Advancing Understory Redevelopment in Forest Reclamation and the Role of Seedling Quality

by

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Abstract

The redevelopment of understories is an often-overlooked aspect of forest restoration following anthropogenic disturbances. In my research I explored the feasibility of actively restoring forest understories through seedling planting. First, I examined the quality of nursery-grown boreal shrub seedlings by testing whether seedling quality attributes that are commonly used to evaluate tree seedlings and their outplanting performance can be applied to shrubs. I followed this up with a field trial where I monitored the survival and growth of nine shrub species that were outplanted onto seven different sites that varied in age since reclamation, canopy tree species, and edaphic conditions. The attributes I selected to evaluate the quality of the shrub seedlings largely did not correlate with performance when seedlings were grown under non-limiting (no stress) conditions and survival was generally very high, except for two species which showed evidence of poor overall seedling quality. This finding suggests that, for some species, nursery cultural protocols need to change in order to produce better planting stock. It was evidenced from the outplanting experiment that larger seedlings typically perform better when exposed to reclamation site conditions. However, when planted in the field and exposed to various environmental stressors, it became clear that site conditions had the greatest influence on seedling performance. Interestingly, understory shrub seedlings survived and grew best on the young reclamation sites that had no tree canopy but also lacked competitive vegetation. While this early response is encouraging for shrub seedling establishment, it might not reflect the ability of these plants to maintain themselves as competitive conditions quickly develop on these sites, negatively impacting subsequent growth and survival. For example, seedlings that were planted on somewhat older (11-16 years) and more competitive reclamation sites experienced the highest mortality. In contrast, seedlings that were planted on older reclamation sites (> 30 years) where a closed tree canopy had developed, survival was high, although growth was slightly reduced compared to the young, open sites, which is likely a reflection of the growing conditions of the understory environment. This study is one of the first to explore active understory redevelopment on reclamation sites and provides crucial observations and information for forest restoration practices that expand beyond tree establishment.

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Chapter 1: General Introduction

1.1 Forest Restoration Commitments and Challenges

Forests cover approximately 31% of the earth's total land surface, or about 4.1 billion hectares. They are among the most biodiverse of all land habitats and provide critical environmental and atmospheric functions such as oxygen production, carbon reduction and storage, flood prevention, and climate regulation. Sadly, forests of today are facing grave threats due to increased human presence, more frequent natural disasters, and widespread industrial development (Millar and Stephenson 2015). In response to this ongoing degradation, global, national, and regional large-scale forest restoration efforts are being proposed with the hopes of returning these affected lands to functioning and productive ecosystems composed of native species that offer an array of economic and ecological services (Jacobs et al. 2015; Löf et al. 2019; Macdonald et al. 2015; Meli et al. 2017). Although these restoration goals are promising and many have been successful (Ratanapongsai 2020; Rorato et al. 2020; Zahawi et al. 2013), recent critical reviews have acknowledged that there are often significant hurdles to overcome such as limitations in seed collection, seedling production, workforce development, and improvements in pre- and post-planting practices (Fargione et al. 2021).

Another deficiency associated with current forest restoration practices is that we do not yet know how to fully restore all levels of forest ecosystems to provide the range of ecosystem services demanded by society. For example, most documented reforestation projects focus on the establishment of trees and tend to either overlook or leave the redevelopment of other important forest components to passive processes (Aerts and Honnay 2011; Forbes et al. 2021; Harris et al. 2012; Morrison and Lindell 2011; Ssekuubwa et al. 2019) Particularly, the redevelopment of forest understories (i.e., the layer of vegetation growing beneath the forest canopy but above the forest floor) is usually left out of restoration research and applications, which is problematic as understories represent most of the plant diversity in many forest ecosystems, contribute significantly to overall structure and function, as well as provide habitat for an array of organisms (Bokhorst et al. 2014; Gilliam 2007; Nilsson and Wardle 2005). In order to take a more comprehensive approach to forest restoration that sees beyond the trees, it is necessary to understand understory dynamics, what influences understory response to and recovery from disturbance, as well as potential understory restoration methods and their associated limitations.

1.2 Understory Functions and Processes

Forest understories are dynamic environments which are largely controlled and regulated by the tree canopy. The seasonality and quantity of light reaching the understory is governed by the type and density of trees in the overstory (i.e., evergreen vs. deciduous) and by spatial factors such as the crown size, the arrangement of leaves, and leaf size (Barbier et al. 2008; Felsmann et al. 2018). For example, in deciduous forests, light availability fluctuates greatly before spring leaf out and after fall senescence (Gill et al. 1998), compared to the understory of evergreen forests, where light conditions are much less variable (Constabel and Lieffers 1996). The canopy also influences understory vegetation by contributing to soil nutrients and fertility (Halpern and Spies 1995; Gillam 2007). It has been long documented that deciduous litterfall is richer in nutrients than evergreen species, where nutrient input via litterfall has been recorded as being 10 to 50% higher for nitrogen and phosphorous and 100 to 400% higher for calcium, magnesium, and potassium (Augusto et al. 2002). Furthermore, soil carbon and organic content are dependent on canopy species, with evergreen forests generally having higher stocks of carbon than deciduous forests (Gärdenäs 1998; Raulund-Rasmussen and Vejre 1995). The canopy also alters hydrological conditions by redirecting precipitation, reducing snow accumulation, and removing soil water through transpiration (Prescott 2002). Evergreens typically intercept more precipitation than deciduous species, especially during periods of dormancy, but also year-round, as conifers have higher leaf area indices (Breda 1999). As a result of all these factors combined, vascular plant species richness is often higher under a deciduous canopy versus an evergreen canopy (Barbier et al 2008).

Understory plants have physiological, morphological, and phenological adaptations that allow them to survive and thrive in their unique environments. For example, understory obligates (i.e., shade tolerant) are typically species with resource conservative traits, are non-competitive, and slow growing (Blondeel et al. 2020; Grime 1977). These species are also slow colonizers and usually rely on clonal reproduction as they produce small volumes of seeds that are generally not dispersed over long distances (Grime 1977). As a result, obligates are typically constrained to older forests with closed canopies which inhibit more competitive species due to resource limitation. Understory obligates tend to allocate more resources to leaves, creating specific morphological leaf traits that can be associated with efficacy of light capture in this environment (Mestre et al. 2017). In contrast, less shade tolerant species are often fast colonizers with resource acquisitive traits, are tall in stature, and produce significant leaf area. These species are more commonly found in newly disturbed or early successional forests or in forests that allow for significant light penetration to the forest floor (Blondeel et al. 2020). In terms of phenological adaptations (i.e., how organisms adapt to seasonal and interannual variations in climate), many understory species of deciduous forests have adopted a shade avoidance strategy known as "phenological escape" (Crawley 1997) where they emerge before the canopy vegetation in the spring to begin photosynthesizing before they become subjected to low light conditions (Richardson and O'Keefe 2009).

1.3 Disturbance and Understories

Disturbances are important aspects of ecological systems, often having profound impacts on ecological processes (Pielech and Czortek 2020). A disturbance can be defined as an event that disrupts the structure of an ecosystem, community, or population, by changing the distribution or availability of resources or physical environment (Pickett and White 1985). Natural and anthropogenic disturbances all have distinct disturbance regimes that vary in severity, intensity, size, seasonality, frequency, and duration. Disturbances can be acute (i.e., short-term), such as an extreme weather event, or chronic (i.e., long-term), such as climate change (Connell et al. 1997). Furthermore, disturbances can also interact with one another, sometimes triggering or promoting an additional event (Burton et al. 2020). The stability of a plant community, often measured by its resistance and resilience to disturbance (Halpern 1988; Sousa 1980), is highly dependent upon the characteristics of the disturbance (Sutherland 1981). Understory plant communities are typically affected by experiencing a reduction in live plant biomass, a release of resources to surviving plants, and new species colonizing the site (Stone and Wolfe 1996). Species that colonize stands affected by severe disturbances, such as stand-replacing fire, are typically those whose seeds and propagules survive the disturbance (Hart and Chen 2006). Changes in understory vegetation community composition are most notably controlled by disturbance severity, with more severe disturbances causing greater changes in understory composition (Roberts 2004). After a severe disturbance, only species that are capable of regenerating from widely dispersed propagules may reappear, resulting in reduced species diversity and establishment of exotic invasives. Post-disturbance recovery and succession of understory species starts with a rapid influx of shade-intolerant and nutrient-demanding species (Su et al. 2019), eventually transitioning into typical understory vegetation as the stand develops and the canopy closes (Hart and Chen 2008; Su et al 2019; Zhang et al. 2014). While this framework provides a good foundation for how understory succession can potentially occur following disturbance, we are seeing more and more evidence that this is often not the case, and that there are many factors and variables at play which determine if and how an understory will redevelop. As understory restoration is typically left to passive processes (Aerts and Honnay 2011; Harris et al. 2012), it is expected that the plant community will colonize either from seeds or underground vegetative propagules that survive the disturbance, be dispersed from vegetated areas adjacent to the disturbed site (Dhar et al. 2018), or from clonal encroachment from surrounding vegetation (Jones and Landhäusser 2018; Rydgren et al. 1998). In some cases, such as when severe disturbances occur, these colonization processes are not as effective (Errington and Pinno 2015), and thus, we must consider other restoration alternatives for situations where understory species may not be able to regenerate without human intervention.

1.4 Understory Redevelopment Considerations and Current Limitations

Although there is currently very little consideration of understories in restoration practices and research, ideas can be drawn from general restoration knowledge and applied to this forest layer. First, it is paramount to set out clear restoration goals for the project at hand, assess the resources available, and address questions such as what results are expected if a passive approach is taken, when intervention (i.e., active restoration) should occur if deemed necessary, and how can restoration resources be used most efficiently (Holl and Aide 2011). In most regions, native propagules are not available at a commercial scale (Macdonald et al. 2015; Smreciu et al. 2013), and nurseries do not yet have the knowledge to develop protocols for the propagation of all desired species. Further, for the species that are presently being produced, there is very little information regarding their general autecology, their seedling quality and nursery cultural requirements, and how they stand up to (often harsh) outplanting conditions. Species that have been studied extensively (i.e., commercially important hardwood and softwood trees) have known, desired morphological and physiological seedling quality characteristics that lead to increased performance upon outplanting, though this has yet to be explored for the understory species the nurseries are now producing and selling at a commercial scale.

1.5 Objectives and Thesis Structure

This thesis consists of two individual research chapters. The overall objective of this work was to provide some preliminary information on the quality and outplanting success of nursery-grown boreal shrub seedlings to help guide the incorporation of understories into future forest restoration efforts.

Chapter 2 examines whether seedling quality attributes that are well studied and applied to evaluate the potential performance and survival of trees seedlings can also be used to assess the potential quality of shrub species. Additionally, seedlings of two different stock years were compared and contrasted to examine product consistency and impacts of nursery cultural practices. The seedlings were assessed by their initial morphological and physiological characteristics, as well as how these characteristics changed over one growing season.

Chapter 3 presents results from a field trial where nine species of shrub seedlings were planted on an array of reclamation sites ranging in age and environmental conditions to evaluate their responses to varying degrees of site explore how the variation in species and seedling survival and growth were related to site soil characteristics, degree of tree canopy closure, and vegetation competition over three growing seasons.

Chapter 4 reviews and synthesizes the key findings of Chapter 2 and 3. Application suggestions and management implications as well as study limitations and potential for future research are discussed.

Chapter 2: Exploring the Applicability of Tree Seedling Quality Measures to Nursery-Grown Boreal Shrub Seedlings

2.1 Introduction

Planting seedlings is a demonstrable method to successfully establish plants, particularly trees, on sites where vegetation has been removed, circumventing the challenges associated with establishing them naturally or artificially from seed (Palma and Laurance 2015). The use of planted seedlings can provide a "head start" in the reestablishment of vegetation in disturbed areas, as seedlings have had a chance to develop their above and belowground organs to readily access resources on a site and to allow them to compete with other, often competitive vegetation (Bebre et al. 2021). Although with greater costs upfront, planting seedlings removes many of the risks and uncertainties associated with the reintroduction of species via seeds, which can end up costing more in the long-term (Palma and Laurance 2015). Seeds are often difficult and expensive to collect, can vary significantly in their viability, and substantial seed quantities would be required for broadcast seeding (Freitas et al. 2019). Once spread on a site, seeds are subjected to a range of additional risks such as erosion through wind and water, predation by insects or other animals, and exposure to poor germination and early establishment conditions (Lamb 2014; Zahawi et al. 2014). Planting nursery-grown seedlings is an effective way of overcoming many of these limiting factors on outplanting sites and provides the ability to target planting positions that are edaphically and climatically appropriate for the species (Landis 2011), often yielding very positive results in terms survivorship and growth, accelerating the recovery of forests (Macdonald et al. 2015; Palma and Laurance 2015).

The overall quality of seedlings being produced by nurseries is a critical component of forest restoration. Methods for assessing seedling quality have continually evolved over time and can vary depending on the type of species being grown and the conditions they are being planted into. Many seedling quality assessment measures were proposed over the years, sending practitioners on a quest for a "silver bullet" or a single, all-inclusive seedling quality test (Puttonen 1996). Height and root collar diameter (RCD) are simple to measure and remain the most common morphological characteristics examined on forest seedling stock (Haase 2008; Jacobs et al. 2005; Rose et al. 1990). While it is well documented that increased height and RCD are correlated to increased field performance (Grossnickle and MacDonald 2018; Haase 2008;

Levy and McKay 2003; Thompson 1985), it is not possible to account for the large variability in species, nursery cultural practices, and site conditions with one single test. There is strong evidence that morphological attributes such as root and shoot morphology, root and shoot biomass, and their balance, can also be useful in predicting field performance (Long and Carrier 1993; Thompson 1985). In addition to seedling morphology, seedling physiological characteristics are also used to evaluate quality. For example, the seedling cold hardiness test can provide a good estimate of stress resistance for a given seed lot, and root growth potential, the ability for the plant to initiate new roots, can be useful when evaluating a seedling's overall vigor (Haase 2008). Quality assessments have since expanded beyond simply measuring a few attributes of individual seedlings to the support the idea of "fitness for purpose" (Ritchie 1984), where a seedling's characteristics are targeted to outplanting conditions rather than producing a standard seedling for all site conditions. This is precisely what the Target Plant Concept (TPC) is predicated on (Dumroese et al. 2016). Seedlings produced with this concept in mind have been cultured to survive and grow on a specific site with specific outplanting conditions (Landis et al. 2010).

The TPC has helped inform and develop seedling quality measures for extensively studied species such as commercially important hardwood and softwood trees. For example, Trembling Aspen (*Populus tremuloides* Michx) seedlings that are being outplanted on moisture-limited sites perform better when they have high initial root to shoot ratios (Landhäusser 2012a). In conifers, seedling survival and growth can be improved with an increased ability to grow roots immediately after outplanting (Grossnickle and Ivetić 2022). Tree nurseries can adjust their cultural practices to select for many of these desired morphological and physiological traits, which has resulted in the production of robust, high quality tree seedlings that have good survivorship and perform well on an array of site conditions (Birkinshaw et al. 2009; Grossnickle and MacDonald 2018). Nowadays, however, the nurseries have graduated beyond solely producing tree seedlings. A variety of shrub species are now being grown and sold at a commercial scale for use in restoration and reclamation projects, such as Bracted Honeysuckle (*Lonicera involucrata*), Blueberry (*Vaccinium myrtilloides),* Northern Red Currant (*Ribes triste*), Mountain Huckleberry (*Vaccinium membranaceum*), Witch Hazel (*Hamamelis virginiana*), among others.

However, unlike for a relatively small number of well-researched commercial tree species, there is very little knowledge about the autecology, nursery cultural requirements, and seedling quality of lesser-known species and their subsequent performance after outplanting on often stressful sites. Assessing and describing seedling quality for these species is a complex task, and so far, there has been virtually no attempts, particularly for boreal forest species.

A reasonable starting point to assess the quality of lesser-known species is using seedling measures and characteristics that are currently used for assessing the quality of commercial tree seedlings and are known to indicate better performance and survival. Height and RCD should be included as they are common and easy to measure. Other measures such as root system size and morphology should be evaluated since seedling survival and growth post-planting are directly related to the ability of the root system to promptly regenerate roots and grow out into the surrounding soil (Grossnickle and Ivetić 2022; Martens et al. 2007; Ritchie 1984). Root volume has been directly correlated with reforestation success for several evergreen and deciduous tree species (Jacobs et al. 2005) and a larger root to shoot ratio (defined as dry weight of root biomass divided by dry weight of shoot biomass) has been seen to result in better chances of survival after outplanting (Landhäusser et al. 2012a,b; Thompson 1985). Measuring carbohydrate reserves can also provide insight into seedling quality as carbohydrate reserves are a critical source of energy for newly establishing seedlings between planting and the start of photosynthesis (Landhäusser et al. 2012b).

This study aims to explore and evaluate the impact of specific morphological and physiological seedling quality characteristics on the survival and growth of nine commercially grown boreal forest shrub species. I explored whether well documented seedling quality attributes that are often related to the performance and survival of tree seedlings such as height, RCD, overall size, root to shoot ratio (RSR), and non-structural carbohydrate (NSC) reserve concentrations in tissues, can be applied to shrub seedlings to predict their survival and growth. Through this research, I hope to 1) identify shrub seedling characteristics that can be related to survival and increased growth and 2) compare and contrast the variability in seedling characteristics within a species and between two different productions years. My findings may be able to provide recommendations to help guide seedling production protocols and assess shrub seedling quality characteristics prior to their use in boreal restoration projects.

2.2 Materials and Methods

2.2.1 Seedling Stock and Initial Quality Characteristics

Shrub seedlings used for this study were dormant frozen stored container stock (-3°C for 6 months), commercially grown from open-pollinated seed sources collected near Fort McMurray, Alberta (57°02'56.6"N 111°28'22.4"W). Seedling stock was grown at Smoky Lake Forest Nursery (54°05'09.9"N 112°14'48.1"W), AB, Canada and was acquired for a 2019 and 2020 planting trial. In 2019, *Cornus canadensis* (Bunchberry), *Lonicera involucrata* (Bracted Honeysuckle), *Vaccinium myrtilloides* (Blueberry), and *Vaccinium vitis-idaea* (Lingonberry) seedling stock was available, while in 2020, the stock included the same four species as in 2019, as well as *Symphoricarpos albus* (Snowberry), *Ledum groenlandicum* (Labrador Tea), *Viburnum edule* (Lowbush Cranberry), *Empetrum nigrum* (Crowberry), and *Linnaea borealis* (Twinflower) (Table 2.1).

In early spring (late May early June), seedlings were thawed for four days at 4°C in a refrigerator. During thawing, the seedling boxes were left open to ensure adequate air circulation. Based on the morphological variability within a species, we sorted seedlings of each species into two or more quality categories using common and easily measured morphological seedling quality characteristics and subjective observations (Appendix Figure A.1 – A.9). Depending on the species, characteristics such as height, root collar diameter, the number of living stems, root system development (plugfill), green leaves (evergreen species), the presence/absence of mold, and an estimation of overall vigor were used to categorize potential quality categories. Category 1 seedlings had characteristics that we considered positive for the survival and early establishment of that species, such as having predominantly green leaves (evergreen species), taller stems, larger RCD, and/or a considerable amount of branching, no visible mold, and sufficient root system development (plugfill). The remaining seedlings that did not meet the quality requirements of Category 1 were separated into one or two more categories, where Category 2 seedlings possessed some of the quality characteristics, while Category 3 seedlings lacked most or all of them. For each species, ten seedlings from each quality category were set aside for destructive sampling to quantify *initial* morphological characteristics such as root, shoot, and leaf mass, and NSC concentrations (see Section 2.2.2).

2.2.2 Experimental Setup and Seedling Measurements

Based on challenges and learnings that occurred during and after the 2019 experiment with four species, I adapted my research protocols for the 2020 experiment, which included a larger selection of species (nine). Since some of the protocols used in the 2020 experiment significantly deviated from those used in 2019, I only explored the inter-annual variation of *initial* seedling stock characteristics of the four species between the two production years (2019 and 2020).

Approximately 50 seedlings per species were planted into 4 L pots (15.0 cm wide, 18.0 cm deep) in late May and filled with a mineral topsoil that was obtained from an agricultural field at the Crop Diversification Centre (CDC) North in Edmonton, Alberta (53°38'45.6"N 113°21'23.2"W). The soil was amended with a slow-release NPK fertilizer (3-6 month, 14N-14P-14K Evergo Division, Nutrien Ag Solutions Inc., Alberta, Canada) at a rate of 40 g fertilizer per 68 L soil. After planting, *initial* morphological aboveground characteristics were measured on each potted seedling. The pots were then fully randomized in a large outdoor area that was covered with a light shade cloth (~ 80% light transmittance) to avoid excessive exposure to direct sunlight. Seedlings were watered to excess when needed, which was typically every two to three days, depending on precipitation frequency. At the end of the growing season in late August 2020, when shoot growth had terminated, seedlings were remeasured and destructively harvested to determine growth and dry mass. Due to mortality and the variability in the seedling stock coming from the nursery, the number of individuals assigned to each quality category was not consistent. While morality was assessed based on the initial number of seedlings, an equal number of seedlings were randomly selected from each quality category for destructive harvest and the subsequent analysis of growth measurements. All plants were separated by tissue type: shoot, root, leaf, and fruit (if applicable). Prior to drying the roots were carefully washed to avoid root loss. The dry mass of all tissues was determined after drying all samples at 70°C to constant weight (approximately 72 hours).

The seedlings that had been initially set aside prior to planting were destructively harvested and measured in the same way as the potted seedlings. In addition, *initial* non-structural carbohydrate (NSC) reserves were quantified for each quality category of each species. For these measurements all seedlings had their shoots and roots separated and, in the evergreen species (*V. vitis-idaea*, *E. nigrum*, *L. groenlandicum*, and *L. borealis*), leaves were separated from the stems. For NSC analyses, samples were heated to 100°C for one hour prior to drying at 70°C (approximately 72 hours). After drying, all samples were weighed and a subsample of each tissue type (shoots, roots, leaves) was ground to a 40-mesh (0.4 mm) using a Thomas mini Wiley mill (Thomas Scientific, Inc., Swedesboro, NJ, USA).

Non-structural carbohydrates were quantified using the procedures outlined in Landhäusser et al. (2018). In short, soluble sugars from tissue samples were extracted with hot 80% ethanol and measured colorimetrically using phenol-sulfuric acid. The remaining starch in the residue was subsequently hydrolyzed to glucose by two digestive enzymes, α-amylase followed by amyloglucosidase, then measured colorimetrically using a peroxidase-glucose oxidase-o-dianisidine solution.

2.2.3 Statistical Analysis

To determine the height and RCD growth of individual plants, the *initial* height and RCD measurements taken from each seedling at planting time were subtracted from the *final* measurement taken on the same plant at the end of the study. To estimate changes in the above- and belowground biomass between the initial and final measuring periods, the average *initial* dry mass (obtained from the initial destructive measurements of seedlings in each category (see Section 2.2.1)) was subtracted from the *final* mass of each seedling for each species and associated quality category. The study was a fully randomized design with category or year as treatment variables. All species were analyzed separately. The design of this study was influenced by the limited seedling availability, and therefore, survival data are absolute and were not statistically compared among categories.

Analyses were performed using R software, version 4.0.2, 64-bit (R Core Team 2022). Assumptions of normality and homoscedasticity were tested using the Shapiro-Wilks test with the shapiro.test function (version 4.0.2; R Core Team 2022) and Levene's test with the LeveneTest function from the car package (version 3.1-0). If these assumptions were not met, transformations were applied (log, exponent, or inverse). Two sample t-tests were used to 1) detect differences in growth between seedling quality categories in the 2020 planting stock, and 2) to identify differences in *initial* seedling characteristics between 2019 and 2020 seedling stock. Since the category assignments differed between 2019 and 2020, *initial* seedling characteristics were averaged for this comparison across all assigned categories with 30 in 2019 and 20 seedlings in 2020.

2.3 Results

2.3.1 *Cornus canadensis*

Across all quality categories, *C. canadensis* seedlings acquired from the nursery in 2020 had more shoots and leaves (16.2 and 79.0, respectively) than seedlings that were produced in 2019 (7.6 and 19.5, respectively) (both $p \leq 0.001$). This difference in aboveground mass was not reflected in root to shoot ratios (RSR), as 2020 seedlings (0.27) had nearly double the RSR of 2019 seedlings (0.16), indicating that the number of roots was overall proportionally greater in the 2020 seedlings ($p=0.05$). Not only did the 2020 seedlings have more root mass, they also contained more carbohydrate reserves. Root NSC reserve concentrations (soluble sugar and starch combined) were more than three times higher in the 2020 seedling stock (10.9% dry weight (DW)) than in the 2019 stock (3.0% DW) ($p \leq 0.001$), while shoot NSC concentrations were 30% higher in the 2019 stock (12.6 % DW) than the 2020 stock (8.4% DW) ($p<0.001$) (Table 2.2; Appendix Table A.1).

In 2020, I separated the *C. canadensis* seedlings into three quality categories, mostly based on the proportion of moldy leaves on individual plants (Appendix Figure A.1). Category 1 seedlings (25% of the seedlings from the nursery) had less than 25% moldy leaves, Category 2 seedlings (54%) had less than 50% moldy leaves with little sign of mold, while Category 3 seedlings (21%) had greater than 50% moldy leaves.

Overall, mortality of the *C. canadensis* seedlings was high (70.3%), but there was clearly a seedling quality effect where only 33.3% of Category 1 seedlings, 85.8% of Category 2 seedlings, and 91.7% of Category 3 seedlings died (Figure 2.1). Since only one seedling in Category 3 survived, this category was removed from the analyses of the growth data. Based on the surviving plants, none of the growth responses were different between the two categories (Table 2.4; Appendix Table A.2) and both categories lost a similar number of shoots and leaf, root, and shoot mass over the growing season (all $p \geq 0.38$). Accordingly, RSR increased in both categories by an overall average of 15.0% ($p=0.673$). Although bunch size (i.e., the lateral spread) increased in Category 1 (70.1 cm^2) seedlings and decreased in Category 2 (-68.7 cm^2)

seedlings, the difference was not significant due to the large variation (p=0.37). No flowers and fruit were produced by *C. canadensis* over the 2020 growing season.

2.3.2. *Lonicera involucrata*

Seedling stock from 2019 and 2020 were comparable in height (33.3 cm and 29.0 cm, respectively; p=0.165) and had similar root collar diameters (RCD) (3.7 mm) and RSR (1.05) (both p≥0.883). However, NSC reserve concentrations of both root and shoot tissues were substantially higher in the 2019 seedling stock (22.6% DW and 14.2% DW, respectively) compared to 2020 (8.5% DW and 9.3% DW, respectively) (both p≤0.001) (Table 2.2; Appendix Table A.1).

Since the 2020 seedling stock was overall uniform, I used initial height as the main measure to separate two potential quality categories (Appendix Figure A.2). Category 1 (18% of seedlings from the nursery) seedlings were taller than 30 cm and Category 2 (82%) seedlings were shorter than 30 cm.

Regardless of category, seedlings had low mortality, 0% in Category 1 and 12.5% in Category 2 (Figure 2.1). At the end of the growing season, the average number of shoots and shoot mass were significantly different between the two quality categories (Table 2.4-2.5; Appendix Table A.2), with Category 1 producing 0.60 shoots and Category 2 producing 2.6 (p=0.008). However, this difference was not reflected in shoot mass growth, where Category 1 seedlings added 2.5 g of shoot mass while Category 2 seedlings added only 1.4 g (p=0.042). Height, RCD, and increases in root and leaf mass were not different between the two quality categories. Averaging across categories, seedling height and RCD increased by an average of 2.6 cm and 3.1 mm, respectively. Root mass increased by 5.3 g and seedlings produced an average of 3.0 g of leaves. Seedlings in both categories more than doubled their RSR during the growing season, with Category 2 seedlings increasing their RSR slightly more (1.6) compared to Category 1 seedlings (1.2) (p=0.095). Only three Category 1 seedlings produced fruit.

2.3.3 *Vaccinium myrtilloides*

Seedlings in 2019 were overall taller (average 20.1 cm) and had more shoots (14.0) compared to seedlings in 2020, which were 15.1 cm tall and had an average of only 4.7 shoots (both p≤0.001). However, RCD and RSR were similar between both years at 2.3 mm and 0.61, respectively (both p=0.19). Both root and shoot NSC concentrations were higher in 2020 seedlings (5.0% DW and 8.3% DW, respectively) compared to 2019 (4.3% DW and 6.6% DW, respectively) (p=0.047; $p \leq 0.001$) (Table 2.2; Appendix Table A.1).

The 2020 seedlings appeared relatively uniform; therefore, I used the number of shoots and height to separate individual plants into two quality categories (Appendix Figure A.3). Seedlings in Category 1 (77% of seedlings from the nursery) were more than 10 cm tall and had more than four shoots, while seedlings in Category 2 (23%) were less than 10 cm in height and had fewer than four shoots.

There was no mortality in either category of *V. myrtilloides* seedlings (Figure 2.1). At the end of the growing season, increases in root and leaf mass were significantly different between the two categories, with Category 1 seedlings gaining 0.60 g of root mass and Category 2 seedlings gaining only 0.14 g (p=0.015). Further, Category 1 seedlings gained 0.70 g of leaf mass over the growing season compared Category 2 gaining only 0.24 g ($p \leq 0.001$) (Table 2.4-2.5; Appendix Table A.2). The quality categories differed marginally in height and shoot mass growth. Category 1 seedlings increased in shoot mass by 0.28 g while their average height decreased by 1.7 cm, and Category 2 seedlings increased shoot mass by just 0.03 g while their average height increased by 0.37 cm (p=0.062 and p=0.087, respectively). All other growth measures were not different between categories (all $p \geq 0.308$). For both categories, number of shoots increased by an average of 3.0, RCD increased by 1.5 mm, and RSR increased from 0.70 to 1.2 (p=0.79). Most seedlings in both categories (66.7%) produced flowers and fruit.

2.3.4 *Vaccinium vitis-idaea*

Seedlings from the 2019 stock were taller; however, the 2020 seedlings had larger RCDs. In 2019, seedlings were on average 9.9 cm tall and had an RCD of 1.0 mm, whereas in 2020, seedlings were 7.0 cm tall and had an RCD of 1.5 mm ($p \leq 0.001$; p=0.003). Root, shoot, and leaf NSC concentrations were all higher in 2019 (3.2% DW, 9.2% DW, and 17.5% DW, respectively) than in 2020 (3.0% DW, 6.1% DW, and 15.4% DW, respectively) (p=0.065, $p \leq 0.001$, p=0.003). The remainder of growth measures were not significantly different between stock years (all $p \geq 0.12$) (Table 2.2; Appendix Table A.1).

The 2020 seedlings had a significant amount of dry, browning leaves. Additionally, many of the seedlings were affected by mold and about one quarter of the batch had loose plugs that fell apart when gently handled (i.e., poor plugfill). Therefore, I used number of brown/moldy leaves and plugfill as the main measures for sorting into two quality categories (Appendix Figure A.4). Category 1 seedlings (80% of seedlings from the nursery) were characterized as having fully green leaves, less than 25% mold, and acceptable plugfill. Category 2 seedlings (20%) had more than 25% mold/dead shoots and loose plugs.

Regardless of category, seedlings had low mortality, 2.9% in Category 1 and 16.7% in Category 2 (Figure 2.1). At the end of the growing season, bunch size (i.e., lateral spread) and height were significantly different between categories (Table 2.4-2.5; Appendix Table A.2). Category 1 seedlings laterally spread significantly more (222.5 cm^2) than Category 2 seedlings (140.1 cm^2; p=0.020). Category 1 seedlings increased in height by 1.1 cm, whereas Category 2 seedlings decreased in height by 0.48 cm (p=0.011). The quality categories differed marginally in shoot mass growth (p=0.073), where Category 1 seedlings increased by 0.19 g and Category 2 increased by only 0.091 g. All other growth measures were not different between quality categories ($p \geq 0.233$). Averaging across both categories, number of shoots increased by 1.7 and root, shoot, and leaf mass increased by 0.28 g, 0.14 g, and 0.62 g, respectively. Root to shoot ratio increased from 0.67 to 1.6 at the end of the growing season (p=0.23). No flowers or fruit were produced.

2.3.5 *Symphoricarpos albus*

Seedlings of this species (and following species) were acquired from the nursery in 2020 only. *S. albus* seedlings had on average 6.2 shoots, were 18.5 cm tall, had an RCD of 2.8 mm, and an RSR of 1.5. Root and shoot NSC concentrations were 13.4% DW and 14.7% DW, respectively (Table 2.3).

Overall, these seedlings appeared healthy and had no obvious deficiencies. Therefore, I used height and branching to split them into two quality categories (Appendix Figure A.5). Category 1 seedlings (76% of seedlings from the nursery) were greater than 15 cm tall and had three or more branches, whereas Category 2 seedlings (24%) were less than 15 cm tall and had less than three branches.

Regardless of category, seedlings had low mortality, 9.5% in Category 1 and 0% in Category 2 (Figure 2.1). Root mass and shoot mass growth were significantly different between categories at the end of the growing season (Table 2.4-2.5; Appendix Table A.2). Category 1 seedlings increased in root mass by 3.2 g and shoot mass by 1.8 g, whereas Category 2 seedlings increased in root mass by only 1.4 g and shoot mass by only 0.60 g ($p=0.024$; $p=0.028$) (Table 2.5-2.6; Appendix Table A.2). Final leaf mass was also significantly different, with Category 1 seedlings having 3.3 g of leaves at the end of the season and Category 2 having just 1.3 g ($p=0.002$). All other growth measures were not statistically different between quality categories ($p\geq0.180$). Averaging across both categories, number of shoots did not change over the course of the growing season, height increased by 0.33 cm, and RCD increased by 2.5 mm. Root to shoot ratio increased from 1.5 to 2.2 ($p=0.49$) and no flowers or fruit were produced.

2.3.6 *Ledum groenlandicum*

L. groenlandicum seedlings had on average 4.3 shoots, were 32.1 cm tall, had an RCD of 3.5 mm, a live crown ratio (length of green leaves on the main stem divided by length of the main stem) of 80.9%, and an RSR of 0.33. Root, shoot, and leaf NSC concentrations were 5.2% DW, 5.8% DW, and 6.8% DW, respectively (Table 2.3).

I separated the seedlings into two quality categories based on height and/or branching and number of dead leaves (Appendix Figure A.6). Category 1 seedlings (17% of seedlings from the nursery) had few dead leaves and either multiple shoots or one large main shoot with green leaves along most of it and Category 2 seedlings (83%) had some dead leaves and minimal branching.

Regardless of category, seedlings had low mortality, 0% in Category 1 and 6.2% in Category 2 (Figure 2.1). At the end of the growing season, none of the growth measures were significantly different between categories ($p\geq0.11$) (Table 2.4; Appendix Table A.2). For both categories, *L. groenlandicum* seedlings lost on average 1.0 shoot, decreased in height by 0.75 cm, increased in live crown ratio by 2.5%, increased in RCD by 2.0 mm, and increased in root, shoot, and leaf mass by 1.0 g, 0.73 g, and 0.36 g, respectively. Root to shoot ratio increased from 0.33 to 0.63 ($p=0.18$) and only one Category 2 seedling produced fruit.

2.3.7 *Empetrum nigrum*

E. nigrum seedlings had on average 8.4 shoots, were 16.0 cm tall, had an RCD of 2.1, 10.0% brown leaves, and an RSR of 0.38. Root, shoot, and leaf NSC concentrations were 2.3% DW, 6.8% DW, and 11.8% DW, respectively (Table 2.3).

Seedlings looked healthy overall, aside from some browning of needles, which I used as the main measure for sorting seedlings into two categories (Appendix Figure A.7). Category 1 seedlings (89% of seedlings from the nursery) had no brown needles, whereas Category 2 seedlings (11%) had greater than 10% brown needles present on an individual plant.

Regardless of category, seedlings had low mortality, 10.0% in Category 1 and 12.5% in Category 2 (Figure 2.1). Leaf mass was the only growth measure that resulted in a significant difference between quality categories, with Category 1 seedlings producing 2.5 g of leaves and Category 2 producing just 1.2 g (p=0.011) (Table 2.4-2.5; Appendix Table A.2). Root and shoot growth were marginally different between categories. Category 1 seedlings produced 1.2 g of root mass and 1.4 g of shoot mass, whereas Category 2 seedlings produced only 0.70 g of root mass and 0.95 g of shoot mass (p=0.062; p=0.071). Averaging across categories, seedlings lost 0.14 shoots and increased in height and RCD by 6.0 cm and 0.22 mm, respectively. Percentage of brown leaves increased by 9.7% over the growing season. Root to shoot ratio increased from 0.41 to 0.66 (p=0.56) and no flowers or fruit were produced.

2.3.8 *Viburnum edule*

V. edule seedlings had on average 1.4 shoots, were 8.4 cm tall, had an RCD of 3.6 mm, and an RSR of 2.7. Root and shoot NSC concentrations were 11.7% DW and 12.7% DW, respectively (Table 2.3).

Seedlings looked robust and healthy overall, so I used height to separate them into two quality categories (Appendix Figure A.8). Category 1 seedlings (56% of seedlings from the nursery) were 8 cm or taller and Category 2 seedlings (44%) were shorter than 8 cm.

Regardless of category, seedlings had low mortality, 14.3% in Category 1 and 3.7% in Category 2 (Figure 2.1). Root collar diameter was only growth measure that was significantly different between quality categories (Table 2.4-2.5; Appendix Table A.2). Category 1 seedlings increased their RCD by 2.2 mm, whereas Category 2 seedlings increased by just 1.3 mm (p=0.056). No other growth measures resulted in significant differences between quality categories ($p \geq 0.139$). Number of shoots remained virtually the same as the initial measurements (1.4), height increased by 3.2 cm, and 1.6 g of leaves, 2.3 g of roots, and 0.55 g of shoots were produced. Root to shoot ratio increased from 2.8 to 3.8 (p=0.90) and this species did not produce any flowers or fruit.

2.3.9 *Linnaea borealis*

L. borealis seedlings had on average 3.9 runners that were 20.4 cm long and an RSR of 0.75. Root, shoot, and leaf NSC concentrations were 6.1% DW, 8.8% DW, and 18.0% DW, respectively (Table 2.3).

While sorting, I noticed the seedlings were attached to the plug either by the main root system or some small adventitious roots, likely due to the trailing and spreading nature of this species. I used this observation as well as overall size and whether the leaves and runners were green and healthy or dry and browning as the main criteria for separating seedlings into three quality categories (Appendix Figure A.9). Category 1 seedlings (21% of all seedlings from the nursery) had green leaves and its main runner originate in the plug and/or many adventitious roots holding the plant onto the plug. Category 2 seedlings (50%) were also green but had only some adventitious roots holding the plant onto the plug. Category 3 seedlings (29%) were brown and dry and had often only a small root attaching the plant and plug.

Overall mortality of the *L. borealis* seedlings was high, 44.4% in Category 1, 41.7% in Category 2, and 62.5% in Category 3 (Figure 2.1). Since only three Category 3 seedlings survived, this category was not considered in the analyses of the growth data. Growth measures that were significantly different between the two categories were shoot mass and leaf mass (Table 2.4-2.5; Appendix Table A.2). Category 1 seedlings decreased in shoot (-0.24 g) and leaf mass (-0.27 g) over the growing season, whereas Category 2 seedlings increased in shoot mass (0.056 g) and leaf mass (0.16 g) (p=0.009 and p=0.037, respectively). There was no difference between categories for the rest of the growth measures ($p \geq 0.19$). Averaging across both categories, seedlings lost (-0.40) runners, decreased in average runner length (-5.4 cm), and slightly increased in root mass (0.012 g). Root to shoot ratio increased from 0.76 to 1.1 and no flowers or fruit were produced.

2.4 Discussion

The separation of seedlings into somewhat arbitrary quality categories based on simple *initial* seedling morphological characteristics ultimately did not lead to improving our ability to predict future survival and growth. When separated into these relatively easily identifiable categories, there were very few examples that led to differences in growth and survival. Growing these seedlings in near optimal and non-limiting conditions might have played a significant role in these findings and has been suggested for other species (Landhäusser et al. 2012a). When we outplanted the same seedling stock using similar proportions of seedling quality characteristics onto field sites that varied widely in edaphic and environmental conditions, we saw much greater mortality (Chapter 3). Although we did not separate and follow the different quality seedling quality categories of individual seedlings in the field, specific combinations of site conditions clearly decreased seedling survival and growth (see Section 3.3 for more detail). Only obvious deficiencies such as poor plugfill and the incidence of mold were clear indicators of poor seedling quality and resulted in significantly reduced survival and growth, most notably for *C. canadensis* and *L. borealis*. Initially, it appeared that the increasing presence of mold resulted in higher mortality for *Cornus canadensis* seedlings; however, this was not the only species affected by mold during frozen storage. *V. vitis-idaea, L. groenlandicum, and E. nigrum* seedlings were also affected. The categories of these species responded in a similar, albeit less pronounced way. All three had slightly greater mortality in the categories that were affected with mold, but differences were small (*V. vitis-idaea*= 6.2%; *L. groenlandicum*= 13.8%; *E. nigrum*= 2.5% between categories), indicating that for *C. canadensis* seedlings, mold was more damaging; however, other factors could have also contributed to its higher mortality.

Storage mold is one of the main diseases affecting conifer seedlings in nurseries (Lilja et al. 2010), and evidently, the same is true for evergreen shrub seedlings. Storage molds are especially a concern for broadleaf evergreens (e.g., *C. canadensis*), as they often have much higher specific leaf area (cm^2/g) than smaller-leaved species such the ericaceous species used in this study. The primary energy source for these types of molds is the carbon-based compounds stored in plant leaves. The mold penetrates the leaf tissues and cells to access stored reserves (mostly soluble sugars and starch) (Schilling et al. 2020), which are extremely important for seedling establishment processes like initiating leaf area and new root growth after outplanting

(Chapin et al. 1990). In addition, *C. canadensis* leaves are in close proximity to the soil, making it difficult to ensure that seedlings are dry before lifting, packing, and freezing (Landis et al. 2010). Plants with high specific leaf areas are also more susceptible to desiccation and cold damage (Xu et al. 2020), which is a concern especially during the hardening phase where seedlings are gradually introduced to conditions similar to what they will experience upon outplanting. To add to this, it is likely difficult to identify when broadleaf evergreen species are fully dormant as they do not display the typical morphological signs of dormancy, such as setting bud or leaf senescence (Camm et al. 1994). If nursery seedlings are not dormant before being put into cold storage (i.e., early lifted), they would not have developed full hardiness (Lindqvist 2001; Weiser 1970), which can lead to a reduction in root growth initiation, stomatal conductance, vigor, and ultimately, outplanting success (Camm et al. 1994).

Linnaea borealis was the other species used in this study that had very poor survival. Since it did not show a significant incidence of mold, it appears that the (lack of) root system development in some of the plants played a significant role in this finding. Upon inspection, roughly 80% of *L. borealis* seedlings (Categories 2 and 3) were only attached to the plug by one or very few roots that appeared to be adventitious and originated from runners produced by this species. Due to its trailing growth habit, many of the lifted seedlings essentially lacked a plug altogether, likely the result of runners crossing empty but soil-filled cavities and establishing adventitious roots in them. In addition, Category 3 seedlings were also brown and often dry (but not moldy), indicating that they might have started dying prior to harvest and storage, perhaps due to the limited access to water because of the poor root connection and the potential damage inflicted by separating them from the blocks during packing. Regardless of category, roughly half of this species perished even when being grown under non-limiting conditions. My initial observations of loose plugs and poor root development appeared to be a good predictor, also indicating that growing this species as a container stock might not be a suitable method. The lack of root development observed in this species negates one of the main benefits of this stock type, which is to produce seedlings with well-developed root systems (Landis et al. 2010). Broadcast or direct seeding this species may be more successful as it appears trailing growth habits are not conducive to container seedling production.

For the remaining species, very few differences in survival or growth were observed among the subjective categories. Although many studies have found a positive relationship between initial seedling height, initial RCD, initial root mass, initial RSR, and subsequent growth (Close et al. 2005; Davis and Jacobs 2005; Grossnickle and MacDonald 2018; Haase 2008; Jacobs et al. 2005; Mattsson 1996) the results from this experiment did not reflect these findings. This is likely true because, first, most of these studies are based on seedlings that were subject to field conditions instead of relatively controlled, non-limiting conditions, like in this experiment. Outplanting sites often expose seedlings to stressful environmental and edaphic factors such as moisture stress, wind, frost, competition from other vegetation for space, light, nutrients, and water (Haase 2008). There appears to be some consensus in the literature that larger seedlings (i.e., increased stock size) typically perform better than smaller seedlings as they are more resistant to many of the site stressors listed previously (Haase et al. 2006; Sutherland and Day 1988), but this is not always the case (Johansson et al. 2015; Lamhamedi et al. 1998). Second, most of these positive relationships between seedling characteristics and increased growth have been observed in conifer seedlings, which differ from shrubs greatly in attributes like growth habit, and are much more uniform overall, so it is not surprising that measures that work for conifer seedlings cannot be readily applied to shrub seedlings. The results of this study show that in most species where height was a measure for separating seedlings into quality categories, the categories that were shorter initially actually increased in height more over the growing season than the categories that were taller initially. Although root collar diameter (RCD) was not used to assign categories as obtaining an accurate measurement is difficult on multi-stemmed species and species that have very small stem diameters such as *L. borealis* and *V. vitis-idaea*, only about half of the categories with larger initial RCDs increased in RCD more over the growing season than the categories with smaller initial RCDs. Another morphological characteristic that is commonly used to predict outplanting performance in tree seedlings is root to shoot ratio (RSR) (Grossnickle and MacDonald 2018; Mašková and Herben 2018). Although my seedlings were not selected based on RSR as it is a destructive measurement, it is interesting to observe how RSRs in these seedlings behaved between the different functional types (i.e., deciduous versus evergreen). All species included in this study increased their RSR over the course of the growing season even under non-limiting conditions. Overall, the deciduous species increased in RSR more substantially than the evergreen species, with *L. involucrata* doubling in RSR, and *V.*

myrtilloides, *S. albus*, and *V. edule* nearly doubling. This finding was reflected in the near-zero or negative height development observed in some species. It is possible that if the shrub seedlings started out with a higher RSR, they could potentially have supported greater increases in shoot growth over the 2020 growing season, which can give seedlings an advantage upon outplanting as they will photosynthesize more effectively and outcompete other vegetation due to their larger size (Grossnickle 2005), which I observed in the field portion of my study. This has been evidenced in deciduous Trembling Aspen (*P. tremuloides*), where seedlings with less shoot mass relative to root mass (high RSR) after the first growing season had high growth rates in the second growing season, which was also associated with high concentrations of root carbohydrate reserves (Martens et al. 2007; Schultz and Thompson 1990). In the nursery, seedlings can be encouraged to put more energy into growing roots instead of shoots by forcing them to terminate shoot growth early while maintaining green leaves during the first growth year. This can be achieved by adjusting cultural practices such as reducing nutrient availability, reducing day length, and/or using an artificial shoot growth inhibitor to induce early terminal bud set (Landhäusser et al. 2012b; Kelly et al. 2015).

This experiment provided me with an opportunity to observe the morphological variation of these shrub seedlings on a couple of different scales – within a species of a specific production year as well as comparing a species' morphological and physiological attributes between two different production years. In 2020, variation in most measured seedling characteristics was between 30-50%, which is a much wider range than what would be acceptable for commercial tree seedlings. Nurseries are contracted to grow tree seedlings under very tight morphological specifications with an allowed variance of only about 6-7%, often leading to a significant cull and the need to produce 10%+ oversow (Dumroese et al. 2009). Shrubs on the other hand can have a much wider range and complexity of growth habits and morphologies than trees, making the application of size specifications based on single stemmed trees more complex. More detailed research on the impacts of these characteristics under more stressful field conditions is needed in order to explore the importance of seedling characteristics in these species.

The results from this study also suggest that inter-annual variation in seedling stock production might play a role in the quality of a seedling. Since we obtained seedling stock of the same four species (*C. canadensis*, *L. involucrata*, *V. myrtilloides*, *V. vitis-idaea*) from the same nursery in 2019 and 2020, we were able to compare *initial* morphological and physiological attributes between two production years. *Lonicera involucrata* and *V. vitis-idaea* did not vary much in their morphological characteristics, but *C. canadensis* and *V. myrtilloides* both significantly differed in number of shoots, root mass, and shoot mass. Some of this variation in morphological characteristics could have been due to human error as a different crew of people measured the different stock years. Regardless of this possibility, the seedlings of these four species were similar in terms of morphology in 2019 and 2020. While the seedlings were morphologically similar between production years, all four species varied significantly in their NSC concentrations during the dormant season. Non-structural carbohydrate reserves are made via photosynthesis and stored in the different plant tissues. Reserves become depleted within a tissue when carbon from photosynthesis is insufficient for metabolic requirements (Smith et al. 2017). The different functional types (i.e., deciduous vs. evergreen) store NSCs differently. Deciduous species replenish their NSC reserves during the growing season, and they are depleted during winter dormancy and at the onset of spring growth, whereas evergreen species' reserves accumulate in the late winter and gradually decrease over the growing season (Smith et al. 2017). Therefore, since deciduous species make all their reserves during the growing season, the significantly different reserve concentrations observed between production years in these species could be the result of one production year being able to photosynthesize more often or for a longer duration than the other. For example, in 2019, *L. involucrata* seedlings had over twice the reserves in their roots and almost twice as much in their shoots than the 2020 seedlings. For evergreen species, their reserves are at their lowest when they go into storage, making the length of time these seedlings are in storage critical as they are not able to replenish their reserves during this time like they would in nature. In *C. canadensis* and *V. vitis-idaea*, the years that had significantly less reserves could have been left in storage for longer than the production year with more overall reserves. Unfortunately, and as mentioned earlier, a comparison of mortality between the two production years was not possible.

2.5 Conclusion

Ultimately, seedling morphological and physiological quality measures that are commonly used on tree seedings cannot be readily applied to shrubs. There were very few differences in survival and growth between the potential quality categories, though most species had high survival and performed well when grown under non-limiting conditions. The species that had exceptionally low survival had glaring deficiencies upon initial inspection, which I attribute to responding negatively to the method in which they were cultured and stored. Most notably, trailing species are not well suited to being grown in containers, and broadleaf evergreen species are particularly susceptible to cold stress/injury and storage molds. Therefore, to account for the large variation in shrub functional types, growth habit, phenology, and dormancy requirements, nurseries might have to adjust their protocols for certain species or groups of species. In addition, the different NSC reserve concentrations observed in this study indicate that even slight variations in nursery processes, such as increasing or decreasing the hardening or storage stages, can have profound and fast effects on seedling quality.

This experiment showed that seven out of nine of the species have the potential to perform well under non-stressful conditions. However, since these seedlings are predominantly produced for use in restoration and reclamation projects, understanding how they perform in more stressful site conditions is crucial and will be explored in Chapter 3 of this thesis.

Tables

Table 2.1: List of boreal shrub species supplied by Smoky Lake Forest Nursery in 2019 and 2020. The first alphanumeric code is the container size (first number: approximate diameter of each cavity (4 cm); second number: approximate depth of each cavity (12 cm/15 cm); letter: number of cavities per block (A=77, D=77)). The numbers with the plus sign are the seedling age (number of years the seedling was grown where it was sown + number of years the seedling was grown after transplant). All seedlings were dormant stock stored frozen at -3°C for 6 months.

Shrub Species	Stock year	Stock type and Size
Cornus canadensis (Bunchberry)	2019 & 2020	412A 1+0 Styroblock® Container Seedling
Lonicera involucrata (Bracted Honeysuckle)	2019 & 2020	412A 1+0 Styroblock® Container Seedling
Vaccinium myrtilloides (Blueberry)	2019 & 2020	412A 1+0 Styroblock® Container Seedling
Vaccinium vitis-idaea (Lingonberry)	2019 & 2020	412A 1+0 Styroblock® Container Seedling
Symphoricarpos albus (Snowberry)	2020	412A 3+0 Styroblock® Container Seedling
Ledum groenlandicum (Labrador Tea)	2020	415D 1+0 Styroblock® Container Seedling
Empetrum nigrum (Crowberry)	2020	412A 1+0 Styroblock® Container Seedling
Viburnum edule (Lowbush Cranberry)	2020	412A 3+0 Styroblock® Container Seedling
Linnaea borealis (Twinflower)	2020	412A 1+0 Styroblock® Container Seedling

Table 2.2: Mean (±SD) of initial 2019 and 2020 seedling characteristics averaged across categories. Different letters indicate a significant difference between stock years. Species with two quality categories have a sample size of 20 and species with three quality categories have a sample size of 30.

	C. canadensis (n=30)		*L. involucrata* (n=30)		*V. myrtilloides* (n=20)		*V. vitis-idaea* (n=20)	
	2019	2020	2019	2020	2019	2020	2019	2020
Number of Shoots	7.6 (2.8)*a*	16.2 (4.2)*b*	-	-	14.0 (5.7)*a*	4.7 (1.7)*b*	16.8 (9.6)*a*	11.2 (3.7)*a*
Number of Leaves	19.5 (13.9)*a*	79.0 (32.7)*b*	-	-	-	-	-	-
Height (cm)	-	-	33.3 (10.4)*a*	29.0 (12.9)*a*	20.1 (2.5)*a*	15.1 (4.1)*b*	9.9 (2.2)*a*	7.0 (1.5)*b*
RCD (mm)	-	-	3.7 (1.0)*a*	3.7 (1.0)*a*	2.4 (0.50)*a*	2.1 (0.98)*a*	1.0 (0.28)*a*	1.5 (0.56)*b*
Root Mass (g)	0.13 (0.14)*a*	0.52 (0.18)*b*	1.1 (0.76)*a*	0.84 (0.63)*a*	0.54 (0.17)*a*	0.22 (0.11)*b*	0.21 (0.16)*a*	0.10 (0.036)*a*
Shoot Mass (g)	0.81 (0.44)*a**	1.9 (0.70)*b*	1.2 (0.82)*a*	0.91 (0.77)*a*	1.2 (0.54)*a*	0.42 (0.28)*b*	0.32 (0.25)*a*	0.16 (0.051)*a*
Leaf Mass (g)	-	-	-	-	-	-	0.97 (0.67)*a*	0.45 (0.21)*a*
Root to Shoot Ratio	0.16 (0.32)*a*	0.27 (0.11)*a*	1.1 (0.39)*a*	1.0 (0.42)*a*	0.51 (0.20)*a*	0.70 (0.59)*a*	1.5 (2.5)*a*	0.68 (0.22)*a*
Root Total NSC %	3.0 (2.2)*a*	10.9 (3.9)*b*	22.6 (8.1)*a*	8.5 (3.9)*b*	4.3 (1.1)*a*	5.0 (1.2)*b*	3.2 (1.4)*a*	3.0 (0.91)*a*
Shoot Total NSC %	12.6 (3.4)*a*	8.4 (2.3)*b*	14.2 (3.9)*a*	9.3 (2.9)*b*	6.6 (1.2)*a*	8.3 (1.0)*b*	9.2 (1.6)*a*	6.1 (2.5)*b*
Leaf Total NSC %	-	-	-	-	-	-	17.5 (2.4)*a*	15.4 (1.8)*b*

* Leaves were collected with shoots

Table 2.3: Mean (±SD) of initial 2020 seedling characteristics averaged across categories.

	C. canadensis (n=30)	*L. involucrata* (n=30)	*V. myrtilloides* (n=30)	*V. vitis-idaea* (n=30)	*S. albus* (n=30)	*L. groenlandicum* (n=30)	*E. nigrum* (n=25)	*V. edule* (n=30)	*L. borealis* (n=14)
Number of Shoots/Runners	16.2 (4.2)	-	4.7 (1.7)	11.2 (3.7)	6.2 (2.7)	4.3 (1.9)	8.4 (2.0)	1.4 (0.68)	3.9 (1.4)
Number of Leaves	79.0 (32.7)	-	-	-					
Height/Runner Length (cm)	-	29.0 (12.9)	15.1 (4.1)	7.0 (1.5)	18.5 (6.7)	32.1 (5.9)	16.0 (1.9)	8.4 (2.0)	20.4 (4.5)
RCD (mm)	-	3.7 (1.0)	2.1 (0.98)	1.5 (0.56)	2.8 (0.59)	3.5 (0.65)	2.1 (0.41)	3.6 (0.65)	
Root Mass (g)	0.52 (0.18)	0.84 (0.63)	0.22 (1.1)	0.10 (0.036)	0.79 (0.40)	0.36 (0.13)	0.19 (0.061)	0.71 (0.28)	0.19 (0.10)
Shoot Mass (g)	1.9 (0.70)	0.51 (0.30)	0.42 (0.28)	0.16 (0.051)	0.60 (0.27)	1.1 (0.31)	0.53 (0.15)	0.28 (0.12)	0.30 (0.17)
Leaf Mass (g)	-	-	-	0.45 (0.21)		1.5 (0.51)	1.1 (0.32)		0.42 (0.40)
Root to Shoot Ratio	0.27 (0.11)	1.1 (0.42)	0.70 (0.59)	0.68 (0.22)	1.5 (0.54)	0.33 (0.075)	0.38 (0.11)	2.7 (1.2)	0.75 (0.38)
Live Crown Ratio (%)	-	-	-	-		80.9 (11.4)			
% Brown Leaves	-	-	-	-			10.0 (8.2)		
Bunch Size (cm^2)	-	-	-	-					
Root Total NSC %	10.9 (3.9)	8.5 (3.9)	5.0 (1.2)	3.0 (0.91)	13.4 (5.0)	5.2 (0.86)	2.3 (0.67)	11.7 (3.5)	6.1 (1.4)
Shoot Total NSC %	8.4 (2.3)	9.3 (2.9)	8.3 (1.0)	6.1 (2.5)	14.7 (2.3)	5.8 (1.5)	6.8 (0.87)	12.7 (1.5)	8.8 (2.0)
Leaf Total NSC %	-	-	-	15.4 (1.8)		6.8 (0.061)	11.8 (1.4)		18.0 (3.2)

Table 2.4: Mean (±SD) growth characteristics of seedlings grown in pots over the 2020 growing season averaged across categories. Asterisks represent significant differences between quality categories for that measure (data displayed in Table 2.6).

	C. canadensis (n=4)	*L. involucrata* (n=10)	*V. myrtilloides* (n=9)	*V. vitis-idaea* (n=10)	*S. albus* (n=6)	*L. groenlandicum* (n=9)	*E. nigrum* (n=7)	*V. edule* (n=10)	*L. borealis* (*n=5*)
Change in Number of Shoots/Runners	-5.0 (5.0)	1.6 (1.5)*	2.9 (0.75)	1.7 (4.8)	0.0 (2.9)	-1.0 (2.5)	-0.14 (1.9)	0.05 (0.57)	-0.40 (2.6)
Change in Height/ Runner Length (cm)	-	2.6 (4.9)	-0.67 (0.76)	0.31 (1.3)*	0.33 (5.0)	-0.75 (6.5)	6.0 (3.0)	3.2 (4.6)	-5.4 (8.9)
Change in RCD (mm)	-	3.1 (1.9)	1.5 (0.80)	-	2.5 (2.9)	2.0 (1.3)	0.22 (0.62)	1.8 (0.94)*	-
Change in Bunch Size (cm^2)	0.7 (200.3)	-	-	181.3 (72.2)*	-	-	-	-	-
Change in Root Mass (g)	-0.14 (0.18)	5.3 (2.0)	0.37 (0.32)*	0.28 (0.18)	2.3 (1.1)*	1.0 (0.45)	0.95 (0.44)	2.3 (1.3)	0.01 (0.11)
Change in Shoot Mass (g)	-1.2 (0.41)	2.0 (1.1)*	0.16 (0.20)	0.14 (0.12)	1.2 (0.73)*	0.73 (0.86)	1.2 (0.46)	0.55 (0.35)	-0.09 (0.14)*
Final Leaf Mass/ Change in Leaf Mass (g)	-	3.0 (1.2)	0.47 (0.22)	0.62 (0.42)	2.3 (0.91)*	0.36 (0.88)	1.9 (0.85)*	1.6 (0.73)	-0.06 (0.27)*
Change in Root to Shoot Ratio	0.15 (0.15)	1.4 (0.60)	0.46 (0.52)	0.89 (1.4)	0.76 (1.3)	0.30 (0.17)	0.25 (0.19)	0.99 (0.96)	0.36 (0.57)
Change in Live Crown Ratio (%)	-	-	-	-	-	2.5 (15.7)	-	-	-
Change in % Brown Leaves	-	-	-	-	-	-	9.7 (12.3)	-	-
Change in Number of Leaves	-10.3 (37.3)	-	-	-	-	-	-	-	-

Table 2.5: Mean (±SD) growth characteristics of seedlings grown in pots over the 2020 growing season that resulted in significant differences between categories.

	L. involucrata (n=10)		*V. myrtilloides* (n=9)		*V. vitis-idaea* (n=10)		*S. albus* (n=6)		*E. nigrum* (n=7)		*V. edule* (n=10)		*L.borealis* (n=5)	
	Category 1	Category 2	Category 1	Category 2	Category 1	Category 2	Category 1	Category 2	Category 1	Category 2	Category 1	Category 2	Category 1	Category 2
Change in Number of Shoots/Runners	0.6 (1.3)*a*	2.6 (1.7)*b*	-	-	-	-	-	-	-	-	-	-	-	-
Change in Height/Runner Length (cm)	-	-	-	-	1.1 (1.1)*a*	-0.48 (1.4)*b*	-	-	-	-	-	-	-	-
Change in RCD (mm)	-	-	-	-	-	-	-	-	-	-	2.2 (1.1)*a*	1.3 (0.78)*b*	-	-
Change in Bunch Size (cm^2)	-	-	-	-	222.5 (75.2)*a*	140.1 (69.1)*b*	-	-	-	-	-	-	-	-
Change in Root Mass (g)	-	-	0.60 (0.49)*a*	0.14 (0.15)*b*	-	-	3.2 (1.6)*a*	1.4 (0.52)*b*	-	-	-	-	-	-
Change in Shoot Mass (g)	2.5 (1.4)*a*	1.4 (0.81)*b*	-	-	-	-	1.8 (1.0)*a*	0.60 (0.46)*b*	-	-	-	-	-0.24 (0.12)*a*	0.056 (0.15)*b*
Final Leaf Mass/Change in Leaf Mass (g)	-	-	0.70 (0.28)*a*	0.24 (0.16)*b*	-	-	3.3 (1.4)*a*	1.3 (0.41)*b*	2.5 (0.98)*a*	1.2 (0.72)*b*	-	-	-0.27 (0.26)*a*	0.16 (0.28)*b*

Figures

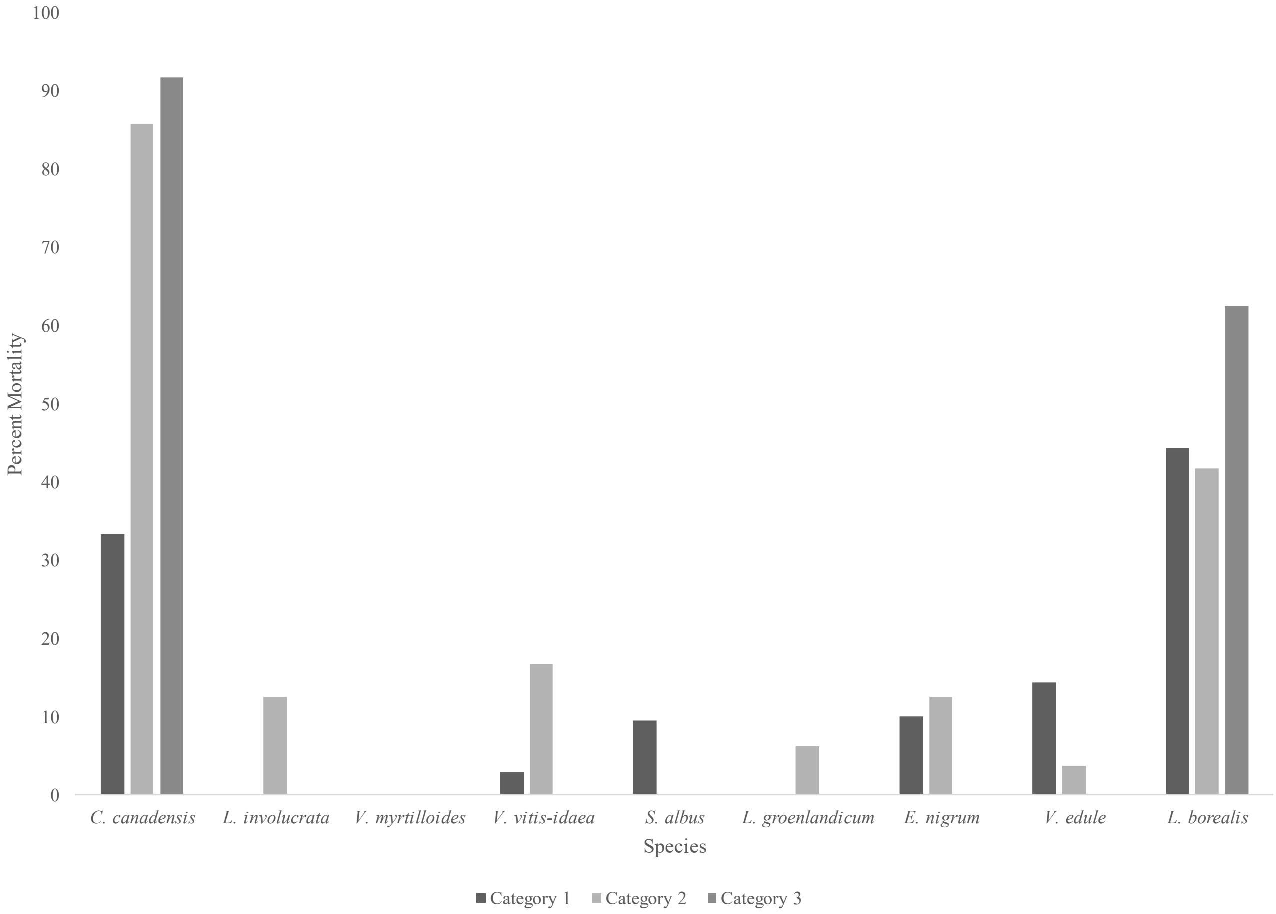

Figure 2.1: Average mortality (%) of seedlings grown in pots over the 2020 growing season.

Chapter 3: Evaluating Outplanting Success of Nursery-Grown Boreal Shrub Seedlings on a Range of Boreal Reclamation Sites

3.1 Introduction

Understories are integral components of forest systems. They contribute greatly to overall biodiversity and nutrient cycles (Gilliam 2007; Nilsson and Wardle 2005), as well as provide habitat for many macro- and micro-organisms (Bokhorst et al. 2014) such as soil invertebrates, amphibians, reptiles, and mammals. In the boreal forest, the understory contains up to approximately 77 species compared to a maximum of six tree species (Hart and Chen 2006). Although understory vegetation contributes relatively little to overall forest biomass, it substantially adds to the annual litterfall returned to the soil, where nitrogen, phosphorous, and calcium are released from plant litter through decomposition and become accessible for plants and microbial uptake (Krishna and Mohan 2017). Understory plants are also extremely important to bird populations as they provide movement pathways, nesting sites, and shelter. Birds play key roles in forest ecosystems as pollinators, seed dispersers, and helping to control herbivore insect populations (Fontúrbel et al. 2021).

Forest restoration efforts are being undertaken across many forest types all over the globe; however, most documented reforestation projects focus on the establishment of trees and tend to either ignore or leave other forest layers to passive processes (Aerts and Honnay 2011; Forbes et al. 2021; Harris et al. 2012; Morrison and Lindell 2011; Ssekuubwa et al. 2019). One unique restoration challenge faced today is the observed lack of understory development in post-mining reclaimed forests. Even upwards of thirty years after site construction, many understories in these reconstructed forests are missing common plants, especially woody species (Dhar et al. 2020; Farnden 2021). The initial expectation was that seeds and propagules from nearby intact forests would colonize and begin passive regeneration as early as the first season after substrate placement (Dhar et al. 2018). However, there is a growing body of evidence suggesting that this is not achievable for severe disturbances such as open-pit mining and temporary drilling pads that require landscape and soil reconstruction (Ash et al. 1994; Handel et al. 1994; Jones and Landhäusser 2017). To be successful, passive understory restoration involves very specific requirements that need to be met (Chazdon 2008; Errington and Pinno 2015). Propagules that are either contained in the cover soil or dispersed from nearby plants are the main sources for vegetation recolonization. The viability of soil propagules is dependent on the severity of soil disturbance, as well as its salvage and storage conditions (Moore and Wein 1977; Qi and Scarratt 1998), while plant-dispersed propagules depend on the maximum distance from a source, which is determined by the dispersal ecology of the species. Encroachment through vegetative propagules primarily only works for small disturbances that are within short distances to an intact vegetation (Jones and Landhäusser 2017; Rydgren et al. 1998). Therefore, the aerial extent, severity of disturbance, and the availability of propagules influence the conditions that should be in place when understories are left to passively regenerate. Actively restoring (i.e., human intervention through seeding or planting vegetation, weeding, burning, and/or thinning (Morrison and Lindell 2011) understories could be an option, though it is not currently practiced, likely because it is considered expensive, native propagules are often unavailable or their dispersal ecology is unknown (Macdonald et al. 2015; Smreciu et al. 2013), and we currently do not have the knowledge to develop protocols for the successful propagation of many species (Macdonald et al. 2015). However, this is starting to change as some tree nurseries are producing seedlings of understory species on a commercial scale for use in forest restoration (see previous Chapter 2). Another major issue is that, at this time, there is very little information available on how, when, and where to integrate these understory species into revegetation efforts. Planting nursery-grown seedlings and direct seeding are the most frequently used active restoration methods in forest restoration (Brancalion et al. 2016; Grossnickle and MacDonald 2018; Palma and Laurance 2015). Although more expensive, planting seedlings typically yields better results (than direct seeding) in terms of increased survivorship and helping to accelerate the structural recovery of forests. Choosing a revegetation method should also take into account other variables associated with active understory restoration such as timing (e.g., open site or closed canopy), edaphic conditions (e.g., soil moisture and fertility), and potential limiting factors.

Since the objective of reclaiming mined areas to self-sustaining ecosystems is relatively new, there are still some unknowns surrounding how site conditions such as landform, when and how it was built, the materials that were used in its reconstruction, and the tree species that were used in revegetation impact the performance of understory plants, however a few localized long-term observational studies have provided valuable insight (Farnden 2021). Current restoration guidance documents (developed for tree seedlings) such as the Target Plant Concept list site conditions such as soil type, local climate, existing vegetation, and comparison to reference sites and potential limiting factors like competing vegetation, soil moisture, and soil temperature (Dumroese et al. 2016) as main predictors of revegetation success (Jacobs et al. 2015). Reclamation site conditions can range from freshly placed soil to decades-old sites with well-established trees which, in some cases, have achieved canopy closure. When dealing with sites with closed canopy conditions, it is important to consider the type, structure, and density of the forest canopy as these determine the amount of sunlight that reaches the understory, which is commonly considered the primary limiting resource that determines understory vegetation cover and/or richness (Barbier et al. 2008; Hofmeister et al. 2009; Jobidon et al. 2004; Wagner et al. 2011). In terms of edaphic conditions, some studies have shown that restoration age has a positive impact on improving water-holding capacity and soil fertility (i.e., soil bulk density, soil organic carbon, total nitrogen, and available nitrogen, phosphorus, and potassium) (Yuan et al. 2019). Some common limiting factors associated with reclamation sites include low-quality rooting media, pre-existing competing vegetation, and herbivory (Riikonen and Luoranen 2018). Because they are already established, the ability to mitigate limiting factors via soil amendments, chemical or mechanical competition control, decompaction, or others is severely diminished. Therefore, a successful revegetation plan should consider site conditions early in the process in order to mitigate as many limiting factors as possible by choosing the appropriate revegetation materials for a given site or by preparing the site in a way that is conducive to the chosen revegetation method.

This field study aims to provide insight into the impact of environmental and edaphic reclamation site conditions on the survival and growth of nine commercially grown boreal forest shrub seedlings (Table 2.1). Shrubs were selected for this experiment because these species are ecologically and culturally important and woody understory vegetation is particularly absent in the reclaimed forests of northern Alberta. To achieve this, I investigated whether soil physical and chemical parameters, vegetation competition, and degree of canopy closure can be related to seedling mortality and growth measures such as height, root collar diameter, and bunch size/lateral spread. Through the results of this research, I hope to 1) identify relationships between shrub seedling performance and site conditions and 2) explore the impact of different temporal stages of reclamation for understory species into planting efforts. My findings may be able to provide guidance for integrating understory species into large-scale restoration forest restoration projects.

3.2 Materials and Methods

3.2.1 Research Area

Most of the research sites (six different locations) were established on Syncrude's Mildred Lake oil sands lease approximately 42 km north of Fort McMurray, Alberta, Canada (57°02'N, 111°35'W). The lease area is located in the central mixedwood subregion of the boreal forest natural region (Natural Regions Committee 2006), which is composed of a mosaic of upland and lowland forests and covers one quarter of the province. On finer textured Luvisolic soils, upland forests are typically mixedwoods dominated by Trembling Aspen (*Populus tremuloides* Michx.) and White Spruce (*Picea glauca* Moench.), while on coarser and well drained Brunisolic soils, Jack Pine (*Pinus banksiana* Lamb.) dominates (Soil Classification Working Group 1998, Natural Resources Committee 2006). Lowland areas are generally either fens or bogs dominated by Black Spruce (*Picea mariana* Mill.) and Tamarack (*Larix laricina* (Du Roi) K. Koch) that have developed on poorly drained Organic soils (Soil Classification Working Group 1998, Natural Resources Committee 2006). This subregion is characterized by cool summers, and long, cold winters, with the majority of precipitation falling during the growing season from May to September (Natural Regions Committee 2006). The average daily temperature for the region is 1°C and average annual precipitation is 418 mm (1987–2010 climate normals, Fort McMurray A* Station, 56°39'N, 111°13'W; Government of Canada 2021).

Additional to the Syncrude research area, this experiment had one reference site located in Thorhild County (54°17'N, 112°46'W), approximately 120 km northeast of Edmonton, Alberta, Canada, which served as an analog of a mature boreal mixedwood forest dominated by Trembling Aspen. This site is located in the dry mixedwood natural subregion of the boreal forest natural region (Natural Regions Committee 2006) and features slightly warmer temperatures and lower annual precipitation levels. Upland soils are Gray and Dark Gray Luvisols and Gleysols and Organic soils in low lying areas (Soil Classification Working Group 1998, Natural Resources Committee 2006). This region has a daily average temperature of 1.6˚C and average annual precipitation is 340 mm (1987–2010 climate normals, Redwater station, 54°00'N, 113°11'W; Government of Canada 2021). In order to evaluate seedling survival and growth in the absence of competition and an overstory, we also planted an open field at the Crop Diversification Center North in northeast Edmonton, Alberta (53°38'N 113°21'W).

3.2.2 Site Construction and Characterization

The six sites on the Syncrude lease were chosen based on their reclamation age (time since planting), overstory species composition, degree of canopy closure, level of competing vegetation, and edaphic conditions. The oldest site included in this study is a 30-year-old reclamation site (SH) that was planted in 1992 with a 3:1 mix of Trembling Aspen (*P. tremuloides*) and White Spruce (*P. glauca*) at a density of 2000 stems per hectare (30-year mixedwood; Appendix Figure A.10). Mature trees on the site were on average 10.5 m tall, while White Spruce saplings and Trembling Aspen suckers were on average 1.6 m tall. Tree canopy leaf area index (LAI; the leaf area (m^2) of the canopy relative to 1 m^2 of ground area) was 4.3 in August 2021, with an understory that was dominated by forbs (predominantly *Astragalus canadensis*, but also *Fragaria vesca* (Strawberry), *Lotus corniculatus* (Birds-foot Trefoil), *Achillea millefolium* (Yarrow), *Taraxacum officinale* (Dandelion), *Lathyrus ochroleucus* (Creamy Peavine), *Aster* sp., and *Melilotus* sp. (Sweet Clover)) while about one third of the ground was void of vegetation (Table A.3). Reconstructed soil at this site is a 100 cm layer of salvaged peat mineral mix (i.e., pre-mining peat deposits that were salvaged and mixed with underlying mineral soil) that was placed over saline sodic overburden in winter 1990-1991.

A second older reclamation site (30-year jack pine (JP); Appendix Figure A.11) was chosen largely based on overstory composition and its more rapidly drained soil conditions. This site was planted in 1992 with Jack Pine (*P. banksiana*) at a density of 3500 stems per hectare. The trees were on average 7.0 m tall, and LAI in August 2021 was 4.5. The dominant ground cover was forbs (predominantly *Astragalus canadensis*, but also *Solidago* sp. (Goldenrod), *Fragaria vesca* (Strawberry), *Lotus corniculatus* (Birds-foot Trefoil), *Achillea millefolium* (Yarrow), *Taraxacum officinale* (Dandelion), *Melilotus* sp. (Sweet Clover), and *Aster* sp.), and approximately one third was void of vegetation. The reconstructed soil at this site is 50 cm peat mineral mix that was placed over tailings sand in winter 1990-1991.

A 16-year-old reclamation site was chosen (16-year spruce/grass (MLSB); Appendix Figure A.12) that was planted in 2006 with an initial 1:1 mix of Trembling Aspen, White Spruce, and 2% White Birch (*Betula papyrifera* Marsh.) at a density of 2500 stems per hectare. Most of the Trembling Aspen died and only White Spruce trees survived that are now on average 2.3 m tall. Leaf area index was 2.2 and ground cover was mostly made up of graminoid species (i.e., grasses) and forbs (predominantly *Astragalus canadensis*, but also *Medicago sativa* (Alfalfa), *Cirsium arvense* (Creeping Thistle), *Crepis sp*. (Hawksbeard), *Solidago* sp. (Goldenrod), *Lotus corniculatus* (Birds-foot Trefoil), and to a much lesser extent, *Achillea millefolium* (Yarrow), *Taraxacum officinale* (Dandelion), *Fragaria vesca* (Strawberry), *Melilotus* sp. (Sweet Clover), *Sonchus* sp. (Sowthistle), and *Aster* sp.). This site's reconstructed soil is 15 cm of peat cover soil and 20 cm of clay subsoil that was placed over tailings sand in 2004.

An 11-year-old reclamation site that was not planted or seeded (11-year sandy site (SHF); Appendix Figure A.13) was selected for its canopy openness and to provide a well-drained contrast soil. Vegetation was predominantly a range of graminoid species, and the reconstructed soil was made up of a 30 cm layer of coarse salvaged forest floor mixture (i.e., FFM, salvage soil material containing the upper organic and mineral horizons of a forest soil) and a 20 cm layer of a sandy subsoil that was placed on top of tailings sand in 2010. The surface soil is comprised of 92.9% sand.

Two young reclamation sites that were planted one year after soil reconstruction in 2019 and in 2020 (new 2019 reclamation site and new 2020 reclamation site, respectively; Appendix Figure A.14-A.15) with a 1:1 mix of Trembling Aspen, White Spruce, and 10% White Birch seedlings at a density of 2000 stems per hectare were selected to observe how a site with these conditions may develop over time. At time of planting, there was no tree canopy cover and most of the site was void of vegetation; however, the 2019 site had significant graminoid cover in 2021. The reconstructed soil at these sites is 35 cm of peat cover soil and 20 cm of clay subsoil that was placed in winter 2018 (new 2019 reclamation site) and 2019 (new 2020 reclamation site). The reclamation substrate the soils were placed on is tailings sand over top of composite tailings. The surface soil was slightly different between the two sites, where the new 2020 reclamation site had a higher proportion of sand, less clay and silt, as well as lower total organic carbon and nitrogen.

The soil at the natural mature mixedwood forest (Appendix Figure A.16) included in this study is a Grey Wooded Orthic Luvisol, which to a depth of 10 cm is comprised of 61.4% sand, 26.8% silt, and 11.8% clay, a bulk density of 0.55 g/cm^3, 12.9% TOC, an EC of 137.2 µS/cm, and a pH of 6.1. The overstory is comprised of approximately 20 m tall 80-year-old Trembling Aspen and the natural understory was predominantly made up of shrubs (*Rosa acicularis* (Prickly Rose), *Symphoricarpos albus* (Snowberry), *Shepherdia canadensis* (Canada Buffaloberry), *Lonicera involucrata* (Bracted Honeysuckle), *Rubus idaeus* (Raspberry), *Amelanchier alnifolia* (Saskatoon), *Ledum groenlandicum* (Labrador Tea), *Viburnum edule* (Lowbush Cranberry), and *Cornus sericea* (Red Osier Dogwood)). Finally, the open field site (Appendix Figure A.17) is an agricultural field with a mineral surface soil comprised of 67.7% sand, 16.2% silt, 16.0% clay. There were no trees or competing vegetation on this site, as seedlings were planted in plastic mulch.

3.2.3 Experimental Set Up and Seedling Measurements

The shrub seedlings used for this study were dormant frozen stored container seedlings (at -3°C for 6 months), commercially grown from open-pollinated seed sources near Fort McMurray, Alberta (57°02’N 111°28'W). Seedling stock was grown at Smoky Lake Forest Nursery (54°05'N 112°14'W), AB, Canada, and was the same seedling material as previously described in Chapter 2 of this thesis (Table 2.1). The seedlings used in this experiment were sorted into their respective quality categories using the same method as described in Chapter 2 (see Section 2.2.1); however, while seedlings assigned to each plot had similar proportions of quality characteristics, I did not keep track of individual seedlings in this study. Ensuring that the proportions of quality categories were equally represented on each site and in each planted plot was important to reduce the risk of seedling quality variation being a potential confounding factor in this study. There was also the possibility of a planting crew effect as in 2019, our team was responsible for planting all sites, while in 2020, due to COVID-19 travel restrictions, a commercial planting company planted the seedlings. Regardless, we boxed up equal numbers of seedling bundles for each site (with the same proportion of quality categories) and they were delivered by truck to Syncrude the following day. Our team only planted the natural mature mixedwood forest and open field in 2020.

For both planting years, five independent plots (at least 20 m apart) were selected at each site and planted with an equal number of individual seedlings (subsample of 8) of the same species. In the spring of 2019, at all sites except the open field (see below), 4 m × 4 m plots were set up, and seedlings (total 64) were randomly planted in eight rows in a 50 × 50 cm grid. In both 2019 and 2020, seedlings at the open field were randomly planted into rows covered with plastic (polyethylene) mulch to reduce competition pressure. In spring of 2020, the plot set up at Syncrude and the natural mature mixedwood forest deviated slightly from the 2019 protocol. In 2020, all individuals of the same species were planted in the same row in a plot. Regardless, plot conditions were uniform, and seedlings were still planted in a 50 × 50 cm grid. After planting, initial morphological characteristics were measured to monitor the survival and growth response of each species to the different site conditions they were planted into. For sites planted in 2019, growth and mortality data for all three growing seasons are available (2019, 2020, and 2021), and two growing seasons of data are available for sites planted in 2020 (2020 and 2021). As we did not plant the reclamation sites in 2020, we were not able to measure initial seedling sizes, and therefore only have growth data for the 2021 growing season. Mortality was unaffected by this, as we assume all seedlings were alive in 2020 upon planting.

3.2.4 Site Environmental/Edaphic Measures

At all five plots at each site, monitoring systems were installed to record rooting zone water dynamics in each year of the experiment. Two types of soil sensors were used to collect soil temperature, soil water potential, and soil volumetric water content (VWC) data: 1) 5TM sensors were installed at a soil depth of 10 cm and were used to monitor soil moisture and temperature, and 2) MPS2 sensors were installed at a soil depth of 10 cm to monitor soil water potential. Daily means from May through September were averaged to determine the average growing season soil temperature and VWC (Table A.4). Weather data for the Syncrude study area such as average growing season precipitation and average minimum and maximum air temperature (Figure 3.5-3.7) for each growing season of the experiment (2019-2021) were downloaded from the Alberta Climate Information Service online interactive Historical Weather Data Viewer.

In June 2021, grab samples of the surface soil (0-10 cm depth) and bulk density samples were taken at the center of each plot at all sites. All grab samples were air dried for one week and prepared for analysis by grinding them down to a fine, homogenized powder (>2 mm) using a TissueLyser bead mill (QIAGEN). Soil samples were analyzed for texture, organic matter content (TOC), salinity characteristics, and plant available nutrients. Texture analysis was performed on a Beckman Coulter LS 13320 Laser Particle Size Analyzer, total organic carbon was measured using the dry combustion method (ThermoScientific, Flash 2000 Organic Elemental Analyzer), pH and electrical conductivity were measured on extracted soil water with a Fisher AR 20 pH/EC Meter, and NH_4-N, P04-P, and K^+ were measured via anions using colorimetric methods (Thermo iCAP6300 Duo ICP-OES; ThermoFisher Gallery Beermaster Plus (Natural Resources Analytical Laboratory (NRAL), University of Alberta)). Bulk density samples were collected by hammering a 331.2 cm^3 metal cylinder into the soil to ensure a consistent and precise volume of soil was collected. Samples were dried in a 70°C oven for 72 hours before being weighed and divided by the volume of the collection cylinder.

3.2.5 Statistical Analysis

All analyses were performed using R software, version 4.0.2, 64-bit (R Core Team 2022). Assumptions of normality and homoscedasticity were tested using the Shapiro-Wilks test with the shapiro.test function (version 4.0.2; R Core Team 2022) and the Levene's test with the leveneTest function from the car package (version 3.1-0). First, two-way Analysis of Variances (ANOVA) were run to identify any species by site interactions. Next, one-way ANOVAs were run to detect differences in species' mortality and growth characteristics among sites. Sites planted in 2019 and sites planted in 2020 were analyzed separately as the seedlings were from different stock years and cannot be directly compared. When statistically significant effects were found at $\alpha=0.05$, post-hoc pairwise comparisons tests were conducted using Tukey's honest significant difference method. To identify differences in seedling response (mortality and growth characteristics) over time, two-way repeated measure ANOVAs were used. If significant site x time interactions were found for a seedling response variable, one-way models were run for all treatment combinations. Next, post-hoc pairwise comparisons (pairwise paired t-test function of the rstatix package) were run using a Bonferroni adjusted α to determine which site x measuring period interactions were significantly different.

In order to descriptively observe differences among site environmental and edaphic variables, I produced a non-metric multidimensional scaling (NMDS) ordination using the metaMDS function from the vegan package of R software. The site variables were scaled/normalized using the Euclidean distance method from the ecodist package.

3.3 Results

3.3.1 Site Environmental and Edaphic Conditions

The youngest reclamation sites (new 2019/2020 reclamation) can be characterized by having the highest electrical conductivity (EC), total organic carbon (TOC), nitrogen (NH4-N), as well as the highest proportion of bare ground out of all reclamation sites (all $p \leq 0.001$) (Table 3.1; Figure 3.1). The 11-year sandy site and the 16-year spruce/grass site had the highest proportion of grass (58.0% and 35.9%, respectively, compared to an average of 6.2% at all other sites), and their soils were predominantly composed of sand (both $p \leq 0.001$). The 30-year mixedwood site also had a relatively high occurrence of forbs (24.7%), and a high leaf area index (LAI) (4.3 compared to 0.93 average) (all $p \leq 0.001$). The 30-year jack pine site also had a high LAI (4.5 compared to 0.90) and high proportion of clay in its soil (37.8% compared to 12.9% average) (both $p \leq 0.001$). The natural mature mixedwood forest can be characterized by having a high proportion of shrubs (29.5% compared to 3.9% average) ($p \leq 0.001$), and the open field had a slightly higher bulk density compared to the rest of the sites (1.3 g/cm^3 compared to 0.80 g/cm^3 average) ($p \leq 0.001$).

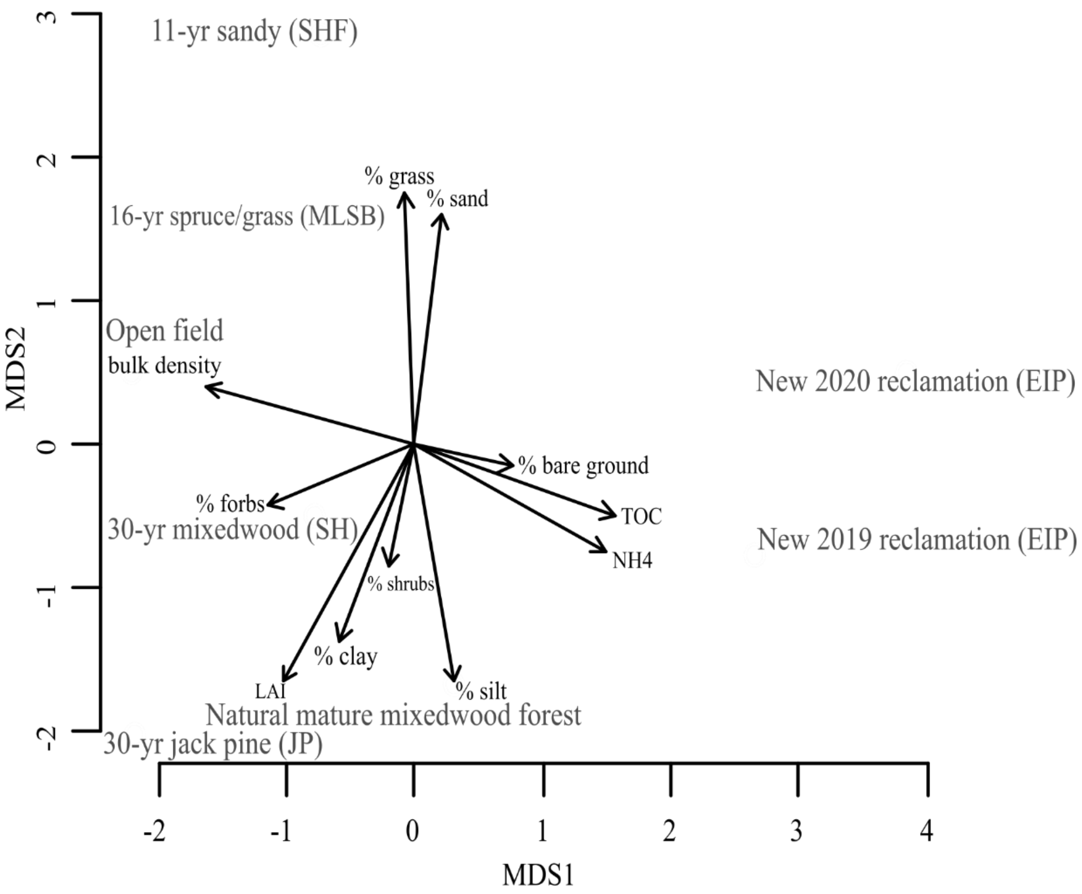

Figure 3.1: Non-metric multidimensional scaling (NMDS) ordination showing each site's environmental and edaphic composition using Euclidean distance, 500 random restarts, and two axes. A solution was reached after 20 iterations (Stress=0.06).

At the reclamation sites, total precipitation was 300.2 mm over the 2019 growing season, 406.8 mm over the 2020 growing season, and just 211.3 mm over the 2021 growing season (Figure 3.2). Average maximum air temperature during the 2019 growing season was 19.1 (°C), 19.3 (°C) in 2020, and 21.2 (°C) in 2021. Average minimum air temperature was 7.6 (°C) in 2019, 8.2 (°C) in 2020, and 8.9 (°C) in 2021. Soil temperature (°C), soil water potential (kPa), and soil volumetric water content (VWC) (m^3/m^3) followed a similar pattern (Table 3.2)

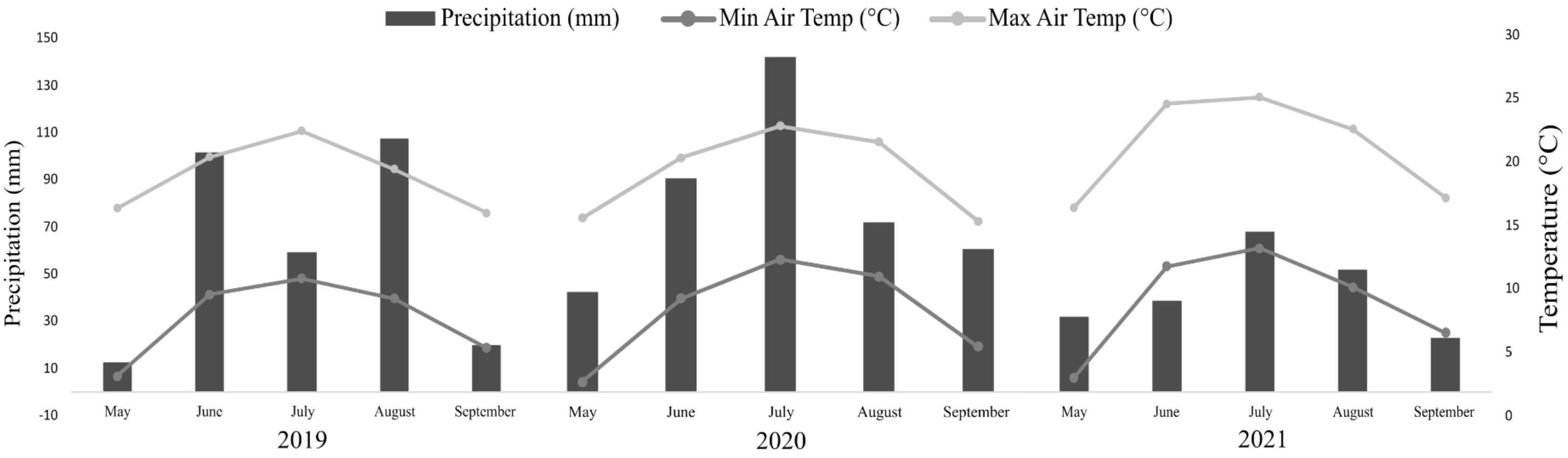

Figure 3.2: Monthly precipitation, average maximum and minimum air temperature at the Syncrude reclamation sites.

3.3.2 Site Differences in Seedling Mortality Across All Species

Across all species and planting years, seedlings survived and grew best on the youngest reclamation sites (new 2019/2020 reclamation sites). Conversely, two sites in particular exhibited the highest overall seedling mortality – the 16-year spruce/grass site (70.7%) and the 11-year sandy site (82.2%). For the seedlings planted in 2019, overall mortality was highest at the 16-year spruce/grass site for all species (*L. involucrata*, *V. myrtilloides*, and *V. vitis idaea*). At this site, *L. involucrata* experienced 50.8% morality compared to an average of 12.9% at all other sites (Figure 3.3; $p \leq 0.001$), *V. myrtilloides* seedlings had 78.2% mortality compared to an average of 22.6% at all other sites (Figure 3.4; $p \leq 0.001$), and *V. vitis-idaea* seedlings had 62.5% mortality compared to an average of 19.6% at the other sites (Figure 3.5; $p \leq 0.001$). *V. vitis-idaea* also experienced high mortality at the open field site (59.3%).

The 11-year sandy site had the highest overall mortality of seedlings planted in 2020; however, mortality was similar between this site and the 16-year spruce/grass site for *V. myrtilloides*, *S. albus* (Figure 3.6), *L. groenlandicum* (Figure 3.7), and to lesser extent, *V. vitis-idaea*. *L. involucrata* seedlings experienced 95.0% mortality at the 11-year sandy site and 76.3% at the 16-year spruce/grass site compared to an average of 23.5% at the other sites ($p \leq 0.001$). *V. myrtilloides* seedlings had the highest mortality at the 11-year sandy site (74.3%) and the 16-year spruce/grass site (73.8%) compared to an average of 21.2% at the other sites ($p \leq 0.001$). *V. vitis-idaea* seedlings had the highest mortality at the 11-year sandy site (95.0%) and 16-year spruce/grass site (88.8%) compared to an average mortality of 38.0% ($p \leq 0.001$). *S. albus* seedlings had 48.8% mortality at both the 16-year spruce/grass site and the 11-year sandy site compared to an average of 9.0% at the rest of the sites ($p \leq 0.001$). There was 100.0% mortality of *L. groenlandicum* seedlings at both the 16-year spruce/grass site and 11-year sandy site, whereas average mortality at the rest of the sites was 45.1% ($p \leq 0.001$). Finally, *E. nigrum* (Figure 3.8) seedlings experienced 80.0% mortality at the 11-year sandy site and 67.5% at the reclaimed 30-year jack pine site compared to 21.3% mortality at the new 2020 reclamation site ($p \leq 0.001$). *V. edule* (Figure 3.9) was not planted at the 11-year sandy site or the 16-year spruce/grass site.

It is worth noting that over the 2021 growing season, there was a substantial increase in seedling mortality for all species at the 11-year sandy site, where *L. involucrata* seedling mortality increased by 58.7% ($p=0.001$), *V. myrtilloides* increased by 54.4% ($p=0.001$), *V. vitis-idaea* increased by 85.0% ($p \leq 0.001$), *S. albus* increased by 35.0% ($p=0.01$), and *E. nigrum* increased by 41.2% ($p=0.005$). *L. groenlandicum* seedling mortality significantly increased at both the 11-year sandy site (51.2%) and the 16-year spruce/grass site (56.3%) over the 2021 growing season ($p=0.003$ and $p=0.002$).

Two sites that were planted in both 2019 and 2020 had higher overall mortality in the 2020 plant, although differences were not statistically significant. These include the 16-year spruce/grass and the natural mature mixedwood forest, where seedling mortality was 14% and 7% higher (respectively) in the 2020 plant compared to the 2019 plant ($p=0.38$ and $p=1.0$). Interestingly, the overall seedling mortality in the reclaimed 30-year mixedwood site was higher after the 2019 plant (26.6%) compared to the 2020 plant (10.4%) ($p=0.90$). Overall mortality at the open field site was comparable between each of the planting years (36.0%) ($p=1.0$).

3.3.3 *Cornus canadensis* and *Linnaea borealis*

Across all sites and planting years, *C. canadensis* seedlings experienced nearly 100.0% mortality. Additionally, although approximately half of *L. borealis* seedlings survived at the new 2020 reclamation site, the high mortality at all other sites made statistical comparison among sites impossible. As a result, the ANOVAs do not include these two species.

3.3.4 *Lonicera involucrata*

Irrespective of planting year, *L. involucrata* seedlings performed the best on the new 2019/2020 reclamation sites and at the open field, where overall seedling mortality was 11.9% and 14.9%, respectively (Figure 3.1). Overall mortality (averaging across planting years) at the reclaimed 30-year mixedwood, reclaimed 30-year jack pine, and natural mature mixedwood forest was comparable at an average of 23.3%. For all sites that were planted in both 2019 and 2020, overall mortality was higher during the 2020 plant, except for at the open field site.

L. involucrata seedlings that were planted in 2019 were tallest (42.5 cm) and had the largest bunch size/lateral spread (10,066.0 cm^2) at the open field site (both $p \leq 0.001$) (Table A.3). Conversely, 2019 seedlings were shortest (24.8 cm) and had the smallest bunch size/lateral spread (391.0 cm^2) at the 16-year spruce/grass site (both $p \leq 0.001$). During the 2020 plant, seedlings were comparable across sites, except at the 11-year sandy site, where seedlings were on average 11.9 cm shorter ($p=0.004$). After the first growing season, seedling bunch size/lateral spread was comparable at the natural mature mixedwood forest, open field, and new 2020 reclamation site; however, in the 2021 growing season, seedlings at the natural mature mixedwood forest significantly increased in lateral spread ($p=0.006$) and were largest among all sites in the end (2,045.5 cm^2) ($p \leq 0.001$).

At the end of the first growing season, 2019 *L. involucrata* seedlings at the 16-year spruce/grass and natural mature mixedwood forest sites produced fruit. No fruit was produced during the second growing season, whereas during the third, seedlings at the open field and the new 2019 reclamation site produced fruit. At the 2020 plots after two growing seasons, fruit was produced at the open field and the new 2020 reclamation site.

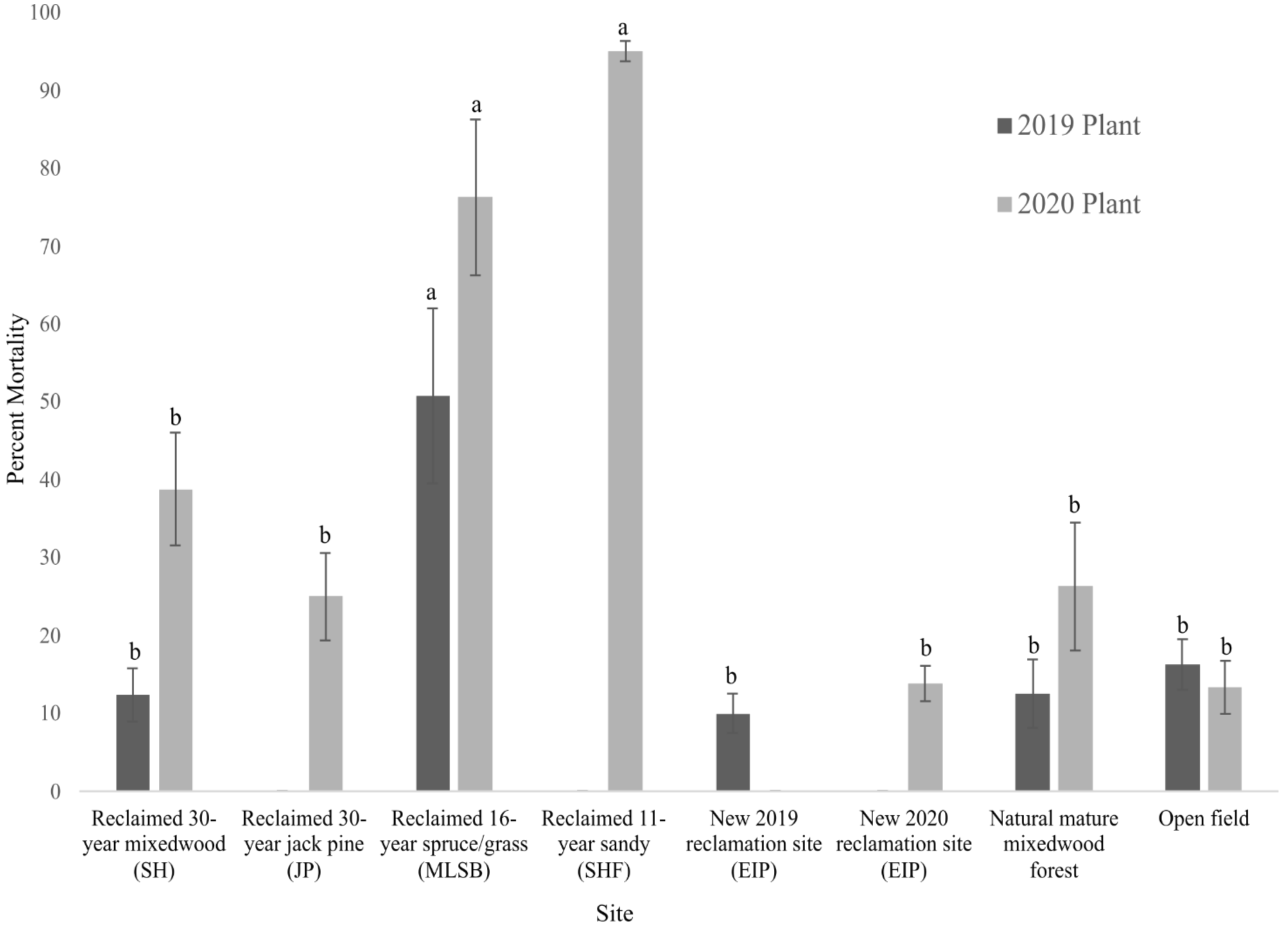

Figure 3.3: Average total mortality (%) of *L. involucrata* seedlings planted in 2019 and 2020. Bars are standard errors and different letters indicate a significant difference between sites for the respective measuring period and planting year ($p<0.05$).

3.3.5 *Vaccinium myrtilloides*

Averaging across planting years, *V. myrtilloides* performed best at the new 2019/2020 reclamation sites, where overall seedling mortality was 13.8% (Figure 3.4). Seedling mortality was on average 21.0% at the natural mature mixedwood forest and the open field, and an average of 28.0% at the reclaimed 30-year mixedwood site and reclaimed 30-year jack pine site. Seedling mortality was comparable at most sites between planting years, except for the reclaimed 30-year mixedwood site where mortality was 16.2% higher during the 2019 plant, as well as the open field, where mortality was 11.7% higher during the 2020 plant.

As of August 2021, seedlings that were planted in 2019 were tallest at the open field (14.8 cm) and shortest at the 30-year mixedwood site (8.5 cm) ($p \leq 0.001$), where seedlings lost a significant amount of height (6.5 cm) over the 2020 growing season ($p=0.009$). The open field was the only site where seedlings planted in 2019 did not reduce height over the course of the experiment (Table A.4). During the 2020 plant, seedlings were tallest at the new 2020 reclamation site (16.5 cm) and shortest at the 11-year sandy site (7.6 cm) ($p \leq 0.001$), compared to average height of 11.7 cm at the other sites. Seedlings at the 11-year sandy, 16-year spruce/grass, and natural mature mixedwood forest sites decreased in height over the two growing seasons.

V. myrtilloides seedlings planted in 2019 produced fruit at all sites at the end of the first growing season, whereas only seedlings at the open field produced during the second and third seasons. At the 2020 plots, during the first season, seedlings at the natural mature mixedwood forest and the open field produced fruit. During the third season, this switched to the open field and the new 2020 reclamation site.

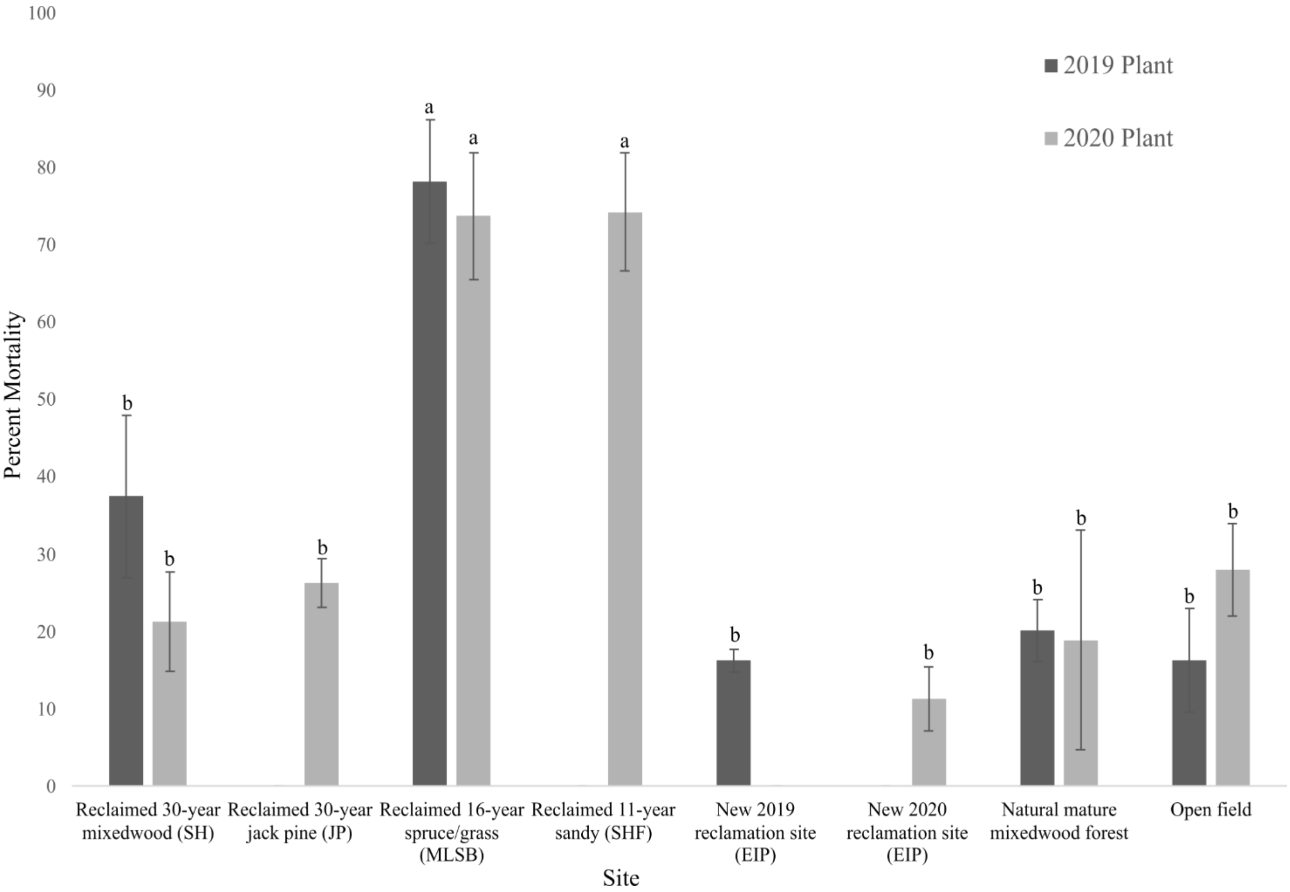

Figure 3.4: Average total mortality (%) of *V. myrtilloides* seedlings planted in 2019 and 2020. Bars are standard errors and different letters indicate a significant difference between sites for the respective measuring period and planting year ($p<0.05$).

3.3.6 *Vaccinium vitis-idaea*

Regardless of planting year, *V. vitis-idaea* performed best at the new 2019/2020 reclamation sites, where there was 16.9% seedling mortality. There was also relatively low seedling mortality at the natural mature mixedwood forest (23.2%) (Figure 3.5). The rest of the sites, including the reclaimed 30-year mixedwood forest, reclaimed 30-year jack pine site, and the open field site had comparable mortality at an average of 45.1%. Overall seedling mortality was higher during the 2020 plant (compared to the 2019 plant) for the reclaimed 30-year mixedwood site, the 16-year spruce/grass site, and the new 2019/2020 reclamation sites (20% higher, 26.3% higher, and 16.2% higher, respectively). Conversely, seedling mortality was 13.0% higher during the 2019 plant at the open field site, and mortality was comparable (23.2% average) at the natural mature mixedwood forest between planting years.

Seedling bunch size/lateral spread was significantly larger at the new 2019 reclamation site (1,327 cm^2) compared to an average of 238.5 cm^2 at the other sites ($p \leq 0.001$) (Table A.5). At the 2020 plots, seedlings at the 11-year sandy site (95.0%) and 16-year spruce/grass site (88.8%) experienced significantly higher mortality than seedlings at all other sites, where average mortality was 38.0%. The 11-year sandy site lost a significant number of seedlings (85%) over the 2021 growing season ($p \leq 0.001$). Similar to the 2019 plots, bunch size/lateral spread at the new 2020 reclamation site (367.0 cm^2) was significantly larger than all other sites (125.5 cm^2 average) at the end of the second growing season. *V. vitis-idaea* only produced fruit at the reclaimed 30-year mixedwood site (2019 plots) after two growing seasons.

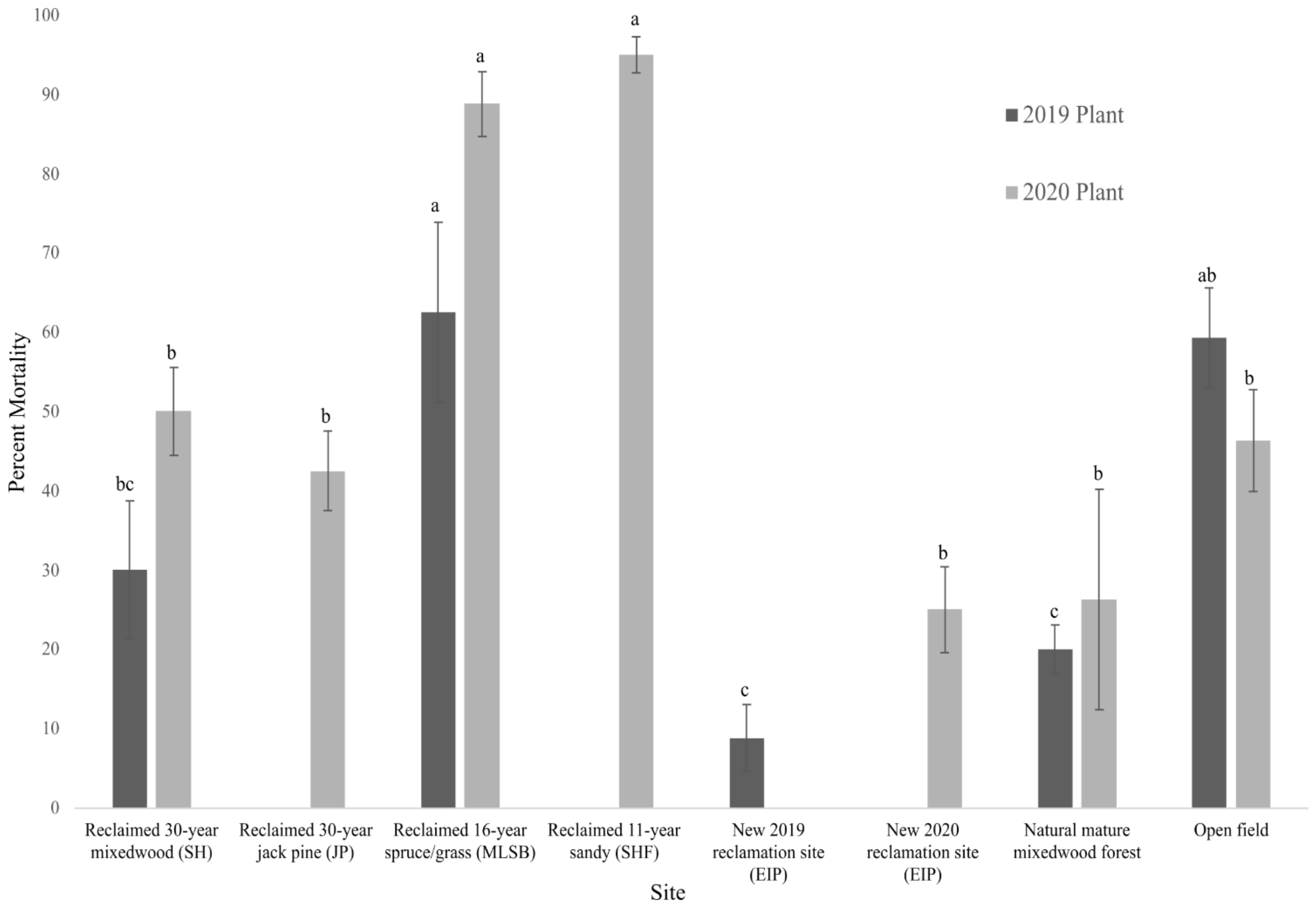

Figure 3.5: Average total mortality (%) of *V. vitis-idaea* seedlings planted in 2019 and 2020. Bars are standard errors and different letters indicate a significant difference between sites for the respective measuring period and planting year ($p<0.05$).

3.3.7 *Symphoricarpos albus*

S. albus seedlings performed best at the new 2020 reclamation site, where seedling mortality was just 3.8% (Figure 3.6). Seedling mortality was comparable (8.2% average) between the reclaimed 30-year mixedwood site and the reclaimed 30-year jack pine site, as well as between the natural mature mixedwood site and the open field site (12.5% average).

Seedling bunch size/lateral spread was largest at the open field (1193.5 cm^2) and smallest at the 16-year spruce/grass site (1.5 cm^2) ($p \leq 0.001$), was comparable between the reclaimed 30-year mixedwood site and natural mature mixedwood forest (773.5 cm^2 average), and comparable between the new 2020 reclamation site and the reclaimed 30-year jack pine site (476.5 cm^2) (Table A.6). Root collar diameter (RCD) at the 16-year spruce/grass site was significantly smaller (1.7 mm) compared to RCDs at the rest of the sites (3.8 mm average). *S. albus* produced fruit at the open field and new 2020 reclamation site after two growing seasons.

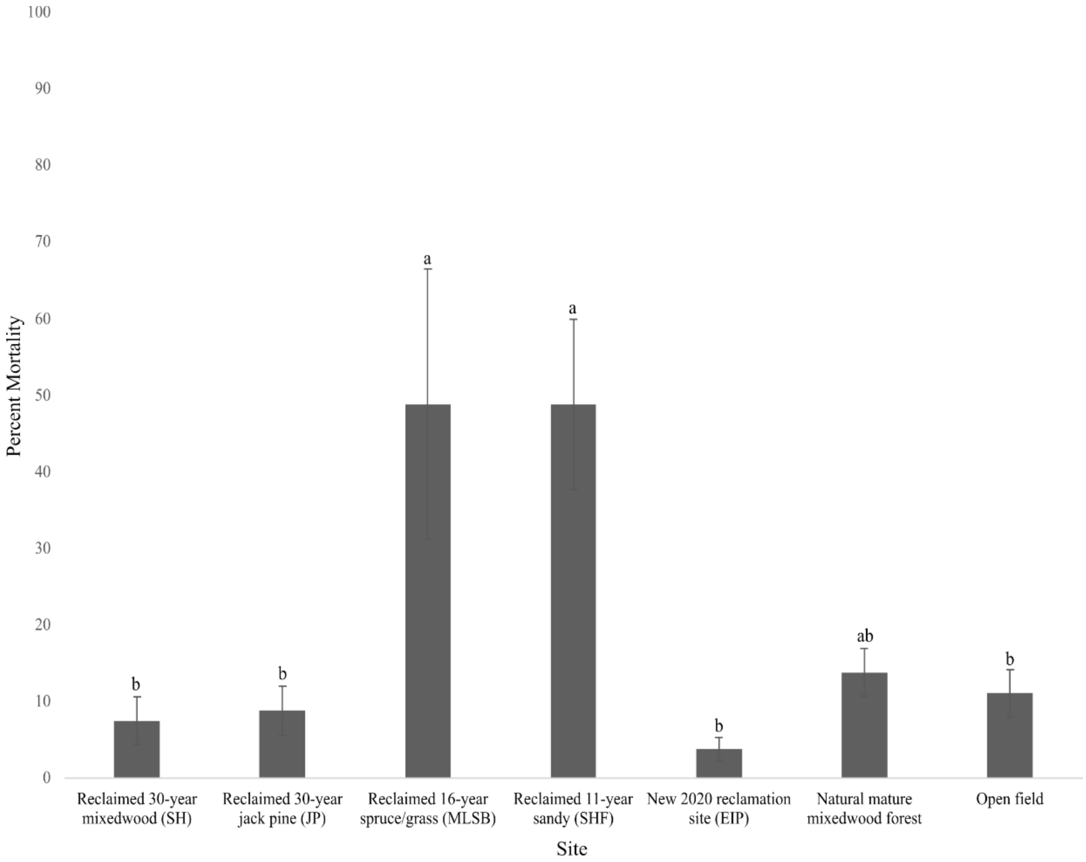

Figure 3.6: Average total mortality (%) of *S. albus* seedlings planted in 2020. Bars are standard errors and different letters indicate a significant difference between sites ($p<0.05$).

3.3.8 *Ledum groenlandicum*

L. groenlandicum performed best at the new 2020 reclamation site, where seedling mortality was 28.8%. Seedling mortality was comparable at the reclaimed 30-year mixedwood site, the natural mature mixedwood forest, and the open field site (44.0% average) (Figure 3.5). The reclaimed 30-year jack pine site had slightly higher seedling mortality at 65.0%.

Out of the sites that had *L. groenlandicum* seedlings remaining at the end of the 2021 growing season, seedlings were tallest at the natural mature mixedwood forest (32.4 cm) and shortest at the open field (19.3 cm) ($p=0.009$), and average height was relatively comparable across the remaining sites (25.4 cm) (Table A.7). Seedling RCD was largest at the new 2020 reclamation site (4.7 mm) compared to the other sites (3.3 mm) ($p<0.001$). Fruit was produced at the open field and mature natural mixedwood forest after the first growing season, and after the second, at these sites again, as well as the reclaimed 30-year mixedwood and new 2020 reclamation site.

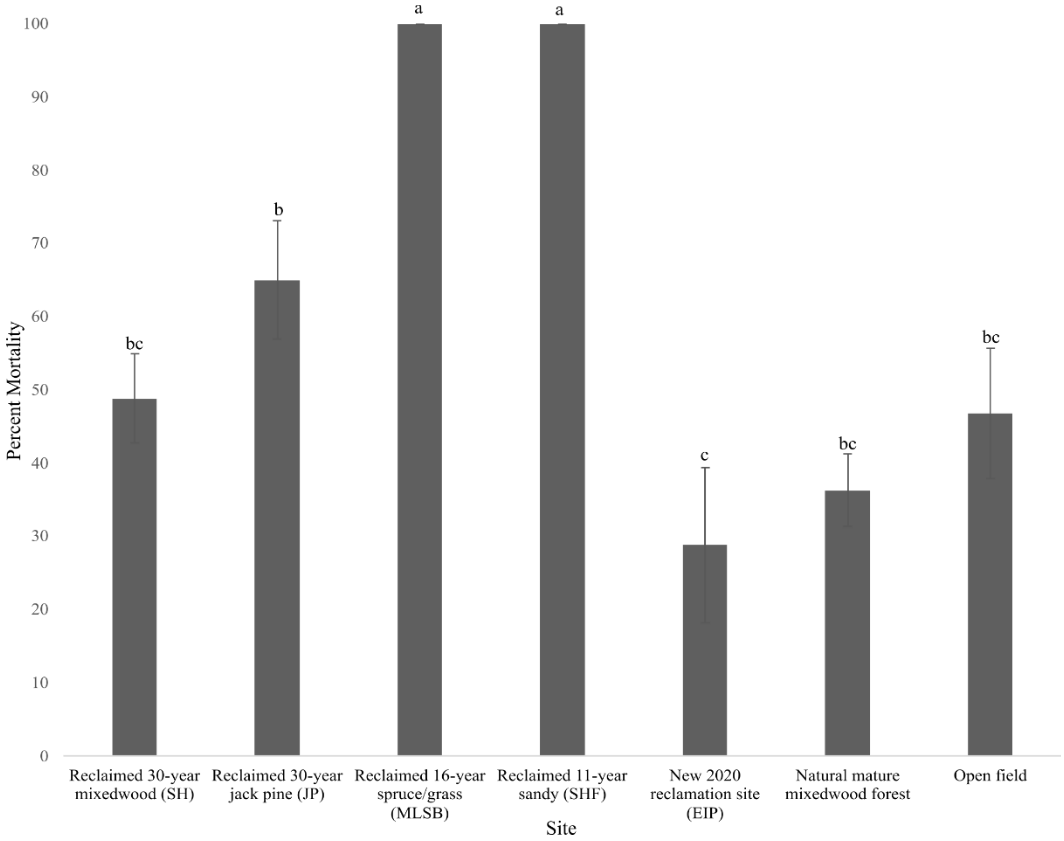

Figure 3.7: Average total mortality (%) of *L. groenlandicum* seedlings planted in 2020. Bars are standard errors and different letters indicate a significant difference between sites ($p<0.05$).

3.3.9 *Empetrum nigrum*

Out of the three sites *E. nigrum* was planted on, seedlings performed the best at the new 2020 reclamation site, which had 21.3% mortality compared to 67.5% at the reclaimed 30-year jack pine site ($p \leq 0.001$) (Figure 3.6). Seedlings were tallest (18.9 cm) and had the largest bunch size/lateral spread (1587 cm^2) at the new 2020 reclamation site ($p=0.01$ and $p<0.001$, respectively) (Table A.8). At the end of the first growing season, there was no difference in seedling height among the three sites *E. nigrum* was planted on; however, seedlings at the new 2020 reclamation site increased significantly in height (4.2 cm) and bunch size/lateral spread (764 cm^2) in 2021 (both $p<0.001$).

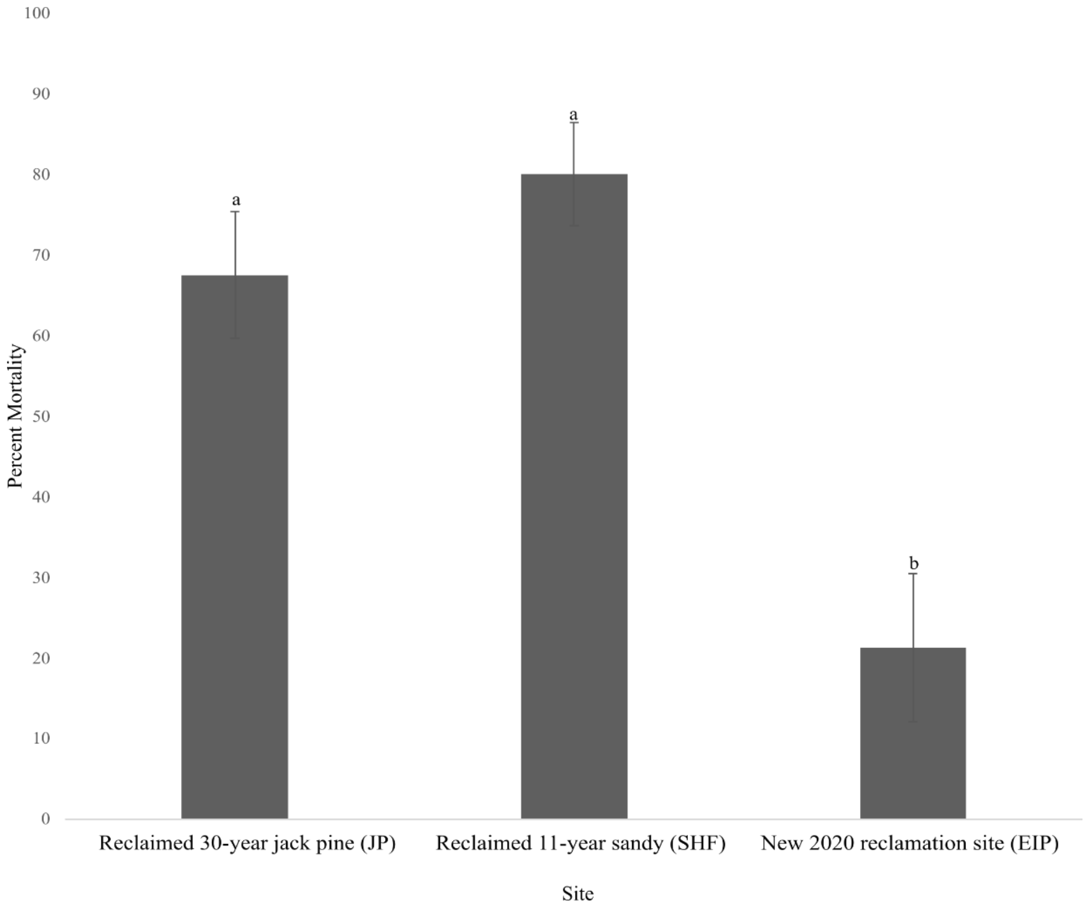

Figure 3.8: Average total mortality (%) of *E. nigrum* seedlings planted in 2020. Bars are standard errors and different letters indicate a significant difference between sites ($p<0.05$).

3.3.10 *Viburnum edule*

Although there was no statistically significant difference in seedling mortality across all sites, *V. edule* performed the best at the new 2020 reclamation site, where mortality was just 5.0%, compared to an average of 17.8% at the reclaimed 30-year mixedwood site, reclaimed 30-year jack pine site, and the natural mature mixedwood forest (Figure 3.7).

After the first year, seedlings were tallest at the reclaimed 30-year jack pine site (20.0 cm) and shortest at the natural mature mixedwood forest (11.2 cm) ($p \leq 0.001$) (Table A.9). Although this pattern remained in 2021, the differences became less significant after the second growing season ($p=0.04$). As of August 2021, seedling RCD was largest at the new 2020 reclamation site (4.2 mm), smallest at the reclaimed 30-year jack pine site (3.3 mm), and comparable between the natural mature mixedwood forest and reclaimed 30-year mixedwood site (3.5 mm average) ($p=0.03$).

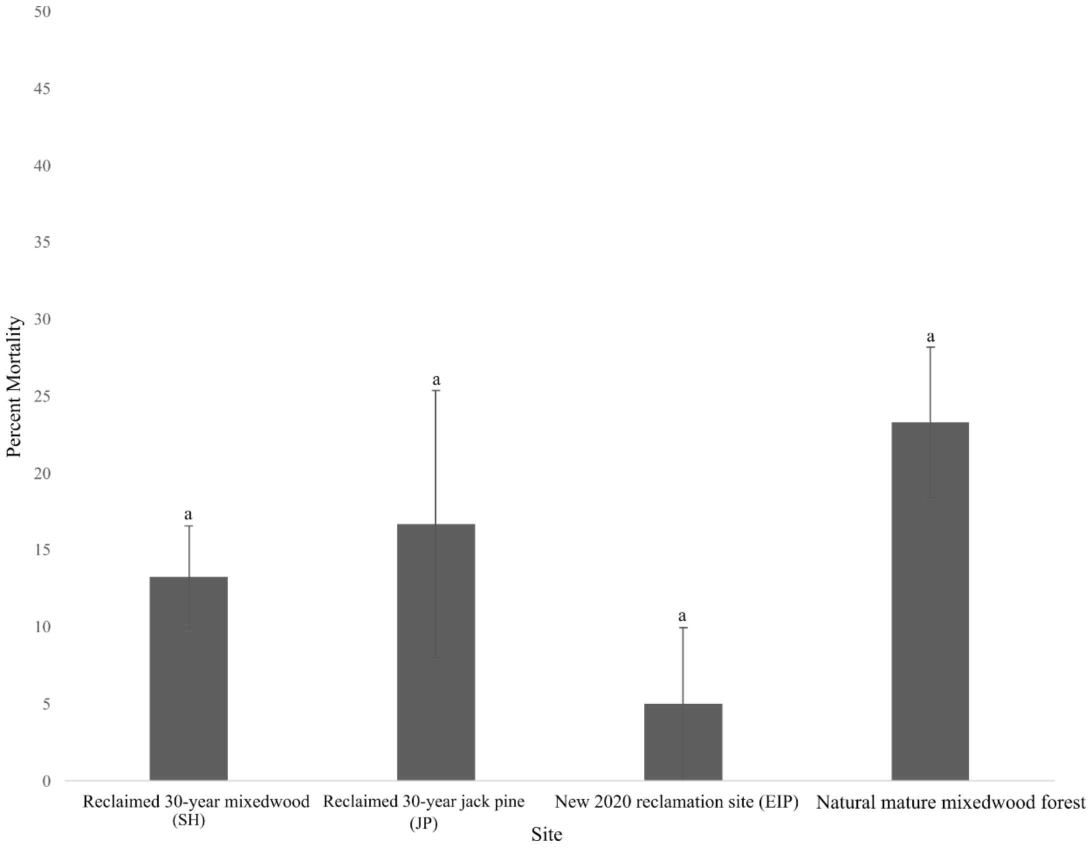

Figure 3.9: Average total mortality (%) of *V. edule* seedlings planted in 2020. Bars are standard errors and different letters indicate a significant difference between sites ($p<0.05$).

3.4 Discussion

At the end of the third growing season, it became quite clear that site conditions played a major role in the performance of all shrub species. However, seedling quality might have played a role in the performance of *C. canadensis* and *L. borealis* (see also Chapter 2). Somewhat surprisingly, the survival and growth for most understory shrub species were greatest at the young reclamation sites (new 2019/2020 reclamation) and the open field site that had no existing tree canopy. This overall response is likely due to an abundance of resources on these sites (e.g., space, nutrients, water, and light). The absence of existing vegetation meant the growth of the planted seedlings was not affected or constrained by limitation of space due to competition and the resource limitation associated with it. In terms of available nutrients, the young reclamation sites had significantly higher soil total organic carbon (TOC), total organic nitrogen (TON), and ammonium (NH4-N) compared to all other sites ($p \leq 0.001$) (Table 3.1)

While this response was true for most species, *V. vitis-idaea* and *L. groenlandicum* did not quite follow this trend. Both these species performed well at the young reclamation sites but performed relatively poorly at the open field site. The young reclamation sites and the open field are similar in their resource availability and absence of vegetation; however, they had large differences in their soil organic content and bulk density (Figure 3.1; Table 3.1). This finding suggests that *V. vitis-idaea* and *L. groenlandicum* may prefer moist organic soils on natural sites (Karlin and Bliss 1983; Ritchie 1955).

The young reclamation sites used in this study provided me with an opportunity to observe how these sites change in composition over a short amount of time. At the end of the 2021 growing season, the majority of the site that was planted in 2019 (new 2019 reclamation) was covered with dense vegetation (predominantly graminoids) compared to the site that was planted in 2020 (new 2020 reclamation) which was still completely void of vegetation. This finding shows the rapid redevelopment of cover through early successional species as early as one year after soil placement, providing a small window of opportunity to establish seedlings prior to competition moving in (Table 3.1; Figure A.14 & A.15). Further development of this vegetation between year three and the latter stages (year 11 and 16) can have a profound negative effect on the performance of planted shrub seedlings if there is no tree canopy in place. The site that experienced the second highest overall seedling mortality – the 16-year spruce/grass site –

had the highest presence of competitive graminoids (Table 3.1; Figure 3.1), which is considered a significant impediment to the establishment of any type of seedlings during forest restoration or regeneration (Schott et al. 2016), often leading to high mortality and poor seedling performance (Löf et al. 2012), likely due to resource limitation (Löf et al. 2007). While the application of traditional competition control methods is possible at the initial stages of forest restoration (prior to planting), it is not advisable when establishing native propagules are to be maintained or during the latter stages of restoration when plants have already been established.

When planted at the older reclamation sites (30-year mixedwood, 30-year jack pine), the shrub seedlings performed relatively well and similarly to the natural mature mixedwood forest. These sites are at an age where trees have reached canopy closure and, therefore, have lower competition in the understory (Su et al. 2019; Hart and Chen 2006). Compared to open sites, however, seedlings were on average less productive (reduced growth) in the understory which likely relates to the lower light levels that were experienced under the tree canopy. This reduced performance response, however, is much less pronounced than the seedling response observed when in direct competition with plants of similar stature, which likely affects multiple resource limitations such as light, water, and nutrients (e.g., 16-year spruce/grass site) (Wagner et al. 2011). Increasing shade can affect other processes as well such as a plant's reproductive capability. Light, temperature, and water availability are the major environmental cues for plants to control the transition to flowering (Bernier et al. 1993) and decreased light levels have been shown to decrease flowering in various species (Ferree et al. 2001; Qin et al. 2022). Most of the shrub species that flowered and produced fruit during this experiment did so at the open field and the young reclamation sites. *V. myrtilloides* and *L. groenlandicum* also flowered on sites that achieved canopy closure, namely the natural mature mixedwood forest and the 30-year mixedwood forest, indicating these species require less light to prompt their reproductive cycles. However, we only monitored these plants for two to three years (2019 plots and 2020 plots, respectively), so their potential for flowering could increase as they get larger and more established.

Seedling quality characteristics such as seedling size and age, although most likely at a much smaller scale, might have also played a role in the survival and growth of the seedlings used in this study. In general, larger seedlings can more readily outcompete vegetation

(Grossnickle 2005) and this was the case for *S. albus* and *V. edule* seedlings in this study, which were 3+0 seedlings. All others were 1+0, meaning *S. albus* and *V. edule* seedlings were grown for two extra years in the nursery. Further, larger plants have more non-structural carbohydrate (NSC) reserves, which newly outplanted seedlings heavily rely on, especially on sites where producing new carbon is challenging due to stress from competition, drought, and extreme soil temperatures (Malik and Timmer 1996; Millard and Grelet 2010; Villar-Salvador et al. 2015). In this study, the larger seedlings generally performed best across all sites (i.e., *L. involucrata*, *V. myrtilloides*, *S. albus*, and *V. edule*). Additionally, NSC reserves may have played a role in the increased mortality observed at the 2020 plots after the first growing season, especially at the 30-year mixedwood and 16-year mixedwood sites. Mortality was also higher overall for *L. involucrata* and *V. vitis-idaea* at the 2020 plots, but lower for *V. myrtilloides*. In Chapter 2 we measured NSC reserve concentrations in roots, stems, and leaves of the different stock types (Table 2.3), and interestingly, the seedling stock that had higher initial NSC concentrations also had higher survival upon outplanting. The observed differences in NSC concentrations between stock types can likely be attributed to differences in nursery cultural practices between the two growing years (see Section 2.4). Many studies have linked seedling field survival and growth in forest restoration projects to increased NSC levels (Marshall 1985; Puttonen 1986; Ritchie and Landis 2010), which was observed in this experiment.

The site with the greatest overall seedling mortality was the 11-year sandy site. I believe the widespread mortality can be attributed to a combination of the site's coarse soil texture (92.9% sand) and the hot and dry weather conditions in 2021 (Figure 3.2). At the reclamation sites, precipitation in June 2021 at the time of planting was only 38.2 mm, compared to 90.3 mm and 101.5 mm in 2020 and 2019, respectively, and air temperatures reached upwards of 40°C. Water stress in newly planted seedlings often leads to mortality or limited early growth (Grossnickle 2005; Kozlowski and Davies 1975). Unlike mature trees that have extensive root systems that can reach deeper soil water resources, newly planted seedlings rely solely on surface soil water (Gates 1980; Marsh 2022). Due to the reduced water holding capacity of the soil, seedlings at this site may not have been able to grow enough roots in 2020 to sustain their water requirements throughout the harsh conditions of 2021. Plant-available water appears to have been a limiting factor on this site, in addition to competitive vegetation. Further, the shrub species used in this study were for the most part generalists, meaning they can typically tolerate a range of environmental and edaphic conditions, but are mostly adapted to mesic moisture and medium nutrient conditions. This could be one reason why, other than the two extreme sites that had the highest mortality across all species (11-year sandy and 16-year spruce/grass), we did not observe major differences in seedling performance across the different sites. For the two extreme sites, other species that are more tolerant to drought and competitive conditions could have been more successful. However, since reclamation site conditions can vary widely from site to site and the opportunity to mitigate site limiting factors is usually limited, selecting appropriate species that can tolerate these specific conditions is an informed approach.

3.5 Conclusion

This understory outplanting experiment is one of the first that explores the potential of restoring understories actively on boreal forest reclamation sites through the planting of understory shrub species. It clearly demonstrated that reclamation site conditions, the age of the sites, and the developing tree canopy play a role in the success of establishing seedlings. As expected, site-limiting factors similar to those that influence tree establishment played a significant role in the success of the planted shrub seedlings. However, specific to the site characteristics, the initial main limiting factor affecting seedling performance was likely abiotic resource limitation (e.g., water, light, nutrients), which later shifted to biotic interactions such as competition. Vegetation that competed directly with the low stature understory species were likely be a major contributing factor on these sites, persisting until a tree canopy creates conditions that are more advantageous to the shade-adapted understory species. In addition, the canopy appears to moderate some of the harsher conditions that the seedlings were exposed to during extreme weather events.

The results from this study suggest the optimal temporal stage of reclamation to incorporate understory shrub species into planting efforts is either soon after soil placement (i.e., concurrently with tree seedlings) or after canopy closure has been achieved. However, it will be important to monitor the young reclamation sites included in this study to determine whether the planted shrub seedlings will be persistent enough to compete with the other vegetation and maintain its presence over time.

Tables

Table 3.1: Mean (±SD) LAI, vegetation competition, annual growing season soil temperature, number of days with an average soil temperature above 5°C, and volumetric soil water content (VWC) at 10 cm below the soil surface, and soil chemistry data. Planted shrubs were not included in percent cover. Different letters indicate a significant difference between sites.

	30-year mixedwood	30-year jack pine	16-year spruce/grass	11-year sandy	New 2019 reclamation	New 2020 reclamation	Natural mature mixedwood forest	Open field
Leaf area index (LAI)	4.3 (0.45)*a*	4.5*a*	0.00*c*	0.0*c*	0.0*c*	0.0*c*	2.0 (0.54)*b*	0.0*c*
Grass % cover	1.9 (2.2)*d*	0.4 (0.5)*d*	35.9 (20.7)*b*	58.0 (16.8)*a*	22.6 (11.5)*bc*	0.0*d*	12.3 (11.7)*cd*	0.0*d*
Forb % cover	24.7 (20.5)*ab*	37.0 (17.2)*ab*	49.0 (17.3)*a*	16.0 (10.2)*bc*	10.0 (7.0)*bc*	0.0*c*	17.0 (10.3)*bc*	0.0*c*
Shrub % cover	11.6 (12.8)*b*	5.0 (0.0)*bc*	0.8 (1.0)*bc*	9.8 (11.7)*bc*	0.4 (0.5)*bc*	0.0*c*	29.5 (17.4)*a*	0.0*c*
Tree % cover	13.5 (6.7)*ab*	16.0 (4.2)*a*	6.3 (6.3)*ab*	0.0 (0.0)*c*	10.8 (5.4)*ab*	0.0*c*	3.4 (6.2)*bc*	0.0*c*
Bare ground %	35.7 (15.6)*abc*	30.0 (12.7)*ab*	2.5 (4.9)*bc*	7.0 (8.4)*bc*	41.6 (16.8)*a*	100.0*c*	19.9 (21.5)*abc*	100.00*c*
Soil EC (µS/cm)	351.7 (263.6)*bc*	175.7 (9.7)*bc*	210.0 (15.1)*bc*	175.9 (200.6)*bc*	413.0 (99.7)*b*	1288.7 (405.2)*a*	137.2 (30.6)*bc*	71.3 (14.6)*c*
Soil pH	7.5 (0.41)*a*	8.0 (0.06)*a*	7.9 (0.23)*a*	6.5 (0.30)*b*	5.2 (0.78)*c*	4.3 (0.19)*d*	6.1 (0.25)*b*	6.2 (0.12)*b*
Soil TOC (w/w%)	2.1 (0.61)*c*	2.3 (0.45)*c*	4.0 (0.81)*c*	2.0 (0.39)*c*	33.2 (14.7)*a*	25.5 (8.1)*ab*	12.9 (8.2)*bc*	3.3 (0.33)*c*
Soil TON (mg/kg)	1.4 (1.2)*c*	0.83 (0.23)*c*	4.9 (0.97)*b*	0.76 (0.27)*c*	1.8 (0.91)*c*	2.3 (1.3)*c*	0.90 (0.22)*a*	3.1 (1.7)*c*
Soil NH_4-N (mg/kg)	4.9 (1.4)*b*	3.1 (0.84)*bc*	3.7 (1.1)*bc*	2.3 (0.54)*bc*	12.4 (3.3)*a*	10.3 (4.2)*a*	7.5 (1.3)*c*	4.0 (0.18)*bc*
Soil PO_4-P (mg/kg)	7.1 (7.3)*b*	2.4 (1.0)*b*	3.3 (4.2)*b*	6.4 (5.6)*b*	1.6 (0.59)*b*	1.0 (0.18)*b*	18.9 (13.6)*a*	31.8 (5.9)*a*
Soil K+ (mg/kg)	141.5 (26.0)*bc*	118.0 (15.7)*bcd*	88.8 (9.4)*cd*	45.1 (6.6)*d*	81.4 (20.3)*cd*	43.8 (16.7)*d*	372.9 (103.9)*a*	174.6 (10.0)*b*
Soil bulk density (g/cm^3)	1.2 (0.07)*a*	1.2 (0.07)*ab*	0.96 (0.12)*b*	1.1 (0.06)*ab*	0.35 (0.25)*c*	0.26 (0.04)*c*	0.55 (0.17)*c*	1.3 (0.05)*a*
Soil clay % (<5µm)	23.7 (3.0)*b*	37.8 (6.4)*a*	11.9 (3.3)*cd*	2.8 (0.46)*d*	16.3 (13.9)*bc*	7.6 (0.85)*cd*	11.8 (3.8)*cd*	16.0 (1.2)*bc*
Soil silt % (5-50µm)	24.1 (5.2)*a*	24.3 (4.5)*ab*	14.2 (3.8)*c*	4.3 (0.59)*d*	27.7 (8.3)*a*	18.7 (5.0)*abc*	26.8 (4.0)*a*	16.2 (0.47)*bc*
Soil sand % (>50µm)	52.2 (3.3)*de*	37.9 (10.7)*e*	74.0 (7.1)*ab*	92.9 (1.0)*a*	56.0 (20.9)*cde*	73.8 (5.5)*bc*	61.4 (6.6)*bcd*	67.7 (1.7)*bcd*

Table 3.2: Mean annual growing season soil temperature, number of days with an average soil temperature above 5°C, and volumetric soil water content (VWC) at 10 cm below the soil surface.

	30-year mixedwood			16-year spruce/grass			New 2019/2020 reclamation			Natural mature mixedwood forest			Open field			30-year jack pine	11-year sandy
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021	2021	2021
Soil temperature (°C)	8.5	4.6	10.0	7.7	4.5	8.1	7.3	4.4	7.0	8.3	4.9	4.7	10.6	6.8	7.2	-	-
Seasonal soil temperature (°C)	13.7	12.7	15.4	15.0	13.4	15.5	14.5	13.5	15.7	10.7	8.9	8.8	17.1	15.8	16.7	15.0	18.7
Soil water potential (kPa)	-66.8	-115.8	-426.0	-54.6	-62.3	-309.5	-35.4	-25.8	-91.0	-74.9	-158.0	-342.8	-14.2	-26.0	-157.9	-375.7	-197.0
Soil VWC (m^3/m^3)	0.18	0.20	0.25	0.19	0.20	0.13	0.20	0.19	0.16	0.18	0.18	0.15	0.26	0.24	0.20	0.17	0.15

Chapter 4: Synthesis and Applications

4.1 Research Summary

The overall objective of this thesis was to assess the seedling quality and outplanting performance of nine nursery-grown boreal forest shrub species. In Chapter 2, seedling quality attributes that have been commonly used to predict potential field performance of tree seedlings were applied to shrub seedlings that were grown under non-limiting conditions. This was followed up by a field trial in Chapter 3, where seedlings of the same species used in the controlled study were outplanted onto reclamation sites of varying ages, degree of canopy closure, vegetation competition, and edaphic conditions.

In Chapter 2, most of the seedling quality measures I applied to the seedlings did not correlate with survival and growth. Since there is currently very little research in this area, I based my subjective quality categories on well documented characteristics that have been related to tree seedling performance (i.e., growth and survival). Several factors could have contributed to why I did not observe similar patterns for shrubs in this study. First, shrubs are much more morphologically variable than trees. We attempted to capture this by including measures that are not typically applied to tree seedlings such as how much the shrub spread out laterally and the number of stems, but still very few differences were observed. Further, we grew these seedlings under non-limiting conditions where they were not influenced by competition for space, light, water, or nutrients. Most studies that explore these relationships and have observed differences in seedling performance based on certain seedling quality characteristics were subjected to field conditions with stresses such as competition for resources. On resource-limited sites, seedlings with a greater competitive advantage such as being taller or having a higher RSR are typically better performers.

In this first study I was able to identify fundamental seedling quality issues with two species – *C. canadensis* and *L. borealis*. I speculate that for *C. canadensis*, incidence of mold and perhaps lifting and storing the seedlings at an inopportune time resulted in increased mortality. Broadleaf evergreen species have several attributes that make them tricky to commercially produce. First, they are especially susceptible to storage mold as their large, retained leaves provide suitable conditions for mold to establish and grow. Additionally, during the hardening phase prior to storage, plants like *C. canadensis* that have high leaf areas often receive more damage from desiccation and cold storage. Finally, at the end of the hardening phase, it is critical to ensure seedlings are fully dormant when they are lifted and put into storage. For *L. borealis*, it appears that poor root development due to the trailing nature of this species led to high overall mortality when grown under non-limiting conditions.

In Chapter 3, it was very evident that reclamation site conditions had an influence on seedling performance. Shrub seedlings generally performed the best (highest survival and growth) at the open sites that had no existing tree canopy and no competing vegetation. The seedlings also performed relatively well at the older reclamation sites, where seedling response was similar to the natural mature mixedwood forest. Performance at the older closed canopy sites was slightly reduced, likely due to the lower light levels in the understory. Because the young reclamation sites were planted one year apart, I was able to observe how these sites might develop over a short period, and it is evident that competitive early successional graminoid species rapidly develop on reclamation sites as early as one year after soil placement. This finding was supported by the conditions at the mid-aged site, where the majority of the ground cover was competitive graminoid species, which appeared to negatively impact seedling survival. The other mid-aged site included in this experiment that had the greatest overall seedling mortality also had a high presence of competitive graminoids; however, I speculate that the high mortality was more so due to a combination of the coarse-textured soil at this site which was magnified by the hot and dry weather conditions of 2021.

Additional to site conditions, seedling quality attributes might have also contributed to the survival and growth of the outplanted shrubs. For example, seedling size appeared to have played a role as larger seedlings generally performed better than small seedlings. Non-structural carbohydrate (NSC) reserve concentrations (measured in Chapter 2) may have also led to increased survival, as evidenced by the 2019 seedlings having higher initial NSC reserves in their roots, stems, and leaves and lower mortality on some sites.

4.2 Research Applications

Applied studies such as this one are crucial for progressing forest restoration research. Through this experiment we were able to identify two shrub species with obvious seedling quality issues which could have been brought on by how they were cultured, suggesting that nursery cultural practices which are successful for commonly grown tree species may not be ideal for all species and need to be adapted. Prior to seedlings reaching customers for use in restoration, reforestation, and conservation projects, they go through an exhaustive process of harvesting, storage, handling, and shipping. It is common in nursery culture to remove the seedlings from their containers (called "lifting"), package them, and put them in refrigerators or freezers until they are shipped to the customer. After harvesting, seedlings are either cooler or freezer stored (together known as cold storage) (Landis 2010), partly to give the nurseries and customers adequate time to schedule growing, processing, and shipping, but also to help decrease the likelihood of some seedling health risks. Although cold storage eases nursery production and staves off some diseases and pests that thrive under nursery growing conditions, seedlings that get cold stored can experience cold injury, desiccation, loss of dormancy, and storage mold. Deciduous species tend to tolerate cold storage well as their lack of foliage during dormancy reduces the risk of desiccation. Particularly, broadleaf evergreen species are known to be more susceptible to cold injury and moisture loss (Landis 2010), and the observations from this study indicate they also might be more susceptible to storage molds as their retained leaves likely provide a suitable environment for fungi to develop. Some modifications the nurseries could make to the hardening and storage of this species include decreasing thaw time to minimize ideal conditions for mold to grow, storing seedlings at the coldest feasible temperature, ensuring foliage is dry before storage, treat their stock with foliar fungicides prior to cold storage, carefully inspect seedlings prior to storage, and inspect stored stock frequently. There is also the option of eliminating cold storage altogether for broadleaf evergreen species, which could be achieved by using a fall planting, avoiding "hot lifting" stock during their active growing phase.

Since we received two different batches of seedlings from two different stock years, we were able to compare and contrast *initial* morphological and physiological attributes as well as outplanting responses. The seedlings were generally similar in morphology between stock years but varied significantly in NSC reserve concentrations while in their dormant state. This difference in NSC reserves appears to have translated to the field, where the stock year with more reserves (2019) had slightly higher survival than the seedling batch with less reserves (2020). This finding highlights the importance of the nursery process, and how slight changes in cultural practices such as the length of time seedlings are able to photosynthesize or how long the seedlings are in storage can have a fast and profound effect on their ability to survive and grow after outplanting.

Although very few differences in seedling survival and growth were observed among the quality categories when grown under non-limiting conditions, larger seedlings typically performed better when exposed to field outplanting conditions. This finding suggests that larger seedlings might be better equipped to tolerate a range of site conditions. However, during the potted growth experiment, we found that the majority of species allocated more energy into growing roots rather than shoots, though this could have been a result of the seedlings not having to compete for resources (i.e., light, space, water, and nutrients). If this also proves to be the case for seedlings that were grown in field conditions, this could be problematic as seedlings need a lot of above-ground tissue in order to effectively photosynthesize and outcompete other vegetation due to their larger size (Grossnickle 2005). In the nursery, seedlings can be encouraged to put more energy into growing roots instead of shoots by forcing them to terminate shoot growth early while maintaining green leaves during the first growth year. This can be achieved by adjusting cultural practices such as reducing nutrient availability, reducing day length, and/or using an artificial shoot growth inhibitor to induce early terminal bud set (Landhäusser et al. 2012b; Kelly et al. 2015). Another option to explore could be giving seedlings a competitive size advantage during the nursery stage. This has achieved positive results for Trembling Aspen (*P. tremuloides*), where seedlings that were nutrient loaded with N-P-K fertilizer at the nursery grew 19% taller than standard seedlings after two growing seasons (Malik and Timmer 1996; Oliet et al. 2009; Schott et al. 2016).

The main limiting factors for the sites included in this study appear to have been competitive vegetation and rapidly draining, coarse textured soil. Vegetation competition is usually combated prior to planting by preparing sites with herbicides, mechanical techniques, or prescribed burning or mulching (Iverson et al. 2008; Löf et al. 2012; Sutton 1993). Traditional competition control methods, however, are not easily applied to sites that have already been established. For example, mechanical techniques and burning are non-selective and would likely disturb and/or damage existing desired vegetation. Selective herbicides could be an option, but there has been a push the past few decades to move away from the widespread use of chemical products in forest restoration due to increased public concern and perceptions of risk (Little et al.

2006). One alternative could be to hand pull the early successional species as they begin establishing on a site; however, this would require a lot of labor. As for mitigating rapidly draining soils that tend to dry out during periods of increased temperatures and minimal precipitation (leading to widespread seedling mortality), incorporating organic matter during reconstruction to increase the soil's water-holding capacity would likely be beneficial for seedling survival.

4.3 Future Research and Study Limitations

Additional research into this topic could include performing a similar experiment that includes more seedling quality categories with more stringent selection criteria to try to capture the wide range of seedling morphological variability we observed in this initial study. If the results of this study also result in very few differences in survival and growth between categories when grown in near optimal conditions, the next step would be to outplant the shrubs into stressful conditions and keep track of each individual seedling's quality category, to see whether differences emerge. Based on these studies, if certain morphological characteristics can be correlated to performance, the nursery could potentially adjust their cultural practices to select for those specific characteristics, ideally resulting in increased seedling quality.

Although the results of the potted experiment showed that the deciduous species put on more root mass (compared to shoot mass) over one growing season, these seedlings did not experience any resource limitations. They were given ample space, their roots systems were completely independent from one another, and they were watered regularly. It would be interesting to observe whether seedling root to shoot ratios developed differently on the outplanting sites where the shrub seedlings had to compete with other vegetation for resources.

Monitoring the young reclamation sites included in this experiment will be necessary to observe whether the planted shrub seedlings become established enough to outcompete early successional graminoids that become widespread on these sites soon after soil placement. Clearly the seedlings performed well when planted prior to competition moving in and initiating resource limitation, but it would be worth monitoring them to see how the seedlings respond when outplanted onto sites one, two, or three years after soil placement. This would give an indication of the window of successful outplanting, because at a certain point, as evidenced by the 11-year and 16-year sites included in this study, seedling survival is highly affected by resource limitation caused by competition.

A main limitation involved with this study was the availability of understory seedlings. Nurseries generally operate as contract growers that grow (or attempt to grow) seedlings based on customer orders. To fulfill orders, they must be placed up to two years in advance (longer for older stock types such as the 3+0). Due to timeline constraints for our study, the seedlings used in this trial were seedlings that were already in production, and therefore, we were not able to plant every species on each site which narrowed our opportunity for comparisons.

Overall, this topic warrants plenty more research and investigation. Nevertheless, I hope that the research presented in this thesis will emphasize the importance of incorporating forest understories into restoration practices and provide answers to some questions regarding timing and how seedlings respond to commonly observed reclamation site conditions.

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Appendix

Chapter 2

Table A.1: Results of Two Sample t-tests ran to identify differences between initial seedling characteristics for 2019 and 2020 stock years. Sample sizes were based on the category with least number of surviving individuals at the end of the experiment.

	C. canadensis (n=30)	*L. involucrata* (n=30)	*V. myrtilloides* (n=20)	*V. vitis-idaea* (n=20)
Number of Shoots	**$\leq$0.001**	-	**$\leq$0.001**	0.90
Number of Leaves	**$\leq$0.001**	-	-	-
Height (cm)	-	0.17	**$\leq$0.001**	**$\leq$0.001**
Root Collar Diameter (mm)	-	0.90	0.19	**0.0025**
Root Mass (g)	**$\leq$0.001**	0.10	**$\leq$0.001**	0.15
Shoot Mass (g)	**$\leq$0.001**	0.14	**$\leq$0.001**	0.12
Leaf Mass (g)	-	-	-	0.21
Root to Shoot Ratio (RSR)	**0.050**	0.89	0.19	0.16
Root Total NSC %	**$\leq$0.001**	**$\leq$0.001**	**0.047**	0.065
Shoot Total NSC %	**$\leq$0.001**	**$\leq$0.001**	**$\leq$0.001**	**$\leq$0.001**
Leaf Total NSC %	-	-	-	**0.0026**

Table A.2: Results of Two Sample t-tests ran to identify differences between quality categories of seedlings grown in pots over the 2020 growing season. Sample size is based on randomly selected individuals from each quality category constrained by the lowest number of survivors in a category. Two quality categories for each species was used in this analysis.

	C. canadensis (n=4)	*L. involucrata* (n=10)	*V. myrtilloides* (n=9)	*V. vitis-idaea* (n=10)	*S. albus* (n=6)	*L. groenlandicum* (n=9)	*E. nigrum* (n=7)	*V. edule* (n=10)	*L. borealis* (n=5)
Change in Number of Shoots/Runners	0.38	**0.008**	0.31	0.34	0.36	0.66	0.40	0.72	0.33
Chage in Height/Runner Length (cm)	-	0.25	0.087	**0.011**	0.60	0.11	0.41	0.14	0.28
Change in Root Collar Diameter (mm)	-	0.96	0.33	-	0.18	0.48	0.80	**0.056**	-
Change in Root Mass (g)	0.79	0.37	**0.015**	0.96	**0.024**	0.66	0.062	0.24	0.19
Change in Shoot Mass (g)	0.40	**0.042**	0.062	0.073	**0.028**	0.57	0.071	0.16	**0.009**
Final Leaf Mass/Change in Leaf Mass (g)	-	0.68	**$\leq$0.001**	0.34	**0.0024**	0.45	**0.011**	0.54	**0.037**
Change in Root to Shoot Ratio	0.67	0.095	0.79	0.23	0.49	0.18	0.56	0.90	0.27
Change in Live Crown Ratio (%)	-	-	-	-	-	0.39	-	-	-
Change in % Brown Leaves	-	-	-	-	-	-	0.64	-	0.30
Change in Number of Leaves	0.57	-	-	-	-	-	-	-	-
Change in Bunch Size (cm^2)	0.37	-	-	**0.020**	-	-	-	-	-

Figure A.1: Category 1, Category 2, and Category 3 (left to right) *Cornus canadensis* seedlings from the 2020 stock year.

Figure A.2: Category 1 and Category 2 (left to right) *Lonicera involucrata* seedlings from the 2020 stock year.

Figure A.3: Category 1 and Category 2 (left to right) *Vaccinium myrtilloides* seedlings from the 2020 stock year.

Figure A.4: Category 1 and Category 2 (left to right) *Vaccinium vitis-idaea* seedlings from the 2020 stock year.

Figure A.5: Category 1 and Category 2 (left to right) *Symphoricarpos albus* seedlings from the 2020 stock year.

Figure A.6: Category 1 and Category 2 (left to right) *Ledum groenlandicum* seedlings from the 2020 stock year.

Figure A.7: Category 1 and Category 2 (left to right) *Empetrum nigrum* seedlings from the 2020 stock year.

Figure A.8: Category 1 and Category 2 (left to right) *Viburnum edule* seedlings from the 2020 stock year.

Figure A.9: Category 1, Category 2, and Category 3 (left to right) *Linnaea borealis* seedlings from the 2020 stock year

Chapter 3

Table A.3: Average growth data of *L. involucrata* seedlings planted in 2019 and 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	2019 Plant					**2020 Plant**			
	Height (cm)			Bunch size (cm^2)		Height (cm)		Bunch size (cm^2)	
	2019	2020	2021	2020	2021	2020	2021	2020	2021
Reclaimed 30-year mixedwood (SH)	34.3 (0.55)_a_	28.6 (1.7)_ab_	30.5 (1.5)_bc_	670.5 (127.7)_c_	847.5 (134.2)_bc_	27.8 (0.94)_a_	27.0 (1.1)_a_	491.0 (124.8)_b_	562.5(107.0)_cd_
Reclaimed 30-year jack pine (JP)	-	-	-	-	-	25.9 (0.49)_a_	26.7 (1.1)_a_	380.0 (38.9)_b_	528.5 (27.6)_cd_
Reclaimed 16-year spruce/grass (MLSB)	33.3 (0.34)_a_*	23.4 (2.6)_b_	24.8 (2.7)_c_	407.0 (74.9)_c_	391.0 (85.4)_c_	20.6 (3.1)_a_	21.9 (2.3)_ab_	250.0 (83.8)_b_	157.0(30.1)_d_
Reclaimed 11-year sandy (SHF)	-	-	-	-	-	21.1 (2.1)_a_	14.4 (3.5)_b_	314.5 (32.7)_b_	122.0 (39.8)_d_
New 2019 reclamation site (EIP)	29.3 (0.83)_b_	28.5 (2.7)_ab_	31.5 (1.2)_bc_*	1,942.0 (520.5)_b_	2,988.5 (519.0)_b_*	-	-	-	-
New 2020 reclamation site (EIP)	-	-	-	-	-	24.4 (0.23)_a_	27.4 (1.1)_a_*	1,057.0(60.1)_a_	1,129.0 (91.6)_bc_*
Natural mature mixedwood forest	31.7 (0.75)_ab_*	35.0 (2.4)_a_	38.9 (2.8)_ab_	1,500.0 (148.2)_bc_	2,339.0 (221.1)_bc_	23.5 (2.0)_a_	26.8 (3.5)_a_	1,034.5 (181.2)_a_	2,045.5 (315.7)_a_
Open field	29.7 (0.84)_b_	33.4 (1.6)_a_	45.2 (2.8)_a_*	5,943.0 (363.2)_a_	10,066.0 (1010.6)_a_*	24.5 (0.67)_a_	27.8 (1.4)_a_*	1,024.5 (39.4)_a_	1,303.5 (100.2)_b_*

Table A.4: Average growth data of *V. myrtilloides* seedlings planted in 2019 and 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	2019 Plant			**2020 Plant**	
	Height (cm)			Height (cm)	
	2019	2020	2021	2020	2021
Reclaimed 30-year mixedwood (SH)	13.2 (1.1)*ab**	6.7 (0.37)*c*	8.5 (1.0)*c*	10.1 (0.78)*b*	10.8 (0.65)*bc*
Reclaimed 30-year jack pine (JP)	-	-	-	11.8 (0.87)*ab*	12.0 (0.62)*abc*
Reclaimed 16-year spruce/grass (MLSB)	14.4 (0.76)*ab**	10.3 (1.1)*b*	10.2 (0.88)*bc*	12.2 (0.59)*ab*	10.3 (0.57)*bc*
Reclaimed 11-year sandy (SHF)	-	-	-	11.5 (0.62)*ab*	7.6 (1.9)*c*
New 2019 reclamation site (EIP)	12.8 (0.71)*b**	9.7 (0.51)*b*	11.1 (0.53)*bc*	-	-
New 2020 reclamation site (EIP)	-	-	-	12.7 (0.88)*ab*	16.5 (0.45)*a**
Natural mature mixedwood forest	16.4 (0.71)*a**	15.2 (0.48)*a*	13.8 (0.83)*ab*	12.8 (1.5)*ab**	11.2 (1.2)*bc*
Open field	14.6 (0.44)*ab**	14.9 (0.83)*a**	14.8 (0.72)*a**	14.7 (0.48)*a**	14.2 (0.45)*ab**

Table A.5: Average growth data of *V. vitis-idaea* seedlings planted in 2019 and 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	2019 Plant		**2020 Plant**	
	Bunch size (cm^2)			
	2020	2021	2020	2021
Reclaimed 30-year mixedwood (SH)	110.5 (21.1)_c_*	141.0 (7.8)*b*	92.0 (12.9)_b_*	88.0 (7.9)*b*
Reclaimed 30-year jack pine (JP)	-	-	181.0 (21.9)*ab*	165.0 (27.7)*b*
Reclaimed 16-year spruce/grass (MLSB)	225.0 (25.1)*bc*	234.0 (42.2)*b*	218.0 (22.2)*a*	62.0 (30.2)*b*
Reclaimed 11-year sandy (SHF)	-	-	161.0 (9.4)*ab*	-
New 2019 reclamation site (EIP)	524.0 (85.9)*a*	1,327.0 (284.9)*a*	-	-
New 2020 reclamation site (EIP)	-	-	211.0 (21.9)*a*	367.0 (37.5)*a*
Natural mature mixedwood forest	235.0 (17.3)*bc*	371.0 (42.5)*b*	195.0 (34.2)*a*	205.0 (9.7)b
Open field	314.5 (22.0)*b*	209.0 (39.9)*b*	209.0 (47.4)*b*	113.5 (13.8)*b*

Table A.6: Average growth data of *S. albus* seedlings planted in 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	Bunch size (cm^2)		RCD (mm)	
	2020	2021	2020	2021
Reclaimed 30-year mixedwood (SH)	354.5 (50.1)*bc*	783.5 (96.6)*b*	2.8 (0.22)*bc*	3.0 (0.09)*b*
Reclaimed 30-year jack pine (JP)	322.0 (81.4)*bc*	496.0 (101.4)*bc*	1.8 (0.12)*bc*	2.5 (0.21)*b*
Reclaimed 16-year spruce/grass (MLSB)	1.5 (0.17)*d*	1.5 (0.23)*d*	1.6 (0.15)*c*	1.7 (0.21)*a*
Reclaimed 11-year sandy (SHF)	233.5 (32.6)*cd*	201.5 (24.1)*cd*	1.9 (0.13)*c*	2.1 (0.19)*b*
New 2020 reclamation site (EIP)	313.0 (34.8)*b*	457.0 (18.5)*bc**	2.5 (0.28)*b*	3.6 (0.17)*b**
Natural mature mixedwood forest	581.5 (116.6)*ab*	763.5 (116.1)*b*	3.9 (0.36)*a*	5.3 (0.48)*b*
Open field	761.5 (59.1)*a*	1,193.5 (105.0)*a**	6.0 (0.46)*a*	6.4 (0.46)*b**

Table A.7: Average growth data of _L. groenlandicum_ seedlings planted in 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	Height (cm)		RCD (mm)	
	2020	2021	2020	2021
Reclaimed 30-year mixedwood (SH)	27.6 (1.4)_abc_	23.8 (2.1)_ab_*	3.7 (1.1)_a_	3.7 (0.16)_b_*
Reclaimed 30-year jack pine (JP)	25.3 (1.0)_abc_	23.9 (1.8)_ab_	2.7 (0.18)_a_	3.3 (0.11)_b_
Reclaimed 16-year spruce/grass (MLSB)	28.8 (2.7)_abc_	-	2.3 (0.12)_a_	-
Reclaimed 11-year sandy (SHF)	23.9 (1.2)_bc_	-	3.4 (0.11)_a_	-
New 2020 reclamation site (EIP)	22.6 (1.7)_c_	28.5 (1.4)_ab_*	3.7 (0.27)_a_	4.7 (0.20)_a_*
Natural mature mixedwood forest	31.4 (1.4)_a_*	32.4 (4.1)_a_*	3.1 (0.34)_a_*	2.9 (0.25)b*
Open field	30.6 (1.1)_ab_*	19.3 (1.2)_b_*	3.7 (0.15)_a_*	3.3 (0.27)_b_*

Table A.8: Average growth data of *E. nigrum* seedlings planted in 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	Height (cm)		Bunch size (cm^2)	
	2020	2021	2020	2021
Reclaimed 30-year jack pine (JP)	15.0 (0.66)*a*	16.0 (0.92)*ab*	509.0 (53.1)*a*	523.5 (30.4)*b*
Reclaimed 11-year sandy (SHF)	15.3 (0.33)*a*	14.1 (0.76)*b*	597.5 (58.2)*a*	393.5 (60.9)*b*
New 2020 reclamation site (EIP)	14.7 (0.99)*a*	18.9 (0.90)*a*	823.0(119.6)*a*	1587.0 (123.6)*a*

Table A.9: Average growth data of *V. edule* seedlings planted in 2020 on different sites in each monitored growing season. Standard errors are in parentheses and different letters indicate a significant difference between sites for the respective measuring period ($p \leq 0.05$).

	Height (cm)		RCD (mm)	
	2020	2021	2020	2021
Reclaimed 30-year mixedwood (SH)	17.6 (1.2)*a*	16.9 (1.5)*a*	3.2 (0.28)*ab*	3.4 (0.23)*ab*
Reclaimed 30-year jack pine (JP)	20.0 (0.97)*a*	20.6 (0.94)*a*	2.9 (0.27)*b*	3.3 (0.13)*b*
New 2020 reclamation site (EIP)	15.6 (1.3)*ab*	15.5 (1.1)*a*	3.8 (0.37)*ab*	4.2 (0.29)*a*
Natural mature mixedwood forest	11.2 (0.85)*b*	15.5 (1.6)*a*	4.1 (0.18)*a*	3.6 (0.09)*ab*

Table A.10: Two-way ANOVA output for total mortality of the four species that were planted on all sites in 2019 and 2020 to identify any species × site interactions.

	2019 Plant				2020 Plant			
	SS	df	F	p-value	SS	df	F	p-value
Species	24233	3	46.8	**≤0.001**	3940	3	4.9	**0.003**
Site	53	4	0.08	0.99	30708	6	18.9	**≤0.001**
Species × Site	12320	12	5.9	**≤0.001**	5573	18	1.1	0.32

Table A.11: Two-way repeated measure ANOVA output for total mortality of the four species that were planted on all sites in 2019 and 2020 to identify any differences in seedling response over time.

	SS	df	F	p-value
Species	5523	2	10.4	**≤0.001**
Site	60417	4	56.8	**≤0.001**
Year × Site	1204	4	1.1	0.34
Year × Species	1970	2	3.7	**0.028**
Site × Species	4917	8	2.3	**0.024**
Year × Site × Species	3829	8	1.8	0.083

Table A.12: One-way ANOVA outputs showing differences in growth and mortality response variable means among sites. Data is from the 2019 plant (three growing seasons). Hyphens used when a measurement was not taken on that species.

	L. involucrata	*V. myrtilloides*	*V. vitis-idaea*
Mortality After one growing season	0.24	0.19	**0.001**
Mortality After two growing seasons	**0.01**	**$\leq$0.001**	**0.01**
Mortality After three growing seasons	**$\leq$0.001**	**$\leq$0.001**	**$\leq$0.001**
Height (cm) After one growing season	**$\leq$0.001**	**0.03**	**-**
Height (cm) After two growing seasons	**0.01**	**$\leq$0.001**	**-**
Height (cm) After three growing seasons	**$\leq$0.001**	**$\leq$0.001**	**-**
Bunch size (cm^2) After two growing seasons	**$\leq$0.001**	-	**$\leq$0.001**
Bunch size (cm^2) After three growing seasons	**$\leq$0.001**	-	**$\leq$0.001**

Table A.13: One-way ANOVA outputs showing differences in growth and mortality response variable means among sites. Data is from the 2020 plant (two growing seasons). Hyphens used when a measurement was not taken on that species.

	L. involucrata	*V. myrtilloides*	*V. vitis-idaea*	*S. albus*	*L. groenlandicum*	*E. nigrum*	*V. edule*
Mortality After one growing season	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	**0.008**	**0.01**	0.33
Mortality After two growing seasons	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**	0.21
Height (cm) After one growing season	0.07	**0.04**	-	-	**0.003**	0.81	**≤0.001**
Height (cm) After two growing seasons	**0.004**	**≤0.001**	-	-	**0.009**	**0.01**	**0.04**
Bunch size (cm^2) After two growing seasons	**≤0.001**	-	**0.003**	**≤0.001**	-	0.06	-
Bunch size (cm^2) After three growing seasons	**≤0.001**	-	**≤0.001**	**≤0.001**	-	**≤0.001**	-
RCD (mm) After one growing season	**-**	-	-	**≤0.001**	0.24	-	**0.03**
RCD (mm) After two growing seasons	**-**	-	-	**≤0.001**	**≤0.001**	-	**0.03**

Figure A.10: 30-year mixedwood site in August 2021.

Figure A.11: 30-year jack pine site in August 2021.

Figure A.12: 16-year spruce/grass site in August 2021.

Figure A.13: 11-year sandy site in August 2021.

Figure A.14: New 2019 reclamation site in August 2021.

Figure A.15: New 2020 reclamation site in August 2021.

Figure A.16: Natural mature mixedwood forest in August 2021.

Figure A.17: Open field site in August 2021.