, was then used to partition variation in each biologically important abiotic factor between distance (*Dist*, variation explained by the distance away from presumed facilitators) and transect type (*Fa*, variation explained by the identity of facilitator, or unfacilitated). The interactive effect (*Dist\*Fa*) explored whether the effect of distance from facilitator on the modeled abiotic factors was dependent on the identity of the facilitator.

If tree island growth is constrained by wind exposure, we expect the vertical height of tree islands to increase with distance from the windward edge (Albertsen *et al.*, 2014). To determine if black spruce tree islands at treeline are expanding, we ran a linear mixed model predicting variation in vertical height (*Height)* as a function of distance from the leeward side (*Distance*; Equation 2; using the 'lm4' package; Bates *et al*., 2015). To account for potential sheltering effects larger tree islands may have, area (*Area)* was included as a random factor (Equation 2). We ran residual diagnostics to test if the model met assumptions.

$$Height \sim Distance + (1|Area) \qquad [2]$$

### 3.3 Results

The three dimensional ordination converged after 100 iterations and had a final stress of 15.18 (stress <20 is considered acceptable; McCune and Grace, 2002). Axis 1 separated treeless patches (high *Kalmia angusitfolia* and *Cladonia* lichen cover) from areas with established conifers (i.e., high conifer cover; Figure 3.3), while axis 2

separated black spruce tree islands from established tamarack individuals (Figure 3.3). It

appears that the biotic environments converge at approximately two meters away from

the base of established individuals at treeline (note overlapping polygons in the centre of

Figure 3.3).
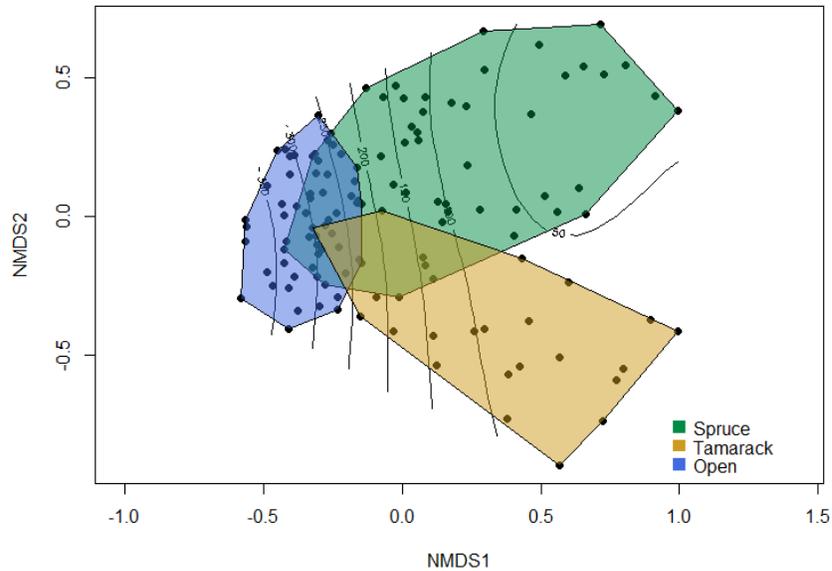


**Figure 3.3** Non-metric multidimensional scaling ordination (k=3, stress= 15.18) of
treeline site plant community composition and abundance grouped by seedling transplant
treatment. Distance (in cm) from established individuals is overlaid on the ordination.
Each point represents the 25x25cm quadrat surrounding each transplanted seedlings,
points closer together have more similar plant species community composition.

Transplant survival at treeline was high across both seasons. Over the course of

the experiment, 95.0% of black spruce seedlings and 92.7% of tamarack seedlings

survived (Figure 3.4). Summer survival was lower than winter survival at treeline for

both species (93.3% and 96.7%, respectively, for black spruce; 86.7% and 98.7%,

respectively, for tamarack; Table 3.1). There were no spatial trends in black spruce or

tamarack seedlings mortality across ordination space, suggesting that neither facilitator

type nor distance from facilitator affected survival (Figure 3.4). Black spruce seedling

health was generally assessed as good and there are no trends in the spatial distribution of

health classifications across ordination space (Figure 3.4a,b). Tamarack seedling health

classifications were more variable than black spruce, with more individuals classified as

fair or poor health (Figure 3.4). Tamarack seedlings ranked in good health occurred

across the ordination; however, there was a cluster of healthy seedlings associated with

high black spruce cover (Figure 3.4c,d).

**Table 3.1** The probability of survival of black spruce and tamarack seedling transplants at treeline over the two seasons, summer and winter, observed across three transect types and along the five transplant positions (n=150).

| *Black Spruce* | | | | | | |
|---|---|---|---|---|---|---|
| | **Summer** | | | **Winter** | | |
| | Open | Spruce | Tamarack | Open | Spruce | Tamarack |
| 1 | 1.0 | 1.0 | 0.8 | 1.0 | 0.8 | 1.0 |
| 2 | 0.8 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 3 | 0.9 | 0.9 | 1.0 | 1.0 | 1.0 | 1.0 |
| 4 | 1.0 | 0.8 | 1.0 | 0.9 | 1.0 | 0.8 |
| 5 | 1.0 | 1.0 | 0.8 | 1.0 | 1.0 | 1.0 |
| *Tamarack* | | | | | | |
| | **Summer** | | | **Winter** | | |
| | Open | Spruce | Tamarack | Open | Spruce | Tamarack |
| 1 | 1.0 | 1.0 | 0.8 | 1.0 | 1.0 | 1.0 |
| 2 | 1.0 | 1.0 | 0.2 | 0.9 | 1.0 | 1.0 |
| 3 | 1.0 | 0.9 | 1.0 | 0.9 | 1.0 | 1.0 |
| 4 | 0.9 | 0.8 | 0.8 | 1.0 | 1.0 | 1.0 |
| 5 | 0.9 | 0.9 | 0.8 | 1.0 | 1.0 | 1.0 |

**Black Spruce**



a. Summer Health

b. Winter Health

**Tamarack**



c. Summer Health
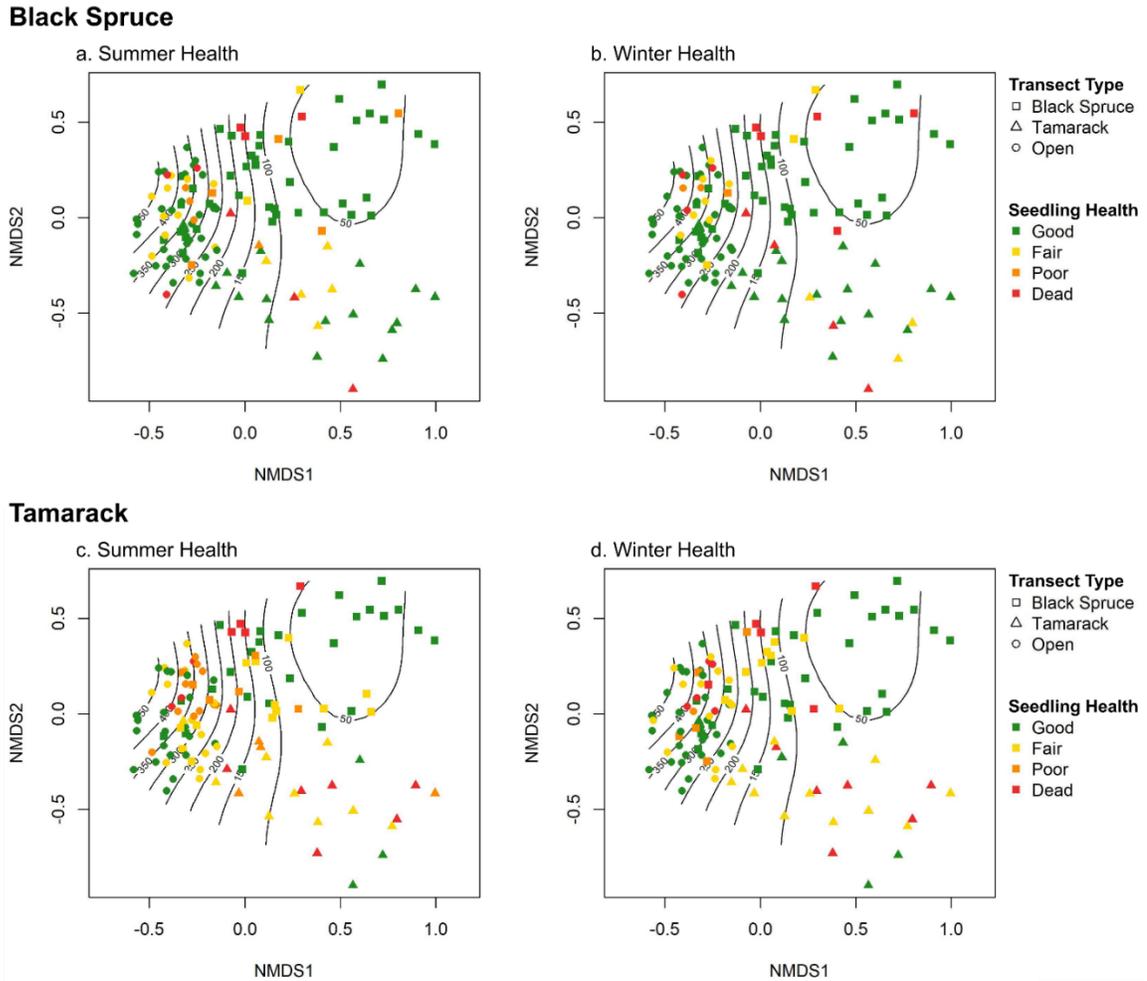
d. Winter Health

**Figure 3.4** Non-metric multidimensional scaling ordination (k=3, stress= 15.18) of treeline site plant community composition and abundance with sites coloured to represent qualitative a) black spruce seedling summer health, b) black spruce winter seedling health, c) tamarack summer seedling health, and d) tamarack winter seedling health. Distance from established black spruce tree islands and tamarack individuals is overlaid on the ordination.

Our linear mixed models indicated that during the 2017 growing season light availability, average minimum soil temperature, and average summer soil moisture changed with distance from presumed facilitators, and that the rate of change was dependent on transect type; average maximum soil temperature changed with distance

from presumed facilitator but rate of change was consistent across transect types (Table 3.2; Figure 3.5). Transect type affected the direction in which light availability changed with distance (Table 3.2). Light availability increased with increasing distance along facilitated transects (0.0038 %·m$^{-1}$ ± 0.0015 %·m$^{-1}$ for black spruce and 0.0035 %·m$^{-1}$ ± 0.0026%·m$^{-1}$ for tamarack), but decreased with distance along unfacilitated transects (-0.0008 %·m$^{-1}$ ± 0.0007 %·m$^{-1}$; Table 3.2). The rate that minimum summer soil temperatures changed with distance from facilitator was greater for black spruce and tamarack transects (0.0058°C·m$^{-1}$ ± 0.0036°C/m and 0.0056° C·m$^{-1}$ ± 0.0048 C·m$^{-1}$ respectively) than for unfacilitated transects (8.80 x 10$^{-4}$ ° C·m$^{-1}$ ± 0.0013° C·m$^{-1}$). Transect type had no effect on maximum summer soil temperature (0.0022° C·m$^{-1}$ ± 0.0017° C·m$^{-1}$ across all transects; Table 3.2). The rate that percent soil moisture changed with distance was dependent on transect type, where rate of change was greater for black spruce and tamarack transects (0.00054 %·m$^{-1}$ ± 0.00037 %·m$^{-1}$ and 0.0013 %·m$^{-1}$ ± 0.0004 %·m$^{-1}$ respectively), than for unfacilitated transects (6.0 x 10$^{-5}$ %·m$^{-1}$ ± 0.0002 %·m$^{-1}$; Table 3.2). Distance from facilitator and transect type didn't affect percent wind interception, where on average seedlings experienced a wind velocity of 0.183 m·s$^{-1}$ ± 0.048 m·s$^{-1}$ (Table 3.2).

Our linear mixed models indicate that winter microclimatic conditions did not vary along transects or across transect types (Table 3.2). The average winter minimum soil temperature across the winter was 2.12°C ± 0.11°C, and the average maximum soil temperature was 2.72°C ± 0.10°C. However, our models indicate that distance from facilitator or transect type had minimal effects on available soil nutrients. Available soil ammonium, important for conifer seedling growth at early

establishment life stages (Staples *et al.,* 1999; Robinson *et al.*, 2001), decreased marginally with distance from established individuals ($8.65 \times 10^{-5}$ µg $NH_4^+ \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1} \pm 0.0024$ µg $NH_4 \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1}$), but did not vary between facilitator types (Table 3.2). The rate of change in available soil magnesium, a necessary element for photosynthetic function (Levitt, 1954), marginally increased with distance along black spruce and tamarack transects (2.00 µg $Mg \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1} \pm 2.95$ µg $Mg \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1}$ and 5.72 µg $Mg \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1} \pm 3.08$ µg $Mg \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1}$, respectively) but decreased with distance along unfacilitated transects (-0.87 µg $Mg \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1} \pm 2.13$ µg $Mg \cdot 10cm^{-2} \cdot$ 255 days$^{-1} \cdot m^{-1}$; Table 3.2). Available soil manganese differed marginally across facilitator types; however, the residuals deviated from assumptions of normality and heterogeneity, so the effect should be interpreted with caution (Table 3.2). Distance from facilitator or transect type had no effect on available soil calcium, potassium, phosphorous, zinc, sulfur or aluminium (Table 3.2; Appendix I). Soil nitrate, iron, copper, boron, lead, and cadmium were below detectable levels (Appendix I).
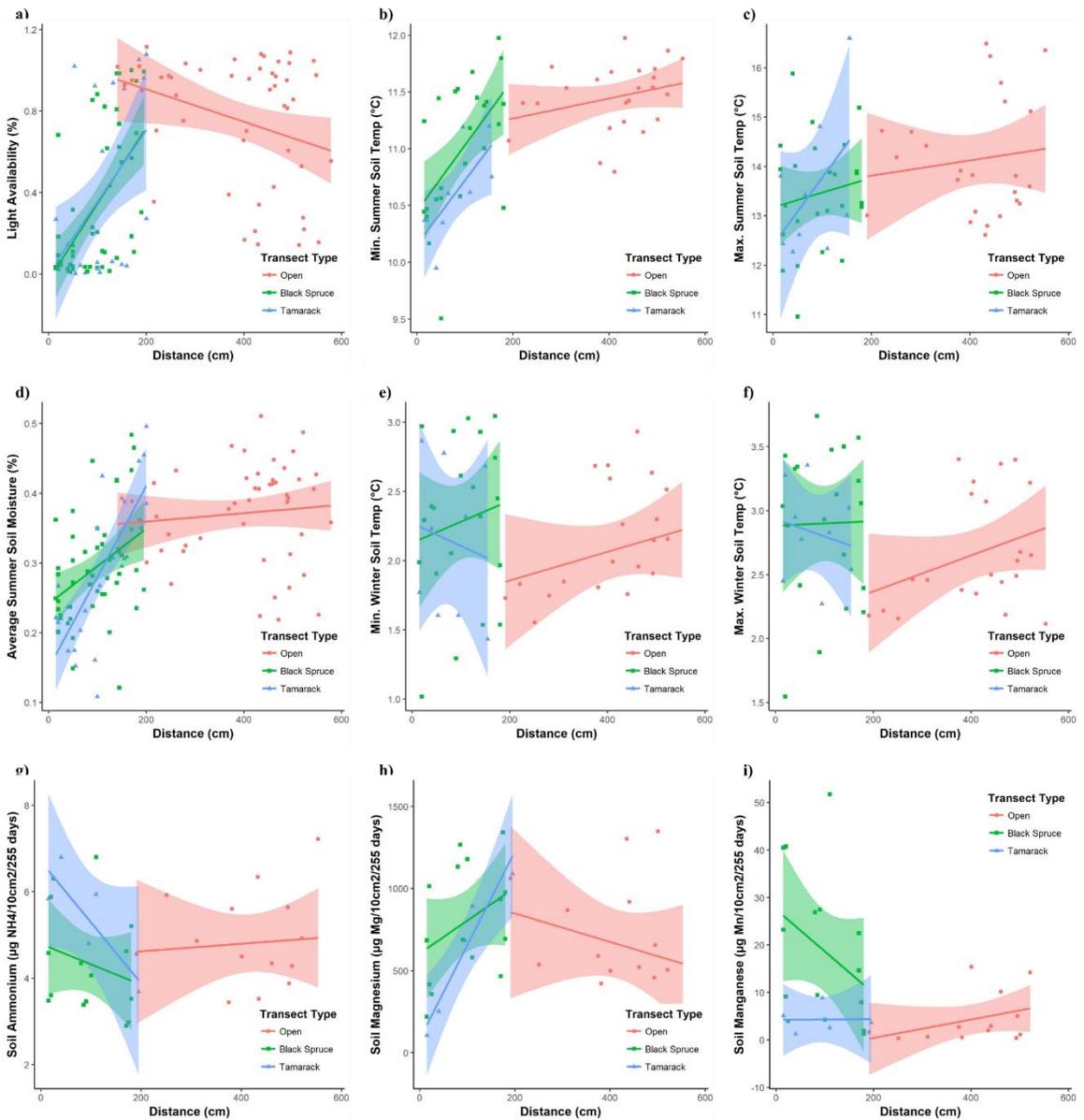
**Figure 3.5** Biologically important abiotic factors: a) light availability, b) average minimum summer soil temperature, c) average maximum summer soil temperature, d) average summer soil moisture, e) minimum winter soil temperature, f) average maximum winter soil temperature, g) soil ammonium, h) soil magnesium, and i) soil manganese as a linear function of distance (cm) away from nearest presumed facilitator at altitudinal treeline in central Newfoundland. Transect type is indicated by colour and symbol. Lines represent linear fit, with shading over the 95% confidence intervals. See table 3.1 for analysis of covariance (ANCOVA) summary.

**Table 3.2** Summary of linear mixed models predicting biologically important abiotic factors with distance from facilitator, facilitator type, and their interaction at altitudinal treeline in central Newfoundland. Analyses of covariance (ANCOVAs), with a type III error, was used to partition the variation within abiotic factors across treatments. Bold values are significant, where *p<0.05, **p<0.01, ***p<0.001.

| | Dist. | | | Trans. | | | Dist. * Trans. | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | MS | F-value | df | MS | F-value | df | MS | F-value |
| *Summer Abiotic Properties* | | | | | | | | | |
| Light Availability | 1 | 2.2516 | **31.433***** | 2 | 0.88641 | **12.375***** | 2 | 1.38284 | **109.913***** |
| Wind Intercept | 1 | 0.0078 | 1.080 | 2 | 0.01113 | 1.54045 | 2 | 0.00105 | 0.14558 |
| Avg. Min. Soil Temp | 1 | 2.4875 | **27.719***** | 2 | 0.07088 | 0.7898 | 2 | 0.45192 | 5.0359* |
| Avg. Max. Soil Temp | 1 | 5.9210 | **7.014*** | 2 | 0.27590 | 0.72444 | 2 | 1.28450 | 0.22867 |
| Avg. Soil Moisture | 1 | 0.1683 | **45.800***** | 2 | 0.03009 | **8.190***** | 2 | 0.06042 | **16.447***** |
| | | | | | | | | | |
| *Winter Abiotic Properties* | | | | | | | | | |
| Avg. Min. Soil Temp | 1 | 0.00012 | 0.0088 | 2 | 0.00925 | 0.07103 | 2 | 0.14786 | 1.135 |
| Avg. Max. Soil Temp | 1 | 0.00310 | 0.04186 | 2 | 0.00640 | 0.0863 | 2 | 0.03258 | 0.43955 |
| Soil Ammonium | 1 | 3.7918 | **6.0311*** | 2 | 1.0588 | 1.6841 | 2 | 1.6872 | 0.08807 |
| Soil Calcium | 1 | 90446 | 1.653 | 2 | 123111 | 2.250 | 2 | 171011 | 3.125 |
| Soil Magnesium | 1 | 659620 | **6.701*** | 2 | 178709 | 1.818 | 2 | 735848 | **3.743*** |

**Table 3.2** (Continued)

*Winter Abiotic Properties Continued*

| | Dist. | | | Trans. | | | Dist. * Trans. | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | MS | F-Value | df | MS | F-Value | df | MS | F-Value |
| Soil Potassium | 1 | 9453.7 | 1.042 | 2 | 5268.5 | 0.581 | 2 | 6536.9 | 0.7206 |
| Soil Phosphorous | 1 | 0.00551 | 0.5486 | 2 | 0.09958 | 0.9919 | 2 | 0.0507 | 0.5057 |
| Soil Manganese | 1 | 61.955 | 1.1435 | 2 | 195.223 | **3.6033*** | 2 | 142.499 | 2.6302 |
| Soil Zinc | 1 | 58.932 | 0.6297 | 2 | 35.236 | 0.3765 | 2 | 3.602 | 0.0385 |
| Soil Sulphur | 1 | 15.790 | 0.1237 | 2 | 39.779 | 0.3117 | 2 | 46.46 | 0.3641 |
| Soil Aluminium | 1 | 37.089 | 0.0279 | 2 | 245.80 | 0.1850 | 2 | 27.894 | 0.0201 |

We detected no trend in black spruce tree island height and position away from leeward side (Figure 3.6; t-value= -1.44, p-value= 0.152). On average, tree islands were 25.58 m² ± 2.83 m² with an average height of 1.02 m ± 0.01 m.
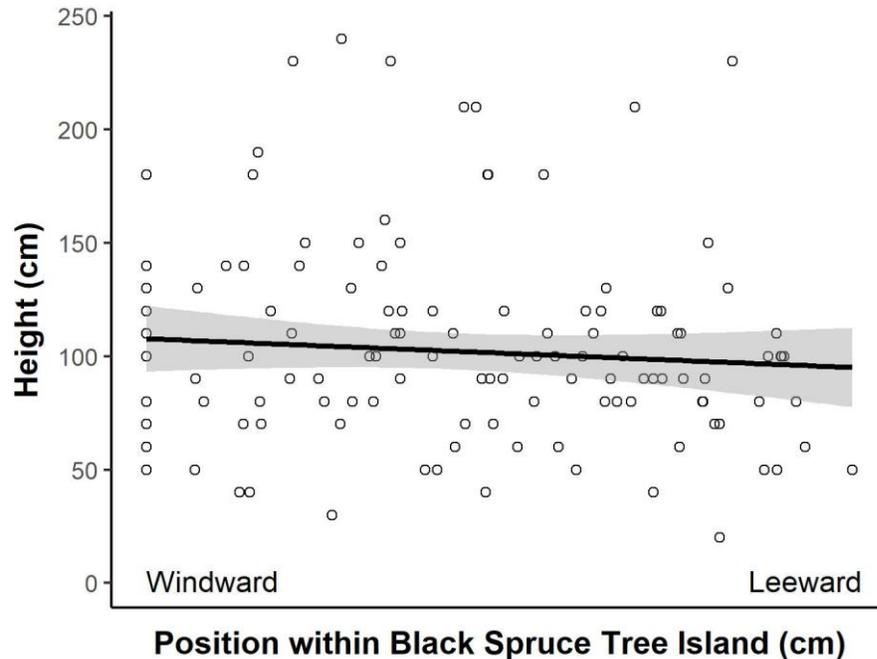


**Figure 3.6** Height of black spruce tree circles as a function of position within black spruce tree islands established at treeline in central Newfoundland (n=17). The line represents the linear model fit and the shaded region represents the 95% confidence interval (t-value= -1.44, p-value= 0.152).

### 3.4 Discussion

Positive feedback loops between established individuals and recruiting seedlings have long been considered the dominant process driving and maintaining tree island patterns at treeline ecotone (i.e., Wilson & Agnew, 1992) and have been proposed as a potential route for treeline range expansion (Holtmeier & Broll, 2017). Our field experiment across alpine treeline ecotone does not provide evidence to support facilitation of seedlings by adults, at least across the first year of the seedling life stage.

Despite changes in both abiotic and biotic environments with distance from tree islands and established individuals, black spruce and tamarack survival in our experiment was high (~90%) and we observed no trends in black spruce or tamarack seedling survival between facilitated and unfacilitated transects. Seedling survival, across the first year, was not a bottleneck to alpine treeline advance along the studied altitudinal gradient; if limitations surrounding seed production, germination, and seedling establishment (reported in Chapter 2) are overcome, our results suggest that recruitment at treeline will likely occur, at least to the seedling life stage studied here. Here, we discuss the effect of established conifers on hypothesized abiotic drivers of seedling survival at treeline.

### 3.4.1 *Solar radiation and subsequent effects*

As predicted, incoming solar radiation at ground level increased with distance away from established black spruce tree islands and individual tamarack, but only to a point, in that we observed a decrease in solar radiation with distance along open transects. This observed peak in solar radiation at intermediate distances from established individuals aligns with the associated transition in the understory biotic community at the treeline site, where *Kalmia* occurrence increases in abundance. *Kalmia* continues to increase in abundance and height (personal observation) with increasing distance away from established individuals, likely driving the decrease in incoming solar radiation along the open transects.

Tree islands and established individuals interact with incoming solar radiation, transforming it into sensible heat and long-wave radiation (Holtmeier & Broll, 2017). Canopies of tall, established vegetation can mediate surface temperatures through

shading, reducing maximum temperatures, and by reducing heat lost through long-wave radiation at night, increasing minimum temperatures (Smith *et al.*, 2003). We found that growing season soil temperature at treeline follow the expected trend, where soil temperature extremes increased with distance away from established individuals. We expected warmer soils to be drier due to increased evaporation; however, soil moisture decreased with distance from established individuals. We speculate that the soils are driest near established conifers due a combination of tree and shrub-driven microenvironments. To elaborate, we hypothesize that transpiration demands of tree islands and established tamarack are higher than the *Kalmia* heath community, while simultaneously, the low-stature *Kalmia* shrub canopy reduces moisture loss via direct evaporation, with the net result of drier soils near established conifers.

Light is a key resource, but radiative extremes associated with unobstructed sky exposure can result in high ground temperatures, soil desiccation, photoinhibition, and increased susceptibility to damaging frosts, potentially jeopardizing seedling survival (Germino *et al.*, 2002). Numerous studies have found that reduction in sky exposure by established vegetation or microtopographic features facilitates seedling establishment and survival at treeline (e.g., Germino *et al.*, 2002; Smith *et al.*, 2003; Gómez-Aparicio *et al.*, 2005; Maher, Germino, & Hasselquist, 2005; Maher & Germino, 2006; McIntire *et al.*, 2016; but see Cranston & Hermanutz, 2013). Despite variation in incoming light, soil temperature extremes, and soil moisture, all biologically important abiotic factors, we observed no changes in black spruce or tamarack seedling growing season survival across

the treeline; suggesting that the variation in microsite environmental conditions equally meet seedling requirements.

### 3.4.2 *Wind and subsequent effects*

Treeline advance requires survival in much windier environments, as individuals colonize treeless habitats where wind is not mitigated by the physical structure of the forest. Strong winds can cause physiological stress, desiccation, and mechanical damage to seedlings (Holtmeier & Broll, 2007). Against expectations, we observed no difference in surface wind with distance away from the leeward side of facilitators. The seedlings were transplanted flush with the soil, nestled within a thick lichen mat, well within the surface boundary layer (Körner, 2016), as evidenced by the drastic decline in wind speed measured between 2 metres and surface level. We expect, therefore, seedlings likely will not experience growing season wind induced stressors until they reach a height that exceeds the protective boundary layer (Körner, 2016).

The interaction between wind and established individuals at treeline has been found to have a larger effect on winter seedling survival than growing season survival (Holtmeier & Broll, 2010; Renard *et al.*, 2016). Tree islands interact with wind to affect the distribution of snow at treeline, where more snow accumulates at the leeward side of established individuals compared to treeless mountain patches (Holtmeier & Broll, 2017). Snow cover provides protection against harsh winter conditions by providing insulation, mediating extreme temperature conditions. We observed no trends in winter seedling survival across treeline, suggesting that there are no changes in snow cover with distance

from established individuals, or at least no changes that influence the size class of seedling studied here. The treeline site is on the lee side of convex topography, where the interaction between topography and wind results in increased accumulation of snow relative to the windward side and crests of convex topography (Holtmeier 2005; Holtmeier & Broll, 2010). We speculate that topographic controls of snow distribution override the effects of established black spruce tree islands and tamarack individuals. Uniform snow cover across treeline is consistent with our findings that average minimum and maximum winter soil temperatures did not vary across treeline.

### 3.4.3 *Secondary effects*

Alpine soils are generally considered nutrient limited, where low soil temperatures constrain microbial activity (Rustad *et al.*, 2001). Growth limitations, where the production of plant cells is less than minimum requirements for renewal of plant tissue or growth, driven by low nutrient availability is considered a mechanism governing the position of tree range limits (Körner, 1998). At the scale of individual trees, soils on the leeward side of established conifers at treeline have been found to have been found to be more nutrient rich than adjacent alpine soils (Holtmeier & Broll, 1992; Cairns, 1999; Liptzin & Seastedt, 2009). Tree islands and established individuals alter soil microclimatic conditions, through interactions with both incoming solar radiation and wind, and organic matter inputs; key controls on microbial decomposition (Cairns, 1999; Liptzin & Seastedt, 2009).

Manipulative experiments at treeline have found positive effects between seedling performance and nutrient availability (Grau *et al.*, 2012; Cranston & Hermanutz, 2013).

Despite changes in the plant community composition and summer soil microclimatic conditions with distance from established conifers at treeline, we found no variation in the majority of soil nutrients (except available soil ammonium and magnesium). Available inorganic nitrogen, a macronutrient essential for plant growth, is thought to be primarily controlled by winter snow cover and therefore, is expected to decrease with distance away from tree islands (Bowman, 1993; Cairns, 1999; Liptzin & Seastedt, 2009). Despite observing no soil temperature differences with distance from established conifers, indicative of a homogenous snowpack, we found available soil ammonium to decrease with distance from established conifers. Contradictory patterns between tree islands, snow cover, and available inorganic nitrogen have previously been observed (positive effect: Cairns, 1999; no effect: Malanson & Butler, 1994; Liptzin & Seastedt, 2009), suggesting that factors other than snow cover, such as organic matter inputs, may control inorganic nitrogen availability (Seastedt & Adams, 2001). Increases in base cations (Ca, Mg, K) on the leeward side of tree islands has been attributed to increased dust deposition driven by tree island-wind interactions (Liptzin & Seastedt, 2009). We found available soil magnesium to decrease with increasing distance from established tree islands but found no changes in available soil calcium or potassium. We speculate that inputs from underlying bedrock may override variation due to dust deposition (Liptzin & Seastedt, 2009). We recommend additional research on how tree islands affect nutrient availability, especially examining summer nutrient availability due to the effect established conifers had on soil microclimatic conditions. High seedling survival, regardless of variation in soil ammonium and magnesium, suggest that nutrient levels are not limiting seedling survival (Jacobs *et al.*, 2014; Trant *et al.*, 2015).

### 3.4.4 *Established tree islands and dieback*

    Island treelines are thought to be the balance between increased recruitment on the leeward side and dieback on the windward side (Harsch & Bader, 2011). Advance in island treelines is therefore equally dependent on increased recruitment as well as the amelioration of factors limiting growth within established tree islands (Harsch & Bader, 2011). Growth limitations have been found to occur year-round but dieback is considered primarily due to winter wind and snow and spring and autumn frosts, where exposed stems are susceptible to mechanical damage by wind and temperature stress (Wardle, 1968; Butler, 2009; Harsch & Bader, 2011). Self-facilitation has been found to alleviate these stressors, where branches on the leeside of tree islands are directly protected from stressors via sheltering from wind and indirectly via tree island-wind-snow interactions (Cairns, 2001; Harsch & Bader, 2011). Therefore, tree island height is commonly observed to increase, and dieback to decrease, from the windward to the leeward side (Marr, 1977; Cairns, 2001). We detected no trend in height across black spruce tree islands from windward to leeward side; symmetrical height suggests growth is not constrained and that tree islands are expanding (Albertsen *et al.*, 2014). We suggest tree islands should be re-surveyed annually to assess if a switch in black spruce growth form from krummholz to erect tree is occurring.

### 3.4.5 *What is hindering treeline advance?*

    Environmental conditions that support one life stage are not necessarily beneficial for other life stages (Cranston & Hermanutz, 2013). Regardless of distance from black spruce tree islands or tamarack individuals and the associated microclimatic changes,

transplant seedling survival was high; however, no naturally occurring seedlings were observed. Previous research along this altitudinal gradient indicates overwhelming constraints on the seed and establishing seedling life stages (as discussed in Chapter 2). Black spruce and tamarack treeline populations produced fewer seeds of low viability; recruitment at treeline is likely dependent of dispersal of seeds from forest populations. If seed limitations are overcome, the majority of seeds dispersed to treeline will be consumed. Bare ground greatly increased germination and seedling establishment over lichen substrates that occur at treeline; however, black spruce seeds that disperse to bare ground are more likely to be consumed. Multiple factors need to align temporally to overcome seed and seedling establishment limitations at altitudinal treeline in central Newfoundland; although, if these series of biotic filters are overcome, seedling recruitment is likely to occur.

While we found no constraints across the first year of the seedling life stage at altitudinal treeline, this does not guarantee survival once individuals surpass the favourable microclimatic conditions of the lichen boundary layer (Körner, 2016). There is currently a lack of knowledge on the growth and survival at the sapling stage, which is particularly problematic because the presence of seedlings beyond current range limits is sometimes concluded as evidence for treeline advance (Harsch *et al.*, 2009; Körner, 2016). The sapling stage is inherently hard to study, especially if no saplings are present at treeline, due to temporal constraints. We recommend repeat surveys of established seedlings at treeline to assess sapling survival once individuals are exposed to atmospheric conditions (Körner, 2016).

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## Chapter 4: Summary and conclusions

Treeline, the ecotone between forest and tundra or alpine ecosystems, is perceived as the thermal limit to tree growth, reproduction, and survival and with the alleviation of climatic constraints, treeline is expected to shift to higher elevations and latitudes (Körner, 1998, 2012; Körner & Paulsen, 2004; Paulsen & Körner, 2014). To date, there has been no uniform trend in treeline response to changing climatic conditions, suggesting that biotic interactions may override thermal controls (Harsch *et al.*, 2009; Hille Ris Lambers *et al.*, 2013). The presence or absence of competitors, consumers, mutualists, and facilitators will affect species' occurrence and abundance and therefore, affect how species' distributions respond to changing climatic conditions (Hille Ris Lambers *et al.*, 2013).

Newfoundland is currently 1.5°C warmer than historical average and is projected to continue to warm, with a shortened winter resulting in an additional 200-400 growing degree days across a longer growing season (Finnis, 2013; Finnis & Daraio, 2018). The projected future climatic conditions support the prediction that treelines in Newfoundland will shift upslope; however, if we are to accurately understand treeline response to Newfoundland's changing climate, we must understand the role biotic interactions have on driving current range limits (Hille Ris Lambers *et al.*, 2013; Hargreaves *et al.*, 2014). Changes in alpine treeline position threatens to displace alpine biota, potentially altering Newfoundland's biodiversity (Holtmeier & Broll, 2007). A loss of alpine ecosystems would have cascading socio- economic and -cultural affects, as alpine ecosystems are primary areas of snow machine use and would alter resident's transportation, hunting, and

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wood collection practises (Waight, 2014). To the best of our knowledge, the research presented in this thesis is the first to examine biotic drivers of altitudinal treeline position on the island of Newfoundland.

This thesis examines biotic drivers of treeline position through the reproduction limitation hypothesis: functional positional of treeline is explained by the failure for trees to recruit beyond range limits (Körner, 1998). Intuitively, treeline advance depends on increased recruitment, the production or dispersal of viable seed and subsequent seedling establishment and survival, at, or beyond treeline (Nathan & Muller-Landau, 2000; Briceño *et al.*, 2015; Johnson *et al.*, 2017). Early-life stages are both most abundant and most vulnerable, and therefore disproportionally affect recruitment (Harper, 1977). This thesis examines the affect biotic interactions at two early-life stages: i) seed and germination limitations (Chapter 2) and ii) seedling survival limitations (Chapter 3).

Seed and germination limitations were approached from the framework that recruitment from seed is comparable to a hurdle race, where a seed needs to pass through several biotic filters for recruitment to occur (Holtmeier, 2009). To quantify the relative importance of predicted biotic interactions on constraining black spruce and tamarack recruitment at treeline, we conducted a series of observational and experimental studies across an altitudinal gradient in central Newfoundland. Recruitment at treeline was simultaneously seed- and establishment-limited. Treeline populations produced fewer seeds than forest populations, and these seeds had low viability. If seed limitations are overcome, we found that establishment limitations, driven by post-dispersal seed predation and unsuitable ground cover for germination, will constrain recruitment. Our

findings highlight the need for multiple factors to align simultaneously if recruitment at treeline is to occur.

Seedlings at treeline have been found to have non-random spatial association, suggesting that amelioration of the environment at microscales will increase establishment and survival (Resler *et al.*, 2005; Malanson *et al.*, 2007). Suitable microsites or 'safe sites' that facilitate establishment are often attributed to taller neighbours, including tree islands (Alftine & Malanson, 2004; Batllori *et al.*, 2009; Renard *et al.*, 2016). Environmental requirements at one life stage are not necessarily beneficial for another life stage (Cranston & Hermanutz, 2013); seedling survival is not guaranteed if seed production and seedling establishment limitations are overcome. To quantify how microclimatic conditions change with distance from facilitators and in turn, how tree islands and microclimate interact to promote seedling survival at range edges, we transplanted black spruce and tamarack seedlings at altitudinal treeline along transects leading away from tree islands, presumed facilitators, and in open areas, presumably void of facilitation. We qualitatively assessed seedling health and quantified a suite of abiotic factors throughout the growing season and following winter. Despite observing unique summer microclimatic conditions on the leeward side of tree islands, we observed no spatial pattern in seedling survival. Seedling survival was high across the forest-treeline ecotone, suggesting that seed and establishment limitations are the bottleneck to recruitment at altitudinal treeline in central Newfoundland.

The principal limitation of this research is temporal scale. Assessments of early-life stage cohorts at only one point of time does not allow for the long term conclusions

on population dynamics at range limits (Körner, 2016). Climate fundamentally controls plant recruitment (Zasada, 1971; Owens & Blake, 1985; Sirois *et al.*, 1999; Walck *et al.*, 2011) and annual variation in climatic conditions will likely result in variation in production of viable seeds, germination, and seedling survival. Long-term monitoring of early-life stages is required to understand the consequences of temporal variation in recruitment at treeline, in particular the effect of mast years, and how production and survival of early-life stages respond to changing climatic conditions. Similar to issues surrounding intrinsic temporal variation, strength of biotic interactions will likely show inter-annual variation. For example, pre- and post-dispersal seed consumption pressures will differ year to year as seed production, food habitats, and seed predator abundance change (Hulme, 1998; Côté *et al.*, 2003). Moreover, there is significant uncertainty in how the magnitude and direction of biotic interactions will change under changing climatic conditions (Hille Ris Lambers *et al.*, 2013). Lastly, short-term studies are insufficient to study how biotic interactions, in particular plant-plant interactions, change with ontogeny (Soliveres *et al.*, 2010). By conducting a series of observational and experimental studies, we were able to examine the effect of predicted biotic interactions across multiple early-life stages. However, we suggest that future research further examine the effects of *Kalmia* on seedling establishment beyond seedling emergence stage (Mallik, 1987; Wallstedt *et al.*, 2002; Zeng & Mallik, 2006), and facilitative effects established tree islands and tamarack individuals have on seedling survival and growth once seedlings grow beyond the protective lichen boundary layer (Körner, 2012, 2016).

This thesis lends support to the reproduction limitation hypothesis, where recruitment at treeline is simultaneously seed- and establishment-limited. However, if

seed and establishment limitations are overcome, seedling survival is high and recruitment is likely to occur. Despite short comings associated with short-term studies, the studies presented in this thesis present invaluable insight into biotic drivers of treeline position and the potential for treeline advance in central Newfoundland. Few studies examining the reproduction limitation hypothesis have examined several limitation mechanisms across multiple early-life stages (but see Wheeler *et al.*, 2011; Dufour-Tremblay *et al.*, 2012; Cranston & Hermanutz, 2013; Kroiss *et al.*, 2015; Kambo & Danby, 2017); by conducting a series of observational and experimental studies, we are able to assess the relative effects of several biotic interactions on recruitment across a series of early-life stages at one site. There is currently uncertainty surrounding how biotic interactions modulate species' distributional response to climate change and how the direction and magnitude of these interactions may be altered by changing climatic conditions (Hille Ris Lambers *et al.*, 2013). Results and discussion presented throughout this thesis provide the first step, determining the relative importance of biotic interactions in governing current species' range limits, and provide a baseline for future studies.

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## Appendix I: Soil nutrients

To investigate how available soil nutrients vary with distance from facilitators and between transect types, we buried separate cation and anion exchange membranes (Plant Root Simulator (PRS®) Probes; Western Ag Innovations Inc., Saskatoon, SK) at three positions (beginning, middle, and end) along a subset of each transect type (n=5 open transects, n=5 black spruce transects, and n=2 tamarack transects). Probes were left *in situ* over the winter (burial period=255 days; Oct 22nd, 2017 to June 4th, 2018), collected the following spring, and sent to Western Ag Innovations Inc. to conduct nutrient supply rate analysis ($NO_3^-$, $NH_4^+$, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd). Nitrate, iron, copper, boron, lead, and cadmium supply rates were all below the detection levels (Table AII.1).

**Table AI.1** Summary of detection levels, as set by Western Ag Innovations Inc. (Saskatoon, SK), and average nutrient supply rate (± standard error), calculated across all transect types (n=12), at three burial positions along the transects (beginning, middle, end) over the burial period (Oct 22$^{nd}$, 2017 to June 4$^{th}$, 2018; 255 days). Nitrate, iron, copper, boron, lead, and cadmium were all below the detection levels. Asterisks denote nutrients that significantly varied across treatment types.

| Nutrient | Detection Level ($\mu g \cdot 10cm^{-2} \cdot 255$ days$^{-1}$) | Transect Position | | |
| --- | --- | --- | --- | --- |
| | | Beginning ($\mu g \cdot 10cm^{-2} \cdot 255$ days$^{-1}$) | Middle ($\mu g \cdot 10cm^{-2} \cdot 255$ days$^{1}$) | End ($\mu g \cdot 10cm^{-2} \cdot 255$ days$^{-1}$) |
| NO$_3^-$ | 2.0 | < detection levels | | |
| * NH$_4^+$ | 2.0 | 5.0 ± 0.32 | 5.0 ± 0.37 | 4.3 ± 0.36 |
| Ca | 2.0 | 531.0 ± 87.12 | 416.3 ± 94.63 | 553.6 ± 99.72 |
| * Mg | 4.0 | 585.0 ± 112.0 | 769.6 ± 106.3 | 769.4 ± 94.17 |
| K | 4.0 | 180.0 ± 34.21 | 149.2 ± 26.90 | 126.9 ± 30.15 |
| P | 0.2 | 1.0 ± 0.07 | 0.9 ± 0.13 | 0.9 ± 0.06 |
| Fe | 0.4 | < detection levels | | |
| * Mn | 0.2 | 13.0 ± 4.60 | 11.4 ± 4.62 | 7.0 ± 2.01 |
| Cu | 0.2 | < detection levels | | |
| Zn | 0.2 | 6.0 ± 1.13 | 12.8 ± 5.19 | 9.9 ± 3.11 |
| B | 0.2 | < detection levels | | |
| S | 2.0 | 21.0 ± 3.60 | 24.9 ± 4.44 | 15.2 ± 2.52 |
| Pb | 0.2 | < detection levels | | |
| Al | 0.4 | 26.0 ± 8.13 | 49.0 ± 20.50 | 23.0 ± 4.99 |
| Cd | 0.2 | < detection levels | | |