Evaluating trembling aspen seedling stock characteristics in response to outplanting and competition

by

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Abstract

Trembling aspen is commonly used in the afforestation of boreal forest reclamation sites; however, the presence of vegetation competition can reduce growth and establishment success. This research examines how initial morphological characteristics influence the above and belowground growth of aspen seedlings planted on sites where vegetation competition could act as a barrier to establishment. In the first study, aspen seedlings were planted in three plots with similar soil conditions but different levels of competition to determine which morphological characteristics were correlated with increased growth. Results show that a high initial root-to-stem ratio is beneficial for improved height growth in plots where competition is removed prior to planting. A high initial root-to-stem ratio did not benefit height growth in the grass treatment which had a high amount of belowground competition. Improving root egress (root development into the surrounding soil) is beneficial for seedling establishment, as there was a significant relationship with height growth. In a second study, seedlings were planted on three reclamation sites with contrasting soil conditions and vegetation features in northwestern Alberta. Seedlings with high root-to-stem ratios outgrew seedlings with low root-to-stem ratios on all three sites, which indicates that a high root-to-stem ratio is an important aspect of early establishment success over a range of site conditions. At an agricultural field where soil moisture was not limiting and competing vegetation was low, morphological characteristics of seedlings had less of an influence on performance. In sites where aboveground competition was tall, seedling height also became a factor for improving growth as tall seedling stock types with a high root-to-stem ratio performed the best. At a site where water and competing vegetation were both limiting factors, stress was likely too high as overall performance was poor across all stock types. The results from this thesis indicate that establishment success can be improved by using aspen with

a high root-to-stem ratio in conjunction with the periodic control of competing vegetation; however, if site conditions are extremely poor, the effects of higher seedling quality will be outweighed by the poor site conditions .

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Chapter 1 – Introduction

1.1. Boreal forest and land reclamation

The boreal forest is the largest terrestrial biome in Canada, occupying one third of its land area (Brandt 2009). Vegetation in the biome is dominated by a small number of widespread coniferous trees (within genera including *Abies*, *Larix*, *Picea*, and *Pinus*) accompanied by other deciduous species (*Populus* and *Betula*) (Brandt et al. 2013). The high-latitude continental climate is generally characterized by long cold winters, short cool summers, and low annual precipitation; however, there is significant variation between regions (Price et al. 2013). In addition to the vast number of lakes, rivers, and wetlands, the boreal forest supplies numerous ecosystem services (e.g., climate regulation, nutrient cycling) as well as both renewable and non-renewable resources (e.g., timber, minerals, oil and gas).

Natural resources present in the boreal forest have prompted various anthropogenic disturbances associated with resource extraction. Timber products are harvested from forestry operations spread throughout the southern boreal zone, extending into certain temperate areas (Brandt et al. 2013). Forest stands are typically clear-cut with mechanical harvesters; however, various silvicultural treatments are becoming increasingly common in an effort to promote sustainable management (Lieffers et al. 1996). Harvested areas can be regenerated artificially (with seeds or planted seedlings) or left to regenerate naturally if site and seedbed conditions are favorable (Brandt et al. 2013). While forestry plays a large role amongst anthropogenic disturbances impacting the boreal forest, the total harvested area is a fraction of the area affected by natural fires annually (Schmiegelow et al. 2006; Brandt et al. 2013).

Mining in the boreal zone includes extraction of minerals and metals, coal, aggregates such as gravel and sand, and bitumen extracted from oil sands. Surface mining operations involves stripping and stockpiling of surface soils while resource extraction is underway; the reclaimed landscape following this type of disturbance is reconstructed from a variety of overburden materials as well as salvaged and stored surface soils (Macdonald et al. 2015). In contrast to fire and forestry, which generally leave the soil undisturbed, surface mining drastically impacts soil properties as soil horizons are excavated and stockpiled. This presents unique challenges for site restoration as excavation causes abrupt changes to soil structure, chemistry, and the propagule bank resulting in a longer recovery period (Macdonald et al. 2015).

While impacts will vary by site, some of the changes to soil conditions can include elevated nitrogen levels (Rowland et al. 2009), increased bulk density from heavy machinery traffic, and reduced soil moisture along with raised soil temperatures due to the absence of aboveground vegetation (McMillan et al. 2007). In situ development associated with oil and gas exploration is characterized by linear features (networks of seismic lines, access roads, pipelines, and well sites) which are less of a large-scale and widespread disturbance (as in mining) but do create heterogeneity of disturbed and undisturbed areas throughout a larger landscape (Burrowes et al. 2010; Jordaan 2012). In 2009, 45% of bitumen was produced with in situ technology while the remaining 55% was recovered through surface mining; however, in situ recovery is expected to account for a larger share of future production as remaining deposits are deep underground (Rahnama et al. 2013). Both of these methods result in significant landscape fragmentation, which can have negative effects on wildlife diversity and ecosystem function (Linke et al. 2005; Machtans 2006).

In Alberta, industrially disturbed sites are required to be reclaimed to an equivalent land capability, which refers to the ability of the land to support land uses similar to what was present prior to the disturbance (Powter et al. 2012). For reclamation sites in the boreal forest, this typically involves reestablishment of a functioning, self-sustaining ecosystem integrated into the surrounding landscape (Macdonald et al. 2015). Following topographic and soil reconstruction, the reestablishment of native vegetation is critical for restoring ecosystem processes (Padilla and Pugnaire 2006; Grant et al. 2007). Planting tree seedlings is an effective way to encourage the early development of a forest with closed canopy conditions, an important step in early forest development for excluding largely non-native and undesirable early successional herbaceous species (Setterfield et al. 2005; Mandryk and Wein 2006), and developing an organic soil layer through increased litter fall (Paré and Bergeron 1996). Trembling aspen (*Populus tremuloides* Michx.) can play a key role in the recovery of disturbed sites as it is an early successional species commonly found after natural disturbances and is able to grow quickly once established.

1.2. Ecology of aspen

Trembling aspen is a deciduous tree species native to the boreal forest in Canada. Its North American distribution ranges from the southwestern regions of Alaska to the coasts of Newfoundland and Labrador, with southern populations found at higher elevations in northern Mexico (Rowe 1972; Barnes 1975; Peterson and Peterson 1992). The species is widespread throughout Alberta, accounting for 75-95% of deciduous trees found in the province, and is the dominant species in natural subregions such as the Dry Boreal Mixedwood (Natural Regions Committee 2006; Gray et al. 2011). Aspen plays an important ecological role in the boreal forest, as it is a keystone species which supports various ecosystem processes (Campbell and Bartos 2001). Litter fall from the aspen canopy improves soil fertility through increased nutrient cycling relative to conifer stands (Cleve and Noonan 1975; Cragg et al. 1977), which subsequently benefits plant understory diversity (Oaten and Larsen 2008). Increased understory and structural diversity in aspen stands has the added benefit of improving diversity of other insect (Schimpf and MacMahon 1985), bird (Rumble et al. 2001), and non-vascular plant species (Crites and Dale 1998). Aspen is a clonal tree species that can reproduce asexually by producing genetically identical suckers that arise from the lateral roots of a parent tree; however, sexual reproduction can occur through mass production of lightweight, wind dispersed seeds (Peterson and Peterson 1992). Seed based establishment was previously thought to be uncommon (Maini 1968), given that seeds are short lived in natural environments and have stringent requirements for germination such as an exposed mineral substrate, sufficient moisture, minimal competition, and concave microsites (Maini 1968; Landhäusser et al. 2010; Pinno et al. 2012). However, recent studies have shown seed based establishment to be common after natural and anthropogenic disturbances (Turner et al. 2003; Romme et al. 2005), as long as the appropriate climatic and substrate conditions are present (Landhäusser et al. 2010; Long and Mock 2012; Schott et al. 2014). Once established, the lateral root system of aspen makes it a fast growing species able to tolerate severe disturbances such as drought (Lieffers et al. 2001) and fire (Brown and DeByle 1987), and allows it to colonize sites characterized by low fertility (Fox et al. 2013), pollution (Winterhalder et al. 2000; Cripps 2003), and soils with heavy metal content (Lozano and Morrison 1981).

1.3. Challenges with seedling establishment

Given its rapid reproduction and phenotypic plasticity, aspen can serve as an important tool for revegetation of boreal sites disturbed by industrial activity. While seed based establishment for aspen can occur on reclamation sites (Schott et al. 2014), container grown seedlings are typically planted to better facilitate canopy closure (Macdonald et al. 2012). However, the problems that limit seed germination can also affect aspen seedling performance (typically determined as growth or survival) as harsh conditions on reclamation sites can limit growth and survival for several years after outplanting (van den Driessche et al. 2003; Martens et al. 2007). This period of reduced growth and survival after outplanting is referred to as transplant/planting shock, which occurs as a result of seedlings acclimatizing to their new environmental conditions (Mullin 1963; Sutton and Tinus 1983). While transplant shock is generally due to restricted root growth or poor root-soil contact preventing the roots from supplying enough water to the shoot (Sands 1984; Grossnickle 2005), it can also be attributed to seedling characteristics or environmental conditions (Close et al. 2005). Drought stress is expected to become increasingly problematic as droughts across the boreal forest are projected to be more frequent and severe over the next 100 years (Wang et al. 2014). Additionally, seedling establishment success may be further impaired by drought as hydraulic damage may impair carbon accumulation in the roots, which could compromise frost tolerance and lead to root death during the dormant season (Galvez et al. 2013).

Competition is another common cause of transplant shock, particularly on older reclamation sites which have not been immediately revegetated after initial disturbance as grasses and early successional herbaceous species are quick to colonize open spaces in the boreal forest (Lieffers et al. 1993; Osko and Glasgow 2010). Aspen seedlings are especially sensitive to competition (Landhäusser and Lieffers 1998), as newly planted seedlings are adjusting to their new environment. Grasses are especially problematic, as their high root-to-shoot ratios allows for a significant competitive advantage belowground over newly planted seedlings (Tilman 1988). Height and diameter growth of aspen seedlings were severely reduced when grown with species such as marsh reed grass (*Calamagrostis canadensis* (Michx.) Beauv.), due to reduced nitrogen availability and soil moisture (Landhäusser and Lieffers 1998; Powell and Bork 2004b; Hu et al. 2015). Belowground competition can also limit root development, as the root biomass of aspen seedlings has been shown to be severely reduced in the presence of grasses (Bockstette et al. 2017). The sod layer forming from grass root and litter near the soil surface has additional impacts on aspen seedlings as infiltration is impeded and nutrients are immobilized (Wilson 1998). In addition, the sod insulates and cools the soil, which can reduce photosynthetic rates and growth (Landhäusser and Lieffers 1998). The physical barrier of the sod, has also been shown to delay suckering in aspen (Landhäusser et al. 2007).

1.4. Seedling morphological characteristics

Seedlings planted on reclamation sites are subject to a wide variety of environmental stresses, therefore ensuring high seedling quality is critical to successful establishment (Mattsson 1997). Production of high quality seedlings is dependent on nursery practices that can optimize morphological and physiological attributes that are linked to improved growth and development on these sites (Wilson and Jacobs 2006), however, these attributes likely have some species specificity. When morphological and physiological characteristics of the seedlings become tailored towards specific site conditions, reforestation success can be improved (i.e. target seedling approach, see Rose et al. 1990). Morphological characteristics such as initial height and root collar diameter were traditionally used to assess seedling quality, as these characteristics were easily measured (Wakeley 1948; Stoeckeler and Slabaugh 1965; Mexal and Landis 1990). It has been suggested that taller seedling stock should, in principle, have better performance in competitive environments (Overton and Ching 1978; Newton et al. 1993; Pope 1993); however, a number of studies have shown that height is a poor predictor of performance for certain species (Chavasse 1977; Tuttle et al. 1988; Thompson and Schultz 1995), including aspen (Landhäusser et al. 2012b). Root collar diameter has been shown to have more consistent positive results over height (Mexal and Landis 1990; Aphalo and Rikala 2003; Wilson and Jacobs 2006); however, it still provides no indication of root system quality (Davis and Jacobs 2005). Root volume and number of first-order lateral roots have been suggested as better indicators of performance for both coniferous (Grossnickle, 2005) and deciduous seedlings (Wilson and Jacobs 2006), as successful seedling establishment is largely dependent on the seedling's ability to grow new roots, develop a proper water balance, and effectively utilize soil nutrients during establishment (Jacobs et al. 2005). Other root-based measurements such as root growth potential and root-to-shoot ratio are useful morphological characteristics which give an indication to physiological traits such as carbohydrate content, nutrient status, and drought avoidance potential (Ritchie and Dunlap 1980; Close et al. 2005; Grossnickle 2012).

1.5. Aspen seedling quality

Previous work with aspen seedlings has indicated that stock types exhibiting high root-to-shoot ratios and total non-structural carbohydrate (NSC) reserves in roots are positive indicators of seedling performance (Martens et al. 2007; Landhäusser et al. 2012b). Martens et al. (2007) identified the importance of these characteristics when naturally regenerated aspen seedlings outgrew nursury produced seedlings that were intially 10 times taller, after two growing seasons. Higher root-to-shoot ratios and NSC reserves in the small seedlings were related to earlier bud set in the first growing season. Completion of aboveground seedling growth better allows for NSC allocation to roots (as well as root growth). Given the indeterminate growth strategy of aspen, ensuring that seedlings set bud is critical for developing higher root-to-shoot ratios and NSC reserves. Water and nutrient withholding, shortening of day length, and shoot growth inhibiting hormones (ex. paclobutrazol) have been found to be effective tools for reducing or terminating shoot growth (Landhäusser et al. 2012b; Schott et al. 2013). Seedlings continue to photosynthesize after shoot growth ceases and can accumulate nutrient reserves for the remainder of the growing season. Improving nutrient reserves in aspen seedlings can result in greater height growth in the first year after outplanting (Landhäusser et al., 2012b; Schott et al. 2016).

Shoot growth inhibitors can also be used in conjunction with fertilization to create nutrient loaded seedlings, potentially providing seedlings with an advantage over competing plants after outplanting (Timmer and Aidelbaum 1996). Fertilizers can be applied at a constant or an exponential rate, where the amount of fertilizer increases over time; however, exponential fertilization has marginal benefits for aspen (Hu et al. 2015) and the low initial application rates have been shown to result in low quality aspen stock (Schott et al. 2013). The growth of nutrient loaded seedlings is optimal when seedlings are planted in the fall or spring, as this is when seedlings will have higher NSC reserves and experience less moisture stress during planting (Landhäusser et al. 2012b). Nutrient loaded seedlings with high root-to-shoot ratios and NSC reserves have been shown to achieve significant height growth on reclamation sites in the first two years after outplanting (Landhäusser et al. 2012b); however, it is still unclear if these characteristics are as significant on nutrient limited or highly competitive sites.

1.6. Research objectives

The aim of this research was to determine how initial seedling characteristics influenced the above and belowground growth of aspen seedlings planted into sites where vegetation competition would be a potential barrier to successful establishment. In the first study, aspen seedlings were planted within three plots with varying levels of competition to determine how competition impacted above and belowground development of aspen seedlings and what initial morphological characteristics were correlated with increased growth. In the second study, seedlings were planted across three sites with contrasting soil and vegetation features in NW Alberta. Multiple comparisons were used to test how a high root-to-stem ratio and seedling size impacted seedling performance. Tall seedlings were hypothesized to perform better on sites with vegetation competition, while seedlings with a high root-to-stem ratio were hypothesized to have better performance (i.e. increased growth and survival) on drier sites.

Chapter 2 – Manipulating aspen planting stock morphology to improve establishment on competitive sites

2.1. Introduction

Trembling aspen (*Populus tremuloides* Michx.) is a widely distributed tree species native to the boreal forest in Canada. Its geographic distribution in North America ranges from the coasts of the Maritime provinces to the southwestern regions of Alaska, with smaller populations reaching as far south as Mexico (Rowe 1972; Peterson and Peterson 1992). It is a clonal tree species that can reproduce asexually through genetically identical suckers that arise from its lateral roots; however, sexual reproduction can occur through production of millions of lightweight, wind dispersed seeds (Day 1944; Maini 1968). In the past, seedling based regeneration of aspen was considered to be rare due to a narrow range of requirements and conditions (Baker 1918; Maini 1968). However, more recent evidence shows that after natural and anthropogenic disturbances (Romme et al. 1997; Turner et al. 2003; Romme et al. 2005), aspen seedling establishment is common, particularly when provided with the appropriate seedbed and climatic conditions (Landhäusser et al. 2010; Long and Mock 2012; Schott et al. 2014). Once established, aspen is a fast growing species that is considered relatively drought tolerant due to its extensive lateral roots system (Lieffers et al., 2001; Martens et al. 2007). These and other characteristics make aspen a desirable species for use in afforestation. In areas where aspen regeneration through vegetative means is not possible, planting aspen seedlings can be useful for facilitating rapid seedling establishment, rather than attempting to create the ideal conditions for natural regeneration through seed (Macdonald et al. 2012).

Planting success of aspen or any other species is dependent on the quality of the planted seedling stock. Seedling quality can be assessed through the evaluation of specific morphological and physiological characteristics and traits. By emphasizing specific traits, seedlings can be produced specific to site conditions that will improve reforestation success (i.e. the target seedling approach, see Rose et al. 1990). Depending on the growing conditions during nursery production, specific morphological and physiological characteristics can be developed in a seedling that are desirable for a seedling to tolerate and overcome environmental stresses early after outplanting (Wilson and Jacobs 2006). However, these characteristics are not often universal and are species and site specific. Easily measured characteristics such as increased shoot length and root collar diameter are commonly used to predict field performance (Chavasse 1980; Mexal and Landis 1990; Mattsson 1997). However, these characteristics are not always the best indicators for individual species. For example, initial height has been shown to be negatively correlated with subsequent height growth for northern red oak (*Quercus rubra* L.) (Thompson and Schultz 1995) and Olga Bay larch seedlings (*Larix olgensis* Henry) (Li et al. 2011), as well as survival rates for loblolly pine seedlings (*Pinus taeda* L.) (Tuttle et al. 1988). Although more difficult to measure, root morphological characteristics such as root mass, volume, and growth potential may be better indicators of performance (Jacobs et al. 2005; Wilson and Jacobs 2006).

When faced with high competition, seedlings are exposed not only to limited resources, but also potentially to conditions that limit above and belowground growing space, which likely exacerbate the effects of planting check (which is the period of reduced growth after outplanting; Mullin 1963). The presence of grasses can be particularly problematic, as aggressive rhizomatous species such as marsh reedgrass (*Calamagrostis canadensis* (Michx.) Beauv.) can rapidly colonize open spaces (Lieffers et al. 1993). Grasses typically have a significant belowground advantage over newly planted seedlings as they have dense fibrous root systems with relatively high root-to-shoot ratios which allow for efficient uptake of water and nutrients (Tilman 1988). Growth limitations of aspen seedlings and sessile oak (*Quercus petraea* (Mattus.) Liebl.) have been well documented when grown with competing grasses (Landhäusser and Lieffers, 1998; Powell and Bork, 2004a, Collet et al. 2006). The accumulation of grass roots and litter near the surface can also have deleterious effects on resource availability, as the sod layer can impede water infiltration, immobilize nutrients, and insulate and keep soils cool (thereby delaying sucker emergence) (Wilson 1998, Landhäusser and Lieffers 1998; Landhäusser et al. 2007).

There have been a number of studies which have documented the effect of competition on aspen seedlings (Landhäusser and Lieffers 1998; Powell and Bork 2004a, b; Donaldson et al. 2006; Rahman et al. 2013); however, there has not been any studies which explore the relationship between morphological characteristics and aspen seedling performance (i.e. growth and survival) when competition is present. Previous work with aspen seedlings has shown that initial high root-to-shoot ratios and carbon reserves in roots were positive indicators of outplanting performance on sites with limited resources such as water and nutrients (Martens et al. 2007; Landhäusser et al. 2012b). The importance of these characteristics were shown when naturally regenerated aspen seedlings outgrew nursery produced seedlings in the second growing season; nursery seedlings were ten times taller but had about eight times lower root-to-shoot ratios (Martens et al., 2007). Producing high root-to-shoot ratios in seedlings with an indeterminate growth strategy is difficult. Water and nutrient withholding, shortening of day length, and shoot growth inhibiting hormones (ex. paclobutrazol) have been found to be effective tools for reducing or terminating shoot growth. Limiting shoot growth while maintaining leaf area allows seedlings to accumulate nutrient and non-structural carbon (NSC, soluble sugars and starch combined) reserves which translates into significantly greater height growth in the first year after outplanting (Landhäusser et al., 2012b; Schott et al. 2016); however, it is still unclear if these characteristics are also beneficial on highly competitive sites.

For this study, different aspen stock types which varied in initial height, root-to-shoot ratio, and root NSC concentration were planted in sites with varying levels of vegetation competition to determine 1) what morphological characteristics benefitted the performance (determined as growth and survival) of aspen seedlings in a highly competitive environment and 2) how the different levels of competition impacted above and belowground development of aspen seedlings.

2.2. Materials and methods

2.2.1. Seedling stock production

Aspen seedlings were grown from seeds collected in the Peace River Dry Mixedwood Lowland region (645-655 m a.s.l.) from open pollinated sources in 2012 (registered seedlot: NNAIT 16-84-23-5-2012 AW). To develop stock types with varying morphological characteristics, different combinations of seedling age, container size, and nursery location were used (Table 2-1). Seedling age (rounded to the nearest month and a half) for each stock type was determined as total number of days from sowing date to the date seedlings were lifted. Containerized seedling production occurred in two locations (Woodmere Nursery Ltd. (WM), Fairview, Alberta (56.064 N - 118.378 W; 647 m a.s.l.) and at the University of Alberta (UA), Edmonton, Alberta (53.526 N - 113.527 W; elevation 677 m a.s.l.). To ensure substrate uniformity, all containers were filled at WM with peat. Different stock types were produced, using two sowing times (April and May of 2013) and two container volumes (volume of 340 and 700 ml using a 615A or 815A styroblock containers, respectively; Beaver Plastics Ltd., Alberta, Canada). Fertilizer, a custom made solution of 1 g/L 10-52-10 (N-P-K) with chelated micro-nutrients, was applied two weeks after seeding. After four weeks, each cell was thinned to one aspen seedling and then fertilized twice per week with a solution of 1.2 g/L 6-15-10 (N-P-K) plus chelated micronutrients until the soil reached saturation. In June, the concentration of fertilizer was doubled (2.4 g/L) for all stock types. Fertilization was terminated on September 13, 2013. Seedlings were initially grown for a total of 9 weeks in the greenhouse at an average temperature of 25°C, relative humidity of 50%, and 20 h extended daylight photoperiod using artificial lighting. After that seedlings were moved outside for the rest of the growing season (with earlier sow dates moved out first). To increase root-to-shoot ratios, a shoot growth inhibitor (Paclobutrazol, Bonzi®, Syngenta, North Carolina, USA) was applied to seedlings (Landhäusser et al. 2012b). The inhibitor was applied by soaking the root systems contained in the styroblocks in a water bath containing a solution of 5 ml of Bonzi per L of water (equivalent to 20 ppm paclobutrazol per liter of water) on July 10 (for April 7 and May 1 sowing dates) and July 15 (May 22 sowing date). Bud set occurred approximately two weeks after treatment (late July). After leaf senescence (November 1) was completed, seedlings were lifted and packaged and placed into frozen storage (-4˚C) until outplanting in the spring of 2014. Variation in growing conditions (particularly during the outdoor period) between the two locations resulted in significant differences between seedling stock from the two growing locations; this resulted in the production of eight different stock types. In addition to the eight manipulated stock types described above, a 'standard' operational nursery stock type was included for comparison. This standard stock type was only produced at the Woodmere nursery under routine operational seedling production procedures for aspen. Seeds were sown into 512A styroblock containers on May 15th, 2013. They were initially grown together with the manipulated stock indoors, but moved outdoors (adjacent to manipulated stock) on July 15, 2013 and pruned. Fertilization occurred through fertigation with the same fertilizer but usually 2-3 times per week when watering was required.

At the time of fall lifting, a representative subset of 10 seedlings from each stock type were sampled to determine morphological traits and carbohydrate reserve concentrations. Height and root collar diameter were measured and roots and stems were separated for their respective dry mass measurements from which root-to-stem ratios were calculated. For leaf nutrient concentrations and stem and root carbohydrate tissue concentrations, dried samples were ground to pass 40 mesh using a Wiley Mill. Water soluble sugars were extracted from root and stem tissues using 80% hot ethanol and quantified colorimetrically using phenol-sulfuric acid. Starch concentration was determined colorimetrically by digesting the residue from the sugar extraction in a mixture of α-amylase and amyloglucosidase enzymes followed by a peroxidase–glucose oxidase/*o*-dianisidine reaction (Chow and Landhäusser 2004). Water soluble sugars and starch concentrations were expressed as glucose equivalents on a percent dry mass basis. To determine the nutrient status of seedlings, total nitrogen, phosphorus, and potassium were measured in green leaves subsampled across different styroblocks from each stock type on September 16 prior to leaf senescense. Total nitrogen in the ground leaves was extracted using the Dumas combustion method and measured with the EA 4010 Elemental Analyzer (Costech Analytical Technologies, California, USA). Phosphorous and potassium were extracted using a nitric acid digestion and the run using the ICP-OES iCAP 6000 (Thermo Fisher Scientific, Massachusetts, USA). Leaf nitrogen, phosphorus, and potassium are reported as percent dry mass.

2.2.2. Site description

In the spring of 2014, seedlings were outplanted in three different sites at the Crop Diversification Center (CDC) North (Edmonton, Alberta 53.642 N - 113.361 W; elevation 651 m a.s.l.); a large agricultural research area in the parkland region of Alberta. Three site types were selected: (1) an open agricultural field that had been in crop production for more than 20 years (control); (2) an active hayfield that had been in production for more than 20 years, and (3) an area in the same hayfield that was plowed prior to planting in the spring of 2014. In the open field, the planting rows were covered with a plastic mulch and periodically weeded in-between rows to minimize competition for the duration of the study. In each of the three sites one planting plot was established. Each planting plot was divided into 10 blocks each containing 4 seedlings (subsample) of each stock type. A total of 360 seedlings were planted in each planting plot. Spacing was 1 m between seedlings in each block and 2 m spacing between blocks.

Mean monthly precipitation and air temperature during the growing season for 2014 and 2015 was obtained from the Oliver Agricultural Drought Monitoring station located at CDC (ACIS, 2016). Soil water potential, water content, and soil temperature were measured hourly with MPS-2 and 5TM sensors (Decagon Devices Inc., Washington, U.S.A.) attached to EM50 data loggers (Decagon Devices Inc., Washington, U.S.A.) and averaged daily. Data loggers were installed at the center of each planting plot at the beginning of May 2015. Soil characteristics for each site treatment were further assessed with six 340 cm^3 cores (2 cores at each depth) collected at three depths (0-5 cm, 10-15cm, 20-25cm) in each of 10 blocks in the fourth week of May. Bulk density for each depth was measured as the dry mass of the soil divided by the volume of the core. As sites were very uniform, samples of two blocks were combined for each depth. Electrical conductivity was determined using the saturated paste method (Slavich and Petterson, 1993). To measure the soil nutrient concentration, 15 samples (5 paired samples from each treatment) were analyzed for Mg^{2+} and S concentrations using the microwave-assisted nitric acid digestion method (Hassan et al. 2007), for NO_3-N and NH_4-N using the 2N KCl method (Jones Jr., 2001), and for total C and N by the Dumas combustion method (Bremner 1996; Nelson and Sommers 1996) using a Costech 4010 Elemental Analyzer System (Costech Analytical Technologies Inc., Valencia, CA, USA). Soil texture was determined using particle size analysis (Bouyoucos, 1962).

Above and belowground plant competition was assessed in each site. Aboveground competition was estimated in the first week of July along a transect diagonally ran across the entire planting plot, with a 0.5 × 0.5 m quadrat placed at the point in each block where the transect crossed. In each quadrat, individual plants were identified to the species level, except for grasses, which were grouped together as Poaceae due to a lack of identifying features present at the time. After species were identified, percent cover was visually estimated to the nearest percentage using cardboard cutouts, which corresponded to 1, 5, and 10% of the quadrat area. The height of each plant was measured and subsequently clipped to determine the dry mass of each species present in the quadrat.

The belowground components of competing vegetation were assessed in all three planting plots using soil cores collected with an auger at four depths (0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm). The aspen root component in these soil cores was estimated to be small, as cores were collected at distances from seedlings (50 cm in plowed and grass treatment and 1 m away in mulch treatment) that were greater than the average lateral root length measured on excavated seedlings two weeks prior. To estimate belowground root density of the competing vegetation, roots were separated from soil cores using the procedure described by Teste et al. (2012). Soil cores were first passed through a 2 mm sieve and coarse root fragments were extracted. The remaining fine root mass was estimated by suspending the soil and organic matter passed through the 2 mm sieve in water. As the fine organic matter was separated from the soil and water using a 250 µm sieve, a subsample was taken and any fine root fragments present were plucked from the subsample. The mass ratio of fine root to extraneous material in the subsample was then scaled up to the total organic mass to determine total fine root mass. Total root mass was dried at 70˚C until weight constancy and weighed to the nearest 0.01g.

2.2.3. Seedling measurements

Initial height and root collar diameter was measured on all seedlings after outplanting in May. As root-to-stem ratio (i.e. ratio of root and stem dry weight) is a destructive measurement, predictive equations for root-to-stem ratio were developed from the initial seedling data to estimate the initial root and shoot mass of outplanted seedlings based on root collar diameter measurements from seedlings sampled prior to planting (Appendices 3-4). The estimated initial root-to-stem ratios from these equations were determined to be representative of the seedlings, as they were not significantly different ($p = 0.33$) from the root-to-stem ratios of seedlings measured prior to planting. Total height, annual height growth, and root collar diameter were re-measured at the end of the growing season in September 2014, 2015, and 2016. Annual height growth was measured from the bud scar to the terminal bud tip of the longest shoot. Although not common, presence of dead or broken seedlings or new suckers arising from seedlings was noted. In the first week of July, leaves were sampled from seedlings in each block and paired samples of two blocks for each stock type from each treatment (5 samples per stock type) were combined for macro and micronutrient concentrations using the microwave-assisted nitric acid digestion method. To obtain more detailed information on the performance of each stock type after two growing seasons, ninety seedlings were also excavated from each planting plot (i.e. one seedling from each stock type per block) in the third week of June 2015. The roots of each seedling were carefully excavated with hand trowels, following each root to minimize breakage and root loss. All leaves were removed from seedlings and dried at 70°C for two days to determine total dry leaf mass. Specific leaf area was estimated by measuring the leaf area of ten leaves collected from each seedling (LI-3100C, LI-COR, Nebraska, USA) and determining their dry mass. Root systems were separated from the shoot and further processed to separate the roots that had egressed from the original plug. Roots and shoots (stem and leaves) were subsequently dried at 70°C for two days. Dry mass of the roots contained in the plug, roots egressed from the plug, and stem and leaf dry mass was determined for each seedling. New root growth was estimated as the difference between total root mass and the average initial root mass (estimated from equations, see above). Root egress was presented as the proportion of root mass growing outside of the plug relative to the total root mass (i.e. egress root mass divided by total root mass).

2.2.4. Statistical analysis

The effect of nursery, sowing date, and container size on initial seedling morphological (height, root collar diameter, root mass, root-to-stem ratio) and physiological characteristics (root NSC and leaf N, P, K concentrations) were analyzed with a three-way analysis of variance (ANOVA). A three-way ANOVA was also used to assess effects of nursery, seedling age, and container size on seedling performance (height growth, root growth, and root egress). Diagnostic plots of fitted values vs. residuals were used to confirm ANOVA assumptions of equal variances and normality. When significant treatment effects were detected, Tukey's HSD test was used for post hoc comparisons.

To compare the differences in soil characteristics, competing vegetation, and the response of stock types in each site treatment (mulch, plow, grass), data were analyzed using two sample t-tests in R (R Core Team 2016). Log or square root transformations were used for data that did not meet assumptions of normality (Shapiro-Wilk test). F-tests were used to compare the variance of each site treatment and Welch's t-test was used if variances were unequal. If the normality assumption could not be met, a non-parametric test was used (Kolmogorov-Smirnov test). Since multiple comparisons were needed to compare each site treatment, p-values were adjusted using the Benjamini–Hochberg correction.

Linear models from the *lm* function in R were used to test the effects of initial seedling characteristics (height, root collar diameter, root mass, root-to-stem ratio, stem and root NSC concentrations, and leaf nutrient concentrations) on seedling performance in response to the different treatments. Regressions were performed on the average stock type values in each treatment. To compare the slopes of significant regression lines, an analysis of covariance (ANCOVA) was used to compare the regression lines for each treatment. Significantly different slopes were indicated by a significant interaction between site treatment and the covariate. Diagnostic plots of fitted values vs. residuals were used to check for equal variances and assess normality. When diagnostic plots showed patterns of unequal variance, a generalized least squares model (from the *gls* function in R; Pinheiro et al., 2016) was fitted with an additional covariance structure to allow for unequal variance. AIC comparisons between the *lm* and *gls* models were used to confirm selection of the appropriate model. A significance level of $\alpha = 0.05$ was used for all analyses.

2.3. Results

2.3.1. Nursery effects on initial seedling characteristics and seedling performance

In general, seedlings produced at UA were larger than seedling grown at WM; at both nurseries seedling stock with an earlier sow date and larger container size also produced larger seedlings (i.e. greater height, root collar diameter, and root mass) (Table 2-1). However, seedlings with earlier sow dates at the UA nursery grew much larger than WM seedlings with the same sow date (nursery and sowing date interaction $p < 0.02$) for all morphological characteristics. For root mass and root-to-stem ratio, there was an interaction between nursery and container size ($p < 0.04$). Sow date had a significant effect ($p < 0.001$) on both root and stem NSC concentrations, where NSC concentrations increased with later sow dates. Additionally, UA grown seedlings had higher stem NSC concentrations ($p < 0.001$).

There was a significant interaction ($p < 0.001$) between nursery and sow date for all three leaf nutrients (N, P, K). Leaf nutrient concentrations generally increased with later sowing dates (Table 2-1). Leaf N was typically greater for seedlings at UA, while leaf P and K were greater for seedlings at WM; however, for the seedlings with the May 22 sow date, the opposite was the case where leaf N was greater for WM seedlings when sown on May 22 (Table 2-1).

Height growth in the first growing season was influenced by container size and seedling age in the mulch and plow treatments ($p = 0.002$) with nursery location having a significant effect only in the plow ($p < 0.001$). In the mulch and plow treatments, height growth in the first growing season was greater in seedlings grown in 340 ml containers and in seedlings that were 5, 5.5, or 6.5 months old. Container size had a significant effect on root growth in all three treatments ($p = 0.02$) with seedlings in the largest container sizes (700 ml) showing the most root growth. Seedling age had a significant effect on root egress in the mulch and grass treatments ($p = 0.006$); however, the response was variable as 5 month old seedlings showed greater egress in the grass treatment while 5.5 and 6.5 month old seedlings had the highest egress in the mulch. There were no significant interaction effects between nursery location, container size, and seedling age for any response variables (refer to Appendix 7 for ANOVA table results of nursery effects on seedling performance).

2.3.2. Soil and competing vegetation characteristics

Soil conditions differed somewhat among treatment areas, where soils in the mulch treatment had a sandier texture in comparison to the plowed and grass treatments (Table 2-2). This corresponded with the higher bulk density in the mulch treatment area. Soil electrical conductivity in the grass treatment was greater than in the mulch to a depth of 15 cm, but was similar at 20-25 cm depth ($p = 0.07$). Volumetric water content throughout the second growing season was significantly higher in the grass treatment than in the mulch or plowed (Table 2-2). Conversely, soil temperature was highest in the mulch treatment throughout the second growing season ($p < 0.001$). Soil water potential was more variable between treatments, as the grass treatment had significantly higher water potential from May to July ($p < 0.001$), but was similar to the mulch during August (Table 2-2). Soil nutrient concentrations in the competition treatments (plow and grass) were generally higher than in the mulch (Table 2-3).

The average height and dry mass of the competing vegetation in the second growing season was lowest in the mulch treatment. There was no significant difference in height for competing vegetation in the plow and grass treatments ($p = 0.99$); however, dry mass was higher in the plowed treatment. Above-ground cover of competing vegetation was lowest in the mulch but not different ($p = 0.76$) between the competition treatments. Root density of competing vegetation was lowest in the mulch treatment and was not significantly different between the two competition treatments at all soil depths ($p = 0.82$; Table 2-4); however, depth had a significant effect ($p < 0.001$) as the first 10 cm of soil in each treatment had higher root density than lower horizons.

2.3.3. Seedling development in each treatment

Seedling mortality was not influenced by stock type and was generally low over the first two growing seasons but increased in the third year. After the first two growing seasons, average mortality across stock types was highest in the plowed treatment (10%) compared with the mulch (0.7%) and grass (2.6%) treatments. In the third growing season, cumulative mortality (i.e. proportion of seedlings that died in the third growing season) was highest in the plowed treatment (18%), lowest in the mulch (0%), with a slight increase in the grass (9%) treatment. Regardless of stock types, seedlings in both competition treatments (plowed and grass) grew significantly less in height (83% reduction), diameter (83% reduction) and total root mass (56% reduction) than seedlings in the mulch treatment (Figure 2-1 a-c). Similarly, leaf production varied greatly between the mulch and the competition treatments, with total leaf dry mass in the second growing season approximately 9 times greater in mulched seedlings compared to the plowed and grass treatments (Figure 2-1 d). However, in these treatments seedlings had higher specific leaf area and root-to-leaf ratios. The grass treatment had the highest specific leaf area (148 cm^2g^{-1}) and root-to-leaf ratio (4.8), while the mulch had the lowest (SLA = 126 cm^2g^{-1} and R:L = 0.7) (Figure 2-1 e-f). Leaf nutrient concentration was generally highest in the mulch plot (except for leaf Mg and Na concentrations); however, there was no difference in leaf nutrient concentration between stock types within each site treatment. Between the two competition treatments, first year height and diameter growth was similar. However, in the second and third year, height and diameter growth was significantly greater in the plowed while root growth was greater in the grass treatment (Figure 2-1 a-c).

Differences in total root mass (Figure 2-1 g) among treatments and across all stock types were similar to the response observed for root mass growth (Figure 2-1 c), as average total root mass was significantly higher in mulch (17.8 g) compared to plowed (8.6 g) and grass (10.0g). Root egress (mass of egressed roots relative to the total root mass) (Figure 2-1 h) was highest in mulch (29%) and lowest in the grass (4%). The higher aboveground growth in the mulch corresponded with lower root-to-stem ratios, as seedlings in the competition treatments had significantly higher root-to-stem ratios (Figure 2-1 i).

2.3.4. Role of stock type characteristics

Within each treatment, most initial morphological characteristics did not influence survival (Table 2-5). Initial height, root collar diameter, and root-to-shoot ratio were found to have a significant linear relationship with 2014 height growth in the mulch and plowed treatments (Figure 2-2 a); however, the slope of these relationships were similar between treatments ($p > 0.09$). Root mass was not significant with height growth in any treatments and no morphological characteristics were significant in the grass treatment. In 2015 and 2016, no other morphological characteristics except root-to-stem ratio had a significant linear relationship with height growth, which only occurred in the plowed treatment (Figure 2-2 b). For belowground development, initial height (Figure 2-3 a), root collar diameter, and root mass had a positive linear relationship with root growth in all treatments and the slope of the relationship in the mulch was greater than that of the competition treatments ($p = 0.02$). Both initial height (Figure 2-3 b), root collar diameter, and root mass (Figure 2-4) had a negative linear relationship with root egress in the mulch and plowed treatment, however, slope of the relationship for both characteristics was similar between treatments (p = 0.08). Seedling development belowground corresponded with aboveground growth, as root egress had a positive linear relationship with total height growth in the mulch and plowed treatment (Figure 2-5), which both had similar slopes (p = 0.63). Initial stem NSC concentrations were only significant with 2016 height growth in the mulch and grass treatments with a difference in slope between treatments, while root NSC concentrations were not significant with any growth measurements.

2.4. Discussion

Increased height growth was associated with a higher root-to-stem ratio in all three growing seasons when competition was present (plowed treatment became competitive in year 1 after weed establishment). This relationship was strongest in the mulch (non-competition treatment) and plow treatments in the first growing season but nonexistent for the mulch and slightly weaker for the plow in the second growing season. Initial morphology did not have any effect on height growth when competition was present after outplanting (grass treatment). Initial height and root collar diameter were significant with first year height growth but this only occurred with low levels of competition (i.e. mulch or plowed treatment in first year). The reduced benefit of seedling morphology towards height growth in the second growing season can be attributed to seedlings responding more to site conditions. Morphological characteristics have a greater effect on performance in the first year, as this affects a seedling's water balance and ability to overcome planting stress (Grossnickle 2012); however, once seedlings are established, growth is influenced more by site conditions rather than morphology (Pinto et al. 2011). Nursery factors (location, container size, and seedling age) had a significant effect on height growth and other performance measures; however, this response can be attributed to the different initial morphological characteristics which arose as a result of the nursery factors. Surprisingly, the relationship between initial root-to-stem ratio and height growth during the third growing season in the plowed treatment remained significant and was stronger than the two previous growing seasons. This could be in response to increased precipitation observed in the third growing season, which would have allowed for greater height growth in high root-to-stem seedlings as these stock types achieved greater root egress in the plowed treatment and likely maintained a high root-to-stem ratio (due to limited aboveground growth). Similar to previous studies (Martens et al. 2007; Landhäusser et al. 2012a), shorter aspen seedlings with a higher root-to-shoot ratio outgrew taller seedlings in terms of height increment. Greater transpirational demand placed on taller seedlings (with more leaf area and lower root-to-shoot ratio) planted in stressful environments (Grossnickle 2005) likely contributed to this response. In addition, seedlings with larger root systems (relative to shoot size) can dedicate more NSC reserves to a smaller number of shoots or branches, resulting in a greater single shoot extension throughout the growing season (Hilbert 1990; Landhäusser et al. 2012b).

Greater root growth was found in taller seedlings in all treatments. It is not surprising that larger seedlings were capable of growing more roots, as they had the photosynthetic capacity with which to support root growth and larger root systems with a greater NSC reserve. In addition, light availability has been shown to be an important factor for improving root development of aspen seedlings (Kelly et al. 2015) and by having greater access to light (being taller than competing vegetation) taller seedlings would be able to potentially allocate more biomass belowground (Brouwer 1962; Johnson et al. 1984). A seedling stock type capable of enhanced root growth could be beneficial for seedlings planted on grassy sites, as grasses typically have a significant belowground advantage over seedlings (Wilson 1998). Water stress (from belowground competition) may cause increased root growth in taller seedlings attempting to improve internal water balance (Rook 1973; Ritchie and Dunlap 1980), as similar results have been seen with Aleppo pine seedlings (*Pinus halepensis* Mill.) planted with competition (Cuesta et al. 2010b).

Root egress was an important growth parameter in this study as there was a positive linear relationship with height growth, consequently, stock types that demonstrate good root egress are also likely better candidates in a competitive environment. Root egress had a negative linear relationship to both initial height and root mass, which suggests that despite the increased root growth in larger seedlings, the majority of root development occurred within the plug volume. Root binding may explain the lower egress observed for the taller UA stock types, which had twice the root mass relative to their WM counterparts prior to outplanting. The increased growth for the UA stock types while in the nursery may have resulted in seedlings outgrowing their containers and led to matting in the roots. Similar results have been observed with larger lodgepole pine (*Pinus contorta* Douglas) (Hellum 1981) and longleaf pine seedlings (*Pinus palustris* Mill.) (South et al. 2005; South and Mitchell 2006) showing greater incidence of root binding (relative to smaller seedlings grown in similar sized containers) which resulted in reduced egress after outplanting.

Manipulating seedling morphology (at least for the stock types evaluated in this study) did not result in substantial improvements when competition was present. Mean values of growth measurements in the competition treatments were typically 2 – 3 standard deviations away from mean values measured in the mulch. Plowing (which was supposed to temporarily alleviate competition) provided little benefit for height growth and in some aspects resulted in poorer aspen seedling performance (higher mortality) as weeds quickly recolonized the site; however, the initial removal of competition appears to be beneficial in allowing initial morphological characteristics to influence growth performance in the first growing season, as these morphological characteristics were not significant with height growth in the grass treatment (where competition was continually severe and prevented establishment). Higher root-to-stem ratios had a stronger effect on first than second year height growth (greater R^2) possibly a result of reduced competition after plowing and the re-establishment of competing vegetation in the second year. While second year height growth was still significantly different between the competition treatments, the overall difference was small (2 cm). By the second growing season, mean grass cover was comparable between the two treatments although the percent cover of Canada thistle (*Cirsium arvense* (L.) Scop.) was higher in the plowed treatment. Thistle has been shown to greatly reduce the survival of aspen seedlings (Musselman et al. 2012; Henkel-Johnson et al. 2016) and likely contributed to higher mortality in the plowed treatment, as smaller seedlings were often shaded by thistle that grew up to 1 m in height. Shading in combination with lower water availability in the plowed treatment (relative to grass) may explain lower root mass in the plowed treatment, as seedlings would have less light and water available for root growth (Ritchie and Dunlap 1980; Kolb and Steiner 1990).

Improvements in performance provided by initial morphological characteristics were either reduced (plowed treatment) or not detected (grass treatment) when competition was present. The effect of morphological characteristics was generally consistent between treatments (i.e. statistically similar slopes between significant regression lines); however, the overall improvement that seedling morphology could provide was diminished (lower intercepts on regression lines) in the competition treatments. Shorter stock types outgrew taller stock types with similar root mass and NSC concentrations (ex. stock type 1 outgrew stock type 6 on average, despite having statically similar root mass and root NSC concentrations), likely due to differences in shoot size (relative to NSC reserve). Generally, competition for light was likely not the main driver of the seedling performance (i.e. growth and survival). In the competition treatments, height growth for the taller stock types was more reduced than the shorter stock types relative to the mulch, likely as a result of both initial shoot size (relative to NSC reserve) and increased water stress (due to belowground competition and increased aboveground demand as a result of a higher root-to-stem ratio). Lower nutrient availability (due to belowground competition) may be another explanation for reduced height growth and the decrease in slope for the relationship between initial height and root growth in the competition treatments, as similar results have been found with beech seedlings showing reduced height and root growth with increasing belowground competition (Curt et al. 2005).

2.5. Conclusion

Manipulating seedling morphological characteristics does not appear to benefit height growth if aspen seedlings are planted directly into grass competition; however, initial root-to-stem ratio appears to be an adequate indicator of height growth if competition is controlled during the establishment year. Initial morphological characteristics becomes less important in the following years as seedlings appear to respond more to prevailing site conditions. Increasing root egress may benefit performance on competitive sites, as root egress had a positive relationship with height growth. While optimizing seedling morphology provided some improvement in growth, this study was not successful in developing a stock type that performed well in a competitive environment. Likely, these extremely competitive sites overwhelm the beneficial characteristics achieved from the quality of aspen seedling stock. Growth measures were significantly lower for all stock types in the competition treatments and improvements in growth provided by morphological characteristics were either diminished or not detected with competition; however, dry conditions during 2014 and 2015 may have contributed to this response. Periodic control of vegetation competition in conjunction with initial high root-to-stem ratio of seedlings may provide optimal results on competitive sites, as shown by the increased growth response observed in the first year of the plowed treatment.

Future work could focus on methods for improving root egress of aspen seedlings, as benefits provided by initial seedling morphology are short lived. On sites with severe competition, site preparation techniques such as mounding, plowing, and herbicide application are necessary, as the quality of seedlings does not appear to counteract adversely competitive site conditions. Aggressive site preparation often leads to reduced vegetation competition for 1-2 years (Haeussler et al. 1999) which could give a good quality seedling enough time to establish and compete successfully with the recovering vegetation.

Tables

Table 2-1: Mean pre-planting characteristics of aspen seedlings from Woodmere and Edmonton. Seeds were collected in 2012 and seedlings grown in 2013. Means within rows followed by the same letter are not significantly different (HSD test). Values in brackets represent one standard deviation of the mean (n=10).

Stock Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Nursery	WM	WM	WM	WM	UA	UA	UA	UA	WM
Container Size (ml)	700	340	340	340	700	340	340	340	220
Sowing Date	Apr 7	Apr 7	May 1	May 22	Apr 7	Apr 7	May 1	May 22	May 15
Age (months)	6.5	6.5	6	5	7	7	6	5.5	5.5
Height (cm)	45.9 **d** (9.04)	28.1 **ef** (5.05)	38.3 **d** (12.1)	18.7 **f** (5.32)	112 **a** (13.3)	85.8 **b** (20.2)	71.2 **c** (14.3)	34.0 **de** (5.16)	37.0 **de** (4.28)
Root Collar Diameter (mm)	4.93 **c** (0.38)	3.59 **de** (0.36)	4.03 **d** (0.52)	3.10 **e** (0.46)	7.56 **a** (1.11)	5.96 **b** (1.02)	5.96 **b** (1.23)	4.02 **d** (0.46)	3.75 **d** (0.31)
Root Mass (g)	6.93 **b** (1.42)	3.25 **cd** (0.73)	4.29 **c** (1.41)	2.01 **cd** (1.06)	17.3 **a** (4.57)	8.23 **b** (5.11)	9.23 **b** (4.39)	3.58 **cd** (1.22)	1.46 **d** (0.41)
Root:Shoot Ratio	3.50 **b** (0.74)	4.42 **ab** (1.20)	3.83 **ab** (1.29)	4.73 **a** (1.56)	1.67 **c** (0.42)	1.51 **c** (0.34)	1.98 **c** (0.34)	3.34 **b** (0.49)	1.20 **c** (0.37)
Shoot NSC (%DW)	13.3 **e** (1.17)	14.2 **de** (1.16)	15.2 **cd** (1.34)	17.3 **ab** (1.71)	15.5 **cd** (0.94)	15.8 **bc** (0.96)	16.6 **abc** (1.64)	17.9 **a** (1.32)	16.5 **abc** (1.39)
Root NSC (%DW)	30.3 **bcd** (2.69)	28.4 **cd** (2.50)	31.4 **abc** (3.09)	33.8 **a** (3.04)	31.8 **abc** (2.69)	31.4 **abcd** (3.76)	32.4 **ab** (3.88)	34.2 **a** (3.55)	27.8 **d** (3.40)
Leaf N (%DW)	2.28 **e** (0.11)	2.31 **de** (0.10)	2.47 **cde** (0.27)	3.22 **a** (0.16)	2.59 **bcd** (0.15)	2.58 **bcd** (0.18)	2.74 **bc** (0.15)	2.78 **b** (0.08)	NA
Leaf P (%DW)	0.36 **ab** (0.04)	0.35 **ab** (0.05)	0.30 **abc** (0.08)	0.29 **abc** (0.06)	0.23 **c** (0.01)	0.24 **c** (0.01)	0.28 **bc** (0.03)	0.36 **a** (0.06)	NA
Leaf K (%DW)	1.89 **a** (0.27)	1.75 **ab** (0.16)	1.52 **abc** (0.45)	1.41 **bcd** (0.24)	0.93 **e** (0.13)	1.08 **de** (0.10)	1.14 **cde** (0.08)	1.41 **bcd** (0.13)	NA

Table 2-2: Site characteristics and mean soil characteristics for surface soils collected at CDC (n=5). Different letters indicate significantly different means between soil underneath the mulch, and soil in the plowed and grass treatment (Benjamini–Hochberg correction, α = 0.05). Values in brackets represent one standard deviation of the mean (soil texture & EC, n=5; bulk density, n=10; VWC, soil temp, and kPa, n=4).

	Mulch	**Plowed**	**Grass**
Geographic location	53°34′N 113°31′ W	53°34′N 113°31′ W	53°34′N 113°31′ W
Elevation (m)	668	668	668
Soil adjustment	none	Plowed spring 2014	None
Site type	Agricultural field	Agricultural field	Agricultural field
Soil texture (%)	Sandy loam	Sandy clay loam	Clay loam
Sand	67.5 (1.72) **a**	47.0 (0.86) **b**	41.3 (2.18) **c**
Silt	19.5 (2.69) **b**	26.5 (2.32) **a**	25.2 (3.77) **a**
Clay	13.0 (3.78) **c**	26.5 (2.69) **b**	33.5 (4.80) **a**
Bulk density (g cm^{-2})			
0-5 cm	1.25 (0.07) **a**	0.84 (0.12) **b**	0.72 (0.12) **b**
10-15 cm	1.24 (0.13) **a**	0.96 (0.08) **b**	0.93 (0.05) **b**
20-25 cm	1.26 (0.15) **a**	1.00 (0.12) **b**	0.96 (0.07) **b**
Electrical conductivity (mS)			
0-5 cm	0.96 (0.12) **b**	1.58 (0.43) **a**	1.73 (0.31) **a**
10-15 cm	1.16 (0.12) **b**	1.39 (0.16) **ab**	2.02 (0.56) **a**
20-25 cm	1.19 (0.16) **a**	1.44 (0.27) **a**	3.47 (3.05) **a**
Volumetric Water Content (m$^{3}_{water}$ m$^{-3}_{soil}$)			
May	0.19 (0.01) **c**	0.24 (0.02) **b**	0.28 (0.02) **a**
June	0.19 (0.01) **c**	0.22 (0.01) **b**	0.29 (0.01) **a**
July	0.20 (0.03) **c**	0.22 (0.02) **b**	0.30 (0.02) **a**
August	0.17 (0.01) **c**	0.20 (0.01) **b**	0.27 (0.01) **a**
Soil Temperature (°C)			
May	12.6 (3.57) **a**	10.6 (2.76) **b**	10.4 (2.63) **b**
June	17.7 (1.97) **a**	15.7 (1.54) **b**	15.6 (1.73) **b**
July	20.6 (1.54) **a**	18.2 (0.86) **b**	17.9 (0.76) **b**
August	18.4 (1.69) **a**	16.5 (1.53) **b**	16.7 (1.39) **b**
Soil Water Potential (kPa)			
May	-47.0 (48.2) **b**	-85.7 (80.1) **c**	-34.5 (41.4) **a**
June	-147 (180) **b**	-301 (261) **c**	-25.5 (12.8) **a**
July	-323 (378) **b**	-451 (407) **b**	-123 (151) **a**
August	-410 (274) **a**	-744 (241) **b**	-332 (107) **a**

Table 2-3: Mean nutrient concentration from surface soils (0-5 cm) collected at CDC. Nutrients marked with an asterisk (*) are presented in w/w%. Different letters indicate a significant difference between the mulch, plowed or grass treatment (Benjamini–Hochberg correction, α = 0.05). Values in brackets represent one standard deviation of the mean (n=5).

Soil nutrients (mg L^{-1})	**Mulch**	**Plowed**	**Grass**
Mg^{2+}	5.75 (1.04) **c**	8.11 (0.88) **b**	12.4 (2.44) **a**
NH_4 - N	1.79 (0.19) **b**	2.80 (0.33) **a**	4.21 (2.13) **a**
NO_3 - N	0.48 (0.08)	0.51 (0.11)	0.48 (0.04)
S	1.39 (0.28) **b**	2.36 (0.47) **a**	4.69 (2.08) **a**
Total C*	0.29 (0.03) **b**	0.49 (0.05) **a**	0.61 (0.10) **a**
Total N*	3.35 (0.33) **c**	6.04 (0.96) **b**	7.60 (1.75) **a**

Table 2-4: Average height, dry mass, percent cover and root density (0-40 cm depth) of competing vegetation measured within quadrats in each site treatment during the second growing season. Percent cover values represent only living vegetation found within quadrats and do not include thatch covering bare ground. Different letters within rows indicate significantly different means between site treatments (a-c) and different letters within columns for root density indicate significantly different means between depths (w-z) (Benjamini–Hochberg correction, α = 0.05). Values in brackets represent one standard deviation of the mean (n=10).

	Mulch	**Plow**	**Grass**
Height (cm)	13.9 (3.92) **b**	32.8 (5.57) **a**	31.3 (8.97) **a**
Dry Mass (g)	3.39 (2.36) **c**	10.7 (5.80) **a**	8.22 (2.99) **b**
Cover (%)	2.00 (1.12) **b**	10.2 (4.77) **a**	7.69 (2.48) **a**
Root density ($mg_{root}\ cm^{-3}{}_{soil}$)			
0-10 cm	1.11 (0.41) **b | w**	8.92 (5.91) **a | w**	6.92 (3.01) **a | w**
10-20 cm	0.86 (0.40) **b | wx**	3.18 (1.77) **a | wx**	2.66 (0.84) **a | x**
20-30 cm	0.56 (0.31) **b | xy**	4.33 (4.93) **a | x**	1.77 (0.48) **a | x**
30-40 cm	0.25 (0.15) **b | z**	1.22 (0.80) **a | x**	1.17 (0.71) **a | x**
Total	2.77 (1.03) **b**	17.6 (11.1) **a**	12.5 (4.05) **a**

Table 2-5: R^2 values for linear models of initial seedling characteristics and survival/growth response for each plot. Significant relationships are bolded ($\alpha = 0.05$). Positive [+] or negative [-] slope is indicated. Third year mortality represents cumulative mortality for that growing season.

a) Mulch Initial characteristics	Height growth 2014	Height growth 2015	Height growth 2016	Root growth	Root egress	Second year mortality	Third year mortality
Height	**0.73 [-]**	0.41	0.26	**0.86** [+]	**0.59** [-]	0.02	0.00
Root collar diameter	**0.57** [-]	0.33	0.33	**0.92** [+]	**0.66** [-]	0.00	0.00
Root mass	0.35	0.23	0.26	**0.81** [+]	**0.69** [-]	0.09	0.00
Root-to-stem ratio	**0.46** [+]	0.36	0.00	0.19	0.02	**0.43** [-]	0.00
Stem NSC	0.01	0.00	**0.47** [+]	0.05	0.10	0.02	0.00
Root NSC	0.00	0.17	0.21	0.02	0.00	0.19	0.00

b) Plow Initial characteristics	Height growth 2014	Height growth 2015	Height growth 2016	Root growth	Root egress	Second year mortality	Third year mortality
Height	**0.55** [-]	0.08	0.18	**0.92** [+]	**0.70** [-]	0.00	0.14
Root collar diameter	**0.47** [-]	0.04	0.14	**0.95** [+]	**0.63** [-]	0.00	0.20
Root mass	0.26	0.00	0.06	**0.91** [+]	**0.47** [-]	0.00	0.30
Root-to-stem ratio	**0.57** [+]	**0.56** [+]	**0.72** [+]	0.12	**0.65** [+]	0.02	0.01
Stem NSC	0.05	0.40	0.19	0.04	0.00	0.18	0.06
Root NSC	0.02	0.00	0.01	0.01	0.00	0.01	0.25

c) Grass Initial characteristics	Height growth 2014	Height growth 2015	Height growth 2016	Root growth	Root egress	Second year mortality	Third year mortality
Height	0.07	0.02	0.12	**0.62** [+]	0.15	0.02	0.28
Root collar diameter	0.01	0.00	0.04	**0.67** [+]	0.12	0.00	0.39
Root mass	0.02	0.04	0.00	**0.55** [+]	0.04	0.00	0.30
Root-to-stem ratio	0.31	0.27	0.19	0.03	0.04	0.22	0.02
Stem NSC	0.21	0.42	**0.47** [-]	0.14	0.04	0.04	0.09
Root NSC	0.00	0.00	0.41	0.02	0.10	0.29	0.07

Figures

Figure 2-1: Mean a) height growth, b) diameter growth, c) root mass growth, d) total leaf mass, e) specific leaf area, f) root-to-leaf ratio, g) total root mass, h) root mass egress, and i) root-to-stem ratio for seedlings across all stock types in each of the site treatments at CDC after two growing seasons. For height and diameter, hatched bars represent growth in 2015 and white bars represent growth in 2016. Error bars are standard error of the mean (n=10). Years are analyzed separately and different letters above bars (height and diameter growth differences within 2014 (A-C) | 2015 (a-c) | 2016 (x-z)) indicate significantly different means (Benjamini–Hochberg correction, α = 0.05).

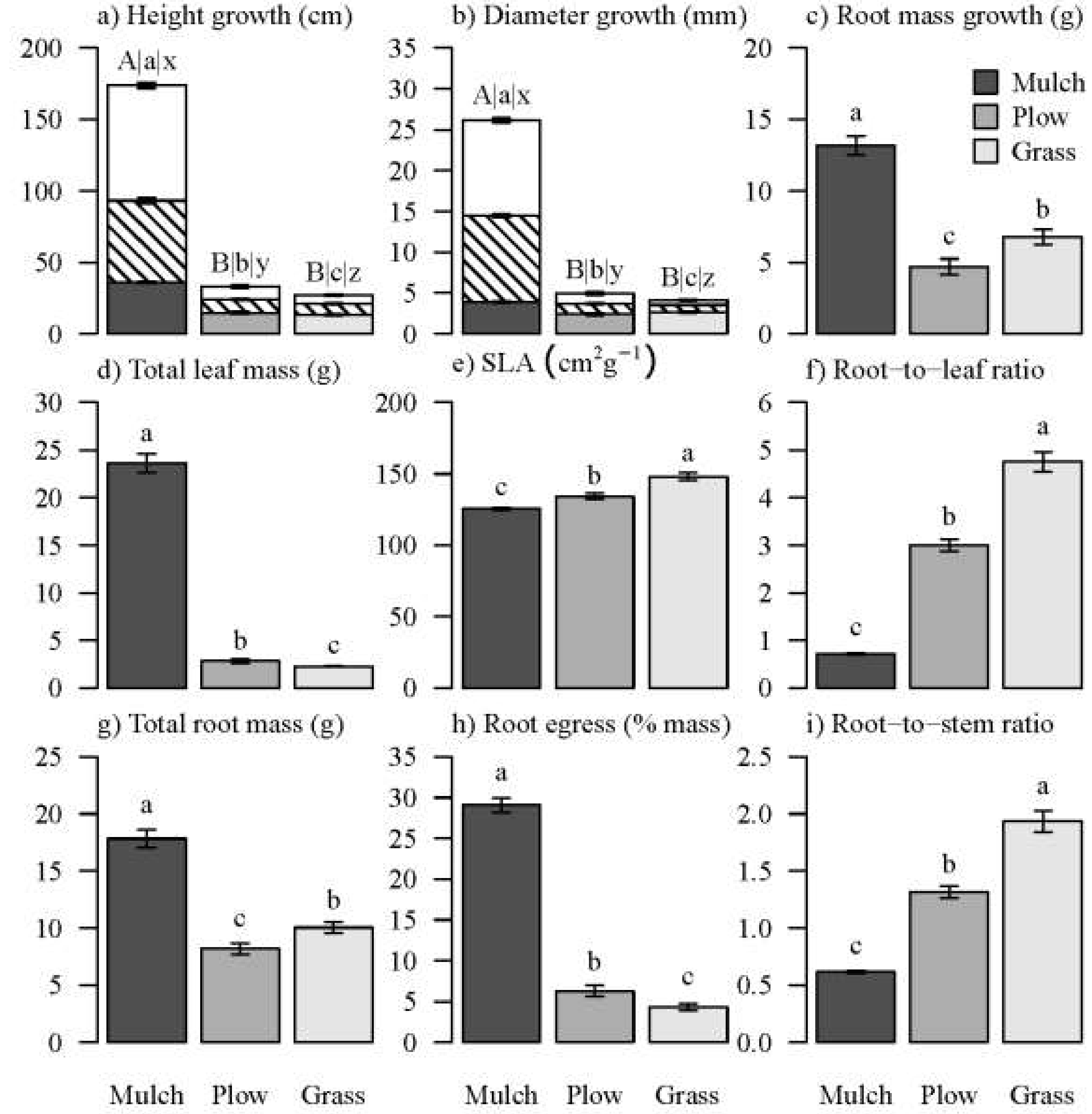


Figure 2-2: Relationship between average initial root-to-stem ratio and average height growth in (a) 2014 (mulch $R^2 = 0.46$, plow $R^2 = 0.57$, no significant relationship in grass) and (b) 2015 (plow $R^2 = 0.56$, no significant relationship in mulch or grass) for nine different stock types in three planting plot. Points represent the mean value for each stock type with error bars representing one standard error of the mean (n=10).

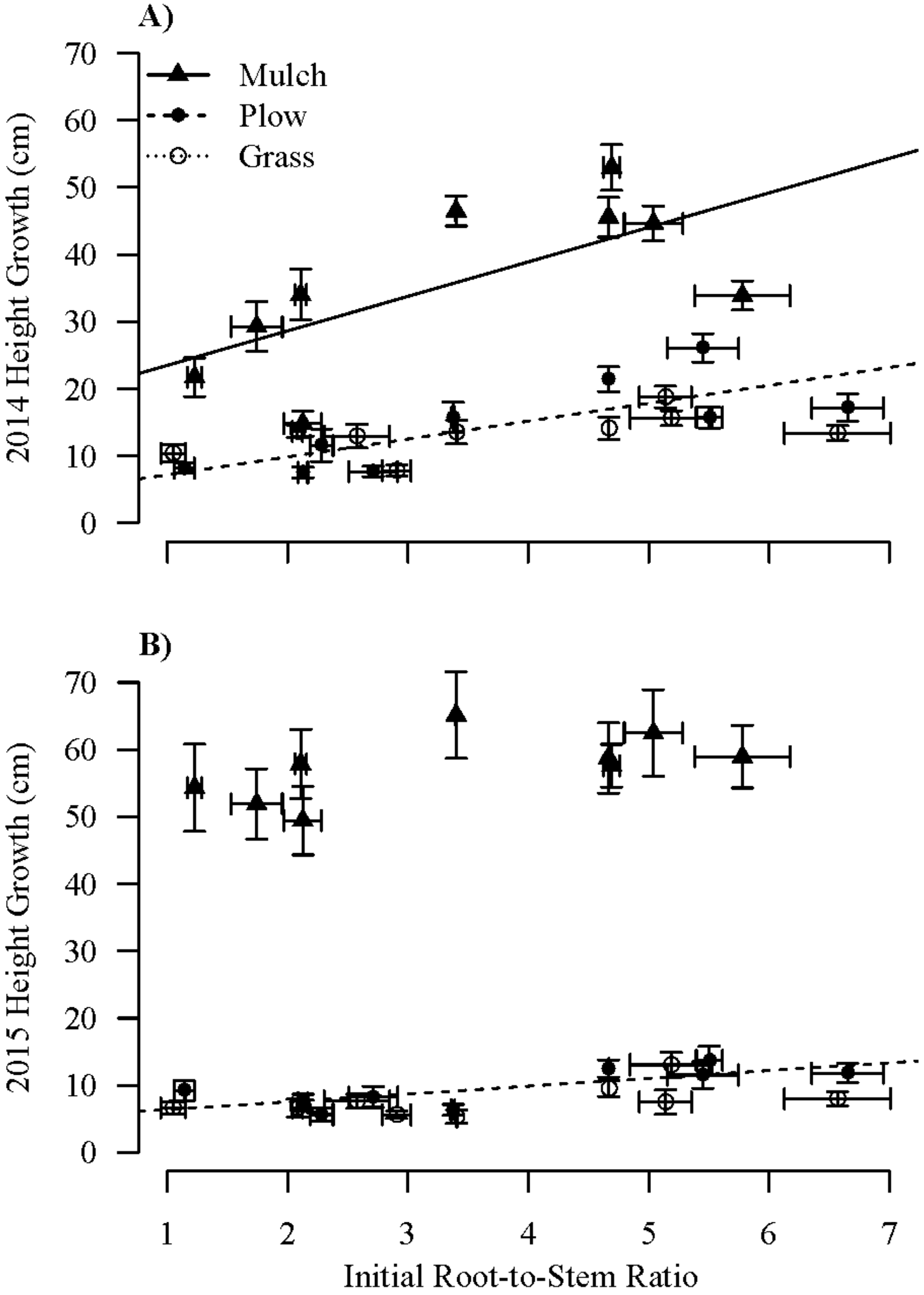


Figure 2-3: Relationship between average initial height and average a) root growth (mulch R^2 = 0.86, plow R^2 = 0.92, grass R^2 = 0.62) and b) root egress (mulch R^2 = 0.59, plow R^2 = 0.70, no significant relationship in grass) for nine stock types on three different planting plots. Points represent a mean value for a stock type, with error bars representing one standard error of the mean (n=10).

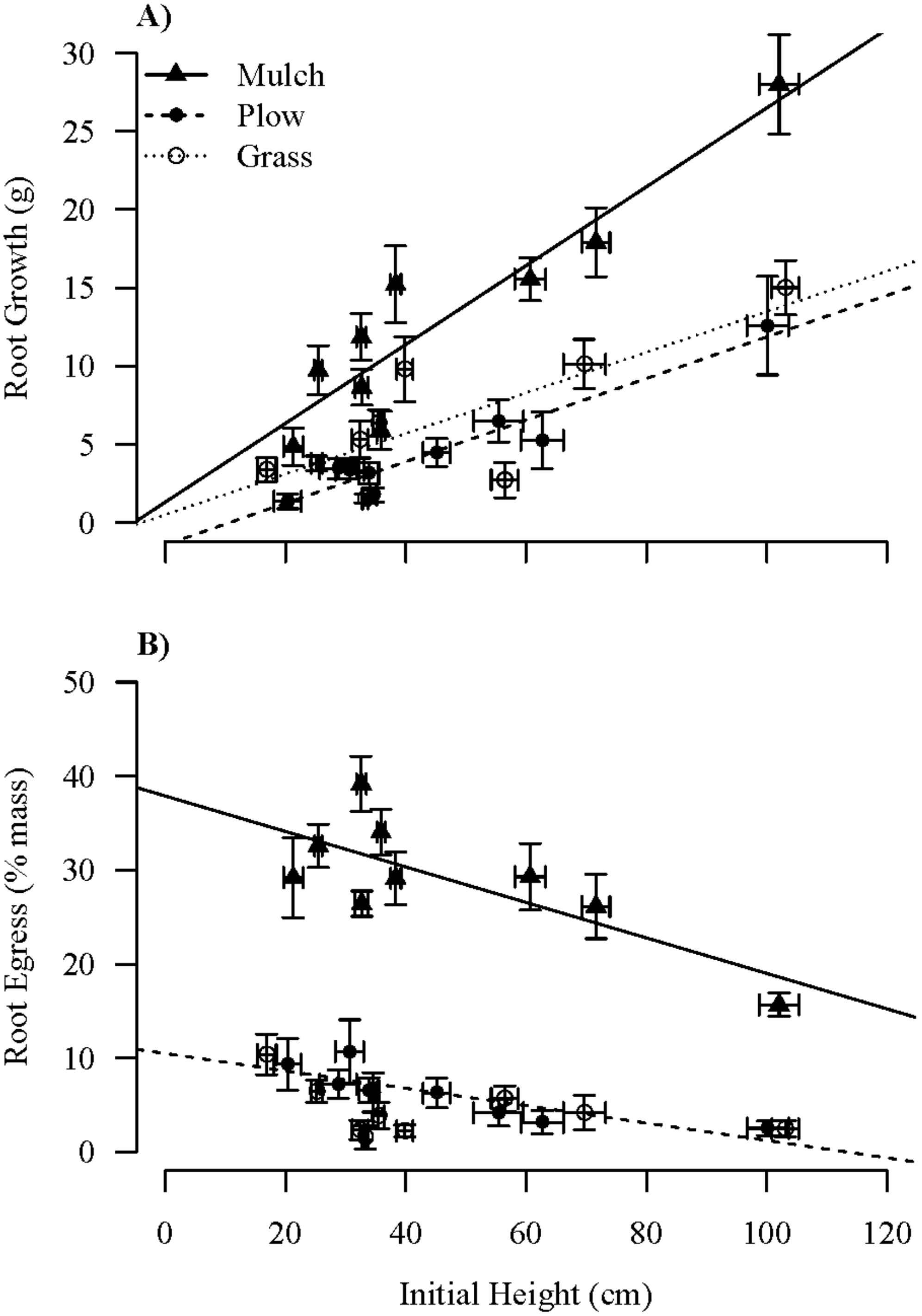


Figure 2-4: Relationship between average initial root mass and root egress (mulch $R^2 = 0.67$, plow $R^2 = 0.47$, no significant relationship in grass) of nine stock types in each planting plot. Points represent the mean value for each stock type, with error bars representing one standard error of the mean (n=10).

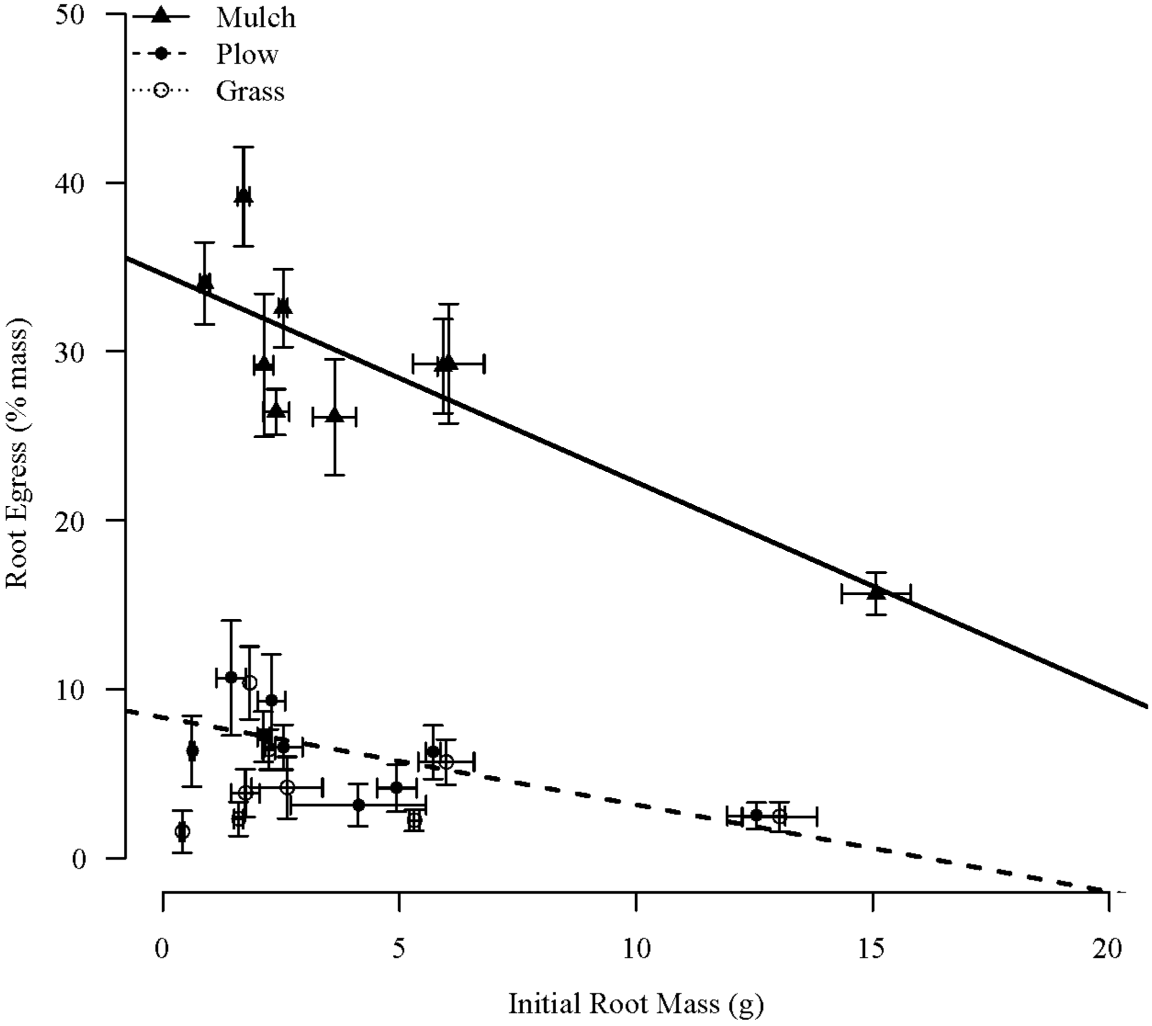
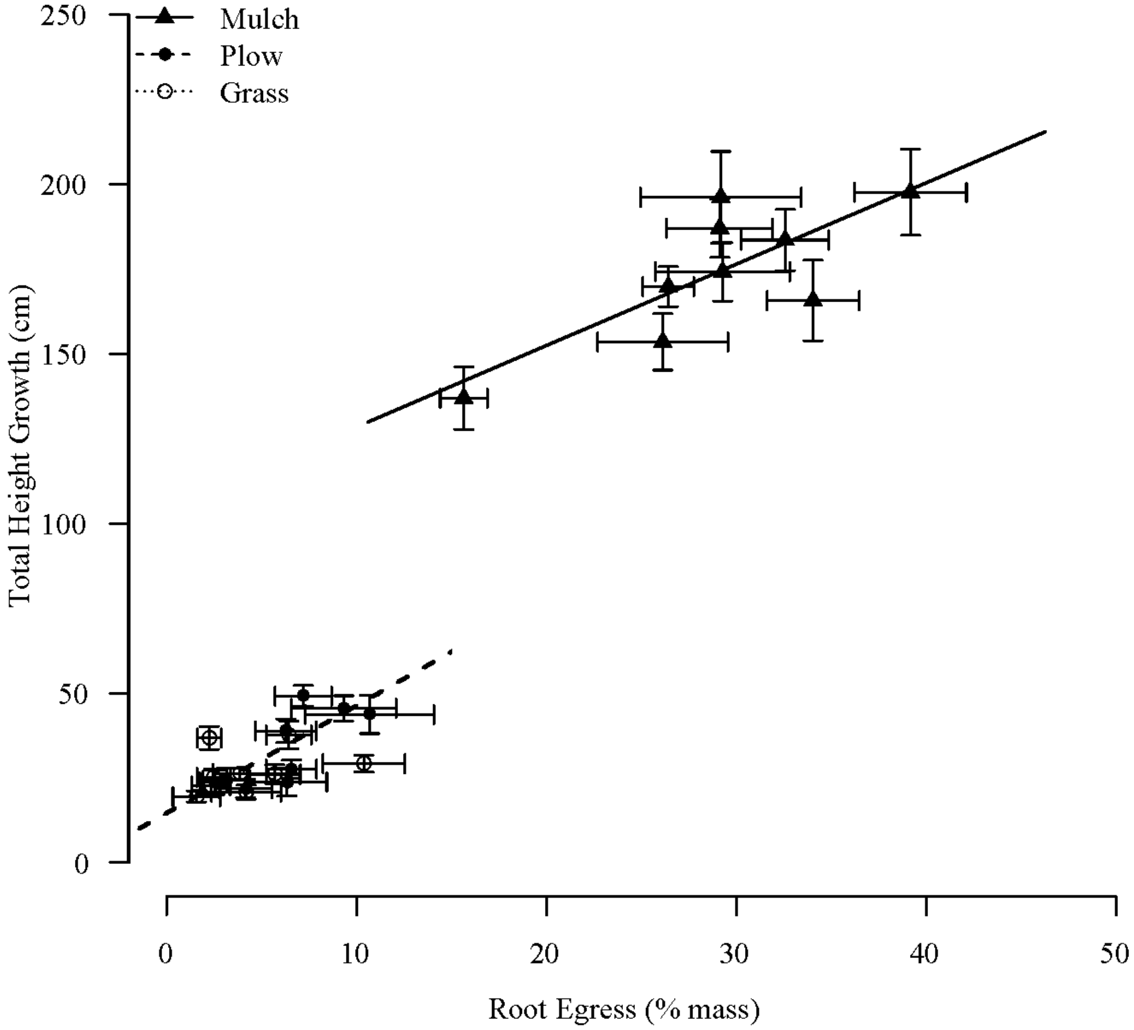


Figure 2-5: Relationship between stock type averages for root mass egress and two-year height growth (mulch $R^2 = 0.60$, plow $R^2 = 0.60$, no significant relationship in grass) in each planting plot at CDC. Points represent the mean value for each stock type, with error bars around each point representing standard error of the mean (n=10).



Chapter 3 - Aspen seedling stock characteristics drive outplanting performance on reclamation sites with contrasting soil and vegetation features

3.1. Introduction

The boreal forest is the largest natural region in Alberta and is host to an abundance of ecosystem services and natural resources. Oil and gas development has expanded rapidly in the region for the last 25 years (Rahnama et al. 2013). As resource extraction continues, there is increasing need for forest reclamation on public land as environmental regulations in Alberta require operators to reclaim their sites to conditions capable of supporting a self-sustaining ecosystem similar to pre-disturbance conditions (Macdonald et al. 2015; Government of Alberta 2016). Trembling aspen (*Populus tremuloides* Michx.) is an ideal species for reclamation as it is fast growing and tolerant to drought (relative to other boreal tree species) once it is established (Lieffers et al. 2001). Moreover, it is a foundational species that supports various ecosystem processes and functions throughout its range, is resilient to disturbance, readily regenerates vegetatively through root suckering, and can quickly achieve canopy closure. Canopy closure is an important aspect in forest dynamics that drives many forest ecosystem processes, as it facilitates forest understory and soil development (Groninger et al. 2007; Barbier et al. 2008; Sorenson et al. 2011). Litter fall from the canopy also contributes to soil nutrient availability through increased organic matter turnover (Cleve and Noonan 1975; Légaré et al. 2004), while increased soil nutrition has also been associated with improving plant understory diversity (Oaten and Larsen 2008).

Challenging environmental conditions (ex. low soil nutrients or water availability) on reclamation sites can reduce aspen seedling growth for several years after outplanting (van den Driessche et al. 2003; Martens et al. 2007). Vegetation competition further contributes to stress and reduces growth of newly established seedlings. Aspen seedlings are particularly sensitive to competition, as grasses such as marsh reedgrass (*Calamagrostis canadensis* (Michx.) Beauv.) are quick to colonize disturbed sites (Lieffers et al. 1993) and can easily outcompete seedlings for resources belowground due to higher root-to-shoot ratios (Tilman 1988; Powell and Bork 2004a). Seedlings planted into these conditions can experience planting shock due to limited root development (Burdett 1990) which can reduce overall establishment success (Landhäusser and Lieffers 1998; Bockstette et al. 2017).

High quality seedlings with characteristics suited to difficult site conditions can be used to mitigate factors limiting growth on some reclamation sites; however, identifying these characteristics is often difficult (Jacobs et al. 2015; Macdonald et al. 2015). Seedling quality is highly dependent on nursery practices that influence morphological and physiological seedlings characteristics which are associated with growth and survival under field conditions (Wilson and Jacobs 2006). Height and diameter have often been used as indicators of seedling quality in conifers (Chavasse 1977; Sutton 1979); however, these appear to be poor indicators of first year growth for aspen seedlings (Martens et al. 2007; Landhäusser et al. 2012b). Martens et al. (2007) observed that naturally regenerated aspen with high root-to-shoot ratios and NSC reserves in roots outgrew nursery produced seedlings that were initially 10 times taller, indicating these characteristics may be a more relevant indicator of seedling quality in aspen. Seedlings with high root-to-shoot ratios and root NSC reserves can be developed through the use of shoot growth inhibitors and black-out treatments (Landhäusser et al. 2012b), or stressful conditions such as mild drought or high vapor pressure deficits (Kelly et al. 2015). Improved performance (i.e. growth and survival) of aspen seedlings with these characteristics has been documented on nutrient poor sites (Landhäusser et al. 2012b) and under drought conditions in a greenhouse (Kulbaba 2014); however, the benefit of these morphological characteristics is still unclear under challenging field conditions (Landhäusser et al. 2012b).

In this study, I evaluated seedling establishment and growth of aspen seedlings on reclamation sites in northwestern Alberta that varied in the degree of interspecific plant competition as well as edaphic conditions. The nine aspen stock types from the previous chapter were classified into five new stock groups which corresponded with characteristics previously identified as indicating improved quality in aspen. Taller stock types were hypothesized to better tolerate competing vegetation on reclamation sites, while stock types with higher initial root-to-stem ratios were expected to perform better (i.e. increased growth and survival) in drier site conditions.

3.2. Materials and methods

3.2.1. Seedling production

The stock types used in this study were produced as outlined in chapter 2 (Table 3-1; refer to section 2.2.1 for additional information on seedling stock type growing conditions, initial seedling measurement protocols, and data analysis of initial seedling characteristics). Aspen seedlings were grown from seed collected in 2012 from open pollinated sources (registered seedlot: NNAIT 16-84-23-5-2012 AW) in the Peace River Dry Mixedwood Lowland region (645-655 m a.s.l.). Seedling production occurred in 2013 in two locations with four stock types produced at the Agriculture Forestry Centre (53.526082, -113.527684, elevation 677 m a.s.l.) in Edmonton, Alberta and four produced at Woodmere Nursery (56.064387, -118.378375, elevation 647 m a.s.l.) in Fairview, Alberta. Seedling characteristics were modified by exposing seedlings to different nursery conditions (see chapter 2), with variations of styroblock size (cell volumes: 700 and 340 ml), and different sowing date (April 7, May 1 or 22) resulting in a total of 8 experimental stock types. A ninth stock type, a conventionally grown standard nursery stock type from the same seed source, was added as to the study. After 9 weeks of establishment and growth in the greenhouse, all stock types were moved outside for the remainder of the growing season (with earlier sowing dates moved out first). To increase the root-to-stem ratio of the seedlings (Landhäusser et al. 2012b), a shoot growth inhibitor (Paclobutrazol, Bonzi®, Syngenta, North Carolina, USA) was applied to seedlings by soaking their roots in a water bath containing a solution of 5 ml of Bonzi per L of water (equivalent to 20 ppm paclobutrazol per liter of water) on July 10 (for the April 7 and May 1 sowing dates) and July 15 (for the May 22 sowing date). Bud set occurred in late July, two weeks after Bonzi application. Following leaf senescence (November 1), seedlings were lifted, packaged, and placed in frozen storage (-3°C) until planting in late May and early June 2014.

At the time of lifting, a random subset of 10 seedlings from each stock type were collected to determine average initial morphological characteristics and nutrient and carbohydrate reserve concentrations. For these seedlings, height and root collar diameter measurements were taken before the roots and shoots were separated for their respective dry mass measurements. Root collar measurements were used in regression models with root and shoot dry mass of these seedlings to generate initial root and shoot mass estimates for seedlings of each stock type planted in the field (see Appendix 4). Leaf nutrient concentrations and stem and root carbohydrate tissue concentrations were extracted from dried samples ground with a Wiley Mill and passed through a 40 mesh. Water soluble sugars were extracted from root and stem tissues with 80% hot ethanol and quantified colorimetrically with phenol-sulfuric acid. Starch concentrations were quantified colorimetrically by digesting the remaining sugar residue with a mixture of α-amylase and amyloglucosidase enzymes followed by a peroxidase–glucose oxidase/*o*-dianisidine reaction (Chow and Landhäusser 2004). Sugar and starch concentrations were measured on a percent dry mass basis. To assess the nutrient status of each stock type, total nitrogen, phosphorous, and potassium were measured in subsampled green leaves (collected on September 16 prior to leaf senescence). Total nitrogen was extracted from ground leaves using the Dumas combustion method and measured with the EA 4010 Elemental Analyzer (Costech Analytical Technologies, California, USA). Phosphorous and potassium were extracted with nitric acid digestion and measured using an ICP-OES iCAP 6000 (Thermo Fisher Scientific, Massachusetts, USA). Leaf nutrients were reported as a percent dry mass.

3.2.2. Site description

Seedlings of the nine stock types were planted at three different sites in the Peace River area: (1) an agricultural field (hereby referred to as ag-field) located 19 km north of Peace River (N 56°23'30" W 117°12'33"), (2) a reclaimed well site situated 69 km northwest of Peace River within mixedwood stands (N 56°48'57" W 117°42'27"), and (3) a reclaimed gravel pit located 40 km northeast of Peace River (N 56°18'10" W 116°33'40"). Prior to planting, the ag-field was rototilled (spring 2014) and the well site was decompacted (fall 2013) using a RipPlow (McNabb et al. 2012). No surface site preparation occurred in the plot located on the gravel pit. A spot application of Roundup (The Scotts Company LLC., Ohio, USA) was used to control Canada thistle (*Cirsium arvense*) in the first summer (2014) in the ag-field plot while no chemical control was used on the other two sites. At each site, an area was divided into 10 blocks with four seedlings from each stock type planted with 1 m spacing between plants in each block (a total of 360 seedlings in each site). Seedlings in the ag-field plot were within a fenced area while the well site and gravel pit were not fenced.

Accurate daily weather data for each site was not available as the nearest weather stations were up to 50 km from each research site. However, regional monthly precipitation and air temperature was estimated by averaging data collected from weather stations closest to the sites (ACIS, 2016). Total precipitation over the first (79.2 mm) and second (156 mm) growing season (May 1 – August 31) was 56% and 14% less than the long term average (181 mm) for the region (Table 3-2). In the second growing season few rainfall events occurred in June and during the remainder of the growing season most rainfall events were less than 10 mm (Figure 3-1).

Hourly soil water content and soil temperature were measured at a depth of 15 cm and averaged daily for each site using EC-5 and 5TM sensors (Decagon Devices Inc., Washington, U.S.A.) connected to EM50 data loggers (Decagon Devices Inc., Washington, U.S.A.) installed the first week of May 2015 at the center of each plot. To further assess soil characteristics, two soil cores (core volume = 98 cm^3) were collected at each of three depths (0-5 cm, 10-15cm, 20-25cm). Soil bulk density was measured for each core. The two soil cores for each depth were subsequently bulked (i.e. one sample with soil subsampled from two separate blocks) for nutrient and electrical conductivity measurements. Electrical conductivity was determined using the saturated paste method (Slavich and Petterson 1993). For soil nutrient concentrations, the 15 soil samples were (5 bulked samples per site) analyzed for K^+, Mg^{2+}, P, and S concentrations using the microwave-assisted nitric acid digestion method (Hassan et al. 2007) and for NO_3-N and NH_4-N using the 2N KCl method (Jones Jr., 2001). Soil texture was determined with particle size analysis using a graduated cylinder and hydrometer (Carter and Gregorich 2008)

Above-ground competition on all sites was assessed in the second week of July with one 0.5 × 0.5 m quadrat placed in each individual block (10 quadrats/site) along a transect running diagonally across the planting plot. In each quadrat, percent cover was visually estimated for each species to the nearest percent. For the lower covers, cardboard cutouts corresponding to 1, 5, and 10% of the quadrat area were used to improve accuracy and consistency of cover estimates. The average height of each species was also measured to the nearest centimeter and subsequently clipped at the ground level to determine the dry mass of each species present in the quadrat. To assess below-ground competition, the total root density of the competing vegetation was estimated in the same 30 vegetation quadrats using 30 cm deep soil cores. To estimate vertical root distribution each core was divided into three 10 cm segments. All cores were collected 50 cm away from a planted aspen seedling to minimize the quantity of aspen roots present, as this distance was greater than the average lateral root length measured at each site. All roots were separated from the cores using the sampling protocol outlined in section 2.2.2.

3.2.3. Seedling measurements

Initial height and root collar diameter was measured on all seedlings after planting. Initial root-to-stem ratios were estimated using the predictive equations based on the initial seedlings measurements mentioned above. Total height, height growth, and root collar diameter after the first and second growing seasons was measured in late August 2014 and 2015. Annual height growth was measured as the shoot extension from the previous bud scar to the terminal bud tip of the longest shoot. The presence of dead or broken seedlings, or new suckers arising from seedlings was noted. To assess the overall performance and growth allocation of each stock type after two growing seasons, ninety seedlings were excavated in each site (i.e. one seedling from each stock type per block) in the third week of July 2015. The roots of each planted seedling were carefully excavated using hand trowels, following each root to minimize breakage and root loss. All leaves were removed from seedlings and dried for two days at 70°C to determine total dry leaf mass. Specific leaf area for each seedling was determined from a subset of 10 leaves that were scanned (LI-3100C, LI-COR, Nebraska, USA) prior to determining dry mass. Root systems were separated from the shoots (stem and leaves), carefully washed and dried at 70°C for two days. To assess root development, roots that had grown outside of the root plug (egressed roots) were removed from the remaining plug (the initial root mass and their growth (ingress) combined) and dry mass for both were measured separately. To explore the functional size of the root system (lateral rooting space) root length was expressed as the average length of the two longest lateral roots. New root growth was calculated as the difference between total root mass and the initial root mass estimated for each seedling from the root collar measurements (see regression models for root mass described earlier). Egress root-to-shoot ratio, which has been proposed as a measure of drought avoidance potential (Grossnickle 2012), was calculated as the mass of egress roots relative to the shoot (stem and leaves). The production of new root mass relative to total plant mass (RG/P = root growth divided by total seedling mass) was also calculated.

3.2.4. Grouping of stock types

To focus our study on fewer key traits that identified our stock types, we grouped the nine seedling stock types by their initial morphological and physiological traits identified in chapter 2 (Table 3-1), Classification and regression tree partitioning (*rpart* package; Therneau et al. 2015) was used to sort the nine stock types into distinct groups using the dominant traits. The classification tree was first grown to its full size but then pruned to minimize the cross-validated relative error to avoid overfitting of the data. In the final version of the tree, four splits were used to define five stock groups, using root-to-stem ratio and seedling height as the distinguishing characteristics.

Initial height of seedlings greater or less than 22 cm was the first split (Figure 3-2). Stock types with an initial height of less than 22 cm were characterized as short seedlings with a high root-to-stem ratio (S-High RSR), as these seedlings had the highest initial root-to-stem ratio

(RSR) among all stock types (Table 3-1). Only stock type 4 was classified into this category. Following the initial split, the next trait separating the remaining stock types taller than 22 cm was an initial root-to-stem ratio separating stock types that had either High RSR (>2.2) or Low RSR (<2.2). In the third split, stock types were separated by seedling height, which sorted stock types into medium (M-High RSR height <43 cm [i.e. stock types 2, 3, 8], M-Low RSR height <50 cm [i.e. stock type 9]) and tall seedlings (T-High RSR height >43 cm [i.e. stock type 1] and T-Low RSR height >50 cm [i.e. stock types 5, 6, 7]). Although the classification tree shows a high proportion of stock type 3 in the T-High RSR stock group, this stock type was placed in the M-High RSR stock group as the average initial height and root-to-stem ratio of stock type 3 better corresponded with the M-High RSR stock group. Overall, the nine stock types were grouped into five stock groups that could be easily defined by a combination of high or low root-to-stem ratio and separated by initial height into a short, medium, or tall size class (Table 3-3).

3.2.5. Statistical analysis

To compare differences in soil characteristics and competing vegetation between sites, two sample t-tests were used in R (R Core Team 2016). Log or square root transformations were conducted when data did not meet assumptions of normality (Shapiro-Wilk test). F-tests were used to compare the variance of each site and Welch's t-test was used if variances were unequal. If normality could not be achieved with data transformations, a non-parametric test was employed (Kolmogorov-Smirnov Test). Since multiple comparisons were required to compare each site, p-values were adjusted using the Benjamini–Hochberg correction.

Non-metric multidimensional scaling (NMDS) was used to characterize the defining features of each site; the vegan package in R was utilized for all NMDS analyses (Oksanen et al. 2016). The NMDS was run on a Bray-Curtis dissimilarity measure, with a stability criterion on 0.00005, a random starting configuration, and the Wisconsin-style double standardized scaling using the *metaMDS* function. Two dimensions were used for the final graph of the ordination, and included 95% confidence interval ellipses and vectors showing site characteristics with significant associations with the ordination ($\alpha < 0.05$). Ellipses for the NMDS were calculated using the *ordiellipse* function and vectors were determined using the *envfit* function.

A two-way analysis of variance (ANOVA) was used to test for significant differences between sites and stock groups on seedling growth responses (mortality, first and second year height growth separately, root growth, egress root mass, root length, egress root to shoot mass ratio, and root growth to total plant mass ratio). Generalized least squares models (*gls* function in R, Pinheiro et al., 2016) were required to meet assumptions of normality and equal variance. A variance covariate was included in each model to control for unequal variances between site or stock group (Zuur et al. 2009). AIC comparisons were used to select the appropriate model based on the lowest AIC. Diagnostic plots of fitted values vs. residuals were used to confirm equal variances and assess normality of all models. When a significant stock group effect was detected, Fisher's LSD test was used to conduct multiple comparisons of growth responses between stock groups within each site using the *contrast* function from the lsmeans package (Lenth 2016). A significance level of $\alpha = 0.05$ was used for all analyses.

3.3. Results

3.3.1. Site conditions

The NMDS ordination with two dimensions summarizes the conditions of each site with a stress value < 0.10 (Figure 3-3). The position of the ellipses shows a distinct separation between the three sites with no overlap. Variation along the first axis separated the well site from the other sites by characteristics of aboveground competition (competing height, grass and forb mass). The ag-field and gravel pit were separated along the second axis largely by soil characteristics as the former was characterized by finer textured soils (silt) with higher nutrient concentrations (soil K and NO_3), while the latter was characterized by coarser soils with higher bulk density and rooting competition.

There were significant differences in edaphic conditions among the three field sites. The soil texture in the ag-field and well site was classified as a silty clay loam while the gravel pit had a loamy texture with significantly more sand (Table 3-4). Most soil nutrient concentrations (NO_3, K, S, Mg) were lowest at the gravel pit compared to the other two sites, except for soil NH_4 and P which were significantly higher (Table 3-5). Coarse material at the gravel pit corresponded with significantly greater bulk density (~1.68 g cm^{-2}) at rooting depth (10-25 cm) relative to the other sites (~1.30 g cm^{-2}). Soil electrical conductivity was highest at the well site (~3.0 dS m^{-1}) and lowest at the ag-field (~1.3 dS m^{-1}) for all depths (Table 3-4). Average volumetric soil water content (at 15 cm depth) over the growing season at the ag-field and well site was twice that of the gravel pit for most of the second growing season (Figure 3-1). Between the ag-field and well site, average volumetric soil water content in May was initially highest at the well site but dropped subsequently below the ag-field for the remainder of the growing season, when volumetric soil water content decreased at the well site. Surface soil temperature (at 15 cm depth) at the ag-field (~18.5°C throughout second growing season) was approximately 3°C warmer than at the well site or gravel pit (Table 3-4).

In the first growing season (May 1 – Aug 31, 2014) competing vegetation was not measured on the three different sites; however, competition on the ag-field and well site was low following site preparation. Aboveground competition was highest on the gravel pit where no site preparation had occurred prior to planting, with sweet clover (*Melilotus officinalis* (L.) Pall.) and graminoids as the prevalent cover at the time of planting (personal communication, A. Schoonmaker). In the second growing season (May 1 – Aug 31, 2015), the average height (106 cm) and dry mass (49.3 g) of competing vegetation at the well site was three times higher than at the ag-field or gravel pit (Table 3-6). Forbs and grasses had similar ($p > 0.05$) aboveground dry mass across all three sites. Two graminoids (*Elymus glaucus* Buckley and *Festuca saximontana* Rydb.) and an introduced forb (*Lappula echinata* Gilib.) were the dominant species (highest dry mass) at the ag-field. Sweet clover and two graminoids (*Agropyron trachycaulum* (Link) Gould ex Shinners and *Bromus inermis* Leyss.) were dominant at the well site. At the gravel pit the dominant species were sweet clover, fringed brome (*Bromus ciliatus* L.), and wild strawberry (*Fragaria virginiana* Duchesne) (see Appendix 8 for full species list). Total cover was similar between the well site and gravel pit, with forb species contributing more to the total cover than graminoids (Table 3-6). Total root mass density of competing vegetation from 0-30 cm soil depth was approximately 9 mg $cm^{-3}{}_{soil}$ and was not different among sites (Table 3-6).

3.3.2. Seedling field performance

Seedling mortality was not influenced by stock group ($p= 0.10$); however, site conditions had an effect on mortality (site effect: $p < 0.001$), which was the highest at the gravel pit (29%) with no difference between the ag-field (5%) or well site (2%) ($p = 0.16$). Height growth (averaged across all stock groups) was greatest at the well site in the first growing season (16 cm, $p < 0.001$) relative to the ag-field and gravel pit (10 cm). However, in the second growing season height growth was highest at the ag-field (21 cm) and lowest at the gravel pit (6 cm) ($p < 0.001$ for all comparisons; Figure 3-4). Similarly, root growth and egress root mass were highest at the ag-field (11 and 4 g, respectively) and lowest at the gravel pit (2 and 0.3 g, respectively) ($p < 0.001$ for all comparisons; Figure 3-5 a-b). Seedlings at the ag-field had the longest average root length (44 cm) relative to those at the well site (24 cm) and gravel pit (13 cm) ($p < 0.001$ for all comparison; Figure 3-5 c). Both egress root-to-shoot ratio (Figure 3-6) and RG/P (Figure 3-7) were similar between the ag-field and well site (egress RSR: $p = 0.66$; RG/P: $p = 0.30$) but lower at the gravel pit ($p < 0.001$).

High RSR stock groups (S, M, and T-High RSR) had greater height growth in the first growing season relative to the Low RSR stock groups (M and T-Low RSR) at the ag-field and well site (Figure 3-4). However, at the gravel pit the stock groups with low RSR had similar height growth to the shorter seedlings with high RSR (S-High RSR) stock group (site by stock group interaction, $p < 0.001$). In the second growing season, height growth was similar between most stock groups at the ag-field ($p > 0.12$); however, the T-Low RSR stock group grew less than the S-High RSR stock group ($p = 0.03$). At the well site the taller stock groups (T-High and Low RSR) had the greatest height growth in the second growing season (site by stock group interaction, $p < 0.001$); however, when examining height growth across both growing seasons, the S and T-High RSR groups had the best overall height growth on the well site (Figure 3-4).

Root growth was highest for the tallest stock groups (T-High and Low RSR) on the ag-field and well site ($p < 0.005$); however, on the gravel pit root growth for the T-High RSR stock group doubled that of the other groups (site by stock group interaction, $p < 0.001$; Figure 3-5 a). Across all three sites egress root mass was highest for the T-Low RSR and lowest for the M-Low RSR ($p < 0.001$); however, the overall difference in egress root mass between stock groups was much smaller on the gravel pit (site by stock group interaction, $p < 0.001$; Figure 3-5 b). Stock group did not have a significant effect on root length ($p = 0.11$; Figure 3-5 c). Differences between stock groups in egress root-to-stem ratio were variable across each site; however, the S-High RSR stock group consistently had a higher egress RSR than the M-Low RSR group (Figure 3-6). There was no difference in RG/P between stock groups on the ag-field, but on the well site and gravel pit high RSR groups typically had higher a RG/P (Figure 3-7).

3.4. Discussion

A high initial root-to-stem ratio was important for the establishment of aspen seedlings as height growth in the first growing season was highest for the high RSR stock groups regardless of site conditions. Root growth and root allocation (egress root-to-stem ratio and RG/P) after two growing seasons also increased for high RSR stock groups (relative to low RSR), although this did not occur at all three sites. High initial root-to-stem ratios are effective for improving height growth as the larger initial root allocation (relative to shoot size) is able to contribute more water and nutrient reserves and increase first year shoot expansion (Hilbert 1990). A initial high root-to-stem ratio would have had an additional benefit in reducing transpirational demands on seedlings during a dry summer (Landhäusser et al. 2012b), as total precipitation in the first growing season was 56% less than the long term average. Seedling size did not influence first year height growth on the ag-field or well site, as comparisons between the S, M and T-High RSR stock groups were similar at these sites. Producing smaller seedlings may have reduced the benefit of a high RSR on the gravel pit, as the S-High RSR stock group had similar height growth to the Low RSR stock groups. The small root mass of the S-High RSR stock may not have had sufficient NSC reserves or the ability to supply enough water during the drought in the first growing season. In addition, the presence of competition also may have limited light availability as this was the shortest stock group (mean height ~19 cm).

Root growth and egress root mass was highest for tall stock groups across all three sites. Taller seedlings were generally expected to perform better belowground as they had greater leaf mass and photosynthetic capacity to support root growth, and larger root systems with a greater quantity of NSC reserves. A high root-to-stem ratio is potentially beneficial towards root growth on drier sites, as the T-High RSR stock group doubled the root growth of all other groups. Transpirational demands associated with a larger shoot also may have prompted increased root growth, as taller seedlings would have allocated more resources belowground to reduce internal moisture deficits during drought conditions (Reich et al. 1980). Similar results have been observed with northern red oak seedlings (*Quercus rubra*), wherein larger seedlings (with a larger initial root mass) showed increased diameter and root growth during a drought year (Franklin and Buckley 2009).

Morphological characteristics were not critical to the overall growth and establishment of seedlings on the ag-field as most stock groups performed comparably. High nutrient availability (relative to the other sites) and low competition on the ag field likely meant that most stock groups had little difficulty growing and establishing. Additionally, the fine textured soil (high water holding capacity) in combination with low evapotranspiration (due to reduced vegetation cover) would have contributed to more consistent volumetric water content in the second growing season, allowing for continuous growth. Increased soil moisture reduces the need for site specific seedling characteristics (Pinto et al. 2011) and nutrient rich sites will reduce the need for a high root-to-stem ratio or increased root NSC reserves towards improving height growth of aspen seedlings (Landhäusser et al. 2012b).

At the well site, stock characteristics had greater influence on seedling performance as aboveground competition increased considerably between the first and second growing season. In the first growing season vegetation competition was initially low as the site had recently been plowed prior to planting; however, in the second growing season vegetation competition increased rapidly as sweet clover and slender wheatgrass proliferated throughout the site (average height ~1 m). When assessing total height growth, the high RSR stock groups grew the most on the well site. A high root-to-stem ratio was essential for improving first growing season height growth, allowing the S and M-High RSR seedlings to reach a sufficient height which prevented outshading from competing vegetation in the second growing season. Seedling size was of secondary importance for improving total height growth, as the taller stock group (T-High RSR) grew more than the small and medium variants in the second growing season. Egress root mass also increased with seedling size, which could be due to the increased photosynthetic capacity of larger seedlings at the well site (Cuesta et al. 2010b). A larger pool of root NSC reserves may have also facilitated egress root growth in larger the stock groups (Timmer and Aidelbaum 1996; Idris et al. 2004), as large seedlings could provide resources to sustain higher and simultaneous growth of roots and shoots (Cuesta et al. 2010a). In contrast, shorter stock groups with less NSC reserves may have prioritized shoot growth at the expense of root development on the well site (Poorter and Nagel 2000).

Poor growing conditions (root competition, dry conditions, high soil bulk density) diminished the potential benefits of seedling morphology at the gravel pit (overall performance measures low relative to other sites). While initial seedling morphology tailored for drier conditions can improve seedling performance (Pinto et al. 2011; Landhäusser et al. 2012b), conditions which become too extreme can minimize any beneficial stock type effects (Rose et al. 1993). The lack of site preparation prior to planting at the gravel pit was another factor limiting the effects of initial characteristics, as seedlings were potentially outshaded and the presence of competing roots may have limited sufficient resource uptake (Bockstette et al. 2017). Most stock groups showed poor growth across various performance measures; however, a high initial root-to-stem ratio substantially improved root growth in drier conditions (T-High RSR doubled root growth of other stock groups on gravel pit). The importance of a high RSR under difficult conditions was also reflected in RG/P (root growth/total plant mass), as Low RSR groups showed reduced belowground allocation relative to plant mass after two growing seasons. Poor root growth of the Low RSR groups could have been affected by other stock characteristics. The M-Low RSR stock had the smallest initial root mass and lowest nutrient reserves of all the stock groups, which would limit root growth (Idris et al. 2004; Landhäusser et al. 2012a). In contrast, the T-Low RSR stock (while having the largest initial root mass) was twice as tall as the T-High RSR stock group. The taller seedlings likely experienced greater transpirational water loss as this stock group generally had higher total leaf mass and leaf area (Haase and Rose 1993). Internal moisture stress due to a low initial root-to-stem ratio was likely another factor contributing to low root growth, as root growth potential will decrease with increasing plant moisture stress (Ritchie and Dunlap 1980)

3.5. Conclusion

Stock type characteristics which improved establishment on reclamation sites were successfully identified. A high root-to-stem ratio appears to be universally important for the early establishment success of aspen seedlings, as the high RSR stock groups consistently outgrew the low RSR stock groups on all three sites regardless of the site limitations or competing vegetation and edaphic factors. For sites where soil moisture is not a limiting factor and competing vegetation is minimal (ex. ag-field) any stock type will likely be sufficient for establishment (so long as competition remains low, at least in the first two growing seasons); however a high root-to-stem ratio is preferred for increasing height growth during the establishment phase. Despite initial height having previously been shown to be a poor indicator of aspen seedling performance (Martens et al. 2007), tall seedlings with characteristics similar to the T-High RSR stock group are recommended for sites with tall aboveground competition. Increased seedling size (while maintaining a high RSR) was shown to be beneficial for improving growth on sites with tall competing vegetation (i.e. well site). On xeric sites such as the gravel pit, initial characteristics may have little influence on seedling performance if conditions become too limiting. Seedlings with a high root-to-stem ratio are recommended since each of the High RSR stock groups had comparable height growth on the gravel pit; however, seedlings with a large root mass similar to the T-High RSR stock group may be needed to improve root growth, which is critical for improving establishment success on dry sites. Improved seedling performance is beneficial in reducing reclamation costs as optimized seedlings will reduce the need to replant. While the benefits of seedling morphology may be limited to the first two years after outplanting (Pinto et al. 2011), it is important to develop a target seedling for each site as morphological characteristics which are incongruous with site conditions may affect growth in subsequent years (see Table 3-8 for recommended stock type characteristics according to site conditions).

While certain stock groups were shown to have improved growth, total height growth after two growing seasons was still relatively low (~40 cm). This suggests that morphological or physiological characteristics play only a partial role in overcoming site conditions that present an array of limiting factors, particularly high competition. When tree planting occurs, management practices should emphasize site treatments that will minimize competition during the early establishment phase. Future work could also examine potential genetic factors which are limiting growth, as aspen is currently adapted to low levels of belowground competition (Messier et al. 2009) and it has been suggested to have potential for greater growth even under optimal conditions if genetic limitations are improved (King et al. 1999).

Tables

Table 3-1: Mean pre-planting characteristics of aspen seedlings from Woodmere and Edmonton. Seeds were collected in 2012 and seedlings grown in 2013. Means within rows followed by the same letter are not significantly different (HSD test). Values in brackets represent one standard deviation of the mean (n=10).

Stock Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Nursery	WM	WM	WM	WM	UA	UA	UA	UA	WM
Container Size (ml)	700	340	340	340	700	340	340	340	220
Sowing Date	Apr 7	Apr 7	May 1	May 22	Apr 7	Apr 7	May 1	May 22	May 15
Age (months)	6.5	6.5	6	5	7	7	6	5.5	5.5
Height (cm)	45.9 **d** (9.04)	28.1 **ef** (5.05)	38.3 **d** (12.1)	18.7 **f** (5.32)	112 **a** (13.3)	85.8 **b** (20.2)	71.2 **c** (14.3)	34.0 **de** (5.16)	37.0 **de** (4.28)
Root Collar Diameter (mm)	4.93 **c** (0.38)	3.59 **de** (0.36)	4.03 **d** (0.52)	3.10 **e** (0.46)	7.56 **a** (1.11)	5.96 **b** (1.02)	5.96 **b** (1.23)	4.02 **d** (0.46)	3.75 **d** (0.31)
Root Mass (g)	6.93 **b** (1.42)	3.25 **cd** (0.73)	4.29 **c** (1.41)	2.01 **cd** (1.06)	17.3 **a** (4.57)	8.23 **b** (5.11)	9.23 **b** (4.39)	3.58 **cd** (1.22)	1.46 **d** (0.41)
Root Mass Ratio	0.77 **a** (0.04)	0.81 **a** (0.04)	0.78 **a** (0.05)	0.81 **a** (0.06)	0.62 **b** (0.05)	0.59 **bc** (0.05)	0.66 **b** (0.04)	0.77 **a** (0.03)	0.53 **c** (0.07)
Stem Mass Ratio	0.23 **c** (0.04)	0.19 **c** (0.04)	0.22 **c** (0.05)	0.19 **c** (0.06)	0.38 **b** (0.05)	0.41 **ab** (0.05)	0.34 **b** (0.04)	0.23 **c** (0.03)	0.47 **a** (0.07)
Root:Stem Ratio	3.50 **b** (0.74)	4.42 **ab** (1.20)	3.83 **ab** (1.29)	4.73 **a** (1.56)	1.67 **c** (0.42)	1.51 **c** (0.34)	1.98 **c** (0.34)	3.34 **b** (0.49)	1.20 **c** (0.37)
Shoot NSC (%DW)	13.3 **e** (1.17)	14.2 **de** (1.16)	15.2 **cd** (1.34)	17.3 **ab** (1.71)	15.5 **cd** (0.94)	15.8 **bc** (0.96)	16.6 **abc** (1.64)	17.9 **a** (1.32)	16.5 **abc** (1.39)
Root NSC (%DW)	30.3 **bcd** (2.69)	28.4 **cd** (2.50)	31.4 **abc** (3.09)	33.8 **a** (3.04)	31.8 **abc** (2.69)	31.4 **abcd** (3.76)	32.4 **ab** (3.88)	34.2 **a** (3.55)	27.8 **d** (3.40)
Leaf N (%DW)	2.28 **e** (0.11)	2.31 **de** (0.10)	2.47 **cde** (0.27)	3.22 **a** (0.16)	2.59 **bcd** (0.15)	2.58 **bcd** (0.18)	2.74 **bc** (0.15)	2.78 **b** (0.08)	NA
Leaf P (%DW)	0.36 **ab** (0.04)	0.35 **ab** (0.05)	0.30 **abc** (0.08)	0.29 **abc** (0.06)	0.23 **c** (0.01)	0.24 **c** (0.01)	0.28 **bc** (0.03)	0.36 **a** (0.06)	NA
Leaf K (%DW)	1.89 **a** (0.27)	1.75 **ab** (0.16)	1.52 **abc** (0.45)	1.41 **bcd** (0.24)	0.93 **e** (0.13)	1.08 **de** (0.10)	1.14 **cde** (0.08)	1.41 **bcd** (0.13)	NA

Table 3-2: Monthly (May to August) precipitation and mean air temperature for 2014, 2015, and long term average (LTA) in the Peace River Region (ACIS, 2016). Data aggregated from the Peace River Airport, Deadwood, and Jean Cote ACGM weather stations.

Month	**Precipitation (mm)**			**Mean Air Temperature (°C)**		
	2014	**2015**	**LTA**	**2014**	**2015**	**LTA**
May	13.6	28.5	29.9	10.1	11.2	11.0
June	36.5	44.5	60.1	14.8	16.0	14.3
July	20.3	43.4	58.8	18.5	17.3	16.1
August	8.8	40.1	32.6	17.0	15.4	15.5

Table 3-3: Mean pre-planting characteristics of stock type groups based on classification tree analysis. Means within rows followed by the same letter are not significantly different (HSD test). Values in brackets represent one standard deviation of the mean (n=4 for S-High RSR, M-Low, and T-High RSR; n=12 for M-High RSR and T-Low RSR).

Stock Type Group	Small High RSR	Medium High RSR	Medium Low RSR	Tall High RSR	Tall Low RSR
Stock Types	4	2,3,8	9	1	5,6,7
Height (cm)	18.7 (5.32) **d**	33.2 (9.52) **c**	37.0 (4.28) **bc**	45.9 (9.04) **b**	89.9 (23.5) **a**
Root collar diameter (mm)	3.10 (0.43) **d**	3.84 (0.49) **c**	3.75 (0.31) **c**	4.93 (0.38) **b**	6.49 (1.32) **a**
Root mass (g)	2.01 (1.06) **cd**	3.71 (1.21) **c**	1.46 (0.41) **d**	6.92 (1.42) **b**	11.6 (6.13) **a**
Shoot mass (g)	0.43 (0.22) **b**	1.01 (0.48) **b**	1.25 (0.26) **b**	2.06 (0.61) **b**	7.06 (4.10) **a**
Root mass ratio	0.81 (0.06) **a**	0.79 (0.04) **a**	0.53 (0.07) **c**	0.77 (0.04) **a**	0.63 (0.05) **b**
Shoot mass ratio	0.19 (0.06) **c**	0.21 (0.05) **c**	0.47 (0.07) **a**	0.23 (0.04) **c**	0.37 (0.05) **b**
Root-stem ratio	4.74 (1.56) **a**	3.98 (1.19) **b**	1.20 (0.37) **c**	3.50 (0.74) **b**	1.72 (0.40) **c**
Root TNC (%DW)	33.8 (3.04) **a**	30.7 (3.66) **b**	27.7 (3.40) **c**	30.2 (2.69) **bc**	31.9 (3.38) **c**
Sugar-to-starch ratio	1.96 (0.37) **b**	1.73 (0.30) **b**	3.89 (1.71) **a**	1.71 (0.49) **b**	2.00 (0.43) **b**
Leaf N (%DW)	3.22 (0.17) **a**	2.53 (0.26) **b**	NA	2.29 (0.12) **c**	2.64 (0.17) **b**
Leaf P (%DW)	0.30 (0.06) **ab**	0.34 (0.07) **a**	NA	0.36 (0.04) **a**	0.25 (0.03) **b**
Leaf K (%DW)	1.41 (0.24) **b**	1.56 (0.31) **b**	NA	1.89 (0.27) **a**	1.05 (0.14) **c**

Table 3-4: Site characteristics and mean soil characteristics for surface soils collected at Peace River sites (n=5). Different letters indicate significantly different means between soils at the agricultural field, well site, and gravel pit (Benjamini–Hochberg correction, α = 0.05). Values in brackets represent one standard deviation of the mean (soil texture & EC, n=5; bulk density, n=10; VWC and soil temp, n=4). Soil temperature and VWC reflect measurements taken at a depth of 15 cm.

	Ag-Field	Well site	Gravel Pit
Geographic location	N 56°23'30" W 117°12'33"	N 56°48'57" W 117°42'27"	N 56°18'10" W 116°33'40"
Elevation (m)	518	572	684
Soil adjustment	Roto tilled spring 2014	Rip plowed fall 2013	None
Soil texture (%)	Silty Clay Loam	Silty Clay Loam	Loam
Sand	16.0 (1.55) **b**	18.6 (0.56) **b**	44.5 (8.08) **a**
Silt	53.0 (2.52) **a**	42.8 (1.97) **b**	39.5 (5.18) **b**
Clay	31.0 (2.29) **b**	38.6 (1.44) **a**	16.0 (3.65) **c**
Bulk density (g cm^{-2})			
0-5 cm	1.23 (0.16) **a**	1.18 (0.18) **a**	1.22 (0.18) **a**
10-15 cm	1.30 (0.12) **b**	1.30 (0.11) **b**	1.65 (0.19) **a**
20-25 cm	1.37 (0.23) **b**	1.30 (0.16) **b**	1.71 (0.22) **a**
Electrical conductivity (dS/m)			
0-5 cm	1.24 (0.17) **b**	2.60 (0.52) **a**	2.08 (0.08) **a**
10-15 cm	1.17 (0.16) **c**	3.37 (0.35) **a**	1.93 (0.14) **b**
20-25 cm	1.00 (0.12) **c**	3.04 (0.36) **a**	1.83 (0.36) **b**
Volumetric Water Content (m^3m^{-3})			
May	0.40 (0.02) **b**	0.42 (0.01) **a**	0.25 (0.04) **c**
June	0.38 (0.01) **a**	0.36 (0.02) **b**	0.18 (0.02) **c**
July	0.40 (0.01) **a**	0.32 (0.02) **b**	0.16 (0.01) **c**
August	0.37 (0.03) **a**	0.30 (0.02) **b**	0.16 (0.02) **c**
Soil Temperature (°C)			
May	14.5 (4.21) **a**	11.5 (3.32) **b**	12.6 (2.38) **ab**
June	19.6 (2.90) **a**	16.4 (2.07) **b**	16.2 (2.02) **b**
July	21.3 (1.64) **a**	18.0 (1.30) **b**	18.0 (1.37) **b**
August	18.7 (2.01) **a**	15.6 (1.70) **b**	16.2 (1.61) **b**

Table 3-5: Mean nutrient concentration from surface soils (0-5 cm) collected at the three sites in 2015. Different letters indicate differences among sites (Benjamini–Hochberg correction, α = 0.05). Values in brackets represent one standard deviation of the mean (n=5).

Soil nutrients (mg L^{-1})	**Ag-Field**	**Well site**	**Gravel Pit**
NH_4	1.30 (0.39) **b**	1.32 (0.53) **ab**	2.01 (0.33) **a**
NO_3	0.38 (0.22) **ab**	0.20 (0.05) **a**	0.12 (0.02) **b**
P	1.22 (0.23) **b**	1.98 (0.12) **a**	2.06 (0.27) **a**
K	8.99 (2.58) **ab**	7.41 (0.50) **a**	5.46 (0.71) **b**
S	1.05 (0.29) **b**	4.72 (2.65) **a**	1.32 (0.16) **b**
Mg	14.3 (1.59) **a**	15.3 (1.01) **a**	7.78 (1.23) **b**

Table 3-6: Average height, total dry mass, total percent cover, and root density (0-40 cm depth) of competing vegetation on the three sites in the second growing season (samples collected July 2015). Different letters (a-c) indicate differences among sites (Benjamini–Hochberg correction, α = 0.05). Different letters (x-y) indicate a difference between functional groups. Values in brackets represent one standard deviation of the mean (n=10).

		Ag-field	**Well site**	**Gravel Pit**
Height (cm)		31.8 (23.2) **b**	106 (25.0) **a**	32.4 (9.10) **b**
Total Dry Mass (g)		10.4 (7.04) **b**	49.3 (19.6) **a**	14.7 (6.24) **b**
	Forb	4.08 (5.57) **c**	24.1 (21.6) **a**	8.23 (5.83) **b**
	Grass	6.34 (7.15) **b**	25.2 (8.24) **a**	6.10 (5.77) **b**
Total Cover (%)		17.9 (8.95) **b**	44.7 (18.1) **a**	52.4 (14.4) **a**
	Forb	11.5 (10.3) **b | x**	35.8 (18.1) **a | x**	39.7 (18.4) **a | x**
	Grass	6.40 (6.55) **a | x**	8.60 (4.70) **a | y**	11.4 (7.41) **a | y**
Root density (mg cm^{-3}_{soil})				
	0-10 cm	4.76 (3.19) **a**	3.52 (2.61) **a**	7.39 (6.46) **a**
	10-20 cm	2.23 (1.21) **a**	1.82 (1.48) **a**	2.69 (1.29) **a**
	20-30 cm	1.14 (0.55) **a**	1.81 (1.99) **a**	1.66 (0.92) **a**
	Total	8.14 (3.57) **a**	7.16 (4.69) **a**	11.8 (6.61) **a**

Table 3-7: Two-way analysis of variance and planned contrasts for seedling growth responses. Analyses for each site examine stock type effects within the site. Italicized fixed effects outline planned contrasts which were done for that particular site when stock type was significant. Values in bold are significant at α < 0.05.

Fixed effect	Mortality			Height growth 2014			Height growth 2015			Root growth		
	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P
Site	2	44.23	< **0.001**	2	49.33	< **0.001**	2	212.50	< **0.001**	2	152.94	< **0.001**
Stock group	4	7.75	0.10	4	206.69	< **0.001**	4	14.72	**0.005**	4	113.87	< **0.001**
Site*stock group	8	13.83	0.09	8	33.74	< **0.001**	8	30.86	< **0.001**	8	36.91	< **0.001**

Fixed effect	Egress root mass			Root length			Egress RSR			RG/P		
	df	χ^2	P	df	χ^2	P	df	χ^2	P	df	χ^2	P
Site	2	227.93	< **0.001**	2	89.69	< **0.001**	2	78.19	< **0.001**	2	66.68	< **0.001**
Stock type	4	52.00	< **0.001**	4	7.54	0.11	4	43.29	< **0.001**	4	45.63	< **0.001**
Site*stock group	8	41.59	< **0.001**	8	14.09	0.08	8	12.62	0.12	8	15.43	0.06

Table 3-8: Summary of recommended morphological characteristics to facilitate stock type selection for challenging site conditions.

Site conditions	Recommended stock group	Height (cm)	Root mass (g)	Root-to-stem ratio
Non-limiting	Any High RSR*	Variable	Variable	Variable
Aboveground competition	T-High RSR	45.9	6.92	3.50
Drought	T-High RSR	45.9	6.92	3.50

*as long as growing conditions are good and competition remains minimal

Figures

Figure 3-1: Daily average volumetric water content. Values averaged from hourly measurements taken with EC-5 and 5TM sensors during the second growing season (May to August 2015) (n = 4). Bars represent daily precipitation for the region averaged from the weather station closest to each site.

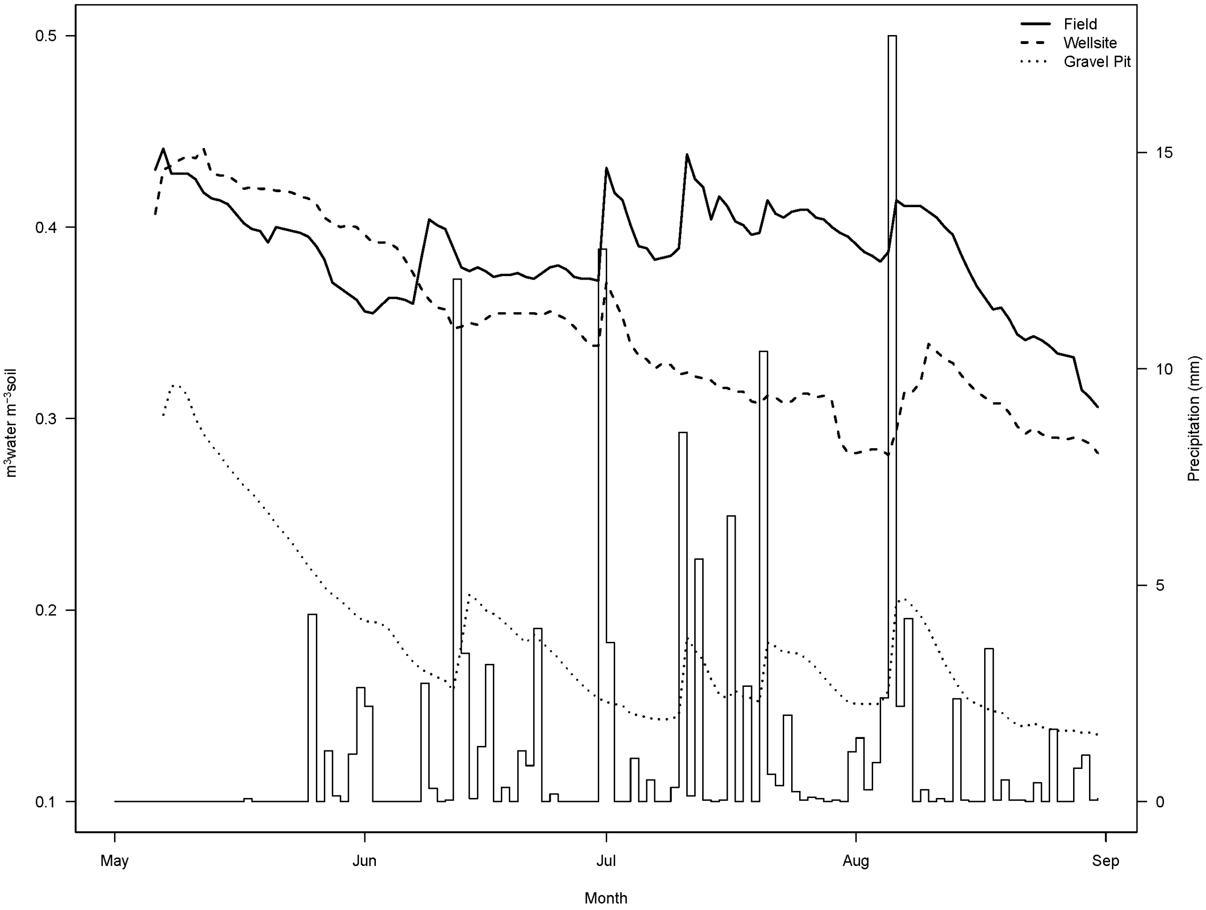
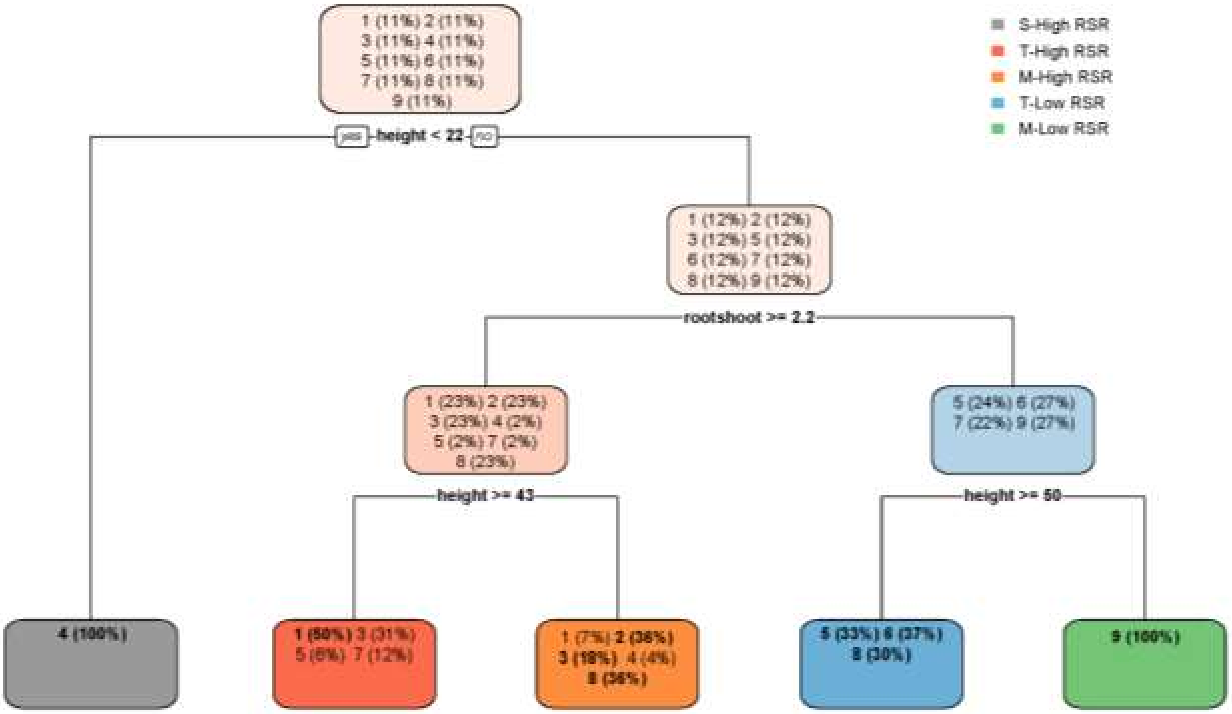


Figure 3-2: Results of the classification tree grouping stock types by initial height and root mass ratio. Numbers in each box refer to stock types which follow the previous classification. Percent values beside each stock type number indicate the proportion of subsampled seedlings from each stock type with the classification characteristics. Bolded numbers in the bottom row indicate the stock type assigned to each stock group.



Note: Although the classification tree shows a higher proportion of stock type 3 in the T-High RSR stock group, this stock type was placed in the M-High RSR stock group as the average initial height and root-to-stem ratio of stock type 3 better corresponded with the M-High RSR stock group.

Figure 3-3: Results of the NMDS ordination of the soil (bulk density; percentage clay, silt and sand; soil nutrition including K, NO3, NH4, P, S) and competing vegetation (vegetation height, aboveground dry mass of grasses and forbs and total belowground root mass) characteristics measured within blocks (stress = 0.09). Ellipses indicate a 95% confidence interval envelope for each site. Vectors are shown for site characteristics which were strongly correlated with one of the ordination axes (α = 0.05).

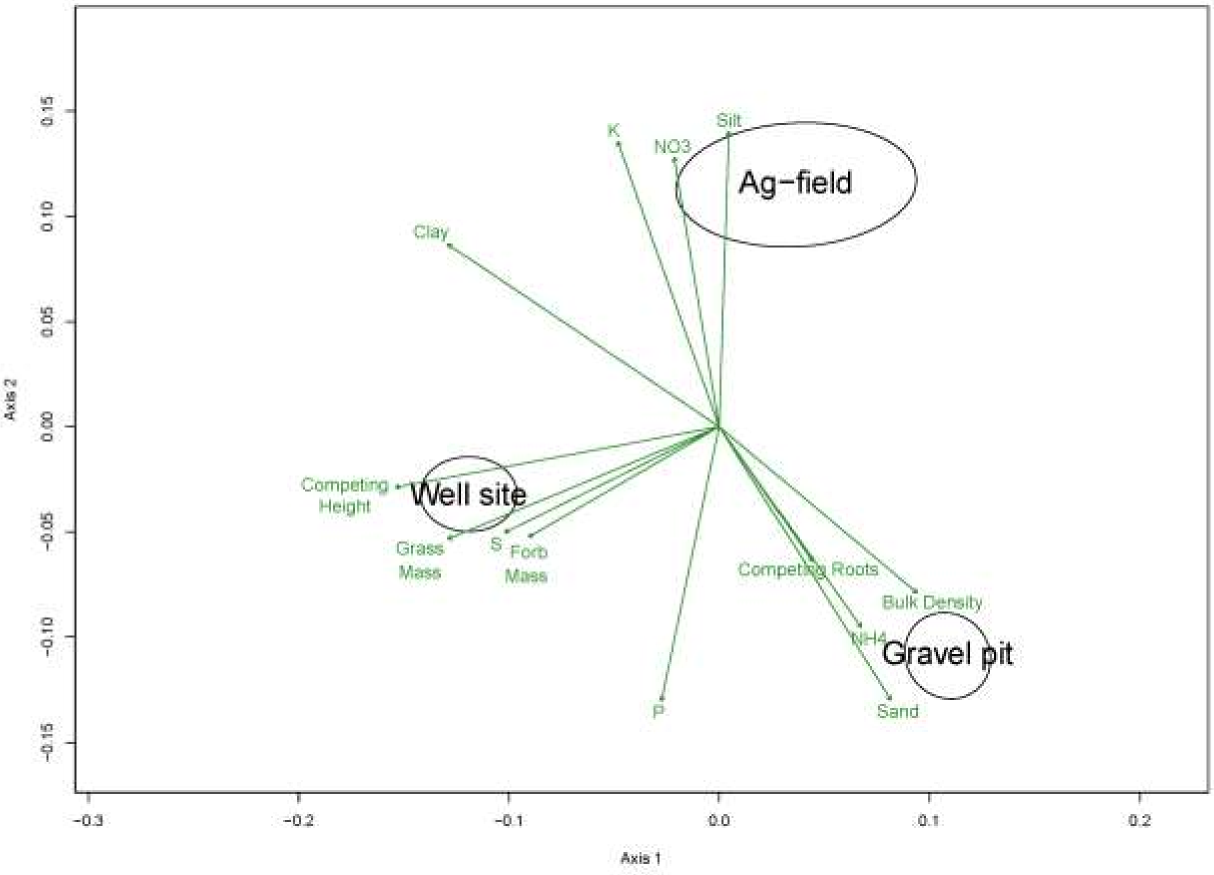


Figure 3-4: Average total height growth between sites and stock types. Error bars represent one standard error of the mean (n=10). Solid colors represent the first growing season (2014) while hatched bars represent the second growing season (2015). Different capitalized letters above the collective bars indicate differences among the site means (upper case letters for 2015 and lower case letters for 2014). Different letters above error bars indicate differences among stock type means within sites (Fisher's LSD test, α = 0.05; upper case letters for 2015 and lower case letters for 2014). Size classes for each stock type group are noted in the legend (S = small, M = medium, T = Tall, RSR = root-to-shoot ratio).

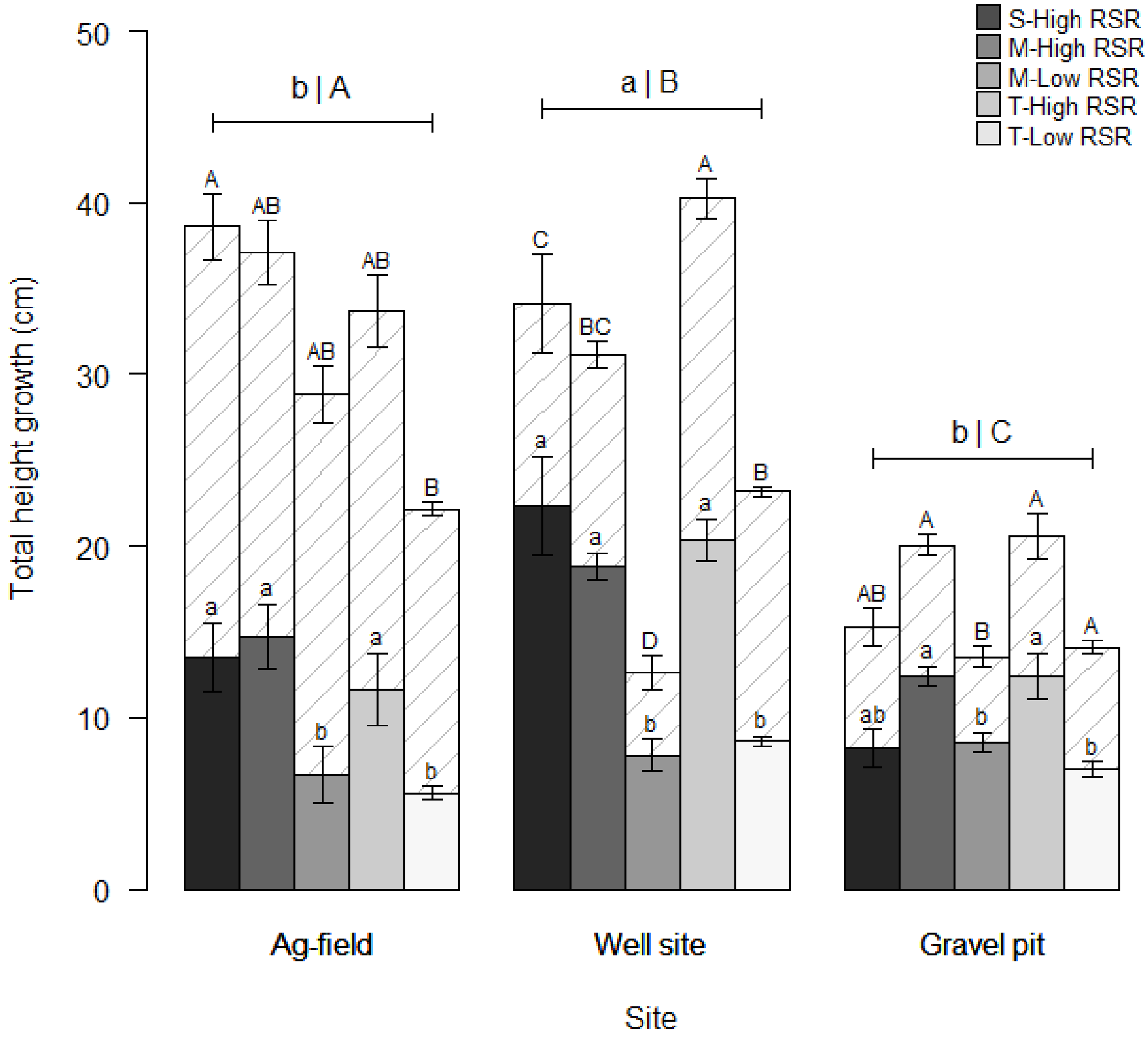


Figure 3-5: Average (a) root growth, (b) egress root mass, and (c) root length between sites and stock types. Error bars represent standard error of the mean (n=10). Different capitalized letters above the collective bars indicate differences among the site means. Different letters above error bars indicate differences among stock type means within sites (Fisher's LSD test, α = 0.05). Size classes for each stock type group are noted in the legend (S = small, M = medium, T = Tall, RSR = root-to-shoot ratio).

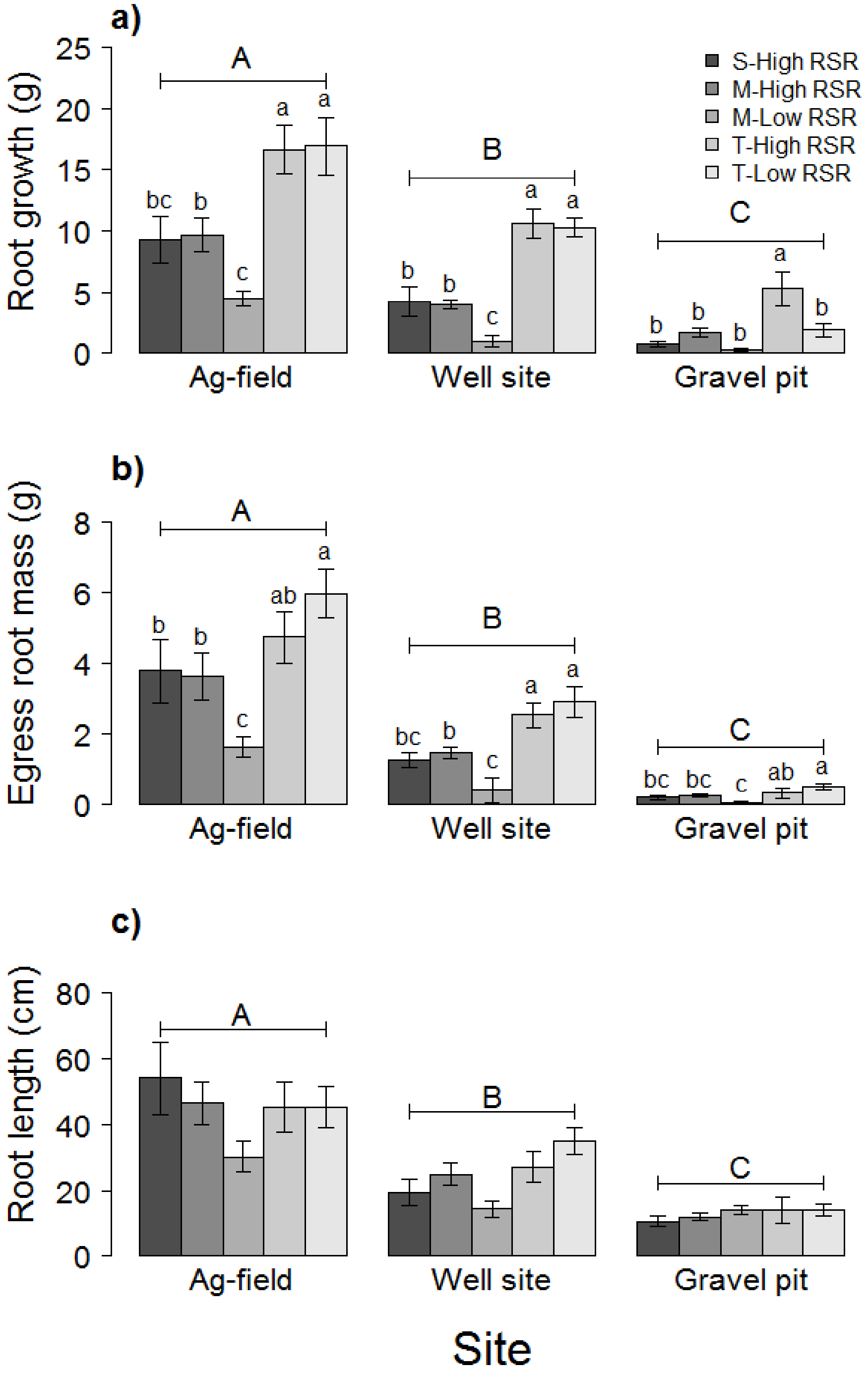


Figure 3-6: Average egress root-to-shoot ratio (egress root mass relative to shoot mass) between sites and stock types. Error bars represent one standard error of the mean (n=10). Different capitalized letters above the collective bars indicate differences among the site means. Different letters above error bars indicate differences among stock type means within sites (Fisher's LSD test, α = 0.05). Size classes for each stock type group are noted in the legend (S = small, M = medium, T = Tall, RSR = root-to-shoot ratio).

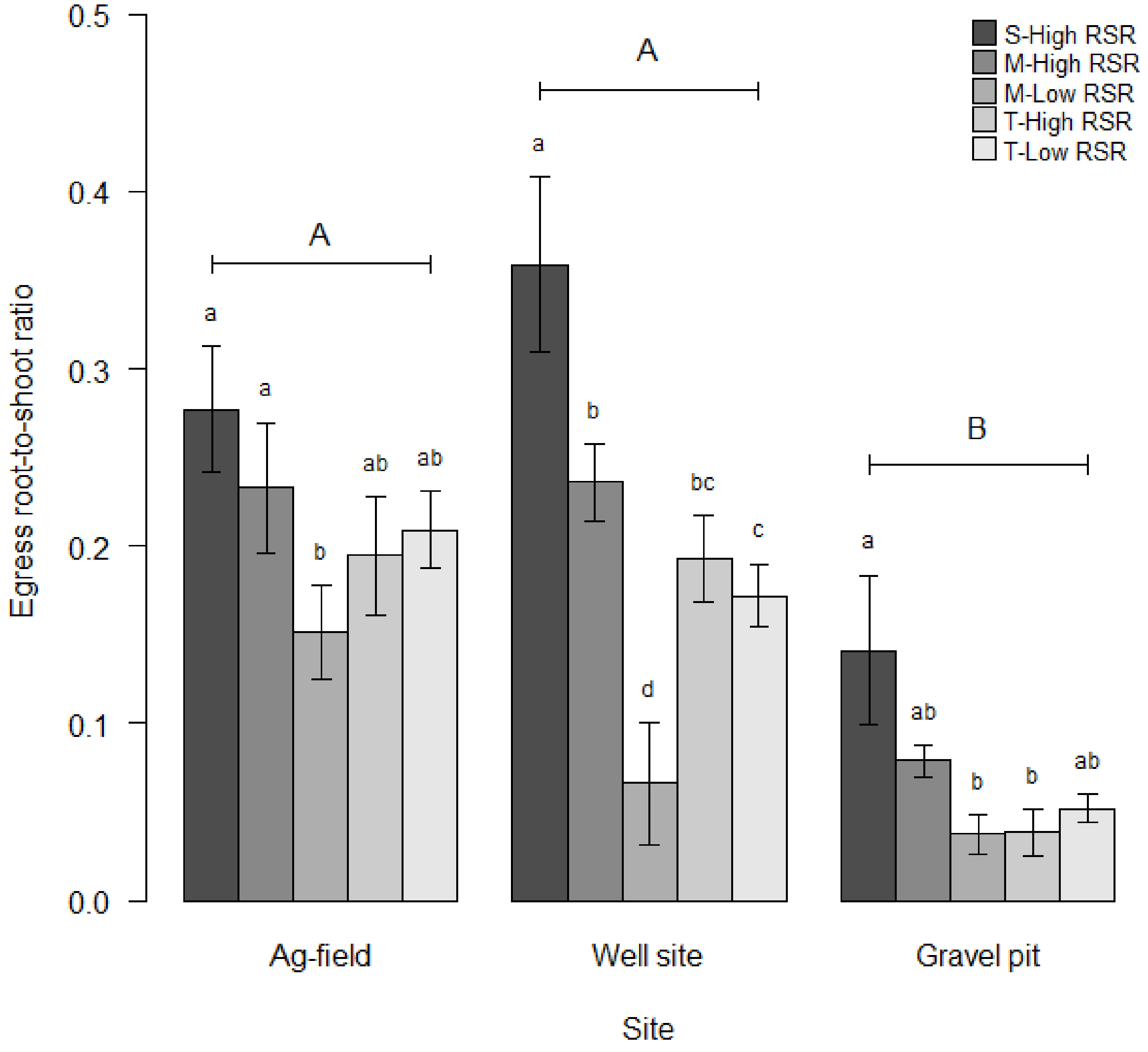
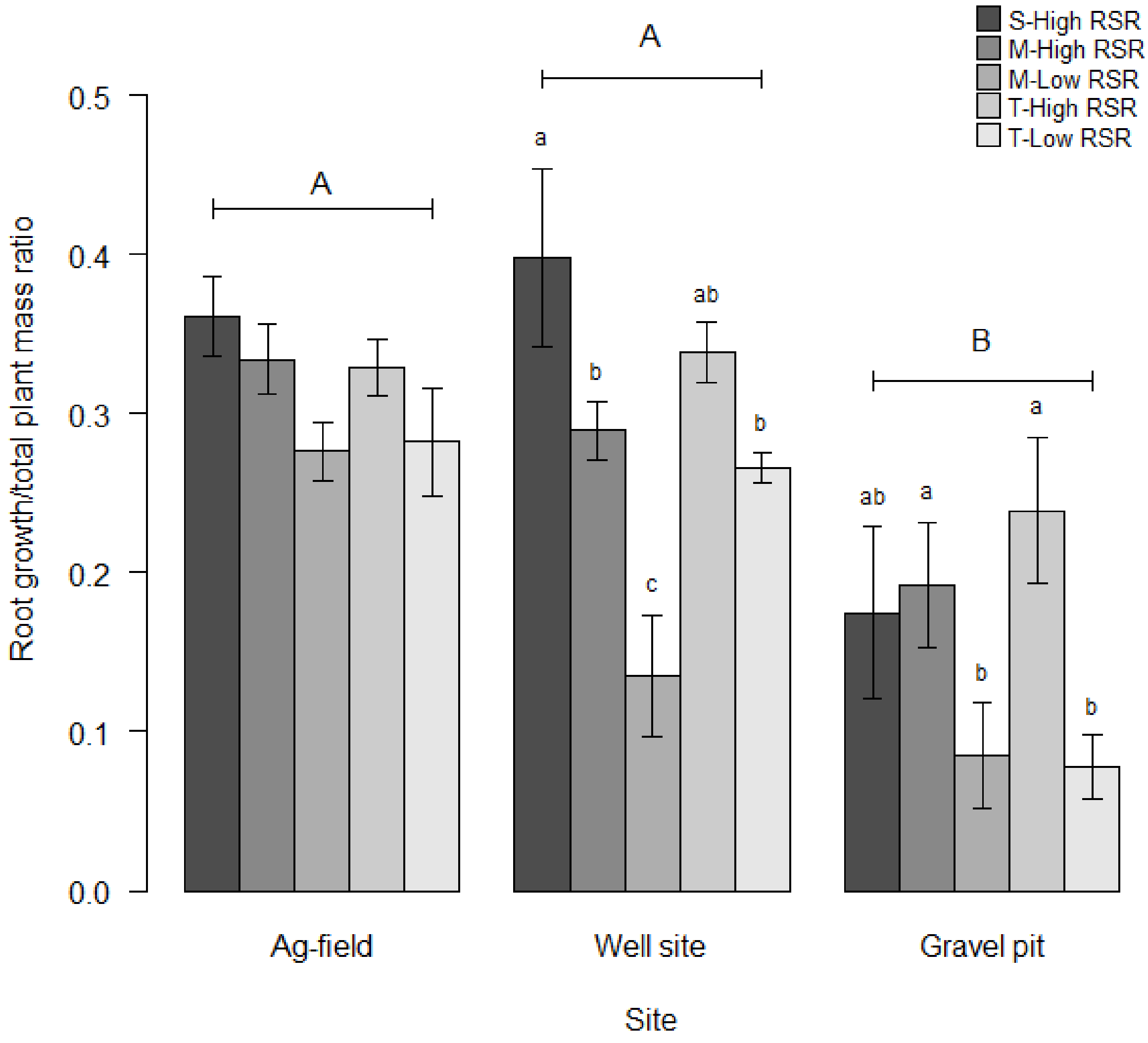


Figure 3-7: Average RG/P (root growth/total seedling mass) between sites and stock types. Error bars represent one standard error of the mean (n=10). Different capitalized letters above the collective bars indicate differences among the site means. Different letters above error bars indicate differences among stock type means within sites (Fisher's LSD test, α = 0.05). Size classes for each stock type group are noted in the legend (S = small, M = medium, T = Tall, RSR = root-to-shoot ratio).



Chapter 4 – Conclusion

4.1. Research summary

The objective of the first study was to identify initial morphological characteristics that would improve performance of aspen seedlings on sites where vegetation competition limited growth and establishment. Stock types varying in initial size, root-to-stem ratio, and root non-structural carbohydrate (NSC) concentrations were planted in three site treatments (mulch, plow, and grass) with different levels of competition. Results showed that a high root-to-stem ratio was related to increased first year height growth; however, this effect was only observed in treatments where competition was removed prior to planting (mulch and plowed treatment). In the grass treatment where competition was present at the time of planting, initial morphological characteristics did not significantly affect height growth. This was likely due to belowground competition from grasses limiting soil water, nutrients, and rooting space available for seedlings. Signs of belowground competition were further demonstrated by the reduction in root egress for seedlings in the grass plot. Improving root egress proved to be important for seedling establishment, as it was related to increased height growth. While increases in height growth could be linked to initial morphology, overall performance of aspen seedlings in the competition treatments was marginal. Competing vegetation reduced overall growth and the effectiveness of initial morphology. The modest improvements in growth observed for seedlings in the plow treatments suggest the importance of periodic control of vegetation in the following years after planting to improve establishment success.

In the second study, the same stock types were planted across three sites in NW Alberta with contrasting soil properties and competing vegetation characteristics. The focus of this study was to assess which morphological characteristics were best suited for contrasting site conditions. The nine stock types from the previous chapter were sorted into five distinct stock groups with identifiable characteristics. At the ag-field, where site conditions were non-limiting, morphological characteristics were marginally beneficial towards seedling performance as most stock groups performed similarly. In situations where competing vegetation was tall, such as at the well site, taller seedlings were shown to perform better (as long as a high root-to-stem ratio was maintained), which contrasts the results of previous studies (Martens et al. 2007) showing poorer performance for taller seedlings. At the gravel pit, where dry conditions and competing vegetation were prevalent, morphological characteristics provided limited benefit towards seedling performance. This was likely a result of poor growing conditions reducing any observable effect on growth. Across all three sites a high root-to-stem ratio was shown to improve height growth in the first growing season, which suggests its importance in improving establishment success regardless of site conditions.

Both studies were successful in identifying the importance of developing aspen seedlings with a high root-to-stem ratio and the benefits it provides on sites with significant vegetation competition. The importance of competition control was also shown as an important step in improving the effectiveness of initial morphological characteristics.

4.2. Management implications

In addition to previous work emphasizing the importance of a high root-to-stem ratio for aspen seedlings (Martens et al. 2007; Landhäusser et al. 2012b; Kulbaba 2014), the results of this thesis have outlined its benefits on sites with vegetation competition. Initial root-to-stem ratio is a useful indicator of aspen seedling quality, as it was related to increased height growth in the first year. Higher root mass allocation relative to the shoot will facilitate increased nutrient and water uptake (Lloret et al. 1999) and allow for greater shoot growth (Landhäusser et al. 2012b) while maintaining a favorable water balance during drought stress (Grossnickle 2012). The effects of root-to-stem ratio on height growth in subsequent growing seasons was more variable between the two studies. A significant effect was observed during all three growing seasons for the plowed treatment in the first study, whereas increased height growth only occurred in the first growing season for the second study. While benefits to height growth may be short-lived, a high root-to-stem ratio was beneficial in reducing mortality and may increase growth if growing conditions improve. Root-to-stem ratio had a stronger effect on height growth in the third growing season in the plowed treatment as precipitation was higher relative to the previous two years.

Site conditions play a large role in determining what stock type characteristics are needed for improving seedling establishment. When competing vegetation is low and soil resources (moisture and nutrients) are not a limiting factor, the need for tailored morphological characteristics becomes superfluous as all high quality stock types will generally be able to grow unimpeded. Taller seedlings with a high root-to-stem ratio are recommended for sites with tall competing vegetation. While each of the High RSR stock groups had similar total height growth, taller seedlings maintained their height advantage after two growing seasons when planted with competition. A high initial root-to-stem ratio in addition to shoot height will help to reduce drought stress that is generally associated with a taller shoot (Kulbaba 2014). On dry sites such as the gravel pit, increasing root growth is a priority to ensure that seedlings can establish a proper water balance (Grossnickle 2005). Stock types with a large root mass are recommended for xeric sites, as this will promote a higher initial root-to-stem ratio and a greater amount of NSC reserves prior to planting. A high root-to-stem ratio in small seedlings (ex. S-High RSR stock group in chapter 3) may not be as beneficial as a smaller root mass may not provide enough reserves and shading from competing vegetation became an issue for small seedlings on the gravel pit.

Key morphological characteristics linked to increased height growth on sites with vegetation competition were identified; however, nutrient loading through the use of shoot growth inhibitors was an additional factor which contributed to overall seedling performance. Nutrient loading can significantly improve root NSC concentrations (Schott et al. 2013) and has been shown to improve height growth (relative to standard seedlings) on reclamation sites (Schott et al. 2016). While differences in height growth between stock types were largely due to morphological characteristics (root NSC concentration was not significant with height growth), overall performance would likely have been poorer without nutrient loading. It is recommended that nutrient loading be used in conjunction with optimized seedling morphology to produce high quality aspen seedlings.

Marginal seedling performance will occur, despite nutrient loading and improved morphology, if site conditions are not conducive to establishment. Vegetation competition severely limited potential growth during the study, as seedlings unrestricted from vegetation competition (i.e. the mulch plot) grew to an average height of 2 meters after three growing seasons. While establishment success can be improved by developing a target seedling with morphological characteristics tailored for specific site conditions, no target seedling will be able to establish if site conditions are initially poor. Significant belowground competition (grass treatment) or dry conditions in conjunction with competition (gravel pit) can severely limit growth and prevent the potential benefits of seedling morphology. Suppression of competing vegetation prior to planting and for up to four years afterwards (Noland et al. 2001) in conjunction with high quality aspen seedlings appears to be the optimal method for improving establishment. The use of selective herbicides to control for undesirable competing plant species could be implemented to reduce overall costs (Henkel-Johnson et al. 2016).

4.3. Future research and study implications

Improvements in height growth for seedlings in the competition treatments could be attributed to a high root-to-stem ratio; however, drought conditions may have reduced its overall effectiveness. During the driest growing season, precipitation was approximately 54% of the long-term average for both study regions. Increased height growth in relation to initial root-to-stem ratio during the third growing season for seedlings in the plowed treatment suggests that tailored seedling morphology would have been more effective under better growing conditions. For seedlings planted at the gravel pit, low precipitation during the establishment year in addition to competition resulted in poor growing conditions and marginal seedling performance.

Measuring seedling growth with competition relative to growth without competition (using control plots on each site) would have been useful to assess tolerance to competition (i.e. degree to which competition reduces growth) and to determine when competition no longer affected seedling growth (i.e. growth in competition plots similar to no competition plots) (Noland et al. 2001). Larger stock types which showed less growth in competition treatments may have had similar growth in treatments without competition, which would imply that the growth of these seedlings was driven more so by biomass allocation than competition. Future monitoring of the growth of these stock types will be useful in determining the amount of time needed for the seedlings to no longer be affected by competition. Studies examining effects of herbaceous competition on eastern white pine (*Pinus strobus* L.) found that competition needed to be controlled for up to four years before growth was no longer greatly impacted (Wagner et al. 1999). While the mulch treatment was useful in highlighting the growth potential of the stock types under optimal conditions, comparisons to the grass and plowed treatments were partially confounded by contrasting soil conditions.

Root egress was an important measure of seedling performance as it was related to increased height growth; however, results were not consistent between the two studies (root egress was not significant with height growth in second chapter). For container grown seedlings, root development outside of the plug relative to shoot dry weight is proposed as a useful measure of survival and drought avoidance potential, as seedlings with minimal root development outside of the plug will have the greatest water stress no matter how large the initial root mass

(Grossnickle and Reid 1984; Grossnickle 2012). Future work could examine potential relationships between morphological or physiological characteristics and root egress, as results were inconsistent in this study.

Belowground competition was a major limiting factor for aspen seedlings in this study, as stock types planted in the grass treatment showed minimal egress. Future studies aiming to improve establishment success of aspen seedlings could examine methods to improve root egress. Site preparation techniques such as mounding are useful for reducing competition and facilitating soil conditions conducive to root development (Sutton 1993) and have been found to improve egress of conifer species such as white spruce (von der Gonna and Lavender 1988). While typically used in a forestry context, studies could examine the efficacy of this method in a reclamation setting.

References

Alberta Climate Information Service (2016) Current and historical Alberta weather station data viewer. https://agriculture.alberta.ca/acis/alberta-weather-data-viewer.jsp. Accessed 20 Jun 2016

Aphalo P, Rikala R (2003) Field performance of silver-birch planting-stock grown at different spacing and in containers of different volume. New Forests 25:93–108.

Baker FS (1918) Aspen reproduction in relation to management. Journal of Forestry 16:389–398.

Barbier S, Gosselin F, Balandier P (2008) Influence of tree species on understory vegetation diversity and mechanisms involved-A critical review for temperate and boreal forests. Forest Ecology and Management 254:1–15.

Barnes V (1975) Phenotypic variation of trembling aspen in western North America. Forest Science 21:319–328.

Bockstette SW, Pinno BD, Dyck MF, Landhäusser SM (2017) Root competition, not soil compaction, restricts access to soil resources for aspen on a reclaimed mine soil. Botany 95:685–695.

Brandt JP (2009) The extent of the North American boreal zone. Environmental Reviews 17:101–161.

Brandt JP, Flannigan MD, Maynard DG, et al (2013) An introduction to Canada's boreal zone: ecosystem processes, health, sustainability, and environmental issues. Environmental Reviews 21:207–226.

Bremner JM (1996) Nitrogen-Total. In: Sparks DL, Page AL, Helmke PA, et al. (eds) Methods of Soil Analysis Part 3—Chemical Methods. Soil Science Society of America Inc., Madison, WI, pp 1085–1121

Brouwer R (1962) Nutritive influences on the distribution of dry matter in the plant. Netherlands Journal of Agricultural Science 10:399–408.

Brown JK, DeByle NV (1987) Fire damage, mortality, and suckering in aspen. Canadian Journal of Forest Research 17:1100–1109.

Burdett AN (1990) Physiological processes in plantation establishment and the development of specifications for forest planting stock. Canadian Journal of Forest Research 20:415–427.

Burrowes A, Marsh R, Teare M, et al (2010) Alberta's energy reserves 2009 and supply/demand outlook. Energy Resources Conservation Board, Calgary, AB 1–232.

Campbell RB, Bartos DL (2001) Aspen ecosystems: Objectives for sustaining biodiversity. Sustaining Aspen in Western Landscapes: Symposium Proceedings 299–307.

Carter MR, Gregorich EG (2008) Soil sampling and methods of analysis, Second Edi. CRC Press, Boca Raton

Chavasse CGR (1977) The significance of planting height as an indicator of subsequent seedling growth [forest and ornamental trees]. New Zealand Journal of Forestry 22:283–296.

Chavasse CGR (1980) Planting stock quality: a review of factors affecting performance. New Zealand Journal of Forestry 25:144–171.

Chow PS, Landhäusser SM (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiology 24:1129–1136.

Cleve KV, Noonan LL (1975) Litter fall and nutrient cycling in the forest floor of birch and aspen stands in interior Alaska. Canadian Journal of Forest Research 5:626–639.

Close D, Beadle C, Brown P (2005) The physiological basis of containerised tree seedling “transplant shock”: a review. Australian Forestry 68:112–120.

Collet C, Löf M, Pagès L (2006) Root system development of oak seedlings analysed using an architectural model. Effects of competition with grass. Plant and Soil 279:367–383.

Cragg JB, Carter A, Leischner C, et al (1977) Litter fall and chemical cycling in an aspen (*Populus tremuloides*) woodland ecosystem in Canadian Rockies. Pedobiologia 17:428–443.

Cripps CL (2003) Native mycorrhizal fungi with aspen on smelter-impacted sites in the Northern Rocky Mountains: occurrence and potential use in reclamation. In: National Meeting of the American Society of Mining and Reclamation and the 9th Billings Land Reclamation Symposium. ASMR, Billings, Montana, pp 193–208

Crites S, Dale MR (1998) Diversity and abundance of bryophytes, lichens, and fungi in relation to woody substrate and successional stage in aspen mixedwood boreal forests. Canadian Journal of Botany 76:641–651.

Cuesta B, Vega J, Villar-Salvador P, Rey-Benayas JM (2010a) Root growth dynamics of Aleppo pine (*Pinus halepensis* Mill.) seedlings in relation to shoot elongation, plant size and tissue nitrogen concentration. Trees - Structure and Function 24:899–908.

Cuesta B, Villar-Salvador P, Puértolas J, et al (2010b) Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species. Forest Ecology and Management 260:71–78.

Curt T, Coll L, Prevosto B, et al (2005) Plasticity in growth, biomass allocation and root morphology in beech seedlings as induced by irradiance and herbaceous competition. Annals of Forest Science 62:51–60.

Davis AS, Jacobs DF (2005) Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests 30:295–311.

Day MW (1944) The root system of aspen. American Midland Naturalist 32:502–509.

Donaldson JR, Kruger EL, Lindroth RL (2006) Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). New

Phytologist 169:561–570.

Fox MD, Tackaberry LE, Drouin P, et al (2013) Microbial community structure of soils under four productivity classes of aspen forests in northern British Columbia. Ecoscience 20:264–275.

Franklin J, Buckley D (2009) Effects of seedling size and ground cover on the first-year survival of planted pine and hardwoods over an extreme drought. In: National Meeting of the American Society of Mining and Reclamation. ASMR, Billings, MT, pp 474–484

Galvez DA, Landhäusser SM, Tyree MT (2013) Low root reserve accumulation during drought may lead to winter mortality in poplar seedlings. New Phytologist 198:139–148.

Government of Alberta (2016) Environmental Protection and Enhancement Act. www.qp.alberta.ca/documents/acts/e12.pdf. Accessed 1 Sep 2016

Grant CD, Ward SC, Morley SC (2007) Return of ecosystem function to restored bauxite mines in Western Australia. Restoration Ecology 15:94–103.

Gray LK, Gylander T, Mbogga MS, et al (2011) Assisted migration to address climate change: recommendations for aspen reforestation in western Canada. Ecological Applications 21:1591–1603.

Groninger J, Skousen J, Angel P, et al (2007) Mine reclamation practices to enhance forest development through natural succession. Forest Reclamation Advisory 1–5.

Grossnickle SC (2005) Importance of root growth in overcoming planting stress. New Forests 30:273–294.

Grossnickle SC (2012) Why seedlings survive: influence of plant attributes. New Forests 43:711–738.

Grossnickle SC, Reid CPP (1984) Water relations of Engelmann spruce seedlings on a high-elevation mine site: an example of how reclamation techniques can alter microclimate and edaphic conditions. Reclamation and Revegetation Research 3:199–221.

Haase DL, Rose R (1993) Soil moisture stress induces transplant shock in stored and unstored 2+0 Douglas-fir seedlings of varying root volumes. Forest Science 39:275–294.

Haeussler S, Bedford L, Boateng JO, MacKinnon A (1999) Plant community responses to mechanical site preparation in northern interior British Columbia. Canadian Journal of Forest Research 29:1084–1100.

Hassan NM, Rasmussen PE, Dabek-Zlotorzynska E, et al (2007) Analysis of environmental samples using microwave-assisted acid digestion and inductively coupled plasma mass spectrometry: Maximizing total element recoveries. Water, Air, and Soil Pollution 178:323–334.

Hellum A (1981) Root egress in lodgepole pine seedlings grown in peat and planted soil. In: Scarratt JB, Glerum CA, Plexman CA (eds) Proceedings of the Canadian Containerized

Tree Seedling Symposium. Canadian Forestry Service, Toronto, Ontario, pp 389–396

Henkel-Johnson D, Macdonald SE, Bork EW, Thomas BR (2016) Influence of weed composition, abundance, and spatial proximity on growth in young hybrid poplar plantations. Forest Ecology and Management 362:55–68.

Hilbert DW (1990) Optimization of plant root:shoot ratios and internal nitrogen concentration. Annals of Botany 66:91–99.

Hu Y-L, Hu Y, Zeng D-H, et al (2015) Exponential fertilization and plant competition effects on the growth and N nutrition of trembling aspen and white spruce seedlings. Canadian Journal of Forest Research 45:78–86.

Idris M, Salifu KF, Timmer VR (2004) Root plug effects on early growth and nutrition of container black spruce seedlings. Forest Ecology and Management 195:399–408.

Jacobs DF, Oliet JA, Aronson J, et al (2015) Restoring forests: What constitutes success in the twenty-first century? New Forests 46:601–614.

Jacobs DF, Salifu KF, Seifert JR (2005) Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. New Forests 30:235–251.

Johnson PS, Novinger SL, Mares WG (1984) Root, shoot, and leaf area growth potentials of northern red oak planting stock. Forest Science 30:1017–1026.

Jordaan SM (2012) Land and water impacts of oil sands production in Alberta. Environmental Science and Technology 46:3611–3617.

Kelly JWG, Landhäusser SM, Chow PS (2015) The impact of light quality and quantity on root-to-shoot ratio and root carbon reserves in aspen seedling stock. New Forests 46:527–545.

King JS, Pregitzer KS, Zak DR (1999) Clonal variation in above- and below-ground growth responses of *Populus tremuloides* Michaux: Influence of soil warming and nutrient availability. Plant and Soil 217:119–130.

Kolb TE, Steiner KC (1990) Growth and biomass partitioning of northern red oak and yellow-poplar seedling: effects of shading and grass root competition. Forest Science 36:34–44.

Kulbaba SP (2014) Evaluating trembling aspen (Populus tremuloides Michx.) seedling stock characteristics in response to drought and out-planting on a reclamation site. M.Sc. thesis, Dept. of Renewable Resources, University of Alberta

Landhäusser SM, Deshaies D, Lieffers VJ (2010) Disturbance facilitates rapid range expansion of aspen into higher elevations of the Rocky Mountains under a warming climate. Journal of Biogeography 37:68–76.

Landhäusser SM, Lieffers VJ (1998) Growth of Populus tremuloides in association with *Calamagrostis canadensis*. Canadian Journal of Forest Research 28:396–401.

Landhäusser SM, Mulak TL, Lieffers VJ (2007) The effect of roots and litter of *Calamagrostis* *canadensis* on root sucker regeneration of *Populus tremuloides*. Forestry 80:481–488.

Landhäusser SM, Pinno BD, Lieffers VJ, Chow PS (2012a) Partitioning of carbon allocation to reserves or growth determines future performance of aspen seedlings. Forest Ecology and Management 275:43–51.

Landhäusser SM, Rodriguez-Alvarez J, Marenholtz EH, Lieffers VJ (2012b) Effect of stock type characteristics and time of planting on field performance of aspen (*Populus tremuloides* Michx.) seedlings on boreal reclamation sites. New Forests 43:679–693.

Légaré S, Paré D, Bergeron Y (2004) The responses of black spruce growth to an increased proportion of aspen in mixed stands. Canadian Journal of Forest Research 34:405–416.

Lenth R V. (2016) Least-Squares Means: The R Package lsmeans. Journal of Statistical Software 69:1–33.

Li GL, Liu Y, Zhu Y, et al (2011) Influence of initial age and size on the field performance of *Larix olgensis* seedlings. New Forests 42:215–226.

Lieffers VJ, Landhäusser SM, Hogg EH (2001) Is the wide distribution of aspen a result of its stress tolerance? In: Shepperd WD, Binkley D, Bartos DL, et al. (eds) Sustaining aspen in western landscapes: Symposium proceedings. U.S. Department of Agriculture, Forest Service, Grand Junction, CO, pp 311–324

Lieffers VJ, Macdonald SE, Hogg EH (1993) Ecology of and control strategies for *Calamagrostis canadensis* in boreal forest sites. Canadian Journal of Forest Research 23:2070–2077.

Lieffers VJ, Stewart JD, Macmillan RB, et al (1996) Semi-natural and intensive silvicultural systems for the boreal mixedwood forest. The Forestry Chronicle 72:286–292.

Linke J, Franklin SE, Huettmann F, Stenhouse GB (2005) Seismic cutlines, changing landscape metrics and grizzly bear landscape use in Alberta. Landscape Ecology 20:811–826.

Lloret F, Casanovas C, Peñuelas J (1999) Seedling survival of Mediterranean shrubland species in relation to root:shoot ratio , seed size and water and nitrogen use. Functional Ecology 13:210–216.

Long JN, Mock K (2012) Changing perspectives on regeneration ecology and genetic diversity in western quaking aspen: implications for silviculture. Canadian Journal of Forest Research 42:2011–2021.

Lozano FC, Morrison IK (1981) Disruption of hardwood nutrition by sulfur dioxide, nickel, and copper air pollution near Sudbury, Canada. Journal of Environment Quality 10:198.

Macdonald SE, Landhäusser SM, Skousen J, et al (2015) Forest restoration following surface mining disturbance: challenges and solutions. New Forests 46:703–732.

Macdonald SE, Quideau S, Landhäusser SM (2012) Rebuilding boreal forest ecosystems after industrial disturbance. In: Vitt D, Bhatti J (eds) Restoration and Reclamation of Boreal

Ecosystems. Cambridge University Press, Cambridge, pp 123–160

Machtans CS (2006) Songbird response to seismic lines in the western boreal forest: a manipulative experiment. Canadian Journal of Zoology 84:1421–1430.

Maini JS (1968) Silvics and ecology of Populus in Canada. In: Maini JS, Cayford JH (eds) Growth and utilization of poplars in Canada, Department. Minister of Forestry and Rural Development, Ottawa, ON, pp 20–69

Mandryk AM, Wein RW (2006) Exotic vascular plant invasiveness and forest invasibility in urban boreal forest types. Biological Invasions 8:1651–1662.

Martens LA, Landhäusser SM, Lieffers VJ (2007) First-year growth response of cold-stored, nursery-grown aspen planting stock. New Forests 33:281–295.

Mattsson A (1997) Predicting field performance using seedling quality assessment. New Forests 13:227–252.

McMillan R, Quideau SA, MacKenzie MD, Biryukova O (2007) Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. Journal of Environment Quality 36:1470.

McNabb DH, Sobze J-M, Schoonmaker AS (2012) Tilling compacted soils with RipPlows: a disturbed soil restoration technique. NAIT Boreal Research Institute, Technical Note 1–7.

Messier C, Coll L, Poitras-Larivière A, et al (2009) Resource and non-resource root competition effects of grasses on early- versus late-successional trees. Journal of Ecology 97:548–554.

Mexal JG, Landis TD (1990) Target seedling concepts: height and diameter. In: Campbell SJ, Landis TD (eds) Target Seedling Symposium. U.S. Department of Agriculture, Forest Service, Roseburg, OR, pp 17–35

Mullin RE (1963) Planting check in spruce. The Forestry Chronicle 39:252–259.

Musselman RC, Shepperd WD, Smith FW, et al (2012) Response of transplanted aspen to irrigation and weeding on a Colorado reclaimed surface coal mine. USDA Forest Service - Research Paper RMRS-RP 1–20.

Natural Regions Committee (2006) Natural regions and subregions of Alberta. Government of Alberta, Edmonton, AB

Nelson DW, Sommers LE (1996) Total Carbon, Organic Carbon, and Organic Matter. In: Sparks DL, Page AL, Helmke PA, et al. (eds) Methods of Soil Analysis Part 3—Chemical Methods. Soil Science Society of America Inc., Madison, WI, pp 961–1010

Newton M, Cole EC, White DE (1993) Tall planting stock for enhanced growth and domination of brush in the Douglas-fir region. New Forests 7:107–121.

Noland TL, Mohammed GH, Wagner RG (2001) Morphological characteristics associated with tolerance to competition from herbaceous vegetation for seedlings of jack pine, black spruce, and white pine. New Forests 21:199–215.

Oaten DK, Larsen KW (2008) Stand characteristics of three forest types within the dry interior forests of British Columbia, Canada: Implications for biodiversity. Forest Ecology and Management 256:114–120.

Oksanen J, Blanchet FG, Friendly M, et al (2016) vegan: Community Ecology Package. In: The R Project for Statistical Computing. https://cran.r-project.org/package=vegan. Accessed 20 Apr 2016

Osko T, Glasgow M (2010) Removing the wellsite footprint: Recommended practices for construction and reclamation on wellsites in upland forests in Boreal Alberta. Edmonton, AB

Overton WS, Ching KK (1978) Analysis of differences in height growth among populations in a nursery selection study of Douglas-fir. Forest Science 24:497–509.

Padilla FM, Pugnaire FI (2006) The role of nurse plants in the restoration of degraded environments the restoration of degraded environments. Frontiers in Ecology and the Environment 4:196–202.

Paré D, Bergeron Y (1996) Effect of colonizing tree species on soil nutrient availability in a clay soil of the boreal mixedwood. Canadian Journal of Forest Research 26:1022–1031.

Peterson EB, Peterson NM (1992) Ecology, management, and use of aspen and balsam poplar in the prairie provinces. Northern Forestry Centre, Edmonton, AB

Pinheiro J, Bates D, DebRoy S, Sarkar D (2016) nlme: Linear and Nonlinear Mixed Effects Models. In: The R Project for Statistical Computing. http://cran.r-project.org/package=nlme. Accessed 13 Jan 2015

Pinno BD, Landhäusser SM, MacKenzie MD, et al (2012) Trembling aspen seedling establishment, growth and response to fertilization on contrasting soils used in oil sands reclamation. Canadian Journal of Soil Science 92:143–151.

Pinto JR, Marshall JD, Dumroese RK, et al (2011) Establishment and growth of container seedlings for reforestation: A function of stocktype and edaphic conditions. Forest Ecology and Management 261:1876–1884.

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Functional Plant Biology 27:1191–1191.

Pope PE (1993) A historical perspective of planting and seeding oak. Progress, problems and status. In: Loftis DL, McGee CE (eds) Proc. Oak Regeneration: Serious problems, practical recommendations. USDA For. Serv., Southeastern Forest Exp. Sta.,. Gen. Tech. Rep. SE-84, Asheville, NC, pp 224–240

Powell GW, Bork EW (2004a) Competition and facilitation in mixtures of aspen seedlings, alfalfa, and marsh reedgrass. Canadian Journal of Forest Research 34:1858–1869.

Powell GW, Bork EW (2004b) Above- and below-ground effects from alfalfa and marsh reedgrass on aspen seedlings. Forest Ecology and Management 199:411–422.

Powter C, Chymko N, Dinwoodie G, et al (2012) Regulatory history of Alberta's industrial land conservation and reclamation program. Canadian Journal of Soil Science 92:39–51.

Price DT, Alfaro RI, Brown KJ, et al (2013) Anticipating the consequences of climate change for Canada's boreal forest ecosystems. Environmental Reviews 21:322–365.

R Core Team (2016) R: A Language and Environment for Statistical Computing. https://www.r-project.org/. Accessed 30 Sep 2014

Rahman N, Schoenau J, Kimaro A (2013) Survival and growth of tree seedlings on reclaimed oil sand site in response to fertilizer and ground cover grass species. In: Soils and Crops Workshop. University of Saskatchewan, Saskatoon, SK, pp 1–11

Rahnama F, Marsh RA, Philp L (2013) The Alberta oil sands: reserves and long-term supply outlook. In: Hein FJ, Leckie D, Larter S, Suter J (eds) Heavy-oil and Oil-sand Petroleum Systems in Alberta and Beyond, AAPG Studi. The American Association of Petroleum Geologists, pp 133–144

Reich PB, Teskey RO, Johnson PS, Hinckley TM (1980) Periodic root and shoot growth in oak. Forest Science 26:590–598.

Ritchie GA, Dunlap JR (1980) Root growth potential: its development and expression in forest tree seedlings. New Zealand Journal of Forestry Science 10:218–248.

Romme WH, Turner MG, Gardner RH, et al (1997) A rare episode of sexual reproduction in aspen (*Populus tremuloides* Michx) following the 1988 Yellowstone fires. Natural Areas Journal 17:17–25.

Romme WH, Turner MG, Tuskan GA, Reed RA (2005) Establishment, persistence, and growth of aspen (*Populus tremuloides*) seedlings in Yellowstone National Park. Ecology 86:404–418.

Rook DA (1973) Conditioning radiata pine seedlings to transplanting, by restricted watering. New Zealand Journal of Forestry Science 3:54–69.

Rose R, Campbell SJ, Landis TD (1990) Target Seedling Symposium: Proceedings, combined meeting of the Western Forest Nursery Associations. In: Rose R, Campbell SJ, Landis TD (eds) General Technical Report (GTR). U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp 1–286

Rose R, Gleason JF, Atkinson M (1993) Morphological and water-stress characteristics of three Douglas-fir stocktypes in relation to seedling performance under different soil moisture conditions. New Forests 7:1–17.

Rowe JS (1972) Forest Regions of Canada. Ottawa, ON

Rowland SM, Prescott CE, Grayston SJ, et al (2009) Recreating a functioning forest soil in reclaimed oil sands in northern Alberta: an approach for measuring success in ecological restoration. Journal of Environment Quality 38:1580.

Rumble M, Flake L, Mills T, Dykstra B (2001) Do pine trees in aspen stands increase bird diversity. USDA Forest Service Proceedings RMRS-P-18 2001 185–192.

Sands R (1984) Transplanting stress in radiata pine. Australian Forest Research 14:67–72.

Schimpf DJ, MacMahon JA (1985) Insect communities and faunas of a Rocky Mountain subalpine sere. The Great Basin Naturalist 45:37–60.

Schmiegelow FKA, Stepnisky DP, Stambaugh CA, Koivula M (2006) Reconciling salvage logging of boreal forests with a natural-disturbance management model. Conservation Biology 20:971–983.

Schott KM, Karst J, Landhäusser SM (2014) The role of microsite conditions in restoring trembling aspen (*Populus tremuloides* Michx) from seed. Restoration Ecology 22:292–295.

Schott KM, Pinno BD, Landhäusser SM (2013) Premature shoot growth termination allows nutrient loading of seedlings with an indeterminate growth strategy. New Forests 44:635–647.

Schott KM, Snively AEK, Landhäusser SM, Pinno BD (2016) Nutrient loaded seedlings reduce the need for field fertilization and vegetation management on boreal forest reclamation sites. New Forests 47:393–410.

Setterfield SA, Douglas MM, Hutley LB, Welch MA (2005) Effects of canopy cover and ground disturbance on establishment of an invasive grass in an Australia Savanna. Biotropica 37:25–31.

Slavich P, Petterson G (1993) Estimating the electrical conductivity of saturated paste extracts from 1:5 soil, water suspensions and texture. Australian Journal of Soil Research 31:73.

Sorenson PT, Quideau SA, MacKenzie MD, et al (2011) Forest floor development and biochemical properties in reconstructed boreal forest soils. Applied Soil Ecology 49:139–147.

South DB, Harris SW, Barnett JP, et al (2005) Effect of container type and seedling size on survival and early height growth of *Pinus palustris* seedlings in Alabama, U.S.A. Forest Ecology and Management 204:385–398.

South DB, Mitchell RG (2006) A root-bound index for evaluating planting stock quality of container-grown pines. Southern African Forestry Journal 5925:47–54.

Stoeckeler JH, Slabaugh PE (1965) Conifer nursery practice in the prairie-plains. Washington, D.C.

Sutton RF (1993) Mounding site preparation: A review of European and North American experience. New Forests 7:151–192.

Sutton RF (1979) Planting stock quality and grading. Forest Ecology and Management 2:123–132.

Sutton RF, Tinus RW (1983) Root and root system terminology. Forest Science Monograph 24:1–137.

Therneau T, Atkinson B, Ripley B (2015) rpart: Recursive Partitioning and Regression Trees. In: The R Project for Statistical Computing. https://cran.r-project.org/web/packages/rpart/rpart.pdf. Accessed 1 Jan 2017

Thompson JR, Schultz RC (1995) Root system morphology of Quercus rubra L. planting stock and 3-year field performance in Iowa. New Forests 9:225–236.

Tilman D (1988) Plant strategies and the dynamics and structure of plant communities, 26th edn. Princeton University Press, Princeton, NJ

Timmer VR, Aidelbaum AS (1996) Manual for exponential nutrient loading of seedlings to improve outplanting performance on competitive forest sites. Suult Stc. Marie, ON

Turner MG, Romme WH, Reed RA, Tuskan GA (2003) Post-fire aspen seedling recruitment across the Yellowstone (USA) Landscape. Landscape Ecology 18:127–140.

Tuttle CL, South DB, Golden MS, Meldahl RS (1988) Initial *Pinus taeda* seedling height relationships with early survival and growth. Canadian Journal of Forest Research 18:867–871.

van den Driessche R, Rude W, Martens LA (2003) Effect of fertilization and irrigation on growth of aspen (*Populus tremuloides* Michx.) seedlings over three seasons. Forest Ecology and Management 186:381–389.

von der Gonna M, Lavender DP (1988) Root egress of white spruce (*Picea glauca*) seedlings after outplanting as affected by patch and mound site preparation. In: Scrivener BA, MacKinnon JA (eds) Learning From the Past, Looking to the Future. Proceedings from the Northern Sliviculture Committee's 1988 Winter Workshop. Northern Silviculture Committee, Prince George, B.C., pp 76–80

Wagner RG, Mohammed GH, Noland TL (1999) Critical period of interspecific competition for northern conifers associated with herbaceous vegetation. Canadian Journal of Forest Research 29:890–897.

Wakeley PC (1948) Physiological grades of southern pine nursery stock. In: Proceedings of the Society of American Foresters. Society of American Foresters, Boston, MA, pp 311–322

Wang Y, Hogg EH, Price DT, et al (2014) Past and projected future changes in moisture conditions in the Canadian boreal forest. Forestry Chronicle 90:678–691.

Wilson BC, Jacobs DF (2006) Quality assessment of temperate zone deciduous hardwood seedlings. New Forests 31:417–433.

Wilson SD (1998) Competition between grasses and woody plants. In: Cheplick GP (ed) Population biology of grasses. Cambridge University Press, Cambridge, UK, pp 231–254

Winterhalder K, Innes JL, Oleksyn J (2000) Landscape degradation by smelter emissions near

Sudbury, Canada, and subsequent amelioration and restoration. In: Innes JL, Oleksyn J (eds) Forest dynamics in heavily polluted regions, Report No. CABI Publishing, Vienna, AT, pp 87–119

Zuur AF, Ieno EN, Walker N, et al (2009) Mixed effects models and extensions in ecology with R. Springer New York, New York, NY

Appendices

Appendix 1: Monthly (May to August) precipitation and mean air temperature for 2014, 2015, and long term average (LTA) at CDC (ACIS, 2016).

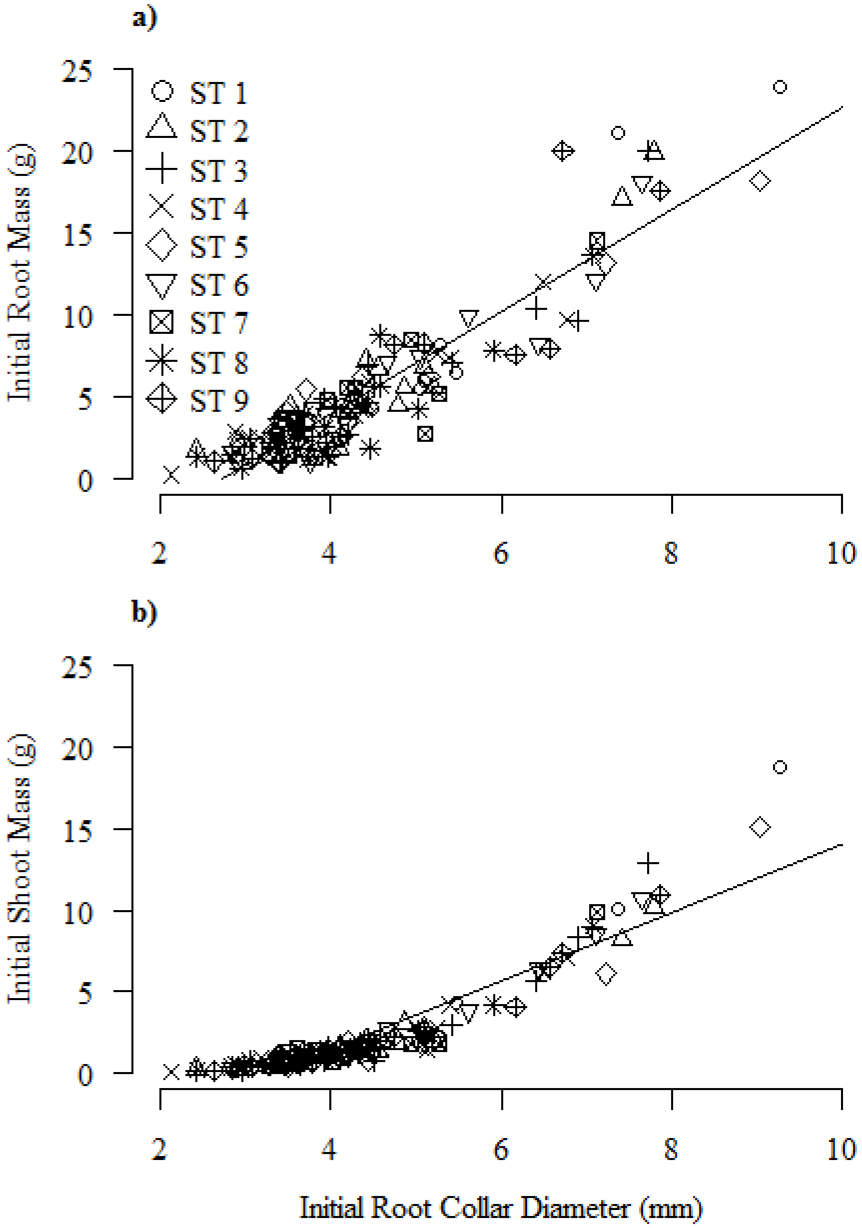
Month	**Precipitation (mm)**				**Mean Air Temperature (°C)**			
	2014	**2015**	**2016**	**LTA**	**2014**	**2015**	**2016**	**LTA**
May	48.3	22.1	81.7	43.2	9.20	10.7	11.6	10.8
June	45.5	53.3	68.8	77.7	14.1	15.8	15.9	14.8
July	120	71.9	84.3	89.0	18.3	17.9	17.2	16.9
August	9.70	27.1	79.3	58.7	16.6	15.9	15.9	15.9

Appendix 2: Mean percent cover, height, and dry mass of plant species found in treatment plots at CDC. Values in brackets represent one standard deviation of the mean (n=10).

Site	**Species**	**Percent cover**	**Height (cm)**	**Mass (g)**
Mulch	*Agropyron* sp.	6.00 (5.21)	11.4 (4.74)	6.56 (5.70)
	Capsella bursa-pastoris (L.) Medik.	1.00 (0.00)	22.3 (21.3)	0.72 (1.00)
	Chenopodium album L.	1.25 (0.50)	15.6 (8.24)	0.65 (0.95)
	Cirsium arvense (L.) Scop.	4.00 (2.65)	12.5 (6.02)	2.88 (2.25)
	Erodium cicutarium (L.) L'Hér. ex Aiton	5.67 (8.08)	8.72 (4.63)	1.89 (3.02)
	Polygonum convolvulus (L.) Á.Löve	2.60 (2.19)	17.7 (7.32)	0.90 (0.84)
	Potentilla norvegica L.	4.50 (4.95)	16.5 (3.54)	2.84 (3.08)
	Senecio sp.	1.67 (0.58)	2.58 (1.23)	0.58 (0.38)
	Taraxacum laevigatum DC.	1.00 (0.00)	7.00 (0.00)	0.10 (0.00)
	Taraxacum officinale F.H. Wigg	7.00 (7.00)	19.8 (4.37)	3.66 (1.52)
	Thlaspi arvense L.	3.22 (3.03)	18.2 (8.93)	3.49 (3.24)
Plow	*Cirsium arvense* (L.) Scop.	12.4 (8.37)	30.7 (8.66)	10.7 (8.09)
	Fragaria vesca L.	1.00 (0.00)	5.00 (0.00)	0.11 (0.00)
	Medicago sativa L.	5.40 (3.21)	29.2 (6.27)	3.73 (2.47)
	Poaceae sp.*	18.1 (10.3)	60.0 (9.61)	22.6 (11.4)
	Taraxacum laevigatum DC.	1.00 (0.00)	10.5 (0.00)	0.27 (0.00)
	Taraxacum officinale F.H. Wigg	2.11 (1.45)	15.3 (6.32)	0.56 (0.55)
	Trifolium hybridum L.	1.00 (0.00)	10.0 (0.00)	0.04 (0.00)
Grass	*Cirsium arvense* (L.) Scop.	3.00 (1.73)	9.33 (6.66)	1.54 (1.52)
	Medicago sativa L.	6.14 (3.76)	35.7 (11.4)	7.25 (5.85)
	Poaceae sp.*	15.2 (7.39)	54.3 (14.4)	18.8 (6.86)
	Potentilla anserina (L.) Rydb.	1.50 (0.71)	13.6 (4.60)	0.53 (0.21)
	Taraxacum laevigatum DC.	1.75 (0.50)	13.9 (6.20)	0.44 (0.45)
	Taraxacum officinale F.H. Wigg	4.29 (2.63)	15.6 (5.76)	2.01 (1.62)

*Grasses present in the plowed and grass treatment include: *Alopecurus aequalis* Sobol., *Bromus inermis* Leyss., *Phalaris arundinacea* L., and *Poa pratensis* L.

Appendix 3: Relationship between initial root collar diameter and initial a) root ($R^2 = 0.91$) and b) shoot mass ($R^2 = 0.97$). Dots coded according to stock types shown in legend. All stock types analyzed together (n=140).



Appendix 4: Slope estimates for predictive equations used to estimate root and shoot mass.

Root mass estimates for each stock type were based on the following equation:
$M_{root} = (2.26+A_n) + (0.94+B_n)*RCD$

Where:
M_{root} = root mass
A_n = Change to intercept for stock type n
B_n = Change to interaction term for stock type n
RCD = root collar diameter

The following coefficients for each stock type are as follows:
Stock type 1: $A_n = 0$ $B_n = 0$
Stock type 2: $A_n = -2.14$ $B_n = -0.07$
Stock type 3: $A_n = -6.01$ $B_n = 1.05$
Stock type 4: $A_n = -5.93$ $B_n = 0.89$
Stock type 5: $A_n = -9.64$ $B_n = 2.32$
Stock type 6: $A_n = -17.92$ $B_n = 3.07$
Stock type 7: $A_n = -12.58$ $B_n = 1.12$
Stock type 8: $A_n = -7.00$ $B_n = 1.12$
Stock type 9: $A_n = -3.35$ $B_n = -0.26$

Shoot mass estimates for each stock type were based on the following equation:
$M_{shoot} = (-3.23+A_n) + (1.07+B_n)*RCD$

Where:
M_{shoot} = shoot mass
A_n = Change to intercept for stock type n
B_n = Change to interaction term for stock type n
RCD = root collar diameter

The following coefficients for each stock type are as follows:
Stock type 1: $A_n = 0$ $B_n = 0$
Stock type 2: $A_n = 3.07$ $B_n = -0.82$
Stock type 3: $A_n = 0.75$ $B_n = -0.14$
Stock type 4: $A_n = 2.44$ $B_n = -0.67$
Stock type 5: $A_n = -12.24$ $B_n = 2.42$
Stock type 6: $A_n = -6.28$ $B_n = 1.44$
Stock type 7: $A_n = -3.02$ $B_n = 0.79$
Stock type 8: $A_n = 1.75$ $B_n = -0.44$
Stock type 9: $A_n = 1.99$ $B_n = -0.41$

*Root and shoot mass estimates that resulted in negative values or a value that was more than 3 standard deviations away from the mean were omitted.

Appendix 5: Average nutrient concentration from aspen leaves collected at CDC on July 7, 2015 (2-year-old seedlings). Different letters indicate significantly different means between the mulch, plowed, and grass treatment (Benjamini–Hochberg correction, $\alpha = 0.05$). Values in brackets represent one standard deviation of the mean (n=10). Leaf nutrients marked with an asterisk (*) are presented in $\mu g\ L^{-1}$.

Leaf nutrients ($mg\ L^{-1}$)	**Mulch**	**Plow**	**Grass**
Ca^{2+}	12.7 (2.53) **a**	11.4 (2.10) **b**	11.6 (2.64) **ab**
Cu^{2+} *	8.67 (5.22) **a**	6.51 (4.71) **b**	2.42 (2.25) **c**
Fe^{2+}	0.67 (2.18) **a**	0.07 (0.02) **b**	0.08 (0.03) **b**
K^{+}	10.7 (1.93) **a**	9.27 (1.60) **b**	7.85 (2.04) **c**
Mg^{2+}	2.18 (0.42) **c**	2.98 (0.76) **b**	3.94 (1.15) **a**
Mn^{2+} *	68.0 (23.6) **a**	65.8 (24.5) **ab**	58.0 (23.1) **b**
Na^{+} *	12.3 (7.02)	15.3 (11.4)	13.7 (11.6)
P	2.28 (0.45) **a**	1.69 (0.38) **b**	1.47 (0.35) **c**
S	2.36 (0.40) **a**	1.68 (0.36) **b**	1.94 (0.71) **b**
Zn^{2+}	0.15 (0.04) **a**	0.05 (0.02) **b**	0.03 (0.03) **c**

Appendix 6: ANOVA results for initial morphological characteristics in response to nursery treatments. The level of significance used is α = 0.05.

Morphological characteristic	Effect	Df	F	P
Height	Nursery	1	251.41	**< 0.001**
	Sow date	3	72.67	**< 0.001**
	Container	1	67.82	**< 0.001**
	Nursery*sow date	2	30.79	**< 0.001**
	Nursery*container	1	2.82	0.10
Root collar diameter	Nursery	1	152.45	**< 0.001**
	Sow date	3	40.12	**< 0.001**
	Container	1	73.53	**< 0.001**
	Nursery*sow date	2	9.20	**< 0.001**
	Nursery*container	1	0.56	0.46
Root mass	Nursery	1	53.44	**< 0.001**
	Sowdate	3	15.54	**< 0.001**
	Container	1	99.61	**< 0.001**
	Nursery*sow date	2	4.64	**0.01**
	Nursery*container	1	17.87	**< 0.001**
Root: shoot	Nursery	1	88.83	**< 0.001**
	Sow date	3	42.08	**< 0.001**
	Container	1	2.07	0.15
	Nursery*sow date	2	4.18	**0.02**
	Nursery*container	1	4.16	**0.04**
Root NSC	Nursery	1	0.77	0.38
	Sow date	3	12.29	**< 0.001**
	Container	1	1.91	0.17
	Nursery*sow date	2	1.37	0.26
	Nursery*container	1	0.75	0.39
Stem NSC	Nursery	1	17.68	**< 0.001**
	Sow date	3	26.58	**< 0.001**
	Container	1	2.70	0.10
	Nursery*sow date	2	1.08	0.34
	Nursery*container	1	0.78	0.38
Leaf N	Nursery	1	0.72	0.40
	Sow date	3	42.13	**< 0.001**
	Container	1	0.05	0.82
	Nursery*sow date	2	21.43	**< 0.001**
	Nursery*container	1	0.08	0.78
Leaf P	Nursery	1	2.24	0.14
	Sow date	3	2.48	0.09
	Container	1	0.05	0.82
	Nursery*sow date	2	11.32	**< 0.001**
	Nursery*container	1	0.20	0.66
Leaf K	Nursery	1	32.02	**< 0.001**
	Sow date	3	0.58	0.57
	Container	1	0.00	0.98
	Nursery*sow date	2	7.73	**0.001**
	Nursery*container	1	2.65	0.11

Appendix 7: Three-way analysis of variance (ANOVA) and mean height growth (2014-2016), root growth, and root egress for each nursery characteristic (nursery location, container size, seedling age) in each site treatment. Italicized numbers represent p-values from the three-way ANOVA (bolded numbers significant at α = 0.05). Numbers in brackets indicate one standard deviation of the mean (n=83). Different letters indicate differences among nursery characteristics within each site (HSD test).

a) Mulch	Height growth 2014	Height growth 2015	Height growth 2016	Root growth	Root egress
Nursery	*0.98*	*0.89*	*0.62*	***0.01***	*0.48*
UA	29.2 (14.8)	56.6 (18.6)	79.6 (25.4)	18.3 (8.96) a	27.6 (12.3)
WM	41.2 (12.5)	57.9 (15.7)	81.2 (23.0)	8.96 (6.07) b	30.3 (8.87)
Container size (ml)	***<0.001***	*0.42*	*0.96*	***<0.001***	*0.05*
220	29.2 (11.5) b	51.9 (16.5)	84.5 (21.9)	5.90 (3.86) c	34.0 (7.67)
340	39.1 (13.7) a	58.8 (16.4)	81.3 (24.0)	11.5 (6.35) b	30.5 (10.4)
700	29.7 (16.8) b	55.9 (19.1)	76.3 (25.5)	21.6 (10.9) a	22.4 (9.58)
Seedling age (months)	***<0.001***	*0.45*	*0.43*	***0.04***	***<0.001***
5	45.5 (9.39) ab	58.7 (16.6)	91.8 (29.0)	4.86 (3.77) c	29.2 (13.4) abc
5.5	37.8 (12.8) bc	58.5 (19.3)	85.3 (21.5)	8.88 (5.19) bc	36.6 (8.71) a
6	33.9 (9.47) c	58.4 (15.1)	79.7 (25.0)	12.1 (5.24) b	27.8 (8.38) bc
6.5	48.8 (10.2) a	60.0 (15.9)	76.5 (24.1)	12.5 (6.92) b	30.8 (8.07) ab
7	18.2 (8.24) d	51.9 (18.1)	75.1 (22.0)	22.9 (9.85) a	20.9 (9.59) c

b) Plow	Height growth 2014	Height growth 2015	Height growth 2016	Root growth	Root egress
Nursery	***<0.001***	***0.04***	***0.03***	*0.13*	***0.02***
UA	9.78 (5.27) b	7.81 (4.14) b	6.77 (6.39) b	6.59 (6.67)	4.19 (4.04) b
WM	18.4 (7.95) a	11.0 (5.64) a	10.5 (7.09) a	3.10 (2.20)	7.97 (7.41) a
Container size (ml)	***<0.001***	*0.22*	*0.36*	***0.02***	*0.22*
220	11.6 (7.69) b	5.65 (3.29)	6.48 (5.23)	1.79 (1.43) b	6.34 (6.61)
340	16.0 (8.54) a	9.80 (4.90)	9.34 (7.29)	4.09 (3.59) b	6.85 (6.89)
700	11.7 (5.68) b	11.0 (6.29)	8.56 (6.95)	8.09 (7.88) a	4.61 (4.44)
Seedling age (months)	***0.002***	*0.87*	*0.71*	*0.27*	*0.52*
5	21.4 (5.89) a	12.5 (4.01)	11.6 (9.19)	1.37 (1.50)	9.33 (8.71)
5.5	13.7 (7.48) b	5.99 (2.91)	5.97 (4.31)	2.48 (1.93)	6.46 (5.39)
6	12.4 (6.89) bc	9.62 (4.77)	10.3 (7.15)	4.85 (3.48)	7.42 (8.65)
6.5	20.9 (7.86) a	12.6 (6.33)	10.5 (5.42)	3.98 (2.44)	6.74 (4.76)
7	7.91 (2.37) c	8.75 (4.71)	7.36 (8.43)	8.52 (8.50)	2.88 (3.27)

c) Grass	Height growth 2014	Height growth 2015	Height growth 2016	Root growth	Root egress
Nursery	*0.91*	*0.43*	*0.63*	***0.04***	*0.35*
UA	12.6 (4.69)	6.56 (3.37)	4.49 (3.80)	8.72 (6.64) a	3.67 (4.23)
WM	14.0 (5.33)	8.75 (4.89)	7.02 (5.50)	5.20 (4.55) b	4.90 (5.37)
Container size (ml)	*0.15*	***0.04***	*0.45*	***<0.001***	*0.10*
220	7.83 (2.62)	5.63 (1.82) b	5.68 (3.36)	1.55 (0.88) c	1.57 (3.93)
340	14.0 (4.97)	7.28 (4.01) b	5.74 (5.30)	5.52 (4.19) b	5.48 (5.32)
700	14.3 (4.73)	10.3 (5.40) a	6.33 (4.59)	12.4 (6.47) a	2.36 (2.35)
Seedling age (months)	*0.10*	*0.86*	***0.02***	***<0.001***	***0.006***
5	14.1 (5.10)	9.53 (3.75)	4.86 (4.13) b	3.39 (2.38) c	10.4 (6.82) a
5.5	10.7 (5.15)	5.48 (2.51)	4.69 (2.87) b	2.80 (2.80) bc	1.95 (3.50) b
6	13.5 (3.16)	7.32 (3.73)	5.33 (4.87) b	4.55 (3.60) bc	4.77 (4.33) b
6.5	17.2 (4.58)	10.3 (6.33)	9.69 (6.61) a	6.80 (5.56) b	4.34 (3.63) b
7	11.7 (4.94)	7.15 (3.06)	4.17 (2.96) b	12.6 (5.70) a	3.33 (4.50) b

Appendix 8: Mean percent cover, height, and dry mass of plant species found in Peace River sites. Values in brackets represent one standard deviation of the mean (n=10).

Site	**Species**	**Percent cover**	**Height (cm)**	**Mass (g)**
Ag-Field	*Chenopodium album* L.	3.00 (1.41)	28.5 (7.78)	1.97 (1.28)
	Cirsium arvense (L.) Scop.	1.50 (0.71)	4.00 (2.83)	0.24 (0.21)
	Dracocephalum parviflorum Nutt.	10.0 (0.00)	31.0 (0.00)	5.22 (0.00)
	Elymus glaucus Buckley	8.00 (4.24)	58.5 (29.0)	9.31 (3.45)
	Festuca ovina L.	6.00 (0.00)	7.00 (0.00)	5.72 (0.00)
	Festuca saximontana Rydb.	8.40 (7.09)	11.8 (7.98)	7.80 (8.07)
	Lappula echinata Gilib.	8.66 (10.0)	35.3 (22.0)	5.15 (6.98)
	Senecio vulgaris L.	5.00 (0.00)	27.0 (0.00)	2.37 (0.00)
	Sonchus oleraceus L.	1.00 (0.00)	33.0 (0.00)	0.21 (0.00)
	Taraxacum officinale F.H. Wigg	8.14 (6.01)	5.85 (3.63)	1.47 (1.32)
	Thlaspi arvense L.	2.33 (1.53)	13.0 (12.5)	0.91 (1.25)
Well site	*Agropyron trachycaulum* (Link) Gould ex Shinners	4.80 (2.97)	92.5 (16.5)	16.6 (9.72)
	Bromus inermis Leyss.	3.71 (3.09)	87.7 (7.80)	10.1 (8.44)
	Equisetum arvense L.	15.9 (11.1)	16.0 (3.87)	4.14 (4.00)
	Melilotus alba Medik.	2.66 (0.58)	73.3 (13.5)	5.07 (3.25)
	Melilotus officinalis (L.) Pall.	16.8 (15.5)	95.8 (61.1)	26.3 (23.3)
	Phleum pratense L.	2.00 (0.00)	72.5 (0.71)	3.14 (1.75)
	Plantago major L.	14.5 (3.54)	36.0 (0.00)	4.13 (0.52)
	Poa pratensis L.	1.60 (0.89)	51.0 (13.6)	1.79 (2.23)
	Rubus idaeus L.	3.00 (0.00)	6.00 (0.00)	0.05 (0.00)
	Sonchus arvensis L.	6.66 (2.89)	16.3 (11.5)	1.79 (1.84)
	Taraxacum officinale F.H. Wigg	2.00 (0.00)	10.0 (0.00)	0.10 (0.00)
	Trifolium hybridum L.	14.6 (8.02)	27.3 (17.0)	7.30 (5.75)
	Trifolium repens L.	2.00 (0.00)	7.00 (0.00)	0.06 (0.00)
Gravel Pit	*Achillea millefolium* L.	4.66 (6.35)	6.33 (2.31)	0.87 (1.33)
	Agrostis scabra Willd.	2.33 (0.58)	32.7 (8.74)	0.80 (0.09)
	Bromus ciliatus L.	9.90 (7.87)	29.7 (9.29)	5.12 (5.97)
	Carex aenea Fern.	4.00 (1.41)	27.0 (1.41)	3.70 (1.51)
	Cirsium arvense (L.) Scop.	1.33 (0.58)	5.66 (1.53)	0.19 (0.10)
	Equisetum arvense L.	3.50 (1.97)	5.00 (1.41)	0.30 (0.14)
	Equisetum sp.	1.00 (0.00)	3.00 (0.00)	0.10 (0.00)
	Fragaria virginiana Duchesne	14.3 (9.49)	5.80 (1.87)	2.67 (1.45)
	Melilotus officinalis (L.) Pall.	14.9 (13.3)	10.0 (10.1)	3.81 (4.60)
	Plantago major L.	2.00 (0.00)	6.25 (3.18)	0.17 (0.11)
	Rubus idaeus L.	10.0 (0.00)	7.00 (0.00)	1.92 (0.00)
	Salix sp.	1.50 (0.71)	10.5 (7.78)	0.37 (0.10)
	Sonchus arvensis L.	6.00 (0.00)	10.0 (0.00)	0.80 (0.00)
	Taraxacum officinale F.H. Wigg	6.11 (3.02)	8.00 (3.35)	1.31 (1.38)

Appendix 9: Fisher's LSD test for multiple comparisons of performance measures between stock groups within each site near Peace River. Values in bold indicate significant differences between two stock groups at α < 0.05.

	Height growth 2014	Height growth 2015	Root growth	Egress root mass	Egress RSR	RG/P
Ag-field						
S-High : M-High	0.62	0.46	0.87	0.88	0.37	0.62
S-High : M-Low	**0.006**	0.49	0.05	**0.03**	**0.01**	0.13
S-High : T-High	0.48	0.54	**0.003**	0.32	0.10	0.57
S-High : T-Low	**< 0.001**	**0.03**	**0.002**	**0.03**	0.14	0.13
M-High : M-Low	**< 0.001**	0.97	**0.04**	**0.04**	**0.03**	0.17
M-High : T-High	0.12	0.90	0.05	0.25	0.28	0.90
M-High : T-Low	**< 0.001**	0.15	**0.004**	**0.02**	0.43	0.16
M-Low : T-High	**0.01**	0.93	**< 0.001**	**0.002**	0.23	0.22
M-Low : T-Low	0.38	0.14	**< 0.001**	**< 0.001**	0.06	0.88
T-High : T-Low	**< 0.001**	0.12	0.90	0.21	0.63	0.22
Well site						
S-High : M-High	0.15	0.26	0.89	0.63	**0.01**	**0.04**
S-High : M-Low	**< 0.001**	**0.02**	**0.01**	0.07	**< 0.001**	**< 0.001**
S-High : T-High	0.47	**< 0.001**	**< 0.001**	**0.006**	**0.001**	0.29
S-High : T-Low	**< 0.001**	**0.04**	**< 0.001**	**< 0.001**	**< 0.001**	**0.01**
M-High : M-Low	**< 0.001**	**< 0.001**	**0.02**	**0.02**	**< 0.001**	**< 0.001**
M-High : T-High	0.43	**0.003**	**< 0.001**	**0.02**	0.22	0.24
M-High : T-Low	**< 0.001**	0.37	**< 0.001**	**0.002**	**0.04**	0.53
M-Low : T-High	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
M-Low : T-Low	0.51	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**
T-High : T-Low	**< 0.001**	**0.04**	0.80	0.41	0.49	0.06
Gravel pit						
S-High : M-High	0.09	0.23	0.35	0.68	0.23	0.76
S-High : M-Low	0.76	0.45	0.66	0.26	**0.04**	0.13
S-High : T-High	0.09	0.12	**< 0.001**	0.34	**0.04**	0.27
S-High : T-Low	0.77	0.21	0.27	**0.02**	0.07	0.08
M-High : M-Low	**0.02**	**0.04**	0.16	0.11	0.25	**0.01**
M-High : T-High	0.85	0.67	**< 0.001**	0.58	0.26	0.26
M-High : T-Low	**< 0.001**	0.96	0.86	**0.04**	0.37	**0.002**
M-Low : T-High	**0.04**	**0.02**	**< 0.001**	**0.03**	0.97	**< 0.001**
M-Low : T-Low	0.21	**0.03**	0.11	**< 0.001**	0.64	0.86
T-High : T-Low	**0.001**	0.71	**0.001**	0.14	0.66	**< 0.001**