Roots in reconstructed soils – how land reclamation practices affect the development of tree root systems

by

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Abstract

Tree root systems are incredibly complex organs that fulfill several vital functions, the main ones being anchorage and uptake of water and mineral elements. They perform these functions in a highly complex and challenging soil environment with heterogeneously distributed soil resources, physical barriers, and often intense competition with neighbouring plants. In adaptation to these challenges, roots systems do not have a genetically pre-determined shape. Instead, root system architecture is, to a large extent, the result of an integrated response to positive and negative environmental cues perceived by the growing root tips. Favorable conditions, such as high resource availability, good aeration and low penetration resistance promote root system development, whereas unfavorable conditions, such as low resource availability, oxygen deprivation and high penetration resistance can restrict it. Competing roots can also restrict root growth in one of two ways, either directly by acting as physical barriers, or indirectly, through local depletion of soil resources. This has some important implications for growing trees, because the resulting spatial arrangement, or architecture, of individual root systems directly determines the soil volume from which they can draw the required resources.

In my dissertation, I used a combination of established and novel techniques to study how changing abiotic conditions and belowground interactions with neighbouring plants act together to shape the root systems and thus determine the rooting space available to planted tree seedlings during the initial stage of seedling establishment in a variety of reconstructed soils. The results revealed that in fertile reconstructed soils, belowground competition with grasses severely restricted root system development for planted aspen seedlings. Moreover, root competition had a much stronger impact on available rooting space than soil compaction, as the competing grass roots were able to rapidly colonize the available rooting space while simultaneously lowering the availability of water and nitrogen. At a second site, with different soils and climatic conditions I was able to show that planting mixtures of functionally diverse tree species resulted in increased belowground productivity or ‘overyielding’ due to niche complementarity, but only on one of two studied soil types, where abiotic conditions did not interfere with seedling phenology.

Overall, these findings highlight the need to consider both rooting space requirements associated with different species and site conditions and potential restraints on this shared resource a key factor in the design of reconstructed soils capable of supporting the successful re-establishment of self-sustaining forests after severe anthropogenic disturbances.

Preface

Chapter 2 of this thesis has been published as 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(7): 685-695. doi: 10.1139/cjb-2016-0301. I was responsible for setting up the field sites, data collection and analysis as well as the drafting of the manuscript. S. Landhäusser, B. Pinno and M. Dyck were involved in developing the idea for the experiment and in the editing of the mauscript.

Chapter 3 of this thesis has been submitted for publication as Bockstette SW, Pinno BD, Landhäusser SM: Responses of planted *P. tremuloides* seedlings to grass competition during early establishment. I was responsible for setting up the field sites, data collection and analysis as well as the drafting of the manuscript. S. Landhäusser and B. Pinno were involved in developing the idea for the experiment and in the editing of the mauscript.

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CHAPTER 1

General Introduction

1.1 Background

Forests make up roughly one third of the terrestrial biosphere and play a crucial role in regulating global climate and element cycles, for example by acting as sinks and sources for atmospheric CO_2 (Dixon et al. 1994; Schimel 1995; Magnani et al. 2007). They are also some of the most biologically diverse terrestrial ecosystems and provide a range of critical ecosystem services such as storage and filtration of water and regulation of water flows, soil formation and stabilization, nutrient retention and cycling as well timber and non-timber forest products (Hooper et al. 2005; Mori et al. 2016). Increasing pressure from a growing human population has resulted in large losses of old-growth forest cover worldwide due to changes in land use, such as conversion to agriculture, or timber and non-timber resource extraction (FAO 2010). In recent years the global rate of forest cover loss has decreased, because deforestation has increasingly been offset by afforestation and reforestation of degraded or marginal land in many parts of the world (Chazdon 2008a; Sloan and Sayer 2015; Köhl et al. 2015). While not as diverse as primary old-growth forests, these secondary forests are playing an increasingly important role in providing critical ecosystem services (Richter et al. 1999; Chazdon 2008b; Rist and Moen 2013). However, depending on the previous land use and the severity of the associated human impact on soils and vegetation, afforestation and reforestation with planted tree seedlings can be challenging as site conditions may have become unfavorable to tree establishment (Burton and Macdonald 2011; Macdonald et al. 2015).

One of the most severe forms of anthropogenic disturbance and a major contributor to degradation and loss of forest cover worldwide is the extraction of mineral resources, through surface mining. The mining process requires the complete removal of all existing vegetation and underlying soils, which are typically salvaged and later used as a growing medium for forest land reclamation (Burton and Macdonald 2011; Zipper et al. 2013; Macdonald et al. 2015). One of the major challenges for successful forest land reclamation after surface mining is the reconstruction of a suitable rooting medium, capable of supporting permanent closed forest cover (Burger et al. 2005; Zipper et al. 2011a, 2013; Macdonald et al. 2015).

Soil reconstruction is the single most important step in the forest reclamation process. It will determine the growing conditions for planted tree seedlings and thus determine the trajectory of forest development for decades. The goal for successful soil reconstruction therefore must be to create a substrate that can store and supply sufficient moisture and nutrients to supply mature forest. Being sessile organisms, trees cannot relocate in search for soil resources, but are restricted to foraging in the limited space accessible to them via their roots (McConnaughay and Bazzaz 1992; Mcconnaughay et al. 1996; Casper et al. 2003). Soil resources are, however, typically heterogeneously distributed, display different degrees of mobility in the soil and their availability often varies drastically in space and time, based on soil materials and other site characteristics (Hodge 2004; Lambers et al. 2008; Croft et al. 2012). The volume of soil that can be freely colonized by an individual tree's roots therefore directly determines the total pool of soil resources to which it has access (Czaran and Bartha 1992; Casper et al. 2003). The main challenges in reconstructing soils that provide sufficient rooting space to growing trees are: (1) the limited availability of high quality substrates for revegetation, (2) the severely altered topography, hydrology and microclimates of post-mining landscapes, (3) the presence of physical, chemical or biological barriers to root growth within the reconstructed soil profile and (4) our limited understanding of belowground processes (i.e. plant-soil and plant-plant interactions) in general, and in human-modified soils in particular.

Increasing our understanding of these important belowground interactions, particularly as they pertain to reconstructed soils, forms the basis for this dissertation.

1.2 The functions of tree root systems and the importance of rooting space

One of the primary functions of root-systems and a key factor in determining overall root system architecture is anchorage and structural support (Fitter 2002). Given their height, trees are particularly prone to windthrow and hence develop the largest root systems of all terrestrial plants with significant investments in structural elements in the form of thick, woody roots (Ennos 2000). Acting as 'beams', these structural roots provide resistance against bending, while finer roots act more like 'cables' due to their tensile strength (Fournier et al. 2006). These fine roots can easily bend but are able to resist considerable amounts of longitudinal forces due to friction at the soil-root interface, an effect which is further increased through mucilage and other excretions that enhance soil contact and adhesion (Ennos 2000; Stokes 2002). Any restriction in rooting space, e.g. due to physical, chemical or biological barriers within the soil could have a negative impact on this primary function of the root system and result in an increased risk of toppling or windthrow.

The second primary function of roots is, of course, the acquisition and uptake of water and mineral nutrients from the soil (Fitter 2002). Being faced with the principal problem of losing water to a dry atmosphere in return for the ability to photosynthesize and fix the carbon required for maintenance respiration and growth, constant water uptake from the soil is critically important for all terrestrial plants. Moreover, the water costs associated with carbon fixation are high. Terrestrial plants typically have to transpire 200-400 molecules of water to fix one molecule of CO_2 (Raven 1984; Stiller et al. 2002). This imbalance underpins the need to maintain large root structures that act largely as water conduits and offers a justification for the high investment costs therein (Stiller et al. 2002). Given the high resource demand of mature trees and the fact that both water and nutrients are spatially and temporally heterogeneously distributed within the soil, there is also a clear need to maintain a large root system, as the size and distribution ultimately define the soil volume in which these resource can be accessed (Stiller et al. 2002; Comas et al. 2013). Barriers that restrict root growth and thus limit the accessible rooting space will therefore directly impact root foraging, resulting in stunted growth, increased susceptibility to drought stress and nutrient deficiencies.

Of course, not all roots play the same role in nutrient and water uptake (Waisel and Eshel 2002). Instead, there is a functional shift from absorption to transport that coincides with an increase in root order. Most water is taken up by unsuberized fine roots. For example, in citrus root systems with a total of eight root orders, first order roots (tips) accounted for 50% and the first three orders combined for over 90% of the total root surface area of the entire root system (Rewald et al. 2011). Large xylem vessels contained in higher-order structural roots act as conduits for rapid water transport from the soil to the leaves (Stiller et al. 2002). Structural roots also play a critical role in the storage and remobilization of metabolically important compounds, such as carbon (Chapin et al. 1990; Loescher et al. 1990; Magel et al. 2000) and nitrogen (Millard and Grelet 2010; Rennenberg and Dannenmann 2015). Although important, transport and storage are considered secondary root functions and as such have no direct impact on root system architecture and no direct correlation with rooting space.

1.3 Root system architecture and developmental plasticity

Root system architecture, i.e. the spatial arrangement of the entire root system, plays a critical role in acquiring growth-limiting resources, such as water and nutrients, as these are typically both spatially and temporally heterogeneously distributed within the soil (Casper et al. 2003). Root system architecture is always the result of an interaction between genetic pre-determination and the various environmental conditions that either stimulated or suppressed the initiation, growth or death of individual roots (Nagel et al. 2009; Hodge et al. 2009; Ingram and Malamy 2010). The trait that allows plants to adapt their root architecture to changing environmental conditions is called developmental plasticity (Malamy 2005; McCleery et al. 2017; Morris et al. 2017).

In plants, the concept of developmental plasticity refers to the fact that embryogenesis does not result in a smaller, but otherwise identical version of the adult plant, but rather in a platform on which all post-embryonic organ development takes place (Malamy 2005; Ingram and Malamy 2010). The blueprint for this platform is genetically pre-determined for each species and genotype, while the actual shape and structure, or phenotype, of an individual is largely a reflection of the environmental conditions it was exposed to during its development (Johannsen 1911). In contrast to animals, post-embryonic organ development in plants is driven by the presence of undifferentiated stem cells in apical meristems. These stem cells differentiate into specialized cells based on positional information rather than cell lineage, allowing them to optimize their morphology by integrating information about encountered environmental conditions into localized growth responses (Malamy 2005; Singh and Bhalla 2006; Hodge et al. 2009). This ability to adjust root system architecture and thus cope with changing soil conditions is clearly an adaptation to a sessile lifestyle, in which the ability to successfully forage and compete for limited resources depends on optimal placement of the respective organs (Ingram and Malamy 2010).

Plants have the ability to optimize root placement through directed root growth driven by external cues perceived by the growing root tip (López-Bucio et al. 2003; Eapen et al. 2005; Monshausen and Gilroy 2009; Roy and Bassham 2014), which trigger a physiological response (e.g. a hormone signal) (Atkins and Emery 2002; Clark et al. 2003; Ingram and Malamy 2010), that either suppresses or promotes root the formation and growth of new roots (López-Bucio et al. 2003; Wang et al. 2009). These directional stimuli are called tropisms and the main ones are gravitropism, chemotropism, hydrotropism and thigmotropism. In the absence of all other external cues, gravitropism allows plants to develop their genotypical architecture. This is because each plant organ has a genetically pre-determined gravitational set point angle, which is determined by species and organ type, but may be modified and overridden by other tropisms (Blancaflor 2003). Thigmotropism, sometimes referred to as mechano-sensing, is one such cue that can override the gravitropic impulse through tactile perception of physical barriers encountered by the root tip as it forces its way through the soil. As an initial response, roots will try force their way through the obstacle using cell elongation and increased turgor pressure. However, should the barrier prove impenetrable, the root tip will slip to one side and navigate the surface of the impeding object using a combination of touch and gravity stimuli to guide it (Semchenko et al. 2008; Monshausen and Gilroy 2009). Because mineral nutrients are taken up from soil solution, hydrotropism and chemotropism are closely linked. Both tropisms are thought to have the ability to override gravitropism enabling plants to preferentially place roots in local soil patches with increased resource availability (Porterfield 2002; Eapen et al. 2005; Barlow 2010; Leitner et al. 2010). This ability to selectively place roots is of particular importance for the acquisition of poorly mobile nutrients such as phosphorus (Wang et al. 2006; Hinsinger et al. 2011). In trees, the use of this selective root placement strategy has been associated with fast-growing, early successional species and a low tolerance for root competition. Conversely, a non-selective strategy aimed at consolidating rooting space to pre-empt competitors from accessing the resources contained within has been linked to high competitive ability and is more typically associated with late-successional species (Bauhus and Messier 1999; Messier et al. 2009; Zangaro et al. 2016).

1.4 Challenges associated with reconstructed soils

The mining process typically begins with the complete removal of all existing vegetation followed by the salvage of suitable soil materials that can later be used as a rooting medium for revegetation purposes. Salvaged soil materials are either directly used for reclamation, or temporarily stockpiled. The entire process of removal, stockpiling and placement of salvaged soil materials typically results in a deterioration in physical and chemical properties as well as a reduction in biological activity (Ghose and Kundu 2004; Anderson et al. 2008; Boyer et al. 2011; Shrestha and Lal 2011; MacKenzie 2013). Once vegetation and soil materials are removed, there are typically still large quantities of non-mineable overburden materials that need be moved. These overburden materials may be piled onto large dumps which ultimately often form part of the reclaimed landscape. Consequently, many post-mining landscapes are drastically modified and characterized by novel landforms with severely altered topography, hydrology and microclimate compared to the surrounding undisturbed landscape (Davis and Duffy 2009; Bernhardt and Palmer 2011; Rooney et al. 2012).

Due to the changes they undergo during the mining and reclamation process, reconstructed soils are typically characterized by increased bulk density and soil strength, altered pH and electrical conductivity, depleted soil organic carbon and nitrogen pools, as well as altered texture and poor soil structure (Indorante et al. 1981; Shukla et al. 2004; Shrestha and Lal 2010, 2011). Reconstructed soils may also differ considerably from undisturbed soils in terms of soil materials, as well as number and thickness of soil horizons. Unlike most natural soils, reconstructed soils are likely to have sharp boundaries between soil horizons as a result of layering during soil placement (Naeth et al. 2011; Leatherdale et al. 2012; Jung et al. 2014). The resulting abrupt differences in texture or density between soil layers can negatively affect soil hydrology and aeration and restrict root distribution throughout the reconstructed soil profile (Chong and Cowsert 1997; Guebert and Gardner 2001; Jin et al. 2013; Jung et al. 2014).

Moreover, the use of heavy machinery during soil reconstruction can result in soil compaction. Characterized by high bulk density and increased soil strength, soil compaction can physically impede root elongation (Kozlowski 1999; Stoltz and Greger 2006; Sinnett et al. 2006, 2008a). Moreover, the reduction in pore space and connectivity can result in reduced infiltration, lower water holding capacity and poor soil aeration (Grant and Koch 2007; Sweigard et al. 2007), creating unfavorable conditions for root growth (Kozlowski 1999; Zou et al. 2001; Kozlowski and Pallardy 2002). In many cases, subsoils are intentionally compacted to form a sealing layer above the fractured overburden or mine tailings materials, with only a shallow layer of loose soil that forms the root-zone for planted trees/vegetation. Apart from providing a growing medium to plants, another main purpose of soil reconstruction is to prevent water percolating through the overburden. The fracturing of overburden material during the mining process drastically increases surface area, which can in turn result in vastly accelerated chemical weathering. This increased weathering can cause significant environmental problems when acids, heavy metals, salts and other potential phytotoxins either accumulate in the root-zone where they can be taken up by planted trees/vegetation (phytotoxins refs) or if they leach into underlying groundwater (Aykol et al. 2003; Schaaf and Hüttl 2005; Geremias et al. 2008; Abreu et al. 2008). Thus the need for sufficient rooting space for growing trees has to be weighed against the need to protect the groundwater from potentially harmful leachates during soil reconstruction.

All of these potential changes in site conditions represent a significant challenge for land reclamation practitioners with regard to soil reconstruction for successful reforestation, especially when considering the fact that there are often no natural analogues to these drastically altered landscapes that could inform decision making when it comes to designing reconstructed soils.

1.5 Thesis outline and objectives

This thesis consists of three individual research chapters. The main objective of this work was to study soil-plant and plant-plant interactions in newly reconstructed soils and how they affect growth and development of planted tree seedlings. The goals were to (1) increase our general understanding of belowground processes during the early stages of ecosystem development and (2) to provide tangible information, based on ecophysiological data, to practitioners engaging in forest land reclamation, as well as afforestation and reforestation activities about soil-plant and plant-plant interactions in different reconstructed soils.

In my first research chapter, I evaluated the individual and combined impact of two treatments: (1) deep tillage and (2) vegetation management on soil physical properties, and soil resource availability for trembling aspen (*P. tremuloides* Michx.) seedlings growing on a reconstructed loamy mine soil affected by severe subsoil compaction as well as intense competitive pressure from herbaceous vegetation. The objectives for this chapter were to determine:

a) the individual and combined impact of subsoil compaction and root competition on resource availability, water uptake and growth of planted aspen seedlings
b) the efficacy of two commonly used management practices, deep tillage and vegetation management (herbicide), in increasing available rooting space and with it access to soil resources for planted aspen seedlings.

In my second research chapter, I examined ecophysiological responses of young aspen seedlings to above- and belowground competition with highly competitive smooth brome (*Bromus inermis* L.) grass. The objective was to study:

c) how planted aspen seedlings and smooth brome compete for growing space during early establishment and
d) how this competition modifies the root and shoot architecture, biomass allocation, as well as morphological and physiological characteristics of aspen seedlings compared to those without grass competition

In my third research chapter, I assessed the impact of intra- and interspecific competition and contrasting soil types on the growth and architecture of seedlings of three important boreal tree species with different functional traits. The species in question were: *Pinus banksiana* (Lamb.) a fast-growing, early successional conifer, *Populus tremuloides* (Michx.) a fast-growing, early successional deciduous species, as well as *Picea glauca* (Moench) Voss a slow-growing, late successional conifer species. The objectives for this study were to:

e) determine if the differences in functional traits among these species would result in increased productivity, or ‘overyielding’, due to niche complementarity when seedlings were grown in mixture

f) assess differences in the type and degree of developmental plasticity displayed by each species in response to mixing and contrasting soil types

CHAPTER 2

Root competition, not soil compaction, restricts access to soil resources for aspen on a reclaimed mine soil[1]

2.1 Abstract

Restricted rooting space in response to soil compaction and belowground competition with herbaceous plants are two main limiting factors for successful reforestation after surface mining. Fine-textured, nutrient-rich soils with adequate soil moisture are particularly susceptible to both of these concerns and while there are recognized ways to manage competition, attempts to alleviate soil compaction through mechanical means have produced varying results. While roots of some herbaceous plants may penetrate compacted soil layers, possibly offering an alternative means to overcome physical restrictions, these potential benefits need to be weighed against negative effects from competition with planted trees. We examined the individual and combined impact of soil decompaction (deep tillage) and management of competing vegetation (herbicide) on soil properties, resource availability, and above- and below-ground growth of aspen (*Populus tremuloides* Michx.) seedlings on a reconstructed mine soil affected by severe subsoil compaction. Our findings suggest that although deep tillage reduced bulk density, this did not increase resource availability and had limited effect on seedling growth. In contrast, competition with smooth brome grass (*Bromus inermis* Leyss.) drastically reduced aspen belowground growth because the grass rapidly occupied available rooting space, while simultaneously lowering the availability of water and nutrients, in particular nitrogen.

2.2 Introduction

In agricultural regions, reclamation after surface mining has often focused on returning disturbed areas to productive crop or pasture land without compensating for incurred loss of forest cover. This was traditionally the case in Alberta, Canada, where the majority of the 660,000 hectares currently under lease for coal extraction falls within the aspen parkland ecoregion, characterized by a mixture of cropland,

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native and tame pastures, and forests dominated by trembling aspen (*Populus tremuloides* Michx.). In recent years, however, there has been a shift in thinking and an increased interest in restoring aspen forest cover to similar levels as found prior to disturbance. Somewhat paradoxically, one of the main obstacles to successful reforestation, on these otherwise productive soils, may be insufficient access to soil resources for growing trees. This is mainly due to two problems common to many reclaimed landscapes: (1) restricted rooting space because of soil compaction and/or (2) intense above- and below-ground competition from herbaceous ground covers dominated by agronomic species.

Persistent excessive compaction of reconstructed soils is one of the most commonly reported barriers to successful revegetation in land reclamation (Burger et al. 2005; Zipper et al. 2011b). Its origin can be linked to the disruption of soil processes during excavation, as well as the degradation of physical, chemical and biological properties during stockpiling (Ghose and Kundu 2004) and the compression by heavy machinery used during final reclamation soil placement (Spoor 2006; Shrestha and Lal 2011). Compacted soils are typically characterized by increased bulk density and high soil strength, which may directly impede root elongation (Kozlowski 1999; Sinnett et al. 2008a). Furthermore, poor soil structure, reduced pore space and a lack of pore continuity (Ashby 2006; Shrestha and Lal 2011) can have wide-ranging implications for water-holding capacity as well as infiltration and drainage, affecting both water and oxygen availability (Chong and Cowsert 1997; Guebert and Gardner 2001). Compacted subsoil layers can impede vertical redistribution of water throughout the soil profile, thus increasing the risk of both drought stress during prolonged dry periods, as well as anaerobic, waterlogged conditions, during wet periods (Cox and McFarlane 1995; Startsev and McNabb 2009). Both of these conditions are essentially inhibitive to root growth (Sweigard et al. 2007). Soil compaction thus has the potential to restrict access to soil resources both directly, by physically impeding root elongation, and indirectly by producing adverse conditions impacting root physiology.

Competition with aggressive ground cover species is another factor that can limit resource availability for trees. Mixtures of herbaceous species, often consisting of non-native grasses and legumes, are frequently seeded onto reclaimed soils prior to tree planting, in order to rapidly establish ground cover and minimize surface erosion (Strong 2000; Skousen and Zipper 2014). Even without active seeding, grasses often quickly colonize these bare soils, either from existing seedbanks or through natural dispersal from nearby seed sources (Johnston 2011). Among the frequently used species are highly competitive perennial agronomic grasses such as smooth brome. Many of these grasses have adopted strategies that rely on oversized, extremely dense root-systems to pre-emptively occupy as much soil volume as possible, thus preventing potential competitors from accessing the resources within (Craine 2006; Frank et al. 2010). This rooting strategy may be particularly detrimental to early successional tree species, such as aspen, as these may have evolved to employ a selective root placement strategy that focusses on colonizing free space while avoiding and spatially segregating from other competitors roots (Messier et al. 2009). Belowground competition with such grasses may thus limit access to soil resources for growing trees, either directly through the depletion of water and nutrients in the soil profile (Tilman 1982, 1987), or indirectly by aggressively occupying the available rooting space (Craine 2006; Frank et al. 2010).

In combination, the adverse effects of conditions such as soil compaction and intense below-ground competition on seedling establishment could either be additive or synergistic and it is likely that some intervention will be required to ensure that the growing demands of the establishing trees are met. Competition can be managed relatively successfully using a variety of strategies including mowing and cultivating, application of various mulches, the use of herbicides, and combinations thereof (Casselman et al. 2006; Salesman and Thomsen 2011; Evans et al. 2013; Henkel-Johnson et al. 2016). There are, however, financial and environmental costs associated with each of these methods which need to be weighed against their benefits. Soil compaction, on the other hand, can be quite difficult to alleviate and should ideally be avoided by using alternative techniques such as traffic management (O'Sullivan 1992), loose dumping (Moffat and Bending 2006) and low compaction grading (Sweigard et al. 2007). Results from studies that attempted to mitigate the effects of compaction are difficult to compare directly, as they vary greatly with changing soil type and implements used. As a general rule, however, it appears that alleviation of soil compaction is both more difficult to achieve and less likely to persist in fine-textured soils due to rapid translocation of unconsolidated soil particles into the newly created pore space (Spoor 2006; Sinnett et al. 2006, 2008b).

In this paper we examine the individual and combined impact of two treatments: (1) deep tillage and (2) vegetation management on soil properties, resource availability and trees growing on a reconstructed loamy mine soil affected by subsoil compaction. We report on the extent and longevity of physical changes induced by deep tillage as well as the impacts of both treatments on soil resource availability (i.e. water and nutrients), tree water-use and growth over the course of the first three growing seasons. We hypothesized that: (1) deep tillage reduces bulk density, increasing available rooting space and access to soil resources; potentially decreasing competitive pressures below ground and benefitting tree growth; (2) suppression of herbaceous vegetation greatly reduces above and below-ground competition resulting in markedly improved tree growth; and (3) the combination of deep tillage and vegetation management has an additive or even synergistic effect on tree growth through increased availability of rooting space and soil resources.

2.3 Materials and Methods

2.3.1 Site description

This experiment was conducted on reclaimed land within the Genesee coal mine lease, approximately 80 km west of Edmonton, Alberta, Canada. The mine was established in 1988 and covers a total area of 7,400 ha. It is situated in a rolling landscape that has largely been converted to agriculture over the last 100 years. Today, agricultural land is interspersed with remnant patches of aspen- and aspen-spruce (*Picea glauca* (Moench) Voss) dominated forest in upland areas, as well as wetlands fringed with balsam poplar (*Populus balsamifera* L.) and willows (*Salix ssp.*) in low-lying areas. The local climate is continental, with warm summers and cold winters. Total annual precipitation averages 500-550 mm (1971-2000). Most precipitation (>400 mm) occurs as rainfall during the growing season between May 1 and August 31, the remainder as snow, particularly between November and January (Alberta Government 2015a). The dominant natural soil types prior to disturbance were moderately well drained Luvisols, as well as imperfectly to moderately well drained Solonetz soils (IUSS Working Group WRB 2014; Alberta Government 2015b).

Our study site was located mid-slope on a reconstructed north-facing hill (2-5% slope) in the northeastern part of the mine (Latitude 53.34° N; Longitude -133.27° W), extending 25 m north-south and about 575 m east-west for a total area of about 1.5 ha. The area under consideration was strip-mined between 1991 and 1995 after suitable topsoil had been salvaged using motor scrapers in 1990-91 and subsequently stockpiled. Soil reconstruction occurred incrementally soon after active mining was concluded and began with leveling of overburden between 1992 and 1995. Subsoil was then placed to a depth of 1-1.4 m as a single lift in 1997 using heavy haul trucks. Areas of high vehicle traffic were subsequently ripped using a subsoiler before the whole area was levelled by crawler tractors to create a uniform surface. Salvaged topsoil was replaced in 1998 to an average depth of about 15-20 cm using the same equipment (Navus Environmental 2010). In 1999, the reconstructed area was seeded with a cover crop consisting of cascade oats (*Avena sativa* L.) and boreal creeping red fescue (*Festuca rubra* L.) in combination with alfalfa (*Medicago sativa* L.) and two clovers (*Trifolium pratense* L.*, T. hybridum*) to reduce surface erosion and promote soil development (Navus Environmental 2010). After establishing a vegetation cover, the area was used for annual hay crops and no soil disturbance occurred until 2010. The reclaimed soil had a loamy texture with a particle size distribution of around 40-50% sand, 30-40% silt and 20% clay and despite having been ripped and subsequently covered by vegetation for 11 years, the subsoil remained highly compacted with average bulk densities ranging from 1.8 to 2.0 Mg ha^{-1}, while bulk density in the topsoil (0-15 cm) averaged around 1.5 Mg ha^{-1} (Sabbagh 2014).

2.3.2 Study design

The study was set up as a split-plot design with six complete randomized blocks (25 × 92 m). Blocks consisted of two main plots, separated by a 20 m wide buffer. Main plots were subjected to one of two levels of soil treatment (deep tillage or no tillage) in 2010. Each main plot, in turn, contained four (9 × 12 m) subplots randomly assigned to one four vegetation covers: (A) aspen, (B) smooth brome, (AB) aspen plus smooth brome, and (C) control (bare soil without vegetation).

The deep tillage treatment, was applied to a depth of approximately 60 cm using a RipPlow® (ForestSoil Science Ltd., Edmonton, AB), mounted onto a Caterpillar D7XR bulldozer. The implement consisted of two individual plows, about 2 m apart, with two widely angled wings, about 60 cm wide at the base. To ensure maximum site coverage, the implement turned around in the buffer strips between main plots and made lapping passes with one plow always in between the two previous furrows.

After repeated herbicide applications (Glyphosate; 5-7% (v/v); Monsanto Canada Inc., Winnipeg, MB, Canada) in 2010 and 2011 to remove all existing vegetation, one year-old trembling aspen seedlings were planted at a regular 1 × 1 m spacing (10,000 stems ha^{-1}) in aspen and aspen plus smooth brome subplots in early June 2012. In the aspen only treatment, all competition was suppressed using a combination of hand-weeding and Glyphosate. Between tree rows, we applied the herbicide using a non-drift roll-on applicator (Danville DEX 30, Danville Industries, Harper, KS, USA) to avoid herbicide accidently coming in contact with aspen seedlings. For spot applications around the bases of stems, we used handheld sprayers. Unvegetated control plots were kept free of vegetation for the entire duration of the experiment. Herbicide applications were repeated as often as necessary, whenever sufficient new growth had emerged.

In aspen plus smooth brome plots, plugs of container-grown smooth brome were inter-planted between established tree rows at 1 × 1 m spacing a few days after tree planting. In smooth brome only plots, grass plugs were planted at a 1 × 0.5 m to achieve the same initial plant density as in aspen plus smooth brome plots.

2.3.3 Plant material and species autecology

Aspen is the pre-dominant tree species in Alberta’s parkland ecoregion. It has the greatest geographical distribution of all North American tree species and is adapted to a wide range of site and soil types (Burns and Honkala 1990a). Aspen are generally considered to be a fast-growing, shade intolerant early successional species. Mature aspen stands often contain a spruce understory which may ultimately take over as a climax species as the relatively short-lived aspen begin to decline. High disturbance frequency may, however, reset this process and ensure almost continuous aspen cover. Aspen root-systems consist of up to 30 m long cord-like lateral roots as well as sinkers reaching depths of up to 3 m deep (DeByle and Winokur 1985) As a clonal species its main means of reproduction is through root suckering (Frey et al. 2003), however, in the context of land reclamation aspen are raised from seed and planted onto reconstructed soils.

Smooth brome is a highly competitive, non-native grass species that was introduced to Alberta for livestock forage, as well as revegetation of mined lands, eroded slopes and roadside ditches and is now naturalized throughout the region (Otfinowski et al. 2007). It is a hardy and drought-resistant cool-season grass that forms dense sods and spreads aggressively through rhizomes, tillers and seeds (Otfinowski et al. 2007). These characteristics give it the ability to rapidly colonize a site and its tendency to form dense monocultures (Salesman and Thomsen 2011), with the potential to displace most native species (Dillemuth et al. 2009; Fink and Wilson 2011). On slightly acid to slightly alkaline, well drained and nutrient-rich soils it can grow to a height of well over one meter and produce equally deep roots (USDA, 2014). Maximum forage yields (i.e. aboveground biomass) of 8.0 Mg ha^{-1} (dry) have been reported (Hanson et al. 1978). At our site, smooth brome grew about 1.3 m tall and achieved complete ground cover after one growing season (personal observation).

Aspen seedlings used in this study were commercially grown container stock from local seed source. Seeds were sown into Styroblock containers in 2011 (PSB 615A; 60 × 152 mm (336 ml); Beaver Plastics Ltd., Edmonton, AB) and grown for one growing season at Smoky Lake Forest Nursery (Smoky Lake, AB) using standard nursery protocols. Seedlings were stored frozen at -3°C until planting in early June 2012. At the time of planting average seedling height was 30 ± 3 cm (SD).

Smooth brome was started from seed in Styroblock containers (PSB 315A; 30 × 152 mm (80 ml); Beaver Plastics Ltd., Edmonton, AB) and grown for 6 weeks (May-June 2012) at the University of Alberta before being planted onto the site at the same time as the aspen seedlings. Plants were grown under natural light at 20°C, watered daily and fertilized weekly. All grass plants were clipped back regularly to a height of about 15 cm to ensure uniform growth.

2.3.4 Rooting space and soil resource availability

In July 2011, prior to the establishment of vegetation subplots, 9 undisturbed soil cores (subsamples) were collected on a regular grid of 3 × 4 m grid in each main plot (n=6) using a hydraulic direct push machine (Geoprobe® model 7730DT, Geoprobe Systems, Salina, KS, USA) equipped with a 100 × 5 cm core sampler. A second round of soil cores, was collected in October 2013 using the same machine but with a slightly wider core sampler (d= 6.36 cm). One core per vegetation plot was collected at a random location within the plot, with the restriction that no cores would be taken closer than 2 m to neighbouring plots. After both sampling efforts, soil cores were immediately separated into three depth increments (0-30, 30-60 and 60-90 cm), labelled, wrapped and subsequently stored in a freezer at -20°C until they could be processed. Cores collected in 2011 were used to assess the initial impact of the deep tillage treatment on bulk density, while the 2013 cores were used to determine the longer-term changes in bulk density associated with both the soil treatment and the different vegetation covers. The 2013 soil cores were also used to estimate root densities (see below).

Nutrient availability was assessed once in late summer of 2012 (August 7-September 7) using ion-exchange resin membranes (PRS^{TM} probes, WesternAG, Saskatoon, SK, Canada). Two pairs of anion- and cation probes each were inserted vertically into the topsoil, in the NW and SE corners of each plot, respectively. The probes were inserted in the center of a square formed by four neighbouring plants. After four weeks, the probes were retrieved and cleaned with de-ionized water to remove any adhering soil particles, before being sent in for analysis.

Soil moisture was monitored in-situ over the entire course of the experiment. MPS-2 matric potential sensors (Decagon, Pullman, WA, USA) were buried at 10 cm depth in the topsoil, where we expected the highest level of root activity to occur. EM50 data loggers (Decagon, Pullman, WA, USA) then recorded soil water potentials (SWP) in each plot every 2 hours.

2.3.5 Vegetation response

Aspen heights and root collar diameters (RCD) were measured each fall (2012-14) after leaf abscission in late September or early October. Each tree in every second row (i.e. 4 out of 9 rows) was measured, representing 45% of the total population. Additionally, two randomly selected seedlings in each plot (N=48) were destructively sampled at the end of the third growing season when most of the leaves had abscised and growth terminated. To determine root mass, seedlings were carefully excavated to include as much of the root-system as possible. To achieve this, we used pitch forks to break up and loosen the soil around the trees followed by hand-held trowels. Excavated seedlings were labelled and bagged in the field and subsequently transported back to the laboratory where they were stored in a freezer at -20°C until they could be processed. Once thawed, each seedling was separated into root and stems (i.e. main stem and branches). Both roots and stems were then thoroughly cleaned under running cold water to remove any soil or other debris and subsequently dried at 70°C until constant weight in order to determine root, stem and total mass.

Water-use of aspen seedlings was measured over the course of the 2014 growing season (May – Sept) using the heat balance method described by Baker and Bavel (1987). Two randomly selected aspen seedlings per vegetation plot (N=24), in three of the six blocks, were equipped with Dynagage® sap-flow sensors (Dynamax Inc., TX, USA). Only seedlings representative of their respective treatment with respect to mean height qualified for selection. Three sizes of sensor were used to accommodate variations in caliper among seedlings (9× SGA5 (5-7 mm), 1× SGA10 (9.5-13 mm) and 14× SGA13 (12-16 mm)). All sensors were installed below the first branch to ensure that sap-flow measurements represented the entire leaf area. Sensors were mounted at least 20 cm off the ground and wrapped in weather shielding as suggested in the manual (Dynamax Inc. 2005). At the end of the measurement period we collected all leaves from 20 of the 24 equipped trees. Four trees had already started shedding leaves and were thus excluded from leaf sampling. The collected leaves were scanned using a LI-3100C Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA) to determine the exact leaf area of each seedling.

Aboveground dry mass of smooth brome was determined in August 2014 using two randomly located clip plots (0.5 × 0.5 m) per plot in which all grass was cut at ground level. The harvested material was then oven-dried at 70°C until constant weight.

Mean root mass per volume of soil (g m^{-3}) was estimated by extracting roots from the same soil cores used to determine bulk density, which had been collected in 2013 using the methodology described above. Core samples were taken from the freezer at random and thawed prior to processing. Roots were extracted using running cold water and sieves with a minimum mesh size of 0.25 mm. Cleaned roots were subsequently oven-dried at 70° C until constant weight to determine dry mass.

2.3.6 Data analysis

To accommodate the fact that our study design included both random (i.e. block) and in some cases repeated (i.e. time) factors, we analyzed all data using linear and generalized linear mixed model analysis. All analyses followed a standard procedure, consisting of (i) assessing residuals for normal distribution and applying appropriate transformations or removal of outliers as necessary, (ii) optimizing model and covariance structure using smallest Akaike information criterion (AIC), (iii) running the analysis, estimating parameters using the restricted maximum likelihood (REML) method and (iv) post-hoc comparisons of significant main effects or interactions ($\alpha=0.05$) using a Sidak correction to control type I error rate.

Treatment effects on bulk density were assessed twice. The initial impact of the deep tillage treatment was analyzed in 2011, i.e. prior to the establishment of vegetation covers, using block as random and depth and deep tillage as fixed factors. Changes to soil physical properties were then re-assessed in 2013, this time adding vegetation as a third fixed factor. Nutrient availability, leaf area, biomass and root density, were all analyzed using block as random and deep tillage and vegetation as fixed factors. Aspen root and stem dry mass were compared using log-transformed (log10) means. Analyses of water availability (2013/14) and sap flow (2014) were done on log-transformed means (untransformed values shown), while effects on mean seedling height were assessed using untransformed data. In both cases, time was added to the model as a repeated factor. All statistical tests were conducted using SPSS 20.0 (IBM Corp., 2011).

2.4 Results

2.4.1 Rooting space and soil resources

In 2011, prior to planting, bulk density at the 0-30 cm soil depth was lower in the deep tillage treatment, than in the untilled control. Below 30 cm, however, bulk densities remained similar, averaging 2.06 ±0.13 (Mg m^{-3} ±SD) across both treatments (*tillage × depth, $p<0.001$*) (Table 2.1). Without the influence of vegetation (control plots), bulk density in the upper 30 cm of the deep tilled did not change between 2011 and 2013, while it decreased in plots that had not been deep tilled. As a result, there was no longer a statistically significant difference in bulk density between the two treatments in 2013 (*tillage × year, $p=0.03$*) (Fig. 2.1a). In 2013, deep tillage no longer had an effect on surface soil bulk density (*tillage, $p=0.32$*); however when comparing soil bulk density across all four vegetation treatments, plots with aspen plus brome had significantly lower soil bulk density than the aspen only and control plots, while brome only plots were not different from the other three treatments (Fig. 2.1b). Below 30 cm soil depth, bulk density remained similar across vegetation covers, with 1.69 ±0.16 at 30-60 cm and 1.83 ±0.12 at 60-90 cm soil depth.

The availability of nitrogen, more specifically nitrate (NO_3^-), responded strongly to the type of vegetation cover (*$p=0.001$*), but was unaffected by deep tillage (*$p=0.77$*). Compared to the control (no vegetation), nitrate availability was reduced by about 43% under aspen and by about 92% under aspen plus smooth brome and smooth brome. Phosphorus (P) availability was similar across all treatments, while deep tillage resulted in lower availability of potassium (K: *$p=0.030$*) when compared to untilled plots (Table 2.2).

The lowest mean soil water potentials, i.e. driest soils, were found under aspen plus smooth brome, followed by smooth brome and aspen cover, while the control (no vegetation) showed the highest mean water potential (*vegetation, $p=0.003$*) (Fig. 2.2). In 2013, soils maintained high water potentials throughout May and June, but became increasingly drier as the growing season progressed, with similar values for July and August and the lowest average potentials recorded in September. Compared to 2013, soils were wetter in May and even more so in September 2014, while being significantly drier in June, July and August (*year x month interaction $p<0.001$*). Despite the different patterns in soil water availability, there was overall no difference in annual mean soil water potentials between years (*year, $p=0.161$*). Furthermore, deep tillage had no impact on soil water availability in both years (*$p=0.094$*).

2.4.2 Vegetation response

Root data from soil cores taken in the second year of the experiment (2013) showed that the average root mass per volume of soil in smooth brome plots was already twice as high as in aspen plots but not different from aspen plus smooth brome combined (*vegetation, $p<0.001$*) (Table 2.3). Furthermore, data from seedlings excavated in 2014 suggested that the overwhelming majority of roots in the aspen plus smooth brome treatment belonged to smooth brome, not aspen seedlings, as these seedlings had on average 87% less total root mass than those without smooth brome (*vegetation, $p<0.001$*) (Table 2.3). Deep tillage had no impact on root mass.

After the first growing season, aspen seedlings across all treatments had the same mean height, averaging 50 ±3 cm (±SD). Starting in the second year, however, aspen growth was negatively affected by smooth brome, resulting in lower mean height for aspen growing in the aspen plus smooth brome treatment (*vegetation × year, $p<0.001$*) (Fig. 2.3). In year 2, seedlings without smooth brome were about

22% taller than their counterparts, with a mean height of 103 ±7.4 cm compared to 85 ±5.2 cm. After three growing seasons, this difference in height had increased to 35%, as aspen without competition reached a mean height of 205 ±6.0 cm, while aspen faced with competition from smooth brome was on average 152 ±16 cm tall. This reduced growth was also reflected in smaller root collar diameters (RCD) for aspen growing with smooth brome, averaging 15.4 ±1.3 mm compared to 25.6 ±1.7 mm in the aspen only treatment.

Aspen stem dry mass was roughly five times greater in the aspen only treatment, compared to aspen growing with smooth brome (vegetation, $p<0.001$). Aspen in untilled plots had about 8% lower stem dry mass than aspen in deep tilled plots (tillage, $p=0.01$). Aspen also had about 83% less leaf area when grown with smooth brome (vegetation, $p<0.001$), while deep tillage had no impact on leaf area (Table 2.4). Smooth brome's aboveground dry mass, in contrast, seemed to be unaffected by competition with aspen seedlings ($p=0.642$), as well as the deep tillage treatment ($p=0.705$) (Table 2.4).

Daily tree water use (sap flow, g day^{-1}) was also unaffected by the deep tillage treatment ($p=0.083$). However, as a consequence of the large differences in leaf area, aspen seedlings in the aspen plus smooth brome treatment had on average about 61% less total sap flow per day than seedlings in the aspen only treatment (*vegetation,* $p<0.001$) (Fig. 2.4). Interestingly, sap flow per leaf area (g cm^2 day^{-1}) was about three times greater for aspen seedlings competing with smooth brome, compared to aspen growing without competition (*vegetation,* $p<0.001$). Time (i.e. month) also had a significant effect on daily sap flow rates ($p=0.036$), as well as sap flow per leaf area ($p=0.020$), with September showing lower flow rates than July.

2.5 Discussion

We hypothesized that deep tillage grants young aspen seedlings greater access to soil resources by improving soil conditions as well as mitigating belowground competition by increasing the volume of soil that can be easily colonized by roots. Our findings indicate that, although restricted to the 0-30 cm soil depth, deep tillage did in fact increase accessible rooting space in 2011 by lowering bulk density to levels generally considered non-restrictive to root growth in loam soils (i.e. <1.8 Mg m^{-3}) (USDA 1999), while untilled soil remained close to or above this threshold with a mean (±SD) of 1.7 ±0.2 Mg m^{-3}. In 2013, three years after the deep tillage treatment, bulk density no longer differed between deep tilled and untilled plots. This was due the fact that bulk density remained similar in deep tilled plots, while it had decreased over time in plots that had not been deep tilled. In 2013, all soils had approached mean bulk densities generally considered non-restrictive to root growth, with restrictive levels of soil compaction only occurring at depths greater than 60 cm. Likely owing to much higher root densities, plots in which smooth brome was present generally had lower mean bulk densities than aspen only plots. Despite the apparent reduction in bulk density, deep tillage did not result in a significant increase in water or nutrient availability. However, while it did not lead to an increase in seedling root or total dry mass, aspen seedlings in deep tilled soil had slightly (8%) greater stem dry mass than seedlings in untilled soil after three years of observation. While similar observations have been made for short-rotation coppice willow on agricultural soil (Souch et al. 2004) and Loblolly pine (*Pinus taeda* L.) on forest soil (Carlson et al. 2006), this apparent absence of a pronounced negative effect of soil compaction on the growth of our tree seedlings, is in stark contrast with much of the existing literature on the effects of soil compaction on reforestation of reclaimed mine soils, where compaction can be both massive and persistent (e.g. Ashby 1998, Miller et al. 2012).

There are several potential explanations why the aspen seedlings in our experiment did not respond more strongly to the deep tillage treatment. First, one might argue that the initial difference in bulk density was not great enough and did not last long enough to cause any real difference in seedling growth due to the naturally occurring reduction in bulk density observed in untreated plots. While it would have been possible to try and increase the efficacy of the treatment through repeated application, it is doubtful if that would have had the desired effect. Such an effort would have drastically increased the degree of soil disturbance, leading to potentially a greater loss of soil structure and an increased presence of water-unstable soil aggregates, which allows for the relocation of soil particles into newly created pores with percolating water (Marks and Soane 1987; Busscher et al. 2002; Spoor 2006). This settling or re-compacting effect after deep tillage has frequently been observed, particularly in fine-textured soils, often occurring within the first year (Moffat and Boswell 1997; Sinnett et al. 2006). Increased mixing of topsoil and subsoil materials during repeated tillage could also have far-reaching implications for aspects such as soil nutrient availability. In our experiment, we neither observed re-compaction over time nor mixing of topsoil and subsoil (Sabbagh 2014). We think that this can be attributed to root activity associated with the vegetation present at the site prior to our experiment, which would have promoted the formation of stable aggregates. Second, the wide variety of soil types that aspen naturally occurs on throughout its large range across North America should also be taken into consideration. Burns and Honkala (1990) suggested aspen may grow on anything from shallow, rocky soils to heavy clays, stating that if deep root placement is restricted they simply develop a shallow root system. Under better conditions, aspen will typically develop a root system, consisting of cord-like lateral roots extending up to 30 m into adjacent open areas, as well as sinkers up to 3 m deep that often follow cracks or root-channels on their way down (DeByle and Winokur 1985). In our experiment, aspen roots were routinely found in core samples from depths greater than 60 cm, suggesting that deep placement of aspen roots was not restricted by nominally high bulk densities. Our site had been occupied by plants for more than a decade prior to the experiment. The large taproots associated with some species previously present at the site, such as alfalfa (*Medicago sativa* L.), may have penetrated deep into the compacted subsoil. The complete elimination of all previous vegetation with herbicide, likely left behind a large number of decaying roots that could have created natural channels to deeper soil layers to be utilized by the tree roots (Van Noordwijk et al. 1991; McKee 2001). Finally, there is the issue that soil compaction may simply not be as uniform as one is likely to assume. Instead, there may be considerable variation in the severity of the problem in both space and time. Soil strength, for example, is known to vary with soil moisture, with wetter soil being easier to penetrate than dry soil (Whalley et al. 2005). At our site we observed considerable swelling and shrinking of the soil, forming deep cracks late in the 2013 growing season when the soil was particularly dry (personal observation).

We had further hypothesized that suppression of herbaceous vegetation eliminates most above and belowground competition, resulting in greatly enhanced tree growth, and that the combination of deep tillage and vegetation management might have an either additive or even synergistic effect on available rooting space, soil resources and tree growth. However, since the impact of deep tillage was limited, the presence of competing vegetation was the most dominant driver of seedling performance. In another study, Casselman et al. (2006) found that hybrid poplar planted onto reclaimed mine soil, showed a significant increase in growth when vegetation control was combined with tillage to reduce soil compaction. However, this increase only occurred at one of their three experimental sites where the substrate lacked topsoil and consisted mainly of shale overburden. Research from Long-Term Soil Productivity (LTSP) sites across North America, assessing the impacts of organic matter removal, soil compaction and vegetation control on forest soils, suggests that compaction had a very limited effect on tree growth, while vegetation control generally resulted in increased growth, particularly on productive sites (Fleming et al. 2006; Ponder et al. 2012).

Our results clearly showed that smooth brome had strongly suppressed aspen growth. It likely did so by rapidly occupying available rooting space, with much higher root densities than aspen, while simultaneously lowering the availability of water and nutrients, in particular nitrate (NO_3^-). Nitrate is the form of nitrogen preferentially taken up by aspen and its limited availability could also have impacted its performance (Landhäusser et al. 2010). Interestingly, seedlings exposed to lower soil water availability in response to competition from smooth brome had higher daily water use per unit leaf area. This could be an indication of physiological adjustments to increase the efficiency of water uptake, such as osmotic adjustments in the roots (Galvez et al. 2013; Villar-Salvador et al. 2015). Other studies on aspen and young hybrid poplar showed reduced growth in the presence of herbaceous vegetation, due to strong competition for water, nitrogen and phosphorus and possibly allelopathic effects (Landhäusser and Lieffers 1998; Kabba et al. 2007; Pinno and Bélanger 2009). Competition may affect these early successional species more than compaction because they pursue a strategy of selective root placement (Bauhus and Messier 1999) and root avoidance (Messier et al. 2009). This approach may be especially incompatible with the aggressive strategy of non-selective root placement employed by smooth brome (Craine 2006; Frank et al. 2010). After three years the complete suppression of competing vegetation resulted in near crown closure of the aspen, creating conditions conducive for the development of a native forest understory. This was clearly not the case for aspen competing with smooth brome as their sparse crowns barely overtopped the grass (Fig. 2.5). It is likely that smooth brome will persist for considerable time in these plots, continuing to impede tree growth and preventing the development of a closed forest canopy.

Plant roots interact with their soil environment in various ways and have been shown to improve soil structure at different scales (Angers and Caron 1998; Yunusa and Newton 2003; Bodner et al. 2014). Recent studies have therefore suggested the use of cover crops as a cost-effective, biological means of improving soil conditions prior to reforestation (Guittonny-Larchevêque et al. 2016). Our findings support the notion that, at this particular site, herbaceous ground covers likely partially mitigated the negative effects typically associated with severe soil compaction through root activity. However, we also showed that, on nutrient rich soils, the presence of highly competitive vegetation, such as perennial grasses, can have negative effects that clearly outweigh these benefits with respect to tree establishment and growth as well as the development of functional forests.

2.6 Acknowledgements

We would like to thank the Genesee coal mine and George Greenhough (Capital Power) for providing field sites, Dave McNabb for the deep tillage equipment, and Eckehart Marenholtz for his technical support in setting up the experiment. We also thank Fran Leishman, Morgane Merlin, Natalie Scott, Ingo Siebert and Adam Kraft for their assistance during various stages of the experiment. We also thank the two anonymous reviewers for their constructive comments and help in improving this manuscript.

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2.7 Tables

Table 2.1 – Deep tillage effect on mean (±SD) dry bulk density (Mg m^{-3}) in 2011

Depth (cm)	Deep tillage	No tillage
0-30	1.42 ± 0.09**c**	1.70 ± 0.21**b**
30-60	1.99 ± 0.05**a**	2.06 ± 0.19**a**
60-90	2.09 ± 0.05**a**	2.10 ± 0.15**a**

Note: Measurements were taken at the end of one freeze-thaw cycle to maximize the efficacy of deep tillage. Letters indicate significant differences.

Table 2.2 – Mean (±SD) soil nitrogen (N), phosphorus (P) and potassium (K) availability from ion-exchange membranes deployed in 2012 (n=6). Letters indicate significant differences.

Nutrient	Treatment effects	p-value	Factor levels	Nutrient availability[†]
N	*deep tillage*	*0.77*	Aspen	229± 159**b**
	vegetation	***<0.001***	Aspen + Smooth brome	32 ± 22**c**
	tillage × vegetation	*0.78*	Smooth brome	41 ± 34**c**
			Control	397 ± 215**a**
P	*deep tillage*	*0.46*		
	vegetation	*0.99*	overall	4 ± 2
	tillage × vegetation	*0.88*		
K	***deep tillage***	***0.03***	deep tilled	22 ± 11**b**
	vegetation	*0.60*	untilled	29 ± 14**a**
	tillage × vegetation	*0.36*		

Note: Different letters indicate significant differences for each nutrient.
[†] PRS™-probe supply rate (µg 10cm^{-2}; burial period: 28days)

Table 2.3 - Mean (±SD) root mass per volume of soil (2013) and whole root system dry mass for aspen seedlings (2014) (n = 6).

Vegetation	**Root mass density** (g m^{-3})	**Aspen root mass** (g/seedling)
Aspen	387 ± 186**b**	192 ± 55**x**
Aspen + Smooth brome	987 ± 375**a**	29 ± 10**y**
Smooth brome	744 ± 387 **a**	-

Note: Different letters indicate significant differences. Analysis for aspen root mass was conducted on log-transformed data; shown are untransformed means.

Table 2.4 – Mean (±SD) aboveground biomass per hectare for aspen and smooth brome and leaf area for aspen in 2014.

	Aboveground biomass (g m^{-2})		**Leaf area** (cm^2)
vegetation	aspen	smooth brome	aspen
Aspen	310 ± 60**a**	-	0.77±0.17**a**
Aspen + Smooth brome	60 ± 10**b**	590 ± 220**x**	0.13±0.05**b**
Smooth brome	-	570 ± 150**x**	-

Note: Different letters indicate significant differences.

2.8 Figures

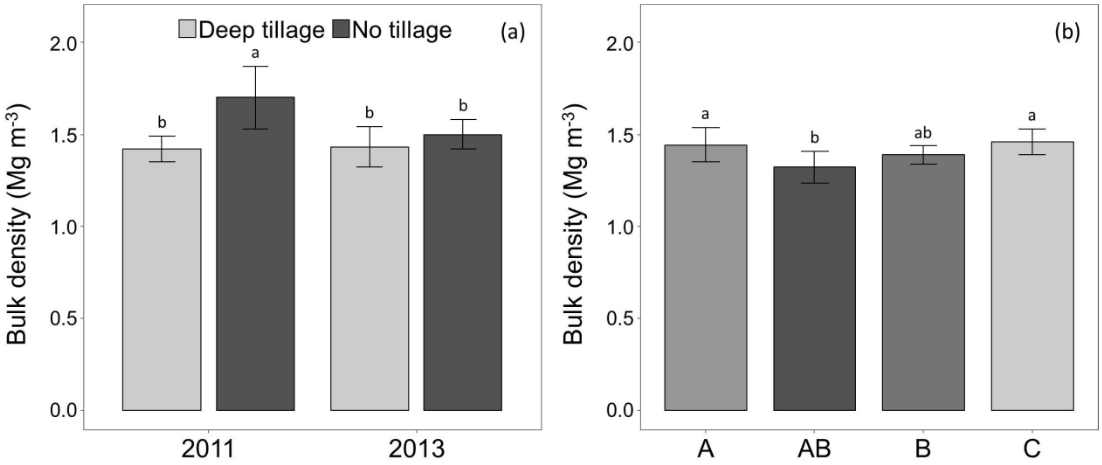

Figure 2.1 - (a) Impact of deep tillage on mean soil bulk density at 0–30 cm soil depth, in 2011 and 2013 without the influence of growing vegetation (n = 6); (b) mean soil bulk density at 0–30 cm soil depth as affected by vegetation cover (A, aspen; AB, aspen plus smooth brome; B, smooth brome; C, no vegetation) in 2013 (n = 6). Lowercase letters indicate significant differences. Error bars represent 95% CI.

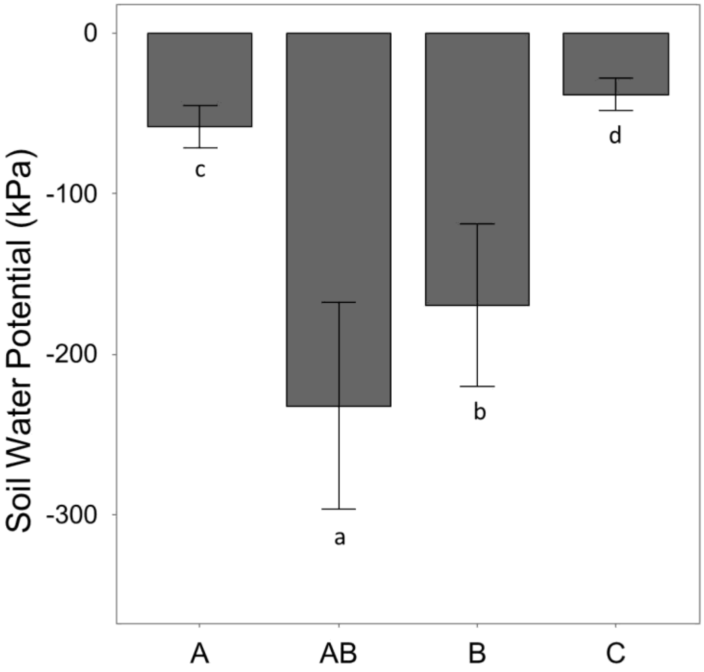

Figure 2.2 - Mean soil water potentials (SWP) (May – Sept) for 2013-and 2014 as affected by vegetation cover (A= aspen; AB= aspen plus smooth brome; B=smooth brome; C= no vegetation) (n=6). Letters indicate significant differences between vegetation covers. Error bars represent 95% CI.

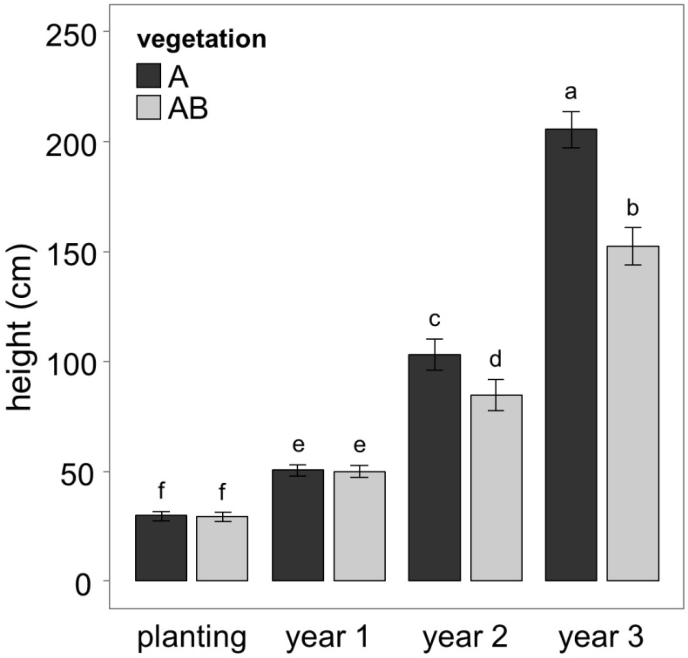

Figure 2.3 – Average total aspen height when growing without (A) or with (AB) smooth brome (n=6). Error bars represent ±95%CI. Different letters indicate significant differences.

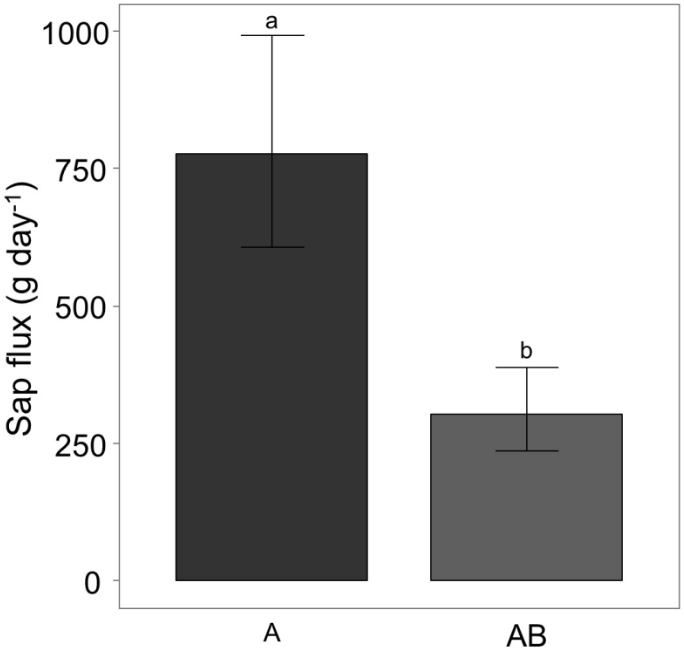

Figure 2.4 – Average daily sap flow rates of aspen seedlings growing with (AB) and without (A) competition from smooth brome during the 2014 growing season. Error bars are ±95%CI (n=3). Letters indicate significant differences.

Figure 2.5 - Three-year old aspen seedlings growing with (AB) (left) and without smooth brome (A) (right) in 2014.

CHAPTER 3

Responses of planted *P. tremuloides* seedlings to grass competition during early establishment[2]

3.1 Abstract

Belowground competition with grasses often plays a critical role during tree seedling establishment, but many underlying mechanisms are not well understood. We used a controlled field experiment to study how trembling aspen (*Populus tremuloides* Michx.) seedlings compete with smooth brome grass (*Bromus inermis* L.) for space during the first three years of seedling establishment and how it affected aspen seedling development. Our study showed that competition with grasses had a limited impact on architectural plasticity of aspen seedlings. Seedlings faced with competition from smooth brome were overall smaller and most architectural parameters, with the exception of shoot height, appeared simply scaled down proportionally. Shoot height changed less than other parameters, because aspen competing with grass allocated relatively more carbon to shoots than roots and adopted a slender shoot morphology in order to quickly overtop the competition. Aspen growing with grass competition had significantly smaller root systems. Both lateral extent and maximum rooting depth were reduced by ~50%. In response to the restricted rooting space, roots of aspen seedlings faced with grass competition had a lower specific root-length. Root carbohydrate reserves were not affected by competition; however aspen roots growing with grass competition had higher soluble sugar concentrations which may be associated with the observed three times higher water uptake efficiency per unit root biomass. Our findings suggest that aspen seedlings have limited capacity for architectural plasticity in response to root competition, but are at least temporarily able to compensate for reduced root system size and rooting space, by optimizing water uptake efficiency.

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[2] A version of this chapter has been submitted for publication (under review): Bockstette, S. W., Pinno, B. D. & Landhäusser, S. M. (2017). Responses of planted *P. tremuloides* seedlings to grass competition during early establishment.

3.2 Introduction

One of the most pervasive competitive struggles among terrestrial plant lifeforms is that between trees and grasses which plays out wherever climatic conditions are suitable for both lifeforms to occur, particularly in the transitional regions (ecotones) between natural grasslands (prairies, savannas) and closed forests. Once established, both forests and grasslands tend to form relatively stable ecosystems with boundaries delineated by mean annual temperature and precipitation (Chapin et al., 2011); however, disturbances, both natural and anthropogenic, that remove existing vegetation can create conditions that lead to a renewed struggle for dominance between trees and grasses and a shift in these boundaries (e.g. Conway & Danby, 2014). These forces are also often in play in afforestation and reforestation where planted tree seedlings typically experience intense above- and belowground competition from fast-growing grasses during the early stages of establishment after disturbance (e.g. Franklin et al. 2012; Henkel-Johnson et al. 2016). The outcomes of these competitive interactions are often hard to predict, as they are driven by a complex interplay of site conditions, as well as the growth strategies and competitive abilities (sensu Grime, 1977) associated with the respective competing species.

Shade-intolerant, early-successional tree species are particularly affected by low light conditions and their survival hinges on their ability to outgrow aboveground competition (e.g. Chapman, 1945). They typically achieve this through high relative growth rates of above and below-ground organs, rapid leaf area development and by selectively placing fine roots and leaves in areas with the highest resource availability (Adams et al. 2013; Pierik et al. 2013). The ability to perceive light levels and composition, for example, allows them to optimize crown architecture and avoid shade (Pierik et al. 2013). As light is typically considered the most limiting factor in forest ecosystems, these species also allocate most of their assimilated carbon to shoot growth which is reflected in generally low root mass fractions (Poorter et al. 2011).

Grasses, of course, also compete for light, especially during germination and early establishment; however, the disproportionate allocation to roots observed in many grasses suggests that most of the competition might occur below ground (Poorter et al. 2011). Several studies have shown that many perennial grasses invest in oversized, extremely dense root systems in an attempt to consolidate rooting space and pre-empt access to the resources within (Mommer et al. 2011b; Ravenek et al. 2016). Some of the most competitive grasses have the ability to colonize soil aggressively through rhizomes, colonizing rooting space as a moving front in what has been called a ‘phalanx-formation’ (Doust 1981; Saiz et al. 2016). Fast-growing early successional trees, in contrast, are more likely to employ a selective root placement strategy, proliferating high root-densities into resource-rich patches within the soil (Bauhus and Messier 1999; Kembel et al. 2008; Bardgett et al. 2014), while attempting to avoid root competition through spatial segregation (Messier et al. 2009). Spatial segregation, however, may not be an option when newly establishing tree seedlings are faced with intense root competition from grasses establishing at the same time. Under such circumstances, these traits are likely to result in severely restricted root system development, reducing the soil volume in which seedlings can access resources.

Competitive interactions are best understood by taking into account both the competitive effects, such as reductions in resource availability, as well as the response observed in the individual plants (Goldberg 1990). While the competitive effects are often simple, mechanistic and relatively easy to quantify, the responses of individuals to competition can be more complex and sometimes difficult to measure. The most frequently reported response to competition is a reduction in plant fitness (survival, growth and reproduction) (e.g. Franklin et al. 2012). However, other responses include changes in carbon allocation to different plant organs (Craine 2006, Poorter et al. 2011), trade-offs between allocation to growth, defence, and reserves (Donaldson et al. 2006, Maguire and Kobe 2015), changes in architectural (i.e. the spatial arrangement of the shoot and root system); morphological (i.e. the properties of individual organs) (Valladares and Niinemets 2007; Hodge et al. 2009; Bardgett et al. 2014; Van de Peer et al. 2017) and physiological traits (Hodge 2004; Bardgett et al. 2014).

Competition has been studied extensively by evolutionary biologists, community ecologists and ecophysiologists; however, many of the underlying mechanisms are still not fully understood, particularly as they relate to below-ground competition (Connolly et al. 2001; Trinder et al. 2013; Wilson 2014). Although a large body of literature exists that explores competitive interactions and mechanisms, only a small fraction of these studies addresses root competition, particularly under field conditions (Wilson 2014). Due to the logistical difficulties associated with studying roots in the field, one area in which knowledge is particularly lacking is how below-ground competition affects the ability of different species to occupy and consolidate rooting space (Casper et al. 2003; Wilson 2014). Fortunately, recent advances in molecular techniques now allow researchers to identify the presence of individual species in mixed root samples and thus gain new insights into spatial aspects of belowground competition (Mommer et al. 2008; Taggart et al. 2011; Randall et al. 2014a).

Forest reclamation sites offer an ideal opportunity to study belowground competition between grasses and early-successional tree species. They offer sizeable areas of bare soil with relatively uniform soil properties and known site history where tree-grass interactions can be studied under relatively controlled field conditions and over the course of several years. Reclamation sites are often rapidly colonized by highly competitive grasses that are either seeded to prevent soil erosion or naturally disperse onto the site from nearby seed sources. Among the species commonly found colonizing these sites, perennial grasses, often invasive exotic species, have been shown to have a particularly strong competitive impact on planted trees (Franklin et al. 2012; Henkel-Johnson et al. 2016; Bockstette et al. 2017). In an earlier study we showed that grass competition with smooth brome (*Bromus inermis* L.) strongly reduced growth of trembling aspen (*Populus tremuloides* Michx.) seedlings by reducing both water and nitrogen availability (Bockstette et al. 2017). The objective of the present study was to examine more closely how aspen seedlings respond to grass competition, with a particular focus on changes carbon allocation to roots and shoots, as well as to reserves.

3.3 Materials and Methods

3.3.1 Site description

The research was conducted on a large reclamation area at the Genesee coal mine, approximately 80 km west of Edmonton, Alberta, Canada. The mine is situated within the transition zone between prairie and boreal forest in gently undulating aspen parkland, characterized by a mix of grasslands and patches of forests dominated by trembling aspen, a fast-growing, shade intolerant early successional species. The local climate is continental, with warm summers and cold winters. Total annual precipitation averages 500-550 mm (1971-2000). Most precipitation (>400 mm) occurs as rainfall during the growing season between May 1 and August 31, the remainder as snow, particularly between November and January (Alberta Government 2015a). For the three-year period of our experiment (2012-2014) the mean annual precipitation was ~510 mm which was slightly below the long-term average of 550 mm (Alberta Government 2016). The dominant natural soil types prior to disturbance were moderately well drained Luvisols, as well as imperfectly to moderately well drained Solonetz soils (IUSS Working Group WRB 2014; Alberta Government 2015b). The study site was located mid-slope on a reconstructed north-facing hill (2-5% slope) in the northeastern part of the mine (53.34° N, 133.27° W), extending 25 m north-south and about 575 m east-west for a total area of about 1.5 ha. More detailed information on the reclamation site and its reconstruction can be found in Bockstette et al. (2017).

3.3.2 Study design

The experiment consisted of 12 blocks (25 × 36 m) which contained three treatment plots (9 × 12 m) randomly assigned to one of three vegetation covers: aspen growing alone (A), aspen growing with smooth brome (AB) and smooth brome growing alone (B). Half of the blocks had been deep tilled in 2010 for a companion study; however this deep tillage treatment had no impact on the vegetation (Bockstette et al. 2017). Deep tillage was therefore not considered a factor in the present study. The entire study site was surrounded by a buffer of 4-5 rows of planted aspen on all sides to minimize edge effects in treatment plots. After repeated herbicide applications (2010/11) to remove all existing vegetation, one year-old container stock of trembling aspen seedlings (see below) were planted at a regular 1 × 1 m spacing (10,000 stems ha-1) in (A) and (AB) plots in June 2012. In the aspen only treatment, all competition was suppressed using a combination of hand-weeding and a non-selective herbicide (Glyphosate; 5-7% (v/v); Monsanto Canada Inc., Winnipeg, MB, Canada). Between tree rows, herbicide was applied using a non-drift roll-on applicator (Danville DEX 30, Danville Industries, Harper, KS, USA) to avoid herbicide accidently coming in contact with aspen seedlings. For spot applications around the bases of stems, handheld sprayers were used. Herbicide applications were repeated as often as necessary, whenever sufficient new growth had emerged.

In plots where aspen grew together with smooth brome, plugs of container-grown smooth brome (see below) were inter-planted between established tree rows at 1 × 1 m spacing a few days after tree planting. In smooth brome subplots, plugs were planted at 1 × 0.5 m to achieve the same initial plant density as plots where aspen grew together with smooth brome. Smooth brome was selected for this study because it is a very hardy and drought-resistant cool-season grass, commonly used in reclamation and agriculture, that forms dense sods and spreads aggressively through rhizomes, tillers and seeds (Otfinowski et al. 2007). It is known for its ability to rapidly colonize a site, displace native species and form dense monocultures (Dillemuth et al. 2009; Fink and Wilson 2011; Salesman and Thomsen 2011).

3.3.3 Plant material

Aspen seedlings used in this study were commercially grown container stock from local seed source. Seeds were sown into Styroblock containers in 2011 (PSB 615A; 60 × 152 mm (336 ml); Beaver Plastics Ltd., Edmonton, AB) and grown for one growing season at Smoky Lake Forest Nursery (Smoky Lake, AB) using standard nursery protocols. Seedlings were stored frozen at -3°C prior to planting in early June 2012. At the time of planting seedlings had a mean height of 30 ± 3 (±SD) cm and an initial root mass fraction ($g_{root}\ g_{total\ mass}^{-1}$) of 0.77 ± 0.04.

Smooth brome was also started from seed in Styroblock containers (PSB 315A; 30 × 152 mm (80 ml); Beaver Plastics Ltd., Edmonton, AB) and grown for 6 weeks (May-June 2012) at the University of Alberta before being planted onto the site at the same time as the aspen seedlings. Plants were grown under natural light at 20°C, watered daily and fertilized weekly. All grass plants were clipped back regularly to a height of about 15 cm to ensure uniform growth.

3.3.4 Root-length density and leaf-area-index (plot-level measurements)

Root-length densities were assessed using soil cores collected in October 2013. One undisturbed soil core was collected per plot (n=12) using a hydraulic direct push machine (Geoprobe® model 7730DT, Geoprobe Systems, Salina, KS, USA) equipped with a 100 × 6.35cm core sampler. Travelling along an east-west transect, the machine collected cores at random locations within each plot with the restriction that no cores would be taken closer than 2 m to neighbouring plots. Core samples were immediately separated into four depth increments (0-15, 15-30, 30-60 and 60-90cm). These were wrapped, labelled and subsequently stored in a freezer at -20°C until they could be processed. Samples were taken from the freezer at random and thawed prior to processing. Using a small core sampler (d= 1.45 cm) one subsample was collected at a random location within each 7.5 cm depth increment for a total of 2 and 4 subsamples for 15 and 30cm long cores, respectively. Roots from bulk samples were extracted using running cold water and sieves with a minimum mesh size of 0.5 mm, while the smaller subsamples were first soaked in water to allow the soil break up and then washed over sieves with minimum diameter of 0.25 mm. Clean extracted roots were scanned with WinRhizo™ (Regent Instruments Inc., Québec City, QC, Canada) to determine root-length density (RLD) (cm_{roots} $cm^{-3}{}_{soil}$). After scanning, roots were dried at 70°C until constant weight to determine root mass. By comparing root-mass per volume of soil (g m^{-3}) from subsamples with those from bulk samples we were able to determine that bulk sampling underestimated root-mass density by approximately 30%. We therefore used a correction factor of 1.3 to estimate actual root-mass density for bulk samples.

In July 2014, plot-level leaf area index (LAI, m^2 m^{-2}) was measured using the LAI 2220C Plant Canopy Analyzer in combination with the FV2200 software (LI-COR Biosciences, Lincoln, NE, USA). Measurements were taken under uniform clear sky conditions using one wand. In plots where brome either grew alone or together with aspen, we took one above- and four below canopy readings following a diagonal transect from NE-SW. In plots where aspen was growing alone, we followed the suggested procedure for small tree plots and took one above- and one below canopy reading looking into the plot from each of its four corners. All measurements were taken using a 90° view-cap. Sky readings were taken at regular time intervals, following the suggested 4A sequence, to allow for scatter-correction when calculating actual LAI. Aboveground biomass for smooth brome was determined by harvesting all standing leaf mass in two randomly located (0.5 × 0.5 m) clip plots per plot, around the same time.

3.3.5 Root identification using species-specific primers

In order to determine the presence of both aspen and smooth brome roots at different soil depths, a second round of soil cores was collected in September 2014. Because the trees were now too tall to operate a vehicle in the plots, we instead used a percussion drill set consisting of a jackhammer and a 1m long coring tool (d = 5 cm) equipped with a plastic liner inside the tool (Eijkelkamp Soil & Water, Giesbeek, NL) to collect two undisturbed core samples per plot. Plastic liners were immediately sealed, labelled and stored in a freezer (-20°C) the same day. Samples were taken from the freezer in random order and allowed to thaw for about one hour at room temperature. The 1m long cores were cut into 10 cm long samples. Roots were extracted under running water using a 250 µm sieve. Extracted roots were carefully cleaned under running de-ionized water to remove any adhering soil particles and subsequently placed on paper towels to remove excess moisture before being stored in labelled aluminum pouches for freeze drying. Freeze-dried root samples were first pooled by plot (n=12) and depth increment and then ground using a TissueLyser II ball mill (Qiagen Inc., Missisauga, ON, Canada) to extract DNA. Total genomic DNA was extracted from ground root samples using a commercial PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) with slight modifications to improve yield and quality. The extracted DNA was further purified by ethanol precipitation. The DNA signatures of the two target species had previously been established at a non-coding chloroplast region, the *trn*L intron intergenic spacer from leaf samples. From the genomic DNA material extracted, segments within the region were amplified by polymerase chain reaction (PCR) using species-specific primers developed in this study. We used a universal forward primer (Primer-C (5'-CGAAATCGGTAGACGCTACG-3') (Taberlet et al. 1991)) and two species-specific reverse primers, Trem404 (5'-ACAGATTCGAGTCGGTTGTC-3') for aspen and Brom284 (5'-GTATGAAACCCCGCTTTCTCT-3') for smooth brome grass. In order to reduce the amount of time and chemicals needed, a multiplex PCR protocol was designed that amplified segments from both aspen and smooth brome simultaneously. This multiplex PCR was done in volumes totaling 25 µL: 6.5 µL autoclaved deionized water, 2.5 µL of forward primer C at 10 µM, 1.5 µL of reverse primer Trem404 at 10 µM, 1.0 µL of reverse primer Brom284 at 10 µM, 12.5 µL of EconoTaq PLUS 2X Master Mix (Lucigen Corp., Middleton, WI, USA), and 1 µL of 1-10 ng/µL of DNA template. Amplifications were performed using an Eppendorf Mastercycler Pro S gradient thermal cycler (Model 6321; Eppendorf Canada, Mississauga, ON, Canada). Thermal cycler conditions: 94 °C for 5 min, 2 cycles of 94 °C for 60 s, 60 °C for 60 s, 72 °C for 80 s, followed by 33 cycles of 94 °C for 60 s, 63–0.4 °C per cycle for 60 s, 72 °C for 80 s and a final extension of 72 °C for 30 min (Taggart et al. 2011). PCR products were visualized right away by gel electrophoresis on a 10 cm long x 15 cm wide gel slab, 1.5 % agarose, with SYBR® Safe DNA gel stain, ran in 2-tiers at 60V for 1 h. Amplicon lengths were compared with a 700 base-pair (bp) ladder run simultaneously on the gel. All 12 samples of aspen gave bands at around 400 bp while all 12 samples of smooth brome gave bands at around 300 bp. Since the bands of aspen and smooth brome were clearly separated on the gel, no further work on precise measurement of the fragment length was required.

The presence of the unique bands corresponding to either of the two species signified their presence in the root materials at the respective depth in the soil. Average maximum rooting depth (2014), as determined by DNA-analysis, was combined with mean lateral root system radius of excavated seedlings (see below) to calculate potential rooting space using a simple cylinder as a spatial representation of the soil volume in which any individual seedling could potentially have roots. We introduce potential rooting space as an architectural measure to establish a direct link between the size of individual root systems, i.e. vertical and horizontal extent of all roots belonging to one individual, and the soil volume in which they can forage for resources. A cylinder was deemed an adequate spatial model for young aspen due to their rooting pattern consisting of long, shallow lateral roots from which vertical sinker roots emerge at roughly regular intervals.

3.3.6 Aspen seedling measurements

Aspen heights and basal diameters were recorded each fall after leaf abscission. Measurements were taken on all trees in every second tree row (i.e. 4 out of 9 rows), representing 45% of the total population in each treatment plot. Seedling mortality during the first three growing seasons was overall low (~13%) irrespective of treatments. After three growing seasons (2014), height and basal diameter data were used to calculate height to diameter (h/d) ratios for individual seedlings. Specific stem length (SSL, cm g^{-1}), was calculated as stem height divided by stem dry mass (Poorter et al. 2011). Both h/d ratio and SSL are morphological parameters used to describe shoot growth form (slenderness) and the relative investment in height growth versus diameter growth, which has been used as an indication of light competition in trees (Erickson et al. 2009; Zhang et al. 2015).

To explore the impact of grass competition on tree water use, aspen sap flow was measured between May and September 2014 using the heat balance method described by Baker and Bavel (1987). In six of the twelve blocks, two representative aspen seedlings (N=24) were randomly selected in treatment plots containing trees and equipped with Dynagage® sap-flow sensors (Dynamax Inc., TX, USA). Three sizes of sensor were used to accommodate variations in caliper among seedlings (9× SGA5 (5-7 mm), 1× SGA10 (9.5-13 mm) and 14× SGA13 (12-16 mm)). All sensors were installed below the first branch to ensure that sap-flow measurements represented the entire leaf area. Sensors were mounted at least 20 cm off the ground and wrapped in weather shielding (Dynamax Inc. 2005). Water uptake efficiency (UE), defined as sap flow per root mass (Leitner et al. 2016), was calculated using growing season average total daily sap flux (g day^{-1}) of individual seedlings divided by their respective total root dry mass (g) (see below). At the end of the measuring period, all leaves of the equipped trees were collected and scanned to determine one-sided seedling leaf area using a LI-3100C Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA). Leaf area data from aspen growing with smooth brome (n=12) were then used to estimate the relative contribution of aspen leaf area to total leaf-area in plots where aspen grew together with smooth brome.

In April 2015, prior to leaf flush, two representative seedlings were randomly selected in each treatment plot and destructively sampled (N=48). Seedlings were carefully excavated to include as much of the root system as possible following roots until they were less than 1mm in diameter. Excavated seedlings were labelled and bagged in the field and subsequently stored in a freezer at -20°C. Once thawed, each seedling was separated into root and stem. Both roots and stems were thoroughly cleaned under running cold water to remove all soil before being dried at 70°C until constant weight to determine root, stem and total woody mass. The length of the three longest lateral roots of each seedling was averaged as a measure of maximum root system radius. To quantify structural allocation to above or belowground parts of the tree, root and stem mass fraction (RMF,SMF, g g^{-1}) were calculated for all 48 excavated seedlings by dividing root and stem dry mass by the total woody mass (root + stem).

Total non-structural carbohydrate (NSC) concentrations, a physiological measure used to quantify the carbohydrate reserve status, were calculated as the sum of starch and total soluble sugars concentrations found both in coarse and fine roots. Coarse and fine roots were analyzed separately as they play different functional roles (i.e. absorption vs. storage). To gain further insight into potential differences in carbohydrate dynamics created by the treatments, we calculated the starch and sugar fractions of total NSC. Each root system was therefore separated into coarse and fine roots using a threshold diameter of 2 mm. These samples were then ground to 40-mesh using a Wiley mill. Ground samples were extracted three times with 80% hot ethanol at 95°C. Extracts obtained in this fashion were analyzed for total soluble sugar concentration using phenol-sulphuric acid. Post-extraction residues were digested using an α-amylase-amyloglucosidase enzyme-mixture to determine starch concentration, followed by colorimetric measurement of the glucose hydrolysate using peroxidase-glucose oxidase-o-dianisidine reagent (Chow and Landhäusser 2004).

3.3.7 Data analysis

With the exception of root-length density and specific root length, all response variables were analyzed using one-way analysis of variance (ANOVA) with vegetation cover as the fixed factor. Individual seedlings were considered independent measures as they were far enough apart that neither their crowns nor roots overlapped. Differences in root-length densities and specific root length were assessed as a two-way ANOVA using vegetation cover and depth as the two fixed factors. As the analyisis in chapter 2 had shown no significant block effects, no random block effects were included in this analysis. Assumptions for ANOVA were tested prior to the analyses and transformations were applied when necessary. Data for seedling root and shoot mass, specific stem length (SSL), leaf-area index (LAI), soil water potential and uptake efficiencies were all log-transformed for the analysis; shown are the untransformed means. Post-hoc comparisons of significant main effects or interactions were conducted using a Sidak correction to control the type I error rate. All analyses were executed using SPSS 20.0 (IBM Corp., 2011). Differences between means were considered significant at $\alpha=0.05$.

3.4 Results

After three growing seasons, aspen without grass competition had a mean leaf-area index (LAI) of 1.2 ±0.5 ($m^2\ m^{-2}$, ±SD) which was much lower than that of smooth brome without aspen (4.6 ±1.3) ($p<0.001$). Aspen and smooth brome growing together had a combined mean LAI of 4.7 ±1.0, which was almost identical to that of smooth brome growing alone ($p=0.983$). Aspen growing with smooth brome had a mean leaf area of 0.12± 0.1 m^2 per seedling which constituted only about 2.6% of total leaf area per plot. Aboveground biomass for smooth brome, a proxy for leaf area, did not differ between smooth brome growing alone (570 ±150 $g\ m^{-2}$) or together with aspen (590 ±250 $g\ m^{-2}$) ($p=0.642$).

Roots extracted from soil cores collected in 2013 revealed that aspen growing alone had lower root-length densities (RLD) than smooth brome growing alone at the 0-15 and 15-30 cm soil depth range, while RLD was similar for both species below 30 cm (vegetation × depth, $p<0.001$). Also, RLD did not change with depth for aspen growing alone, whereas it decreased rapidly between 0 and 60 cm depth for smooth brome. The combined RLD of aspen growing with smooth brome was statistically identical to that of smooth brome growing alone at all depths (Fig. 3.1). When growing alone, both aspen and smooth brome had similar mean specific root-lengths (SRL, $cm\ g^{-1}$), with means of 104±40 and 102±39, respectively. However, aspen and smooth brome growing together had a 15% lower combined mean SRL (88±30) than either species growing alone (vegetation, $p=0.015$). Specific root-length was lowest at the 0-15 cm soil depth, while remaining similar below 15 cm (depth, $p<0.001$).

DNA-analysis of root samples revealed that aspen growing alone reached an average maximum rooting depth of 0.8 ±0.1 m, while aspen growing with smooth brome only reached a maximum rooting depth of 0.4 ±0.3 m ($p<0.001$) (Table 3.1). However, variability in maximum rooting depth was greater for aspen growing with smooth brome (CV=75%) than for aspen growing alone (CV=12.5%). Maximum rooting depth of smooth brome was not affected by the presence of aspen, ($p=0.701$) as roots were consistently found at the maximum sampling depth of 1 m in all soil cores.

Aspen growing with smooth brome were on average shorter, had a smaller root collar diameter, lower stem dry mass and less leaf area per individual seedling than aspen growing alone ($p<0.001$ for all) (Table 3.1). Aspen growing with smooth brome also had more slender stems than aspen growing alone ($p<0.001$) with mean height to diameter ratios (cm cm^{-1},) of 99 ±10 and 80 ±3, respectively. Likewise, specific stem length, i.e. height per unit shoot dry mass (cm g^{-1}), was three times greater for aspen growing with smooth brome (2.7±0.6) than for aspen growing alone (0.8±0.2) ($p<0.001$).

Total root dry mass was significantly lower for aspen growing with smooth brome ($p<0.001$) (Table 3.1). Aspen growing with smooth brome also had a lower mean root mass fraction (RMF, g g^{-1}) and higher stem mass fraction (SMF, g g^{-1}) than aspen growing alone ($p<0.001$) (Fig. 3.2). Root systems of aspen seedlings growing together with smooth brome had an average radius of 1.0 ± 0.5 m, compared to 2.1± 0.8 m for aspen growing alone ($p<0.001$). In combination with maximum rooting depth (see above), this resulted in a potential rooting space of 11.1 m^3 for aspen growing alone, compared to 1.3 m^3 for aspen growing with smooth brome (Fig. 3.3).

Total non-structural carbohydrate (NSC, soluble sugars plus starch) concentrations in roots of aspen growing alone did not differ from those found in aspen growing together with smooth brome ($p=0.224$), but fine roots (d<2 mm) generally had significantly higher NSC concentrations than coarse roots ($p<0.001$) Aspen growing together with smooth brome showed a significantly lower starch fraction and higher sugar fraction (%NSC) than aspen growing alone ($p<0.001$) (Table 3.2). Root water uptake efficiency i.e. the mean daily total sap-flow rate (g day^{-1}) per unit root mass (g), was about four times greater in aspen growing with smooth brome (11.99±9.5) than in aspen growing alone (2.99±1.2) ($p<0.001$).

3.5 Discussion

We used a controlled field experiment to study how planted aspen seedlings compete with smooth brome grass for growing space during the first three years of seedling establishment and how this interaction affects aspen seedling root and shoot architecture, morphology and physiology. Our study showed that competition with grasses had a minimal impact on architectural plasticity of aspen seedlings. Seedlings faced with competition from smooth brome were overall smaller, but all architectural parameters, with the exception of height and root collar diameter, appeared to be simply scaled down proportionally. Aspen growing with grass competition had significantly smaller root systems; however, both lateral extent and maximum rooting depth were each reduced by ~50% in response to competition, thus the overall spatial proportions of the root system did not change. Maximum rooting depth was about 40% of root system radius both for aspen growing alone and together with smooth brome, suggesting a fixed coarse architectural pattern rather than architectural plasticity in response to root competition, at least during this early stage of seedling growth. It should be noted that our findings pertain to planted aspen seedlings growing in a simultaneously establishing grass cover. The responses might be different when looking at belowground interactions in natural ecotonal grassland-forest ecosystems. Kalliokoski et al. (2008) presented radial extent and maximum rooting depth for three boreal tree species (*Betula pendula*, *Picea abies* and *Pinus sylvestris*) at three developmental stages (sapling – pole – mature). Using their data to calculate the relationship between radial extent and maximum rooting depth revealed that *B. pendula* and *P. abies* maintained a fixed pattern throughout all three stages, while in *P. sylvestris* maximum rooting depth increased more rapidly than radial extent. To our knowledge, no study has explored this relationship for planted aspen seedlings.

Aspen are thought to employ a selective root placement strategy, preferring to proliferate roots into resource-rich patches with little direct competition (Bauhus and Messier 1999). A *Populus* hybrid (*P. deltoides* × *balsamifera*) has also been shown to have low tolerance for root competition. In a split-pot study, the hybrid suffered reduced fine root mass and altered root morphology (lower specific root-length, SRL) in the presence of competing grass roots (Messier et al. 2009). In our study, we also found that specific root-length (SRL) was lower when aspen and smooth brome grew together. Furthermore, we observed lower SRL near the soil surface where root competition was likely most intense. Moreover, in a previous study we demonstrated that smooth brome led to a significant reduction in water and nitrate availability in the topsoil (Bockstette et al. 2017). Nitrate is highly mobile in the soil solution and becomes less available at shallow depths as soils become increasingly dry (Lynch 2013; White et al. 2013). Faced with high root densities and resource depletion near the soil surface, vertical stratification through deep root placement is often thought to be one of the main mechanisms allowing trees to successfully compete with herbaceous vegetation (Casper and Jackson 1997; Schroth 1998; Balandier 2005). Based on their findings it would have been plausible to expect aspen seedlings to invest in deeper roots, potentially at the expense of reduced lateral spread, in order to minimize direct root competition and increase nitrate and water uptake (Lynch 2013; White et al. 2013); however, this is not what we observed in our experiment. It is, however, important to note that while the average maximum rooting depth was reduced in response to root competition, there was greater variability in maximum rooting depth for aspen growing with smooth brome. This likely indicates that individual seedlings experienced different levels of root restriction when competing with smooth brome. Based on the observed root system radius and maximum rooting depth, potential rooting space for aspen was reduced by 88% in response to grass competition. This reduction in rooting space was closely reflected in an 87% reduction in total root mass as well as an 81% lower stem mass and an 84% lower leaf area.

Aspen is considered a very shade-intolerant species (Burns and Honkala 1990b; Kobe and Coates 1997; Reich et al. 1998a) and while most tree species can tolerate more shade when they are small, becoming less shade-tolerant with increasing size, this trend was not found for aspen (Kneeshaw et al. 2006). Landhäusser and Lieffers (2001) observed 100% mortality of aspen seedlings transplanted into understory light conditions (~25% light transmission) after one growing season. Competition for light is considered size-asymmetric, meaning that larger individuals have a disproportionate advantage over smaller individuals, because of their ability to intercept incoming light (e.g. Craine and Dybzinski, 2013). Accordingly, aspen in our study showed greater plasticity in shoot traits in response to aboveground competition for light. Lower root mass fractions (RMF) and increased stem mass fractions (SMF) in aspen growing with grass competition indicated preferential allocation to shoot growth. In combination with altered shoot morphology (lower h/d ratios, higher SSL), this allowed aspen seedlings to maximize height growth in an attempt to outgrow smooth brome (Poorter et al. 2011; Van de Peer et al. 2017; Setiawan et al. 2017). After three growing seasons, aspen seedlings growing with grass competition were thus only 23% shorter than those without; however, it took seedlings three years to outgrow the shade created by smooth brome's dense canopy (Bockstette et al. 2017). Our findings agree with those of Reich et al. (1998) who found that nine boreal tree species, ranging from shade-intolerant aspen to shade-tolerant white cedar (*Thuja occidentalis*), all increased allocation to shoot over root, had higher specific leaf area, but lower specific root-length in response to low light.

Reduced light availability and drought stress have been shown to lead to a depletion in non-structural carbohydrate (NSC) reserves in roots of aspen (Galvez et al. 2011, 2013; Maguire and Kobe 2015). Despite the above- and below-ground competition from smooth brome, no depletion in root NSC reserves was observed in our study. However, we found higher sugar fractions in roots of aspen growing together with smooth brome. We attributed this shift in NSC towards soluble sugars to a potential osmotic adjustment, in response to reduced water and nitrogen availability in the presence of smooth brome (Kozlowski and Pallardy, 2002; Sanders and Arndt, 2012). Osmotic adjustment has been shown to play a role in maintaining cell turgor at low water potentials in aspen and other *Populus* species and allows for water uptake at lower soil water potentials (Galvez et al. 2013; Gebre et al., 1998, 1994). Therefore it is probable that this osmotic adjustment also contributed to the higher root water uptake efficiency observed in aspen when growing with smooth brome.

In summary, our findings suggest that trembling aspen seedlings exposed to intense competition with smooth brome grass showed very little architectural plasticity, especially with regard to root architecture. Preferential allocation to shoot growth and altered stem morphology allowed aspen seedlings to maximize height growth and overtop smooth brome within three years; however, growth rates were still much lower compared to aspen growing without grass. Aspen seedlings were able to maintain similar concentrations of root NSC reserves with or without competition, but shifted the form of NSC to sugars rather than starch when faced with competition. It appears that aspen growing with grass competition were able to partly compensate for reduced root system size and rooting space, through higher water uptake efficiency.

3.6 Acknowledgements

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3.7 Tables

Table 3.1 - Root and shoot characteristics (mean, ±SD) for three-year old individual aspen seedlings growing with and without competition from smooth brome (n=24). Competitive response shows the net change in each parameter due to competition. Letters indicate significant differences.

Parameter	**Aspen alone**	**Aspen with brome**	**Competition response**
Shoot			
Height (cm)	205 ±11**a**	152 ±17**b**	-26%
Basal diameter (mm)	26 ±2**a**	15 ±1**b**	-42%
Stem dry mass (g)	312 ±70**a**	59 ±16**b**	-81%
Leaf area (m2)	0.77 ± 0.4**a**	0.12 ± 0.1**b**	-84%
Root			
Root system radius (m)	2.1 ±0.8**a**	1.0 ±0.5**b**	-52%
Maximum rooting depth (m)	0.8 ±0.1**a**	0.4 ±0.3**b**	-50%
Root dry mass (g)	214 ±59**a**	28 ±10**b**	-87%

Table 3.2 - Total non-structural carbohydrate (NSC) reserves (mean, ±SD), starch and sugar fractions in coarse (d>2mm) and fine root (d<2mm) tissues of aspen seedlings excavated in April 2015 (n=24)**.**

Parameter	Tissue	Aspen alone	Aspen with brome
total NSC (% dry weight)	coarse roots	9.7 ± 3.0^{b}	9.0 ± 1.7^{b}
	fine roots	12.5 ± 2.8^{a}	12.0 ± 2.1^{a}
starch-fraction (% NSC)	coarse roots	16.6 ± 11.2^{a}	4.6 ± 3.9^{b}
	fine roots	17.7 ± 11.3^{a}	8.3 ± 6.2^{b}
sugar-fraction (% NSC)	coarse roots	83.4 ± 11.2^{b}	95.4 ± 3.9^{a}
	fine roots	82.3 ± 11.3^{b}	92.7 ± 6.2^{a}

3.8 Figures

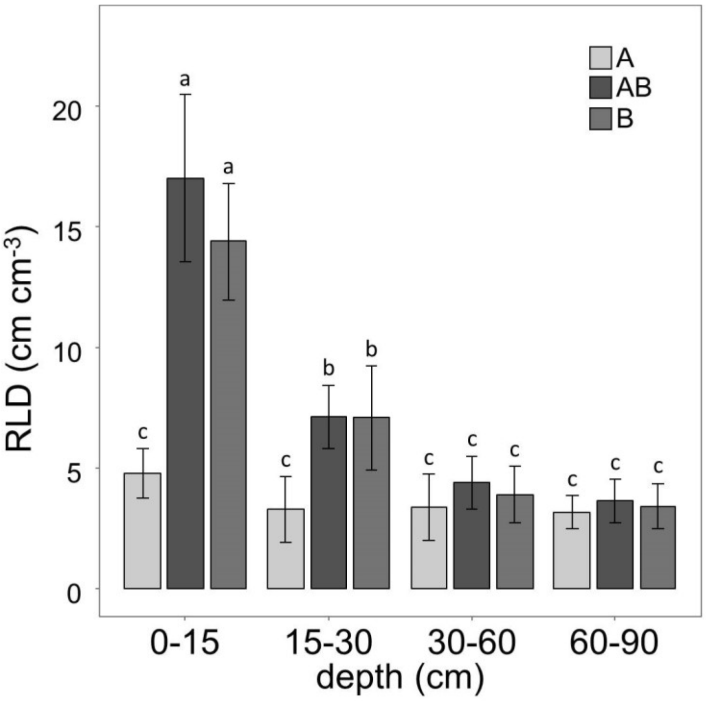

Figure 3.1 – Root-length density (RLD) by depth for aspen growing alone (A), aspen growing with smooth brome (AB) and smooth brome growing alone (B). Letters indicate significant differences between means. Error bars represent 95% CI, (n=12).

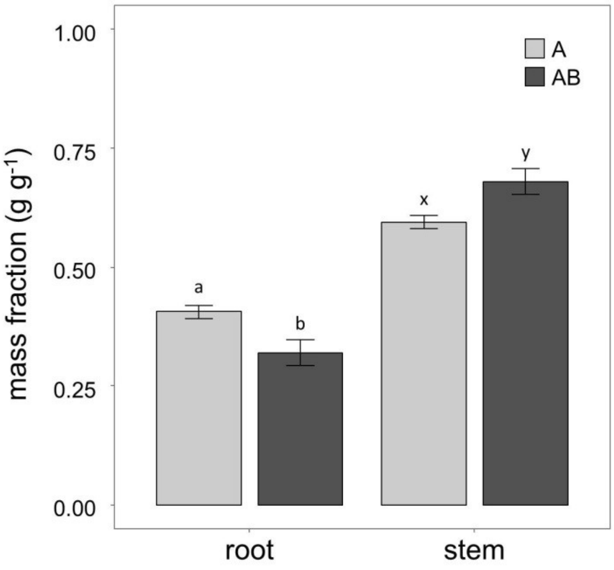

Figure 3.2 – Root and stem mass fractions for aspen growing alone (A) and together with smooth brome (AB) (n=24). Letters indicate significant differences. Error bars represent 95% CI.

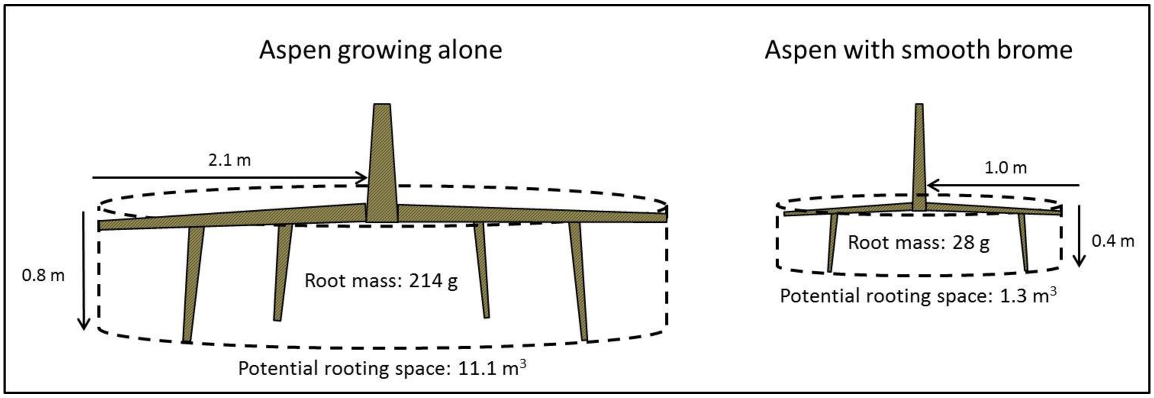

Figure 3.3 – Root-system architecture and potentially accessible rooting space for aspen seedlings growing with and without smooth brome.

CHAPTER 4

Temporal niche segregation and belowground overyielding in young tree mixture on a reconstructed soil

4.1 Abstract

Mixed forests are highly complex systems where the interplay of positive and negative interactions affects the fitness of individuals and thus drives community composition. The outcomes of these interactions are thought to depend on species' functional traits which are modified by abiotic conditions. While interactions among functionally similar species are more likely to be competitive and may ultimately result in the displacement of one or more species, co-occurrence of functionally different species can result in increased ecosystem productivity, or overyielding, due to complementary resource requirements, as well as different spatial or temporal niches. Overyielding due to niche complementarity has been shown in experimental grasslands and agro-forestry systems, but is less well studied in mixed forests. Moreover, studies on overyielding in tree mixtures have predominantly focused on increased aboveground productivity. We planted three functionally different tree species both in mixture and in monospecific plots on two contrasting soil types to (1) test whether mixing results in aboveground and belowground overyielding due to niche complementarity (2) determine how the response to mixing, i.e. the plasticity in architecture and/or biomass allocation, differs among species and soil types. After four growing seasons, we found that mixing resulted in belowground overyielding but the occurrence of this effect depended on soil type. The observed overyielding was the result of reduced belowground competition due to differences in phenological patterns, i.e. temporal niche segregation, among two of the three species. This effect was not observed in a second soil type where unfavorable edaphic conditions such as excessive soil moisture and low soil temperatures delayed the start of the growing season and thus negated phenological differences among species.

4.2 Introduction

Mixed forests are highly complex ecosystems where a constantly changing interplay of positive and negative interactions among biotic and abiotic components, both above and below ground, exerts a strong control over the growth and fitness of individual trees and thus plays a critical role in shaping community composition during all stages of forest development (Casper and Jackson 1997; Michalet and Touzard 2010; Morin 2011; Cavard et al. 2011; Aschehoug et al. 2016). Due to their higher biodiversity, mixed forests are not only considered to be more resilient to environmental change (Wardle et al. 2000; Hooper et al. 2005; Pretzsch 2005), but also have the potential to be more productive than mono-specific stands (Hooper and Vitousek 1997; Hooper et al. 2005; Pretzsch 2005; Morin et al. 2011).

The outcomes of interactions among neighbouring individuals growing in mixture are thought to depend largely on the functional traits associated with each species, such as differences in shade-tolerance, phenology, or rooting patterns (Hooper et al., 2005). While interactions among tree species with similar growth strategies and resource use patterns are more likely to be competitive and may ultimately result in the displacement of functionally similar species (Stubbs and Wilson 2004; Valverde-Barrantes et al. 2013), positive interactions may result in increased ecosystem productivity, or overyielding (Hooper et al. 2005). Such positive interactions arise either from facilitation , i.e. if one species improves the growing conditions for another species, or through reduced competition due to niche segregation, i.e. if co-occurring species occupy spatially or temporally separate niches within the ecosystem (Silvertown 2004; Michalet and Touzard 2010; Fichtner et al. 2017).

Overyielding due to niche complementarity has been demonstrated in experimental grasslands (e.g. Hooper and Dukes, 2004; Marquard et al., 2009; Wilson, 1988) and agro-forestry systems (e.g. Schroth, 1998, 1995) but has been less well studied in mixed forest stands (Scherer-Lorenzen et al. 2007; Meinen et al. 2009a). Moreover, studies on overyielding in tree mixtures have often focused on increased aboveground productivity due to vertical stratification among the crowns of co-occurring tree species with different degrees of shade tolerance (e.g. Chen et al., 2003; Kelty, 2006; Sapijanskas et al., 2014).

Only a handful of studies have explored belowground niche segregation and overyielding in mixed forests and these studies have produced mixed results. Büttner and Leuschner (1994) found vertical niche segregation belowground in mature stands of *Fagus sylvatica* L. and *Quercus petrea* (Matt.) Liebl., but did not examine if this resulted in overyielding, compared to monocultures. Several studies have explored vertical belowground niche segregation between *Fagus sylvatica* and *Picea abies* (L.) Karst.; however, the results were inconclusive. While some authors suggest that belowground overyielding, e.g. increased root biomass, occurred in mixed stands (Schmid 2002; Schmid and Kazda 2002; Rewald and Leuschner 2009), others found decreased biomass in mixture (Bolte and Villanueva 2006). Meinen et al. (2009b, 2009c) compared a total of 24 temperate forest stands across a species-richness gradient and found neither significant spatial segregation among co-occurring species nor any evidence of belowground overyielding.

A possible explanation for the apparent lack of niche segregation and belowground overyielding in these species-rich forests may be found in the fact that these were mature old-growth stands consisting mostly of functionally relatively similar, late-successional tree species. Niche segregation, particularly belowground, may be a more common occurrence during earlier stages of forest development where functionally different, i.e. early and late successional species, establish concurrently. For example, Lei et al. (2012a, 2012b) suggested that due to the rapid colonization of available rooting space, both belowground competition and overyielding due niche segregation may play an important role in the development of young mixed forest stands. However, despite their recognized importance, many belowground interactions in young tree species mixtures are still poorly understood (Lei et al. 2012b).

In adaptation to early successional environments with limited nutrient cycling, early successional tree species are thought to rely on rapid colonization of the available rooting space within the soil, whereas late successional species are more likely to have shallower root-systems that are better adapted at capturing nutrients from decomposing litter (Gale and Grigal 1987). Fast-growing early successional trees are also more likely to show a high degree of plasticity in response to environmental cues, combined with a low tolerance to root competition (Messier et al. 2009). These species are also more likely to employ a selective root placement strategy, preferentially proliferating roots in resource-rich patches within the soil (Bauhus and Messier 1999; Kembel et al. 2008; Bardgett et al. 2014). Conversely, slow-growing late successional tree species, are typically more tolerant of stress, including root competition, are more likely to employ a non-selective root placement strategy and typically show a low degree of developmental plasticity in response to external cues (Bauhus and Messier 1999; Messier et al. 2009). Developmental plasticity and the ability to perceive both abiotic and biotic components of the soil environment enable plants to alter root-system architecture, i.e. the spatial arrangement of their roots, (Malamy 2005; Barlow 2010) as well as allocation to roots, stem and leaves to optimize resource acquisition and use (Poorter et al. 2011; Freschet et al. 2015).

Sites that are being reclaimed to forest land after anthropogenic disturbances, such as surface mining, offer an excellent opportunity to study belowground interactions in young tree species mixtures under natural but still relatively controlled conditions, over extended periods of time and at relatively large spatial scales. Due to the severity of the disturbance and the fact that soils have to be entirely reconstructed, reclamation sites also offer fairly uniform soil conditions, simple plant communities (i.e. relative low number of species, similar age etc.) and few biological legacies compared to undisturbed natural sites.

As part of a large-scale reclamation study in Alberta, Canada, three co-occurring, but functionally different tree species common to the boreal forest region were planted in a replacement series (de Wit 1960; Jolliffe 2000) in mixed and monospecific plots on two contrasting reconstructed soil profiles. The chosen species were *Pinus banksiana* Lamb., a fast-growing, early successional conifer, *Populus tremuloides* Michx., a fast-growing, early successional broadleaved deciduous species, and *Picea glauca* (Moench) Voss, a slow-growing, late successional conifer. Early seedling development was monitored over the first four growing seasons to determine how intra- and inter-specific competition, along with contrasting soil conditions affected aboveground and belowground growth and productivity, as well as seedling architecture and biomass partitioning.

Specifically we wanted to test the hypotheses that: (1) regardless of soil conditions, mixed species planting results in aboveground and belowground ‘overyielding’ due to niche complementarity among the three species and (2) the two early successional species, *P. banksiana* and *P. tremuloides* will show a higher degree of plasticity in architecture and/or biomass allocation in response to inter-specific competition and contrasting soil types than the late successional *P. glauca*.

4.3 Materials and Methods

4.3.1 Site description

This experiment was conducted as part of the Aurora Soil Capping Study (ASCS), a large-scale (~40 ha) multidisciplinary reclamation experiment located at the Aurora North mine (Syncrude Ltd.), approximately 80 km north of Fort McMurray, Alberta, Canada (57°19'48"N 111°31'20"W). The overall aim of this larger study is to test the impact of different soil capping prescription over lean oil sand overburden on reclamation outcomes. The mine is one of seven currently active surface mines, extracting bitumen. The sites is situated within Alberta's mixed boreal forest, where the pre-disturbance landscape is gently undulating, consisting of a roughly even mix of upland forests as well as bogs and treed fens in lowlands (Natural Regions Committee 2006). The local climate is warm summer continental (Köppen-Geiger classification: Dfb), with short warm summers and long cold winters (Kottek et al. 2006). Total annual precipitation averages 400-450 mm (1971-2000). The short growing season, with daily mean temperatures above 5°C, typically lasts from around mid-April until the end of September (Alberta Government 2015a). Upland stands of *P. tremuloides* and *P. glauca* are typically associated with finer-textured Luvisols, while *P. banksiana* stands are mostly found on coarse-textured, rapidly draining Brunisols (IUSS Working Group WRB 2014). Stands of *Picea mariana* (Mill.) BSP and *Larix laricina* (Du Roi) K. Koch are typically found on thick organic peat soils, characteristic of poorly-drained lowlands (Natural Regions Committee 2006). Given its high organic matter content as well as its local abundance, salvaged peat, mixed to varying degrees with underlying mineral soil, has historically been used as the principal topsoil for revegetation of overburden and mine tailings in this region (Rowland et al. 2000; Jung et al. 2014). More recently, selectively salvaged and directly placed forest floor material (FFM) salvaged from upland forest sites, typically comprising the organic litter, fermenting litter and humus, as well as the upper mineral soil layers that make up the A horizon, has been introduced as an alternative topsoil material (Naeth et al., 2013).

The ASCS was also designed to test questions relating to tree growth and understory development in response to various reconstructed soil profiles and planting strategies. For our study, we focussed on two contrasting soil profiles reconstructed either with peat, salvaged from a depth of up to 3 m, or with forest floor material (FFM) salvaged from upland P. banksiana stands as topsoil materials prior to mining. Both topsoil materials were salvaged in 2011 and directly placed onto the site without stockpiling, to preserve many of the propagules contained in these materials (Jones 2016). Below these topsoil horizons, both reconstructed soil profiles included two subsoil horizons, one consisting of a selectively salvaged Bm horizon and the other of salvaged C horizon material from native Brunisolic soils prior to mining. The

Bm material was salvaged from depths ranging from 15-50 cm, while the C horizon material was salvaged to a depth of 250 cm. All subsoil materials were salvaged in the winter 2007/8 and subsequently stockpiled until 2011 when soil reconstruction for the study began. More detailed information on macronutrient availabilities associated with each of the soil materials used in this study can be found in Appendix A (Table A1).

4.3.2 Study design

Both reconstructed soil profiles had a total depth of 1.5 m and comprised either peat or FFM as topsoil materials, overlaying two subsoil horizons consisting Bm and C horizon material (Fig. 4.1). Each soil profile was replicated in three 1 ha plots randomly located across the entire study site. Each main plot, in turn, contained four subplots (25 × 25 m) randomly assigned to one of four tree covers: monospecific *P. tremuloides*, *P. banksiana*, and white spruce *P. glauca* (single), as well as an even mixture of all three species (mixed). All tree plots were planted at a regular 1 × 1 m spacing (10,000 stems ha^{-1}) in May 2012. All seedlings used in this study were commercially grown, one-year-old container-stock produced from open-pollinated seed, sourced locally from the Fort McMurray area. Standard nursery protocols were used and dormant seedlings were stored frozen over winter prior to planting in 2012. Initial seedling characteristics, at the time of planting, are presented in Appendix A (Table A2).

4.3.3 In-situ root measurements

To allow for a non-destructive in-situ observation of root-density and distribution, each tree plot was equipped with one minirhizotron tube in 2012. Minirhizotron tubes were installed at the center of a randomly selected square formed by four neighbouring trees, pointing randomly at one of the four seedlings. All minirhizotron tubes were inserted at a 45 degree angle to a maximum depth of 85 cm. After four growing seasons (October 2015), a total of 85 images were taken at 1 cm intervals along each tube using the BTC-2 ICAP camera system (Bartz Technology Corp., Carpinteria, CA, USA). Each image was subsequently analyzed using Rootfly (Wells and Birchfield, Clemson University, SC, USA) to determine total root length (cm). Root-length density (RLD), i.e. root length per volume of soil (cm cm^{-3}) was then calculated for each image using its width and height (18 × 13 mm) with a depth of field of 0.785 mm, as suggested by Taylor et al. (2014). The reported root-length densities for mixed plots represent a combination of all roots present in each window; no distinction between species was attempted. It should also be noted that there was some volunteer vegetation colonizing the FFM soil type; however, overall cover was low (6.2%) and did not differ among the tree species plots (Jones 2016). Our reported root densities may thus be slightly inflated, but the comparisons among tree plots can be considered valid.

4.3.4 Destructive sampling

At the end of the fourth growing season (2015), two randomly selected seedlings per species were destructively sampled in all single and mixed species plots to assess differences in seedling architecture and allocation to root, stem and leaves in response to growing conditions. In order to be considered for excavation seedlings needed to fulfil two criteria: (1) the seedlings had to be representative of the plot average with respect to height and crown size and (2) the two seedlings needed to be at least 10 m apart to be considered independent of each other. The chosen seedlings were then carefully excavated using a combination of pitchforks and hand trowels, taking care to extract the root-system as intact as possible. Excavated seedlings were then wrapped in plastic bags and frozen at -20°C prior to being taken back to the lab for further measurements. Individual seedlings were taken out of the freezer in random order and thawed prior to determining shoot height, root collar diameter and height/diameter (h/d) ratio, as well as maximum crown and root-system radius. Crown radius was determined by measuring the diameter at the widest point of each crown, while the radial extent of each root-system was estimated by averaging the lengths of the three longest lateral roots.

Populus tremuloides leaves were removed immediately after thawing and dried at 70° C until constant weight, except for a small subsample of randomly selected leaves from each seedling which was scanned, using a LI-3100C Leaf Area Meter (LI-COR Biosciences, Lincoln, NE, USA), and subsequently dried and weighed to determine specific leaf area (SLA, cm^2 g^{-1}). To facilitate the removal of needles, all conifer seedlings were allowed to air-dry in large open paper bags for three days prior to processing. Prior to drying, a small subsample of fresh *P. banksiana* and *P. glauca* needles was collected from each seedling. Fresh needles were scanned using a flat-bed scanner (EPSON Perfection V700) and projected needle area was determined using ImageJ image analysis software (https://imagej.nih.gov/ij/). This method likely resulted in a systematic underestimation of needle areas due to the fact that needles are not flat. However this was not a problem for this study since we only intended to compare needle area within the same species. Total leaf/needle dry mass (g) and the specific leaf area (SLA, cm^2 g^{-1}) for each species were used to estimate the total one-sided leaf/needle area for each seedling. Once leaves and needles were removed, roots and stems were separated and dried at 70° C until constant weight. Root, stem and leaf mass in combination with total seedling dry mass, were used to calculate root, stem and leaf mass fractions (RMF, SMF, LMF; $g_{leaf,\ root,\ stem}$ g_{total}^{-1}) for each excavated seedling.

4.3.5 Root identification using species-specific primers

DNA extracted from root samples was used to determine the impact of topsoil materials and neighbour species identity on the local maximum rooting depths for all three tree species. A percussion drill set, consisting of a jackhammer and a coring tool (100 × 5 cm) equipped with a plastic liner (Eijkelkamp Soil & Water, Giesbeek, NL), was used to collect 1m long undisturbed soil core samples in August 2015. Soil cores were collected at the point of minimum distance (0.5 m) between randomly selected pairs of neighbouring trees that were representative of the plot in terms of mean height. A total of two cores were collected in each single-species plot, whereas in mixed-species plots two cores were collected for each possible neighbour species combination (i.e. *P. tremuloides - P. banksiana, P. tremuloides - P. glauca* and *P. banksiana - P. glauca*).

Plastic liners were sealed immediately after extraction, labelled and stored in a freezer at -20°C. Frozen soil cores were taken from the freezer in random order and allowed to thaw for about one hour at room temperature. The 1m long cores were then cut into 10 cm depth increments and roots contained within each core section were carefully extracted under running water using a 250 µm sieve. Extracted roots were carefully cleaned under running de-ionized water to remove any adhering soil particles. Clean roots were placed on paper towels to remove excess moisture, then temporarily stored at -20°C in labelled aluminum pouches and subsequently freeze-dried. Freeze-dried root samples were weighed and then used to extract DNA in order to determine presence/absence of each of each species at each depth increment. To increase the likelihood of detection, freeze-dried root samples were pooled by plot, neighbour species combination and depth increment and subsequently ground in a TissueLyser II ball mill (Qiagen Inc., Missisauga, ON, Canada). Total genomic DNA was extracted from the ground root samples using a commercial PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) with slight modifications to improve yield and quality. The extracted DNA was further purified by ethanol precipitation. The presence of unique bands corresponding to either of the species signified their presence in the root materials at the respective depth in the soil. The DNA signatures of the three target species were established at a non-coding chloroplast region, the *trn*L intron intergenic spacer. From the genomic DNA material extracted, segment within the region was amplified by polymerase chain reaction (PCR) using species-specific primers. We used a universal forward primer (Primer-C (5’-CGAAATCGGTAGACGCTACG-3’) (Taberlet et al. 1991)) and three species-specific reverse primers developed in this study, Trem404a (5’-CAGATTCGAGTCGGTTGTC-3’) for *P. tremuloides*, Glau277 (5’-CACATTCCCACTTTTTAGGTG-3’) for *P. glauca* and Bank240 (5’-CTCTCTCCATTTTTGAGGTGT-3’) for *P. banksiana*. The PCR was done in volumes totaling 25 µL: 5.5 µL autoclaved deionized water, 2.5 µL of forward primer C at 10 µM, 2.5 µL of reverse primer

(Trem404a or Glau277 or Bank240) at 10 µM, 12.5 µL of EconoTaq PLUS 2X Master Mix (Lucigen Corp., Middleton, WI, USA), and 2 µL of 1-10 ng/µL of DNA template. Amplifications were performed using an Eppendorf Mastercycler Pro S gradient thermal cycler (Model 6321; Eppendorf Canada, Mississauga, ON, Canada). Thermal cycler conditions: 94 °C for 5 min, 35 cycles of 94 °C for 60 s, 60 °C for 60 s, 72 °C for 80 s, followed by a final extension of 72 °C for 30 min. PCR products were visualized right away by gel electrophoresis on a 10 cm long x 15 cm wide gel slab, 1.5 % agarose, with SYBR® Safe DNA gel stain, ran in 2-tiers at 100V for 35 min (See Protocol of PCR Amplicon Visualization by Gel Electrophoresis). Amplicon lengths were compared with a 700 base-pair (bp) ladder ran simultaneously on the gel. PCR run with reverse primer Trem404a produced amplicons of around 400 bp from aspen DNA extracts, but none from spruce and pine extracts. PCR ran with reverse primer Glau277 produced amplicons of around 270 bp from spruce DNA extracts, but none from aspen and pine extracts. PCR run with reverse primer Bank240 produced amplicons of around 270 bp from *P. banksiana* DNA extracts, but none from *P. glauca* and *P. tremuloides* extracts. A more precise measurement of the amplicon lengths was done using fluorescent amplified fragment length polymorphisms (FAFLP) (Randall et al. 2014b). First, PCRs were rerun on the DNA extracts of all three target species using primer sets VIC-C/Trem404a, VIC-C/Glau277 and VIC-C/Bank240, respectively. The forward primer VIC-C is a 5' fluorescently labelled primer C. After PCR, the products were processed for FAFLP. The fragment lengths measured from *P. banksiana*, *P. glauca* and *P. tremuloides* were 265, 276 and 393-398 bp, respectively.

4.3.6 Soil temperature and water availability

All single and mixed species plots were equipped with MPS-2 soil water potential sensors and EM50 data loggers (Decagon, Pullman, WA, USA) in 2013 to monitor how soil type and planting treatment affected water availability and soil temperature. Sensors were inserted at the center of each reconstructed soil horizon and soil water potentials and soil temperature were recorded every two hours over the course of each growing season (May – September). Readings from all depths were used to calculate a mean daily temperature for the entire soil profile. Mean daily soil temperatures >5ºC were added up over the course of the growing season to calculate growing degree days (GDD_5). This base temperature was chosen because it has been shown as a critical threshold below which, particularly for aspen, root physiological activity is significantly reduced (Wan et al. 1999; Landhäusser et al. 2001).

4.3.7 Spatial and temporal niche segregation

Aboveground niche segregation was assessed using mean seedling heights (vertical) and crown radii (horizontal) measured on the two excavated seedlings per species and plot (n=6). Horizontal belowground niche separation was determined using the mean root-system radii measured on the same excavated seedlings. Given the plant spacing of 1 × 1 m, neighbouring trees were considered to have horizontally overlapping spatial niches (above- and belowground) if the sum of their average crown or root-system radii were > 1 m.

Vertical belowground niche separation was assessed using a combined approach. DNA analysis of mixed root samples (see above) was used to determine the presence of one or both species in each 10 cm depth increment of each core sample (n=3). The resulting maximum rooting depths for each species were averaged and compared for each neighbour species combination and soil type. Any significant difference in maximum rooting depth between neighbouring species was considered indicative of vertical belowground niche segregation. Alongside the DNA analysis, minirhizotron image were analyzed to calculate mean root-length densities for each 10 cm depth increment in all three monospecific plots as well as the mixtures on both soil types. We used the resulting root density profiles to visually assess the depth(s) at which root-length densities peaked for each individual species as well as in mixture on both soil types. This additional approach was chosen in case the DNA-analysis revealed no vertical separation between neighbouring individuals. In that case, root densities peaking at different depths for each species could still indicate some degree of vertical niche segregation when all three species grew in mixture. The root-length densities presented for the mixtures represent a combination of all three species.

In order to detect any potential complementarity in soil resource use, i.e. temporal niche segregation, between the different species, we first calculated the daily mean soil water potentials (2015) for the first and second soil horizon in all monospecific plots on both soil types (n=3). We considered a drop in daily mean soil water potential below field capacity (-33 kPa) to indicate the onset of water uptake by the planted seedlings at the beginning of the growing season. We then compared the mean Julian dates on which this threshold was first crossed, using any significant differences in the onset of water uptake as an indication of temporal niche segregation among species. Likewise, we tracked and compared the dates on which daily mean soil water potentials dropped below wilting point. This was done to determine the duration of the period during which seedlings could readily extract water from varying depths throughout the reconstructed soil horizons.

4.3.8 Assessment of overyielding in mixture

Because our experiment was designed as a replacement series with three species, where densities are the same in mixed and monospecific plots, relative yields (RY) in mixed plots were calculated as:

$$RY = Y_{mixed} / Y_{mono}$$

$$\text{with } Y_{mono} = (Y_1+Y_2+Y_3) / 3$$

where Y_{mixed} is a measure of multispecies productivity in mixed species plots, Y_1-Y_3 are measures of productivity for each individual species grown in monospecific plots and RY values >1 indicate overyielding (de Wit 1960; Weigelt and Jolliffe 2003; Kikvidze and Armas 2010). Due to the fact that our assessments of productivity were not based on actual yields, i.e. a complete harvest of all trees in each plot, but on a relatively small sample, we additionally compared means using one-way ANOVA to ensure the calculated mean productivity in mixture was actually statistically different from that averaged across the three monospecific plots (n=3). Consequently, we only considered overyielding to occur where Y_{mixed} / Y_{mono} resulted in values >1 and Y_{mixed} and Y_{mono} were statistically different from each other.

4.3.9 Statistical analyses

Differences in abiotic conditions and overall seedling growth performance were assessed using soil type as predictor variable. Subsequently, we tested whether growing in mixture would result in significant differences in seedling architecture and biomass allocation, compared to seedlings grown in monospecific plots. This test was conducted separately for each species and both soil types using planting treatment (mixed vs. monospecific) as predictor variable. To determine if the responses to mixing were modified by soil type, we also compared the mean relative differences (%) in architectural traits and allocation patterns between seedlings of each species growing in mixture and seedlings grown as a single species using soil type as predictor variable. The comparison of relative, rather than absolute differences was chosen to avoid any confounding effects arising from potential differences in seedling size between the two soil types.

The individual species' maximum rooting depths, as determined through DNA analysis, were compared for each species pairing in mixture on both soil types using species as predictor variable. The mean Julian dates on which soil water potentials first dropped below field capacity in each of the monospecific plots on both soil types were compared using species as predictor variable.

Overyielding was assessed separately for each soil type. Aboveground overyielding was assessed by comparing mean shoot mass (g seedling^{-1}) and mean leaf area (m^2 seedling^{-1}), averaged across all three species growing in mixture (Y_{mixed}), with mean shoot mass and leaf area in the three monospecific plots

(Y1-Y3) (n=3). Belowground overyielding was likewise assessed using mean root mass (g seedling^{-1}) as well as mean RLD (cm cm^{-3}).

All statistical tests were conducted using one-way ANOVA. Due to low replication we used $\alpha=0.1$ to determine significant effects. Fisher's LSD test was used for comparisons of main effects. All statistical analyses were done using SPSS 20.0 (IBM Corp., 2011).

4.4 Results

4.4.1 Differences in abiotic conditions among soil types

The contrasting topsoil materials exerted a strong influence on edaphic conditions, resulting in two reconstructed soil profiles with very distinct characteristics. Due to its high organic matter content, soil reconstructed with peat generally had much higher moisture retention than soil reconstructed with sand-textured FFM material. During the 2015 growing season (May – September), for example, soil reconstructed with peat was not only significantly wetter ($p=0.033$) but also about 4°C cooler ($p=0.019$) than soil reconstructed with FFM (Table 4.1).

As a result of this high moisture content, soil reconstructed with peat also took about 14 days longer to warm up in the spring than soil reconstructed with FFM. In 2015, soil reconstructed with peat first reached a critical daily mean soil temperature >5°C on May 12, while soil with FFM had already reached a mean temperature of 11.2°C. Over the course of the entire 2015 growing season, this faster warm up was reflected in the 50% higher growing degree days (GDD_5) in soil reconstructed with FFM compared to soil reconstructed with peat ($p=0.019$) (Table 4.1).

4.4.2 Individual species' response to growing in mixture

Four-year old *P. banksiana* growing on soil reconstructed with peat had the same mean height, root-collar diameter (RCD) as well as leaf area and crown radius when grown in mixture or as a single species (Table 4.3). *Pinus banksiana* seedlings growing in monospecific plots on peat did, however, have a 48% greater root system radius (126 cm) than those in mixed plots (85 cm) ($p=0.074$). There were no differences in mean stem, leaf, or root mass and no differences in the stem, leaf and root mass fractions (SMF, LMF and RMF, g g^{-1}) between *P. banksiana* grown in monospecific and mixed plots on peat (Fig. 4.2). When growing in FFM, *P. banksiana* seedlings had the same mean height and leaf area, as well as crown and root system radius in mixed as in monospecific plots (Table 4.2). However, *P. banksiana* seedlings growing in mixed plots had a 23% higher mean RCD ($p=0.006$) and consequently a lower mean h/d ratio than those in monospecific plots ($p=0.098$). *Pinus banksiana* growing on soil reconstructed with FFM also had a 27% greater total seedling mass in mixed than in monospecific plots (*p=0.10*). Specifically, both stem mass (*p=0.033*) and root mass (*p=0.086*) were higher for *P. banksiana* growing in mixture, while leaf mass did not differ significantly. There were no differences in biomass allocation for *P. banksiana* growing in monospecific or mixed plots on FFM (Fig. 4.2).

Picea glauca growing on soil reconstructed with peat showed no difference in height, RCD and seedling dry mass, or any of the other measured parameters among seedlings in mixed or monospecific plots (Table 4.3). Consequently, there were also no differences in allocation to root, stem or leaf mass (Fig. 4.2). When growing in FFM, mean height, RCD and h/d ratios, as well as leaf area, crown, and root-system diameter did not differ between *P. glauca* seedlings grown in mixed or monospecific plots (Table 4.3). *Picea glauca* growing in mixed plots on soil reconstructed with FFM did, have a lower mean root mass than those in monospecific plots (*p=0.036*) (Table 4.2). However, RMF, SMF and LMF did not differ between *P. glauca* growing in monospecific or mixed plots on soil reconstructed with FFM (Fig. 4.2).

Populus tremuloides growing in peat showed no significant differences in any of the measured architectural parameters in response to growing in mixed rather than monospecific plots (Table 4.2). The exception was a trend towards a higher mean root-system radius for *P. tremuloides* in mixed (4.6 ±1.4 m) than monospecific plots (3.3 ±1.2 m) on soil reconstructed with peat (*p=0.11*). *Populus tremuloides* growing on peat also had the same biomass allocation pattern in mixed and monospecific plots (Fig. 4.2). When growing on soil reconstructed with FFM, *P. tremuloides* seedlings did not differ in any of the measured architectural parameters when growing in mixed or monospecific plots (Table 4.2). However, *P. tremuloides* growing on soil reconstructed with FFM did show differences in biomass allocation in response to mixing, with a 16% higher RMF (*p=0.004*) and 10% lower SMF (*p=0.031*) in mixed plots compared to monospecific plots, while LMF did not differ (Fig. 4.2).

4.4.3 Overyielding in mixture

Based on our findings, there was no indication of either aboveground or belowground overyielding when the three species were grown in mixture on soil reconstructed with peat. Averaged across species, the average seedling in mixture had the same mean shoot and root mass as the average seedling grown in monospecific plots (Table 4.3). Likewise, mean leaf area and root-length density did not differ between mixed and monospecific plots on soil reconstructed with peat.

When growing as a mixture on FFM, we found evidence of overyielding, but only belowground. Root mass was on average 45% higher in the mixed species plots, with an average of 196 g of roots per seedling in the mixed plots compared to 135 g in the monospecific plots (*p=0.016*). Similarly, the mean root-length density was 50% higher in mixed (21 cm cm^{-3}) than in monospecific plots (14 cm cm^{-3}) on soil reconstructed with FFM (*p=0.016*). However, there was no indication of aboveground overyielding when the soil was reconstructed with FFM (Table 4.3).

4.4.4 Spatial niche segregation

On soil reconstructed with peat, there was still complete horizontal segregation among the crowns of all three species when growing in mixture after four growing seasons. Given the mean crown radii of 37 cm for *P. banksiana*, 23 cm for *P. glauca* and 40 cm for *P. tremuloides* (Table 4.3) and the plant spacing of 1 × 1 m, the gap between neighbouring crowns, averaged 40 cm between *P. banksiana* and *P. glauca*, 23 cm between *P. banksiana* and *P. tremuloides* and 47 cm between *P. glauca* and *P. tremuloides*. When grown in monospecific plots on peat, the horizontal gap among neighbouring crowns averaged 18 cm for *P. banksiana* and 58 cm for *P. glauca*, while the crowns of *P. tremuloides* in overlapped by 6 cm.

There was already some degree of vertical stratification in the developing canopy when all three species grew in mixture on soil reconstructed with peat. With a mean height of 140 cm, *P. tremuloides* seedlings were on average 46 cm taller than *P. banksiana* and 63 cm taller than *P. glauca* (*p=0.012*) (Table 4.2).

With mean root-system radii of 85 cm *P. banksiana*, 122 cm for *P. glauca* and 455 cm for *P. tremuloides*, there was clearly no horizontal separation among the root systems of all three species when growing in mixture on peat (Table 4.3). There was also no horizontal separation among neighbouring root systems when each species was grown in monospecific plots, with mean root-system radii of 126, 119, and 329 cm for *P. banksiana*, *P. glauca* and *P. tremuloides*, respectively (Table 4.2).

Moreover, the DNA analysis of mixed root samples revealed no clear vertical segregation among the three species when grown in mixture on soil reconstructed with peat. There were no significant differences in maximum rooting depth, i.e. no vertical separation among root systems, when *P. banksiana* grew next to *P. glauca*, or *P. glauca* next to *P. tremuloides* (Fig. 4.3). There was, however, a trend towards vertical separation, i.e. different maximum rooting depths where the root systems of *P. banksiana* (83±6 cm) overlapped with those of *P. tremuloides* (47±31 cm) (*p=0.11*).

Pinus banksiana and *P. glauca* showed no difference in maximum rooting depth in response to mixing, with an average depth of 72 and 49 cm, respectively (both *p>0.31*). However, *P. glauca* was the only species that showed a significant change in maximum rooting depth in response to neighbour species identity. When *P. glauca* grew next to *P. tremuloides* it had a maximum rooting depth of 37 cm, while when growing next to *P. banksiana* it reached a maximum rooting depth of 80 cm ($p=0.092$).

The analysis of minirhizotron images taken in 2015 indicated that, despite the vertical overlap among the root systems of neighbouring trees, there were differences in fine root distribution patterns and the depths at which root-length densities peaked for each of the three species in monospecific plots (Fig. 4.4). *Pinus banksiana* growing in monospecific plots on soil reconstructed with peat had a mean root-length density (RLD) of 9 cm cm^{-3}. Root-length densities were relatively uniform in the first and second soil horizon (peat and Bm), with a peak in RLD (17 cm cm^{-3}) occurring at the boundary between the Bm and C subsoil horizons at 50-60 cm soil depth. *Picea glauca* grown in monospecific plots as a single species on soil reconstructed with peat had a similar root distribution and reached the same mean RLD as *P. banksiana* (9 cm cm^{-3}); however, *P. glauca* RLD peaked at a shallower depth (30-40 cm), just below the boundary between peat and Bm, reaching an RLD of 18 cm cm^{-3}. *Populus tremuloides* growing in monospecific plots on soil reconstructed with peat generally had a low mean RLD (5 cm cm^{-3}), with peak RLD (25 cm cm^{-3}) occurring within the peat at 10-20 cm soil depth. Moreover, *P. tremuloides* maintained very low root densities in the subsoil.

When all three species were grown together in mixture on soil reconstructed with peat, the combined root distribution pattern indicated two distinct peaks, one at 10-20 cm in the peat (21 cm cm^{-3}) and a second at the transition between Bm and C subsoil at 50-60 cm soil depth (24 cm cm^{-3}).

Although seedlings growing on soil reconstructed with FFM were already significantly taller than their counterparts on peat, there was still clear horizontal segregation among neighbouring crowns when all three species were grown in mixture. However, here the mean distances between neighbouring crowns were generally lower than what had been observed on peat. With mean crown radii of 53 cm for *P. banksiana*, 20 cm for *P. glauca* and 45 cm for *P. tremuloides* (Table 4.2), the gap between neighbouring crowns averaged 27 cm between *P. banksiana* and *P. glauca,* 35 cm between *P. glauca* and *P. tremuloides* and only 2 cm between *P. banksiana* and *P. tremuloides*. With a mean height of 228 cm, *P. tremuloides* were about 80 cm taller than *P. banksiana* which were, in turn, 80 cm taller than *P. glauca* ($p<0.001$), indicating that there was already vertical stratification in the canopy of mixed plots on FFM.

The mean root-system radii of 126 cm for *P. banksiana*, 83 cm for *P. glauca* and 269 cm for *P. tremuloides*, indicated no horizontal separation among the root systems of all three species growing in mixture on soil reconstructed with FFM (Table 4.2). Similarly, there was no horizontal separation among neighbouring root system in the monospecific plots on FFM, with mean root-system radii of 126, 119 and 329, respectively (Table 4.2).

Using DNA markers to determine presence/absence of roots belonging to each of the three species, we found no indication of vertical segregation among the root systems of neighbouring seedlings growing in mixture on FFM, irrespective of which two species grew next to each other (Fig. 4.3). Moreover, mixing had no effect on maximum rooting depth for any of the three species. On average, *P. banksiana* reached a maximum rooting depth of 77±15 cm, while *P. glauca* reached 58±12 cm and *P. tremuloides* 83±20 cm on soil reconstructed with FFM.

The minirhizotron images revealed that, on soil reconstructed with FFM, *P. banksiana* grown in monospecific plots generally had a high mean RLD throughout the whole soil profile (21 cm cm^{-3}), with RLD peaking at 39 cm cm^{-3} above the transition between the Bm and C horizon at 40-50 cm soil depth, followed by a marked drop in RLD around 70 cm soil depth (Fig. 4.4). *Picea glauca* growing in monospecific plots on FFM reached a mean RLD of 11 cm cm^{-3} and RLD peaked once at 20-30 cm soil depth (18 cm cm^{-3}), at the transition between FFM and Bm, followed by a second smaller peak at 70-80 cm (13 cm cm^{-3}) in the C horizon. *Populus tremuloides* growing in monospecific plots on FFM had a mean RLD of 10 cm cm^{-3} and the root distribution pattern displayed two distinct peaks in mean RLD, one at the transition between FFM and Bm (16 cm cm^{-3}) and a second one just below the boundary between the Bm and C horizon at 50-60 cm depth (17 cm cm^{-3}). When all three species were grown together in mixture on soil reconstructed with FFM, the mean RLD for the entire soil profile was 21 cm cm^{-3} and RLD peaked once below the transition between the FFM and Bm horizons (39 cm cm^{-3}) and again at 70-80 cm (32 cm cm^{-3}) in the C horizon.

4.4.5 Temporal niche segregation

On soils constructed with peat, there was no detectable difference in the onset of water uptake in the spring among all three species. Averaged across all three species growing in monospecific plots on peat, soil water potentials (mean ±SD) dropped below field capacity on JD153 (±22 days) in the first horizon (peat) and on JD175 (±24 days) in the second horizon (Bm). With the exception of a five-day period (JD 188-192) in one of the monospecific *P. banksiana* plots, soil water potentials never dropped below wilting point in the first two soil horizons of both mixed and monospecific plots, when soil was reconstructed with peat (see also Fig. A1).

On soil reconstructed with FFM, in contrast, there were clear differences among species with respect to the timing of the onset of water uptake in the spring. Daily mean soil water potentials in monospecific

P. banksiana plots first fell below field capacity in the first horizon (FFM) on JD123 (±1 day), significantly sooner than in *P. tremuloides* plots, where field capacity was first reached 23 days later on JD146 (±3 days) (*p=0.028*). *Picea glauca* plots reached this point on JD134 (±13 days), which was not different from *P. banksiana* (*p=0.188*) or *P. tremuloides* (*p=0.149*). Soil water potentials in the second soil horizon (Bm) first dropped below field capacity on JD135 (±2 days) for *P. banksiana* in monospecific plots, which was 22-23 days sooner than for both monospecific *P. glauca* and *P. tremuloides* plots (both *p=0.001*), where soil water potentials in the second soil horizon dropped below field capacity on JD155 (±1 day) and JD156 (±5 days), respectively. In mixed plots on soil reconstructed with FFM, field capacity was reached on JD 131 (±9 days) in the first soil horizon, which was not significantly different from the monospecific plots. However, in the second soil horizon field capacity was reached on JD 147 (±5 days), which was 13 days later than in monospecific *P. banksiana* plots (*p=0.007*), but 8-9 days sooner than in both monospecific *P. glauca* (*p=0.033*) and *P. tremuloides* plots (*p=0.029*). In the first two soil horizons of *P. banksiana* plots on FFM, daily mean soil water potentials dropped below wilting point as early as JD 134 (±9days) and JD 166 (±27days), while in monospecific *P. tremuloides* plots wilting point was on average reached 41 days later on JD 175 (±13days) in the first soil horizon and only in one plot in the second horizon on JD 188. The first soil horizon of monospecific *P. glauca* plots reached wilting point on JD 154 (±24days), which was not different from monospecific *P. banksiana* and *P. tremuloides*. Only in one *P. glauca* plot, was wilting point reached in the second soil horizon on JD 186. In the first soil horizon in mixed species plots, wilting point was reached around the same time as in *P. banksiana* and *P. glauca* plots (JD 145 ±6days), but 30 days earlier than in monospecific *P. tremuloides* plots. Soil water potentials in the mixed plots never dropped below wilting point in the second horizon throughout the 2015 growing season (see also Fig. A2).

4.5 Discussion

In this study we had hypothesized that, regardless of soil type, the three tree species used in this study were functionally different enough to result in aboveground and belowground overyielding due to niche complementarity when planted in an even mixture. However, we found that, at this early stage of forest development, overyielding only occurred belowground and only on one of the soil types tested. The observed belowground overyielding in mixed plots was only observed on soils reconstructed with FFM and appeared to be mainly driven by an increase in root mass in *P. banksiana* and to a lesser extent *P.*

tremuloides, and despite the lower root mass observed for *P. glauca* in mixture. The resulting overall increase in root mass likely also explains the higher mean RLD found in mixed plots on FFM (Table 4.2). Increasing root mass has been shown to be a more effective way to increase root densities than changing root morphology, i.e. specific root length (g cm^{-1}) (Freschet et al. 2015).

For *P. banksiana* growing on FFM, mixed species planting resulted in higher stem, root and total seedling mass. This increase in productivity indicates that, despite the same overall stem density, intra-specific competition in *P. banksiana* plots exceeded inter-specific competition in mixed plots. Our soil water data suggest that *P. banksiana* seedlings in monospecific plots experienced considerable water stress early in the growing season, with soil water potentials dropping below wilting point (-1500 kPa) on May 14 in the first and June 15, 2015 in the second soil horizon. In the first soil horizon of mixed plots on FFM, soil water potentials also dropped below wilting point around the same as in *P. banksiana* plots (May 16, 2015); however, soil water potentials in the second horizon never reached wilting point at any time during the 2015 growing season, suggesting less competition for water in mixed plots. Height-diameter ratios are commonly used as an indication for the degree of aboveground competition and lower h/d ratios are typically associated with reduced competitive pressure (e.g. Erickson et al., 2009; Setiawan et al., 2017; Van de Peer et al., 2017). The lower mean h/d ratio found for *P. banksiana* in mixed plots on FFM, therefore suggests intra-specific competition also exceeded inter-specific competition aboveground, despite the fact that *P. tremuloides* in mixed plots were already significantly taller, thus potentially reducing light availability for *P. banksiana*.

Despite a trend towards greater root mass, there was no significant increase in overall productivity for *P. tremuloides* growing in mixed plots on FFM. Instead, *P. tremuloides* seedlings simply allocated relatively more to root and less to stem mass, while maintaining the same leaf mass fraction. These findings suggest that *P. tremuloides* might have experienced relatively less aboveground, but more belowground competition when growing in mixed species plots on FFM. The presence of *P. banksiana* and *P. glauca* likely resulted in increased competition for water, while simultaneously reducing light competition, when compared to monospecific *P. tremuloides* plots. Poorter et al. (2011) used published data to create dose-response curves and quantify the impacts of a whole variety of environmental stimuli, including drought stress, on allocation to leaf, stem and root mass. They found that plants respond to prolonged water stress with an increase in root mass fraction, at the expense of stem mass fraction, while leaf mass fraction generally remains stable. The resulting increase in root mass relative to leaf mass, i.e. higher root:leaf mass ratio, has been shown to be an effective mechanism to increase drought resistance in hybrid poplars (Tschaplinski et al. 1998). It should also be noted that, given the uniform age and size of *P. tremuloides* seedlings in the mixed and monospecific plots, it is unlikely that the observed change in allocation was a sign of allometric growth, or ontogenetic drift (e.g. Reich, 2002; Weiner, 2004). Instead, it indicates a plastic response to increased belowground competition in mixed plots on FFM.

The lower mean root mass found in *P. glauca* in mixed plots on FFM suggests that for this species inter-specific competition also exceeded intra-specific competition. However, although this reduction occurred belowground, our soil water data suggest that the reason was likely not increased belowground competition. In the first soil horizon of *P. glauca*, both field capacity and wilting point were reached around the same date as in mixed plots on soil reconstructed with FFM. Moreover, in the second soil horizon in the *P. glauca* plots, wilting point was reached on June 8, 2015, whereas soil water potentials never dropped below wilting point in the second horizon in mixed plots. It is therefore more likely that the reduced productivity was due to increased aboveground competition for light with the significantly taller *P. banksiana* and *P. tremuloides*. In a study conducted in mixed boreal forest in Quebec and Ontario, Canada, young *P. tremuloides* have been shown to reduce light availability and thus negatively affect the growth of shade-tolerant *Picea mariana* (Mill.) BSP during early stages of forest development (Cavard et al. 2011).

Both *P. banksiana* and *P. tremuloides* are fast-growing resource-acquisitive species, while *P. glauca* is a relatively slow-growing and resource-conservative species (Reich et al. 1998b). Based on findings from a large-scale biodiversity experiment comprising 24 tree species, Fichtner et al. (2017) suggested that the underlying mechanisms for overyielding in tree mixtures differ based on functional types. Fast-growing, resource-acquisitive species appear to benefit mostly from reduced competition when growing in mixture, while overyielding in slow-growing, resource conservative species can be attributed to some form of facilitation. Our findings seem to support this notion, as the observed overyielding in mixed plots on FFM appeared to be mainly driven by reduced competitive pressure for the two fast-growing species, particularly *P. banksiana*.

In our study, the root-systems of all three species overlapped both horizontally and vertically in the interspaces between the planted seedlings, thus indicating a lack of clear spatial segregation in this zone. However, minirhizotron images taken in monospecific plots revealed distinct root distribution patterns for each of the three species. Fine-root densities peaked at different depths for each species, suggesting that there may have been some degree of vertical separation in mixture with respect to the depths at which each species maintained its highest fine-root densities. On FFM, these complementary rooting patterns appeared to have resulted in a more complete occupation of the entire soil profile, which was reflected in the higher mean root-length density in mixed plots. More importantly though, the reduced competition in mixed plots on FFM seemed to be mostly driven by distinct temporal resource use patterns, arising from phenological differences among species, most notably between the evergreen *P. banksiana* and the deciduous *P. tremuloides*. The slow drop in water potentials observed in *P. tremuloides* due to the need to produce new foliage each spring, created a temporal niche during which *P. banksiana* seedlings experienced less water stress than those growing in intra-specific competition.

Interestingly, planting a mixture of the same three species on peat did not result in overyielding, even despite the fact that we found some vertical segregation between *P. banksiana* and *P. tremuloides*. Our soil water data suggest that the lack of overyielding was caused by an absence of the distinct temporal patterns in water uptake that was observed on soil reconstructed with FFM. They also indicate that the soil profile reconstructed with peat remained much wetter and colder than the soil profile reconstructed with FFM. Excessive moisture and low soil temperatures represent two major limiting factors for root growth in the boreal forest as they have been shown to reduce microbial activity and the associated nutrient cycling as well as impede root water uptake (Landhäusser et al. 2001; Wan et al. 2001; Peng and Dang 2003; Morris and Blackwood 2015). The prevalent conditions in soil profiles reconstructed with peat therefore likely prevented temporal niche segregation among the tree species. Our findings stand in contrast to other studies which found that trees showed better initial growth performance on peat soils than on FFM, when peat was mixed with underlying mineral soil (Pinno et al. 2012). This mixing tends to improve drainage and thus eliminate some of the negative effects associated with pure peat soils (Moskal et al. 2001; Hemstock et al. 2010; Ojekanmi and Chang 2014).

It should also be noted that the relationships observed at this early stage of forest development can be expected to shift in favor of the shade-tolerant *P. glauca* with increasing stand age (e.g. Cavard et al., 2011). After only three growing seasons, *P. tremuloides* did not yet appear to facilitate the growth of *P. glauca*. However, mature *P. tremuloides* have been shown to facilitate the growth of shade-tolerant *P. glauca* by improving soil conditions and nutrient cycling through litter inputs, suppressing herbaceous competition, reducing exposure to environmental extremes and reducing pest pressure (Man and Lieffers 1999; Légaré et al. 2005; St. Clair et al. 2013). Based on tree ring analysis in mature stands of mixed boreal forest, Cavard et al. (2011) found that the facilitative effect of *P. tremuloides* typically becomes apparent as the trees reach an age of about 40-50 years.

We had further hypothesized that all three species would show some degree of plasticity in architecture and/or biomass allocation in response to inter-specific competition, as well as to the different soil conditions. Overall we observed some form of plasticity in response to mixed species planting, between the two soil types, in all three species. *Pinus banksiana* displayed plasticity in mean root-system radius in response to mixing on peat, where mean root-system radius decreased, and on FFM, where mixing had the opposite effect. On FFM, *P. banksiana* also showed plasticity in its height-diameter ratio, despite there being no difference in mean height between mixed and single species plots. However, *P. banksiana* did not display any plasticity in biomass allocation in response to mixing and the two soil types. In contrast, *P. tremuloides* did not show any architectural plasticity in response to mixing on either soil type. It did, however, display plasticity in biomass allocation in response to mixed species planting on FFM. *Picea glauca* did see a decrease in total root mass when growing in mixed species plots on FFM, but not on peat. However, the fact that stem, leaf and total mass were also nominally lower, even if the differences were not statistically significant, suggests that this decrease in root mass in response to mixing on FFM was more likely a sign of reduced productivity rather than plasticity in allocation. *Picea glauca* in mixed plots on peat did show plasticity in local maximum rooting depth with respect to neighbour species identity, doubling maximum rooting depth when growing next to *P. banksiana* rather than *P. tremuloides*.

Based on the literature we had expected the two fast growing species, *P. banksiana* and *P. tremuloides*, to show a greater degree of plasticity than the slow growing *P. glauca* both in response to soil type and inter-specific competition (e.g. Bauhus and Messier, 1999; Messier et al., 2009). However, in our study, *P. banksiana* displayed the highest degree of plasticity, while *P. glauca* and *P. tremuloides* displayed similar degrees of plasticity, albeit in different form.

In summary, our findings show that each of the three species displayed unique plasticity in their response to growing in mixed rather than monospecific plots and that these responses were modified by the contrasting soil conditions associated with the two reconstructed profiles. Moreover, we were able to show that a mixture of P. *banksiana, P. glauca* and *P. tremuloides* can result in overyielding, particularly belowground, during the early years of stand development. The observed overyielding was found to be a consequence of temporal, rather than spatial niche segregation, likely driven by differences in phenology (i.e. deciduous vs. evergreen) between the three species. However, the occurrence of this temporal niche segregation appears to be dependent on favourable growing conditions that allow for unimpeded seedling growth and root system development for these inter- and intra-specific interactions to play out. Where soil conditions, such as excessive moisture content and low soil temperature limited growth and physiological activity (i.e. water uptake) at the beginning of the growing season, particularly for the two conifer species, no temporal niche segregation occurred and consequently no overyielding was observed.

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4.7 Tables

Table 4.1 – Mean (±SD) soil water potential, temperature and growing degree days (GDD_5, >5ºC) for the two reconstructed soil profiles during the 2015growing season (May-September).

soil type	**mean water potential (kPa)**	**mean soil temperature (°C)**	**GDD_5**
FFM	-622±293**a**	15.2±0.4**a**	1129±42**a**
peat	-76±43**b**	11.5±1.7**b**	749±167**b**

Note: Different letters indicate significant differences (α=0.1, n=3).

Table 4.2 - Architectural parameters (mean ±SD) of 4-year old seedlings grown either in monospecific plots or mixtures on two contrasting soil types (n=6). Different letters indicate significant differences in response to mixing within soil types (α=0.1). No comparisons were made between soil types.

		P. banksiana		***P. glauca***		***P. tremuloides***	
Soil type	**Parameter**	**monospecific**	**mixed**	**monospecific**	**mixed**	**monospecific**	**mixed**
peat	height (cm)	104±22	94±29	68±16	77±16	138±38	141±46
	RCD (cm)	2.7±0.5	2.2±0.8	2.0±0.7	2.2±0.4	2.0±0.5	1.9±0.7
	h/d ratio (cm cm^{-1})	39±5	43±6	36±8	35±3	71±12	74±8
	total mass (g)	426±252	346±245	242±133	238±94	448±321	464±359
	stem mass (g)	182±119	153±116	102±56	99±34	159±114	165±121
	leaf mass (g)	193±111	152±99	101±59	100±48	57±39	64±57
	root mass (g)	52±26	41±30	40±20	40±13	232±172	236±190
	leaf area (m^2)	0.9±0.1	0.7±0.5	0.4±0.3	0.4±0.2	0.6±0.4	0.7±0.6
	crown radius (cm)	41±12	37±15	21±5	23±7	53±41	40±27
	root-system radius (cm)	126±40**a**	85±31**b**	119±29	122±24	329±116	455±135
FFM	height (cm)	140±10	149±10	71±9	70±9	231±59	228±61
	RCD (cm)	3.0±0.2**b**	3.7±0.2**a**	1.8±0.4	1.5±0.4	2.9±0.4	3.1±0.9
	h/d ratio (cm cm^{-1})	46±3**a**	41±7**b**	42±12	49±12	79±10	74±18
	seedling mass (g)	649±160**b**	825±179**a**	219±51	174±54	848±273	1057±541
	stem mass (g)	290±69**b**	393±76**a**	89±24	71±26	420±167	466±253
	leaf mass (g)	294±74	347±70	102±22	82±25	117±37	137±69
	root mass (g)	66±19**b**	102±41**a**	29±8**a**	20±5**b**	311±73	454±225
	leaf area (m^2)	1.3±0.3	1.6±0.3	0.5±0.1	0.4±0.1	1.3±0.4	1.5±0.8
	crown radius (cm)	47±17	53±17	22±5	20±5	64±34	45±21
	root-system radius (cm)	109±145	126±36	85±26	83±17	241±28	269±64

Table 4.3 - Above- and belowground productivity (mean ±SD) averaged across all three species growing in monospecific plots (Y_{mono}) or mixture (Y_{mixed}) on both soil types (n=3). Letters indicate significant differences in productivity between mixed and monospecific plots (α=0.1). Relative yield (RY) values >1 indicate overyielding in mixture. * RY values automatically set to 1.0 if the difference between Y_{mono} and Y_{mixed} was statistically non-significant.

Soil type	**Compartment**	**Parameter**	$\mathbf{Y_{mono}}$	$\mathbf{Y_{mixed}}$	**RY**
peat	aboveground	shoot mass (g seedling^{-1})	264±150	244±158	1.0*
		leaf area (m^2 seedling^{-1})	0.63±0.4	0.60±0.4	1.0*
	belowground	root mass (g seedling^{-1})	108±69	105±80	1.0*
		root length density (cm cm^{-3})	8±8	10±9	1.0*
FFM	aboveground	shoot mass (g seedling^{-1})	437±77	499±54	1.0*
		leaf area (m^2 seedling^{-1})	1.0±0.2	1.1±0.1	1.0*
	belowground	root mass (g seedling^{-1})	135±19**b**	196±20**a**	1.45
		root length density (cm cm^{-3})	14±2**b**	21±2**a**	1.53

4.8 Figures

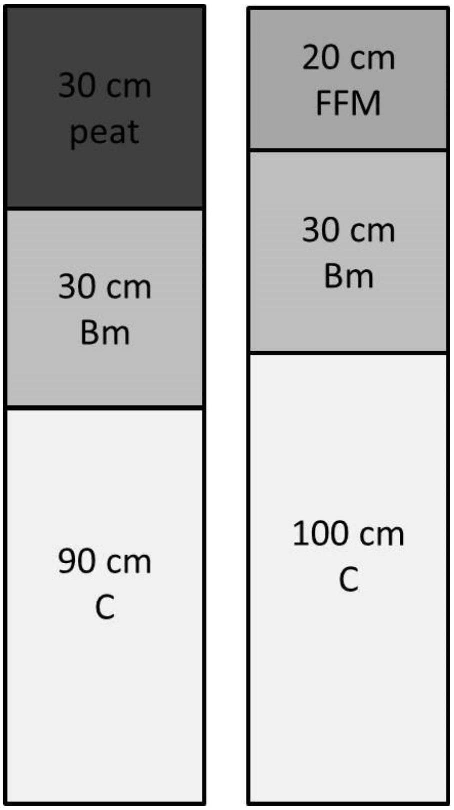

Figure 4.1 - The two reconstructed soil profiles used for this study, comprising two contrasting topsoil materials, peat salvaged from lowlands and sand-textured forest floor material (FFM) salvaged from upland forest, as well as two subsoil materials, Bm salvaged from a shallow depth and C material salvaged from depths >1 m.

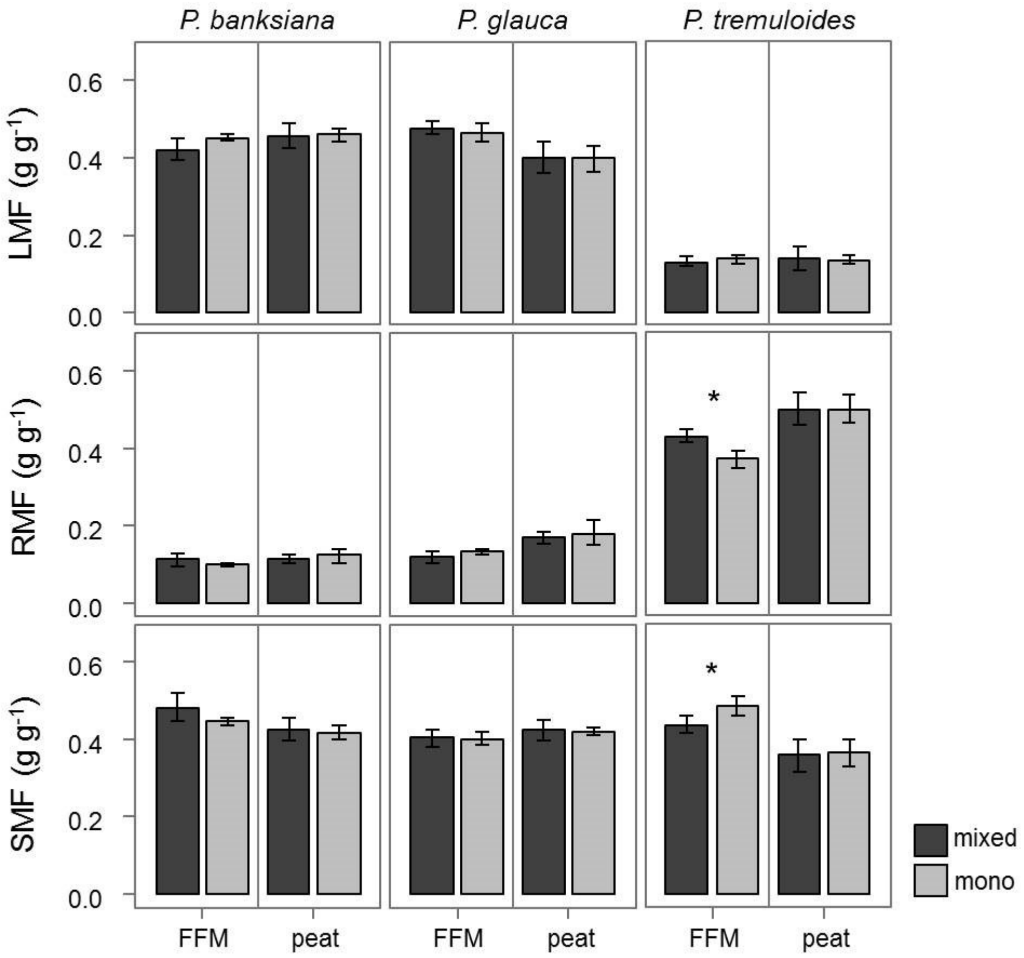

Figure 4.2 - Leaf, root and stem mass fractions (SMF, RMF, LMF, g g^{-1}) for *P. banksiana*, *P. glauca* and *P. tremuloides* growing in mixed or single species plots on on peat and forest floor material (FFM). Error bars represent 90% CI. Asterisks indicate significant differences.

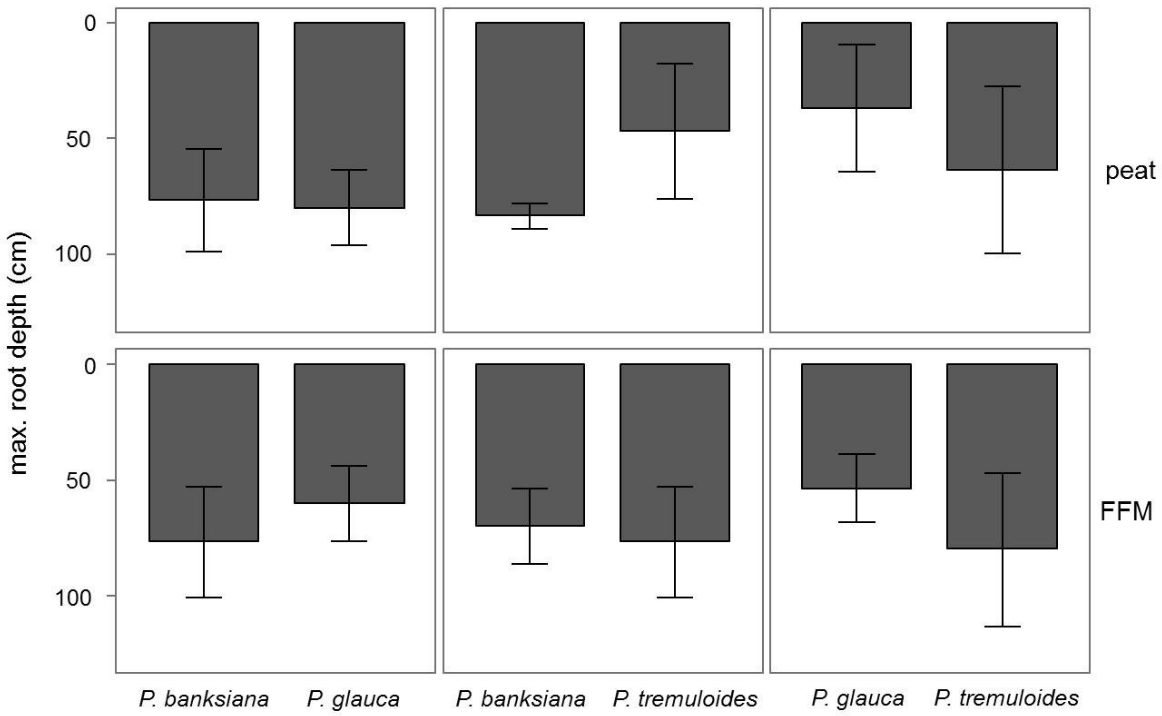

Figure 4.3 – Maximum rooting depths for each of the three different species combinations found in mixed plots on peat and forest floor material (FFM) as determined by DNA-analysis. Error bars indicate 90% CI.

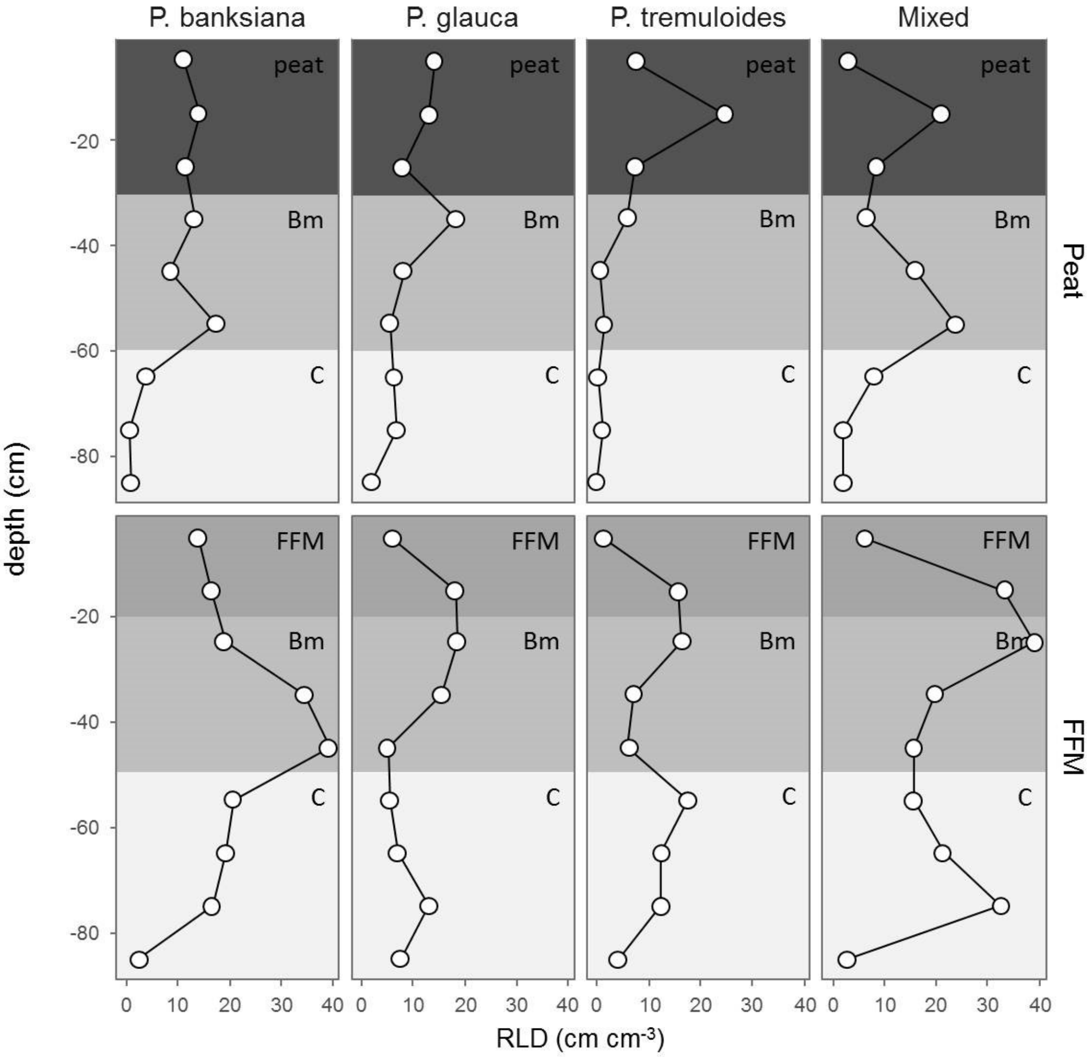

Figure 4.4 - Mean root-length densities (RLD, cm cm^{-3}) by soil depth for *P. banksiana*, *P. glauca* and *P. tremuloides* in single species plots, as well as mixed plots on peat and forest floor material (FFM) (n=3). Root-length densities were obtained from minirhizotron images taken at the end of the third growing season (2015).

CHAPTER 5

General discussion and conclusion

5.1 Summary and synthesis

The main goal of my thesis was to increase our knowledge of belowground processes that affect the growth and development of planted tree seedlings during the first years of establishment under challenging site conditions. To this end, I studied how soil conditions and root competition in different reconstructed soils interact to shape architectural, morphological and physiological traits of tree seedlings of three important boreal species with contrasting functional traits. All research presented here was conducted in Alberta, Canada on forest reclamation sites with reconstructed soils after surface mining.

In the following paragraphs I will summarize and synthesize the main findings of this dissertation, suggest management implications, briefly describe the limitations of the present work, and make some recommendations for further research.

In my first research chapter, I examined the individual and combined impact of two unfavorable soil conditions, severe soil compaction and intense root competition, on soil resource availability and seedling growth of planted aspen. The objectives were to determine:

a) the individual and combined impact of subsoil compaction and root competition on resource availability, water uptake and growth of planted aspen seedlings
b) the efficacy of two commonly used management practices, deep tillage and vegetation management (herbicide), in increasing available rooting space and with it access to soil resources for planted aspen seedlings.

The results of this study revealed that in fertile soils, belowground competition with grasses can restrict rooting much more than subsoil compaction and therefore have a much stronger negative impact on the growth of planted tree seedlings. We were able to show that smooth brome grass was able to colonize the available rooting space at a much faster rate than the planted aspen seedlings. We also demonstrated that the presence of smooth brome grass led to a strong in reduction in soil resource availability, specifically water and nitrogen. Our findings also showed that aspen root development was severely suppressed competing grass roots were present. The observed reduction in seedling growth was therefore assumed to be a consequence of both resource (water and mineral nutrients) and non-resource (rooting space) competition below ground. Lastly, the experiment showed that, at this particular site, deep tillage was not an effective means to improve soil physical conditions through a long-term reduction in bulk density and soil strength. Moreover, soil compaction did not appear to impede vertical root placement for either species.

My second research chapter was a follow-up expanding on the findings of the first experiment which clearly showed the strong impact of resource and non-resource competition from smooth brome grass on establishing aspen seedlings. The goal for the second experiment was to study in more detail the ecophysiological responses of planted aspen seedlings to intense above- and belowground competition. The objective for my second research chapter was to study:

g) how planted aspen seedlings and smooth brome compete for growing space during early establishment and
h) how this competition modifies the root and shoot architecture, biomass allocation, as well as morphological and physiological characteristics of aspen seedlings

Our findings showed that smooth brome quickly dominated growing space aboveground, as well as belowground and that aspen seedling growth was drastically reduced as a consequence of this competition. Aspen seedlings faced with grass competition were overall much smaller than those without, but showed otherwise no differences in root or shoot architecture. Our results also showed that aspen employed a growth strategy which allowed them to outgrow aboveground competition with smooth brome within three years. The seedlings achieved this by preferentially allocating to shoot growth and by having slender shoots with higher height-diameter ratio compared to seedlings establishing without grass competition. However, belowground aspen roots were still surrounded by grass roots on all sides, as smooth brome roots reached more than 1 m deep into the soil, while aspen had an average rooting depth of 0.4 m. We were also able to show that smooth brome dominated the first 30 cm of the soil with much higher root densities than aspen and that as a consequence of this intense competitive pressure both lateral root spread and maximum rooting depth were reduced by 50% when seedlings were faced with grass competition.

We achieved this by using a combination of traditional techniques, such as extraction of root core samples and the careful excavation of entire tree root systems, and state-of-the art molecular techniques that allowed us to reliably distinguish morphologically hard to distinguish roots in mixed samples. To our knowledge, this experiment was the first to use DNA analysis of mixed root samples in combination with species-specific markers to study the depth distributions profile of directly competing tree and grass roots under field conditions.

My first two chapters clearly showed that belowground competition for rooting space and the resources contained within can have a strong negative impact on the growth and development of tree seedlings planted onto newly reconstructed soils. However, it is well known that resource availability depends strongly on soil type and climate, while resource and rooting space requirements can be expected to vary among species. Moreover, interspecific interactions do not necessarily have to competitive in nature but may also be facilitative or at least neutral. Rooting space requirements are thus likely to vary in accordance with abiotic site conditions and the functional traits associated with the co-occurring species. To further explore these belowground processes I conducted another experiment in which three boreal tree species belonging different functional groups were planted either in monospecific or evenly mixed plots on two contrasting reconstructed soil profiles to test the effects of interspecific interactions under different abiotic conditions on seedling growth and productivity. The findings of this study are presented in my third research chapter. The objectives were to:

a) determine if the differences in functional traits among these species would result in increased productivity, or ‘overyielding’, due to niche complementarity when seedlings were grown in mixture
b) assess differences in the type and degree of developmental plasticity displayed by each species in response to mixing and contrasting soil types

The study showed that mixing of functionally different tree species resulted in increased belowground productivity or ‘overyielding’ due to niche complementarity, but only on one of the two studied soil types, where abiotic conditions did not inhibit seedling physiology. The underlying mechanism causing this effect was found to be a difference in phenology among the planted species which resulted in different temporal resource use patterns. On soil reconstructed with FFM, coniferous jack pine started taking up water several weeks sooner than deciduous aspen seedlings. In monospecific jack pine plots we had observed intense competition for water within the first weeks of the growing season. Because jack pine only constituted only a third of the trees in mixture, there were thus several weeks of reduced intraspecific competition for jack pine early in the growing season, when aspen did not yet take up any water. This period of reduced competition was sufficient to result in increased growth, particularly root growth, for jack pine seedlings in mixture which, in turn, caused the observed belowground overyielding. In contrast, when the soil was reconstructed with peat, the low temperature and/or excessively high moisture contents early in the growing season reduced root physiological activity for all three species and thus overrode the differences in phenology. Using a combination of techniques we were able to show that there was no clear spatial segregation among the root systems of neighbouring trees grown in mixture further supporting the notion that different temporal niches were responsible for the observed overyielding. To our knowledge, this is the first study that provides evidence of this phenomenon in young boreal tree mixtures.

The main limitation for this work can be seen in the limited number of sites and species studied, the generally low replication and the relatively short observation periods that focussed exclusively on early establishment of planted. More research, including other soil types and species, as well as longer observation periods is needed to further improve our understanding of the important belowground processes highlighted in this work.

5.2 Management implications

My research can provide tangible information based on ecophysiological data to help inform industry and government about the varying impacts of soil reconstruction and vegetation management practices applied during reforestation after anthropogenic disturbances on planted tree seedlings. Specifically, the findings presented in this work underpin the need to consider the rooting space required by and available to planted tree seedlings a key factor in the design of reconstructed soils. In order to reconstruct soils that provide sufficient rooting space, it is critical to reconcile the projected resource availability of the reconstructed soil with the resource requirements of the various plant species that will ultimately be part of the restored forest ecosystem. Resource availability can be projected based on the knowledge of the physical and chemical (i.e. abiotic) conditions associated with different soil materials used for soil reconstruction. Because soil materials typically undergo some degree of change during the mining and reclamation process it is critical that soil conditions be assessed and monitored prior to and for some time after soil reconstruction. Long-term effects of climate change should also be taken into consideration and adjustments should be made as projections become more accurate over time. Our research showed that on relatively resource-rich soils, intense belowground competition can have a strong negative impact on the development of planted tree seedlings by restricting root development and reducing resource availability. Thus the consumptive requirements of the species present at various stages of forest development also need to be considered and should therefore routinely be measured. Both resource requirements and the strategies employed to acquire and compete for these resources can be expected to vary with site conditions and species' functional traits.

For example, we showed that fast-growing early successional tree species are particularly affected by intense belowground competition form highly competitive perennial grass species, which may therefore have to be controlled during the period of seedling establishment to ensure healthy root system development. Conversely, we also showed that planting mixtures of tree species can be a good strategy that can result in increased productivity, but only if the species in question have complementary functional traits and when the abiotic conditions allow these differences to materialize.

5.3 Recommendations for further research

Despite the recognized importance of belowground processes in shaping forest ecosystems at all stages of development, our understanding of the complex plant-plant and plant-soil interactions is still quite limited. This is due to the elusive nature of roots, as much as it is due to the technical difficulties associated with observing belowground processes, without affecting them. Until recently, studying roots typically required destructive sampling, which not only disturbs the soil environment but also disrupts the very processes researchers want to study. Fortunately recent advances in non-destructive root study methods such as electrical capacitance (e.g. Ellis et al. 2013; Dietrich et al. 2013), ground penetrating radar (Addo-Danso et al. 2016), magnetic resonance imaging (MRI) (Nagel et al. 2009; van Dusschoten et al. 2016), or X-ray computer tomography (Tracy et al. 2012; Mairhofer et al. 2015), as well as novel molecular techniques that allow researchers to identify roots belonging to different species mixed species assemblages (Frank et al. 2010; Mommer et al. 2011a; Randall et al. 2014a) have significantly enhanced the toolkit available to root researchers. These novel techniques could be used in combination with environmental monitoring and physiological measurements to expand on the findings of this dissertation and explore questions such as:

1. How do root architectural, morphological and physiological traits contribute to belowground competitive ability and how do they vary within and between species and functional groups?
2. What is the relative importance of resource vs. non-resource competition and how does it shift with substrate texture and chemistry, as well as species' functional traits?
3. How do belowground processes change with stand development?
4. What is the role of mycorrhizal networks in mediating belowground interactions among and within species and does that role change along gradients of resource availability.

5. What are the actual rooting space requirements for a variety of mature forest stands along a gradient of soil and climatic conditions?

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Appendix A

Table A1 - Macronutrient availabilities (µg 10 cm^2) (±SD) obtained from ion-exchange membranes (PRS^{TM}-probes) buried at the centre of each soil horizon for 34 days (July 04-August 07, 2013). Different letters indicate significant differences (n=3, α=0.1).

	NH4	**NO3**	**P**	**K**	**Ca**	**Mg**	**S**
FFM	6.0 ±1.0 **a**	8.0 ±4.0 **b**	3.1 ±1.2 **a**	130.3 ±22.3 **a**	657.3 ±228.1 **b**	112.7 ±28.0 **ab**	63.0 ±46.0 **b**
peat	5.7 ±2.1 **a**	51.0 ±36.1 **a**	0.4 ±0.2 **b**	22.0 ±5.2 **c**	2401.7 ±193.4 **a**	173.3 ±32.6 **a**	1070.7 ±109.7 **a**
Bm	6.2 ±1.0 **a**	19.7 ±10.3 **b**	0.9 ±0.2 **b**	61.2 ±28.8 **c**	558.5 ±228.1 **b**	70.2 ±23.4 **b**	164.5 ±127.4 **b**
C	7.8 ±4.9 **a**	26.0 ±13.4 **b**	0.6 ±0.3 **b**	27.8 ±3.8 **c**	644.9 ±182.6 **b**	112.7 ±82.9 **ab**	161.5 ±160.2 **b**

Table A2 – Initial seedling characteristics at the time of planting (May 2012).

Parameter	***P. banksiana***	***P. glauca***	***P. tremuloides***
Shoot height (cm)	18.2 ±2.8	29.3 ±5.5	29.7 ±8.6
Shoot mass (g)	2.2 ±0.7	4.5 ±1.6	0.7 ±0.4
Root mass (g)	1.3 ±0.5	2.9 ±1.4	2.5 ±1.2

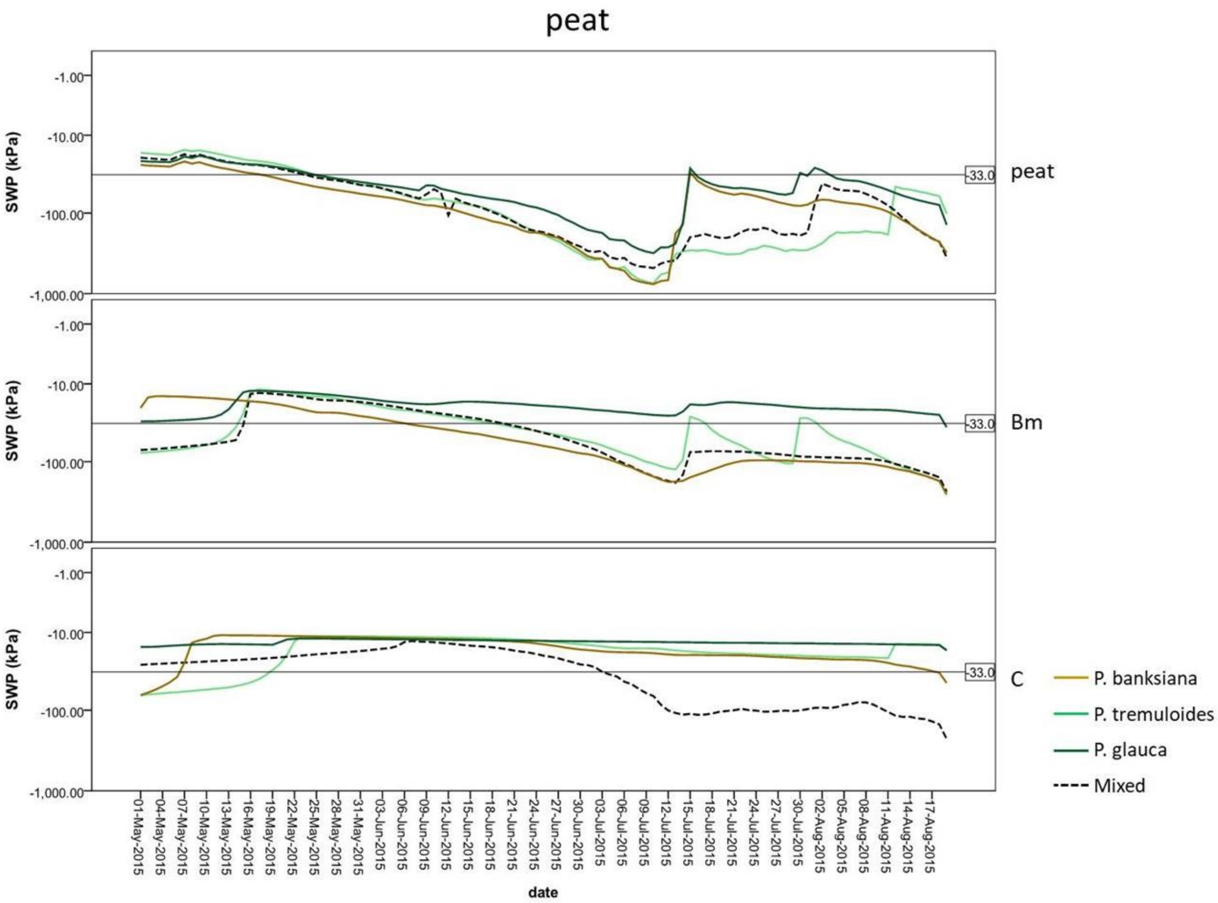

Figure A1 – Daily mean soil water potentials (SWP, kPa) for all four vegetation covers on soil reconstructed with peat. The horizontal line indicates field capacity (-33 kPa).

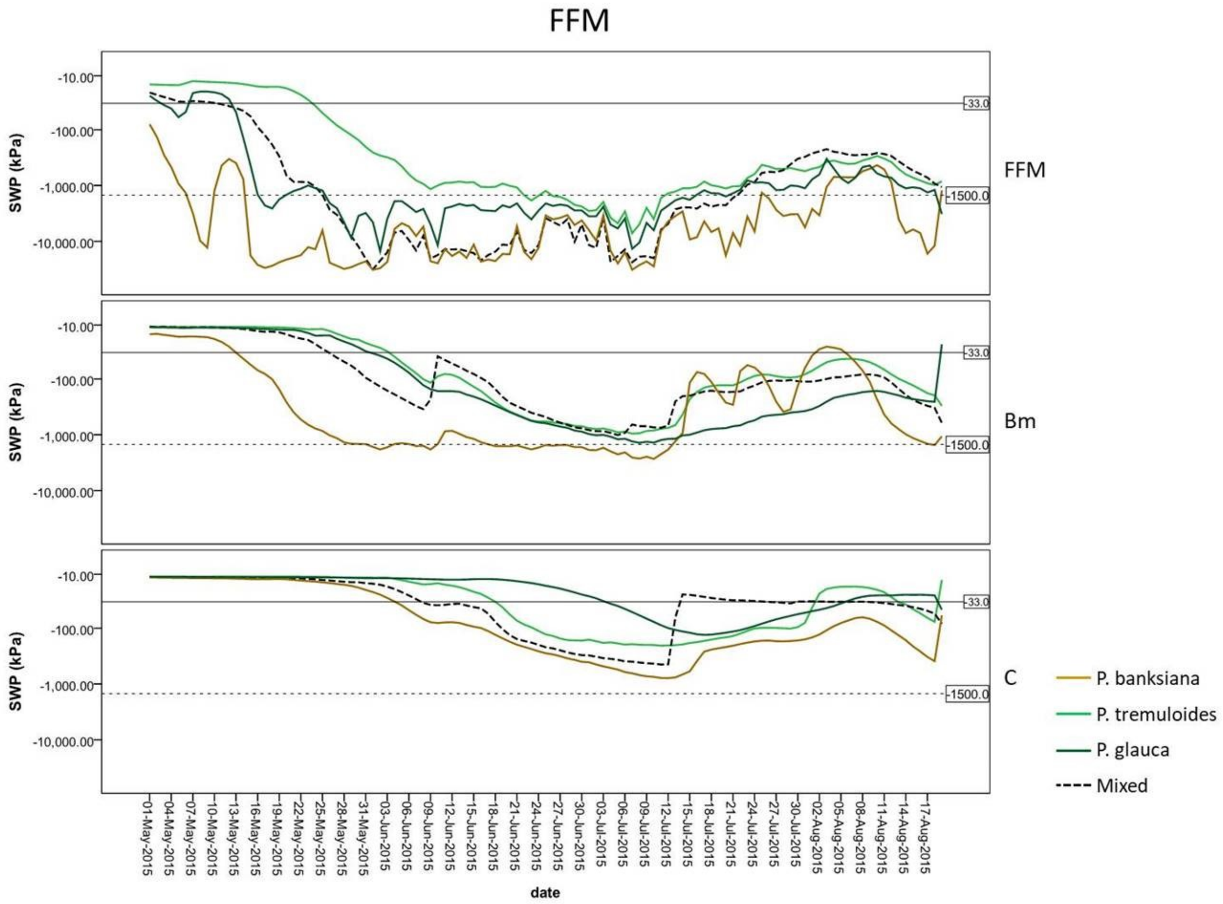

Figure A1 – Daily mean soil water potentials (SWP, kPa) for all four vegetation covers on soil reconstructed with forest floor material (FFM). The soilid horizontal line indicates field capacity (-33 kPa) and the dotted line indicates permanent wilting point (-1500 kPa).