If we knew what it was we were doing, it would not be called research, would it?

- Albert Einstein -

University of Alberta

Transfer of live aspen roots as a reclamation technique
– Effects of soil depth, root diameter and fine root growth on root suckering ability –

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Land Reclamation and Remediation

Department of Renewable Resources

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Fall 2012
Edmonton, Alberta

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Abstract

A transfer of live trembling aspen (*Populus tremuloides* Michx.) roots including the surrounding forest floor was conducted to examine aspen's ability of vegetative regeneration to establish a boreal forest on a reclamation site. Forest floor was salvaged at two depths (15 cm and 40 cm) from a natural aspen stand and placed onto a reclamation site at those same depths. Root density, root characteristics, sucker density and height were assessed. Two controlled studies investigated the importance of root diameter and fine roots in relation to burial depth to the suckering success and root survival. Root fragments that produced emerged suckers were located in the upper 20 cm of the soil and mostly had diameters between 1 and 4 cm. Higher sucker densities, taller suckers and lower sucker mortality at the 40 cm treatment suggest that increased salvage depth (good mineral soil-root contact) is a prerequisite for successful suckering.

Acknowledgements

First, I must thank my patient supervisors Dr. Simon Landhäusser and Dr. Victor Lieffers. I am very lucky to have had such knowledgeable and kind supervisors guiding me over the course of my degree. Simon, heartfelt thanks for your tremendous support in difficult times, thank you for your never ending encouragement and for always having time to answer my many questions; no matter how big or small. Thank you, for letting me move south during the writing process, and putting up with all the difficulties that came along.

Financial support for my studies and attendance at conferences was greatly appreciated and generously provided by grants from Natural Sciences and Engineering Research Council of Canada (NSERC), Capital Power, Shell Canada, Suncor Energy, and Syncrude Canada, and Virginia Tech.

I would also like to thank everybody, who was involved in the forest floor transfer at the Genesee coal mine. Special thanks go to Dan Kuchmak, who answered my many questions and who made the whole transfer a fun experience despite the cold, long hours, and several hiccups. Especially the pizzas directly delivered to the research site were greatly appreciated.

Thanks to all the people in the silviculture lab and summer students who helped me in several ways during my time at the University of Alberta. I am most grateful to Eckehart Marenholz, Stefan Stängle, Pak Chow, Jean Linsky, Ryan Sherritt, Jordana Fair, Kaitlin Schott, and Devin Goodsman for their support during field and lab work. Devin, thanks for your patience, advice and help, whenever I had questions about statistics.

I would like to thank my family for supporting me to start my new life so far away. It is comforting to know that you are just a phone call away, whenever I am lost in translation. Sascha, you are the best brother in the world. Thank you, for your unconditional support and for always believing in me no matter what; I would not know what to do without you. Heartfelt thanks to Jack and Ruth Baskin, who were always supportive, encouraging, non-judgemental, and are wonderful people I am most grateful to have met and in my life.

And finally, all my thanks and love to Eric, who cheered me on and calmed me down in all the right places, joined me in the field, listened to me talking about statistics, graphs, tables and hypothesis with a great amount of patience, and always believed.

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Chapter 1: Introduction

1.1 Reclamation in the boreal forest region

In the northern hemisphere the boreal biome expands in a more or less continuous band around the world covering approximately 920 million ha (FAO 1995). About a third of the boreal zone lies within Canada (Natural Resources Canada 2009*a*). It is bounded in the north by the tree line and in the western south by the aspen parkland region and in the eastern south by the temperate hardwood forests (Larsen 1980).

Economically and ecologically, the boreal forest is of significant importance. About 900,000 Canadians are employed by industries depending on its resources. Revenues from the energy sector and taxes paid by the companies that extract and produce coal, oil and gas contribute greatly to Alberta's GDP (Environment Canada 2009). The boreal forest does not only regulate the Earth's climate and purifies water; it also hosts a diverse ecosystem of vegetation and wildlife with about 140,000 species of animals, plants and micro-organisms (Bonan and Shugart 1989).

The development of these diverse ecosystems is influenced by several local and regional environmental elements such as temperature of soil and air, moisture, soil development, nutrient availability, light conditions (photoperiod/day 16 – 24hrs), and topography (Bonan and Shugart 1989). Roughly one third of Alberta is covered by boreal forest and its diversity is very distinct from northern to southern Alberta and brings about major changes in the distribution of trees and forest types. The northern parts of the province are mostly dominated by coniferous species such as pine (*Pinus spp.*), spruce (*Picea spp.*), fir (*Abies spp.*) and larch (*Larix ssp.*) with broad leaved species such as trembling aspen (*Populus tremuloides* Michx.), poplar (*Populus spp.*) and birch (*Betula spp.*) occurring in pure stands or mixed with conifers. This tree composition changes gradually towards the southern edge of the boreal zone reaching the aspen parkland region, where broad leaved species dominate forest stands (Larsen 1980).

Disturbances play an important role in the dynamics of the boreal forest and ecosystems are adapted to recover quickly. Insect infestation, outbreak of diseases, windthrow, and especially fire are the most common natural disturbances contributing to the diverse composition and structure of ecosystems (Larsen 1980). However, there are no natural analogues for anthropogenic disturbances such as agriculture, harvesting, and resource mining and the necessary infrastructure such as pipelines and road networks to access these resources. Industrial exploration such as surface mining has severe impacts on boreal forest ecosystems, where large areas of the forest are currently cleared and ecosystem functions and processes are deeply disrupted. As a result the natural and/or assisted reclamation and recovery of these post-industrial forest lands often resulted in at best simplified and homogenized ecosystems.

One of the greatest challenges in natural and assisted ecosystem recovery in the boreal forest region is the climate. The boreal zone is known to be a climate-limited ecosystem characterized by short, cool summers and long, cold winters. With average growing season temperatures of 13°C to 16°C, the length of the growing season varies from less than 160 days in the north to about 185 days in the southern part of the boreal region. From south to north, average winter temperatures range from -12°C to -24°C (Government of Alberta – Acroclimatic Atlas of Alberta 2010) resulting in frozen upper soil layers and discontinuous permafrost in some areas with poor soil drainage and thick insulating organic soil layers as a result of slow decompositions rates. Due to low average temperatures during the growing season, potential insufficient warming of these soils results in slow tree growth and high seedling mortality. Reason for this is the reduced ability for water uptake as cold soils limit root penetration, increase water viscosity and lower hydraulic conductivity in trees (Hammond 2009).

In addition to the low temperatures the boreal climate is characterized by sparse annual precipitation [350 – 600 mm depending on elevation (Government of Alberta – Acroclimatic Atlas of Alberta 2010)], which falls as a majority during the growing season (Natural Regions Committee 2006). Compared to soils of temperate ecosystems, boreal forest soils are pedogenetically young as glaciers in Alberta disappeared seven to ten thousand years ago. Since then, the cold climate only allowed limited weathering of soils and caused a slow breakdown of organic materials resulting in nutrient poor soils (Hammond 2009). The cold and dry environment, in combination with the low supply of nutrients, are conditions, to which boreal tree species generally are well adapted to. However, coupled with severe disturbances such as surface mining that disturbs the natural structure of soils, their hydrology and nutrient cycling processes, the re-establishment of vegetation and particularly forests on these disturbed sites can be a difficult and tenuous process.

Land reclamation in Alberta

The boreal forest region of Canada holds large quantities of resources such as coal, conventional and non-conventional oil and gas, and a variety of other minerals (Natural Resources Canada 2009*b*). The surface mining of these resources has enormous impacts on the environment bringing about major changes of the landscape. This is especially the case for surface mining (open pit mining, strip mining) compared to in situ mining techniques for oil and gas that generally cause less disturbance to the soils and hydrology in an area (Alberta Chamber of Resources 2011).

The extraction of natural resources has a long history in Alberta, where the first commercially extraction of coal started in the 1860s near Lethbridge and since then has been playing an important role in Alberta's economy. Currently, Alberta has seven active coal mines, which are mostly located in the central plains region. These mines produce between 30 and 35 million tons of coal each year and provide employment for over 2,500 people (Alberta Chamber of Resources 2011). The extraction of oil sands has been initiated more recently (1967) near Fort McMurray but expanded rapidly. As of 2010 there are 91 oil sands projects (most of them in situ), but four are currently actively extracting oil sands via surface mining. A total area of 4,802 km² holds deposits of oil sands that can be accessed by surface mining (Alberta Chamber of Resources

2011) and 715 km² of this area has already been disturbed (Government of Alberta 2011).

The reclamation of these post mined lands is controlled with the Land Surface Conservation and Reclamation Act 1973 and the Environmental Protection and Enhancement Act 1993, where mining companies are required to return the land to equivalent land capability compared to pre-disturbance conditions (Government of Alberta 1993). According to the Conservation and Reclamation Regulation, Alberta Regulation 115/1993, the term "equivalent land capability" is defined as follows: "The ability of the land to support various land uses after conservation and reclamation is similar to the ability that existed prior to an activity being conducted on the land, but that the individual land uses will not necessarily be identical."

This rather vague definition has brought about several issues in the reclamation of disturbed sites. It gives mining companies some freedom to leave the reclamation concept subject to their own discretion. In the past that often resulted in reclaiming pre-mining forests with plant communities dominated by persistent (often non-native) herbaceous species or to agricultural land, since it was less expensive, uncomplicated and quick. More recently, trials to establish boreal ecosystems often failed as trees failed to establish and as seeds of most boreal understory and shrub species are not for sale (Lanoue and Qualizza 2000; Alberta Native Plant Council 2010). Further, the natural invasion from adjacent areas was prevented (or very slow) due to competition from non-native cover crop vegetation that was planted to control soil erosion in the first year of reclamation (Grant et al. 2008). This use of non-native species created problems in the natural revegetation process and long-term effects might include the permanent change in species composition subsequently leading to new successional trajectories and modified ecosystem processes (Mandryk and Wein 2006).

The transformation of formerly forested sites into agricultural land in the past is a problem that has far reaching consequences. This is especially true for the most southern part of the boreal forest; the dry mixedwood natural subregion.

Historically, mosaics of grass-, shrubland, and large aspen dominated forests with shrubby understory, scattered with white spruce (*Picea glauca*) occupied this region (Moss 1932). Since then, heavy human impact mostly through agriculture and more recently through the resource extraction industry has drastically changed the landscape, fragmenting and removing the native vegetation cover (Morton 1938; Curtis 1956; Simonson and Johnson 2005). The ongoing loss and the fragmentation of productive forestland by human activities resulted in several drastic ecological issues including loss of habitat and biodiversity, isolation of ecosystems, soil erosion and depletion of soil nutrients as well as changes in microclimates (e.g. Burgess and Sharpe 1981; White and Mladenoff 1994; Van Apeldoorn et al. 1994). The loss and fragmentation of forest is probably one of the most significant land management challenges within Alberta's forested areas. New and more effective ways to reclaim post mined lands into functional boreal forest ecosystems are needed more than ever.

Various research has been undertaken on the early establishment of forest plant communities and trees in the northern part of the province at the Athabasca oil sands (e.g. Renault et al. 1998; MacKenzie 2006; MacKenzie and Naeth 2010). However, forest reclamation in the dry mixedwood natural subregion has received little attention and is associated with several challenges as it has the warmest and driest climate of any of the boreal natural subregions (Natural Regions Committee 2006).

In forest land reclamation the ultimate goal is the restoration of disturbed sites with native tree and understory species, establishing maintenance-free, functional and healthy forest ecosystems. Since forest ecosystems are complex and dynamic, it is essential to understand the processes of vegetation establishment on disturbed sites and their interactions.

1.2 Re-establishment of boreal forest vegetation

Ecosystems rely on succession to re-cover after a disturbance has damaged the function or interrupted processes of the ecosystem. Plant succession describes a series of changes in vegetation composition, structure and diversity over time and is strongly linked to the natural disturbances that dominate these ecosystems (Connel and Slatyer 1977; Walker and del Moral 2003). However, the vegetation recovery and composition depend on propagule availability (seed, spores, and plant vegetative parts) through seed dispersal from adjacent areas or present in the soil, which in turn is predetermined by the pre-disturbance species composition of the stand and adjacent areas (Fyles 1989; Pennanen et al. 2004; Johnstone and Chapin 2006). Further, the severity and seasonal timing of the disturbance can affect the survival of these propagules (Wang 2003; Rydgren et al. 2004). Suitable microsites and environmental conditions that allow germination and early seedling growth, competition among and within species for light, nutrients and moisture, as well as mortality are processes that determine structure and diversity of the forest (Fyles 1989; Chen and Popadiouk 2002; Pennanen et al. 2004; Johnstone and Chapin 2006).

Tree species that initially re-vegetate a boreal forest site include early successional species such as trembling aspen, balsam poplar (*Populus balsamifera*), paper birch (*Betula papyrifera*), and jack pine (*Pinus banksiana*) (Larsen 1980). These tree species are fast growing, shade intolerant, have advantageous regeneration methods [e.g. vegetative reproduction (only aspen, poplar, and birch), prolific seed production, seed dispersal capability] and are known to reoccupy sites rapidly after disturbance. Over the course of time these species become interspersed or dominated by coniferous species that are more tolerant to shade and are longer-lived, e.g. spruce (*Picea spp*.) (Bergeron 2000; Chen and Popadiouk 2002). As a result of the continuous cycle of disturbances and succession, diverse mosaics of forest stands comprised of early, mid-, and late successional plant communities characterize the boreal forest region.

In natural forest stands the revegetation of sites is usually known as secondary succession as the area has been previously occupied and therefore has the propagules to re-colonize quickly. Years of nutrient cycling and weathering processes have substantially modified and improved the fertility and structure of the soil providing excellent conditions for plant establishment. In addition, propagules of the pre-disturbance vegetation stored in the soil are usually readily available and regenerate rapidly.

These conditions do not apply for sites that have been recently prepared for reclamation. Soils of these sites are constructed, undeveloped and have been altered little by pedogenesis; consequently the recovery of forest ecosystems may be considered more similar to primary succession. Under natural conditions the formation of forest ecosystems may take hundreds of years, during which the simultaneously formation of soils and establishment of plants takes place. Pioneer species of forbs, grasses, shrubs and trees will gradually be replaced, and dominated by mid-, and late successional species until climax communities have established (Mann and Plug 1999; Svensson and Jeglum 2003; Merila et al. 2010; Nossov et al. 2011). Shortening of these primary successional pathways to provide quick tree cover is one of the most important and challenging steps towards successful reclamation of forest lands. Research needs to be conducted in order to improve current reclamation practices with new techniques that allow rapid establishment of boreal forest ecosystem.

Forest reclamation methods

In the surface mining industry the vegetation, topsoil (surface soils) and subsoil (subsurface geological material; overburden) are commonly removed prior to the extraction of the natural resource. Depending on jurisdiction, the surface soils are sometimes selectively salvaged and the upper soil horizons (forest floor (LFH) with the A and B horizons) are separately stockpiled from the lower C horizon soil material. These materials can be stored for several months, but more often for several years in stockpiles, and are placed back onto the land after mining operations have moved on (McMillan et al. 2007). The establishment of understory and overstory plant species contained in the propagule bank in these stockpiled materials is very slow and often unsuccessful as propagule viability significantly decreases during soil storage (Iverson and Wali 1982; Koch et al. 1996; Rokich et al. 2000; MacKenzie and Naeth 2010). As a result reclamation sites have to be seeded and planted with trees and other plant species. Unfortunately seeds of most native boreal understory species are not commercially available (Lanoue and Qualizza 2000; Alberta Native Plant Council 2011), which makes it almost impossible to rapidly establish cover of native species. Instead, mostly barley (*Hordeum vulgare* L.) has been used for initial plant cover to control soil erosion in the first years as it can easily be outcompeted by native vegetation in the following years (Burger and Zipper 2002; Davis et al. 2005; Halofsky and McCormick 2005; Williams and Crone 2006). However, the natural re-colonization of native forest plants from dispersed propagules from surrounding undisturbed land, and reclaimed areas is very slow. The invasion of these species is affected by several factors (e.g. distance to seed source, seed dispersal capability) and has only been successful with some native grasses and forbs. In addition, the natural ingress also includes non-native, weedy forbs [e.g. scentless chamomile (Tripleurospermum perforatum syn. T. inodorum)] and grasses [e.g. smooth brome (Bromus inermis)], which can expand quickly and thus prevent native species from establishing (Hardy BBT Limited 1990; Mandryk and Wein 2006). The rapid development of a continuous tree canopy cover could reduce the establishment and expansion of these non-native and weedy shade-intolerant species on reclamation sites.

Traditionally, nursery grown seedlings of fast-growing species such as aspen, poplar and pine are planted to achieve quick canopy cover. However, after outplanting these seedlings often perform poorly in “new” reclamation soils. This planting check is usually caused by outplanting stress as a lack of water and nutrients, resulting in slow growth and high mortality rates (Steneker 1976; Nambiar and Zed 1980; Burdett et al. 1990; Martens et al. 2007). Creating suitable substrate (top- and subsoil) is a challenging task since the capability and quality of the reconstructed soil greatly determines the success or failure of forest ecosystem establishment. The topsoil has to serve as plant rooting medium, storage and supply of nutrients and water, whereas the subsoil provides anchorage to trees by allowing for deep root penetration, and access to stored water. In the past, research has been conducted dealing with different placement depths (e.g. 20 versus 40 cm) and soil types (e.g. peat-mineral mix) of topsoil and their influence on soil formation processes and vegetation establishment (e.g. Bowen et al. 2005; Rowland et al. 2009; Alberta Environment 2010; Mackenzie and Naeth 2010). For example, Rowland et al. (2009) compared the degree of establishment of natural-like ecosystems between two types of topsoil (peat-mineral mix versus subsoil) and found that the peat-mineral soil cap including several fertilization treatments was most successful. However, it was also reported that the ecosystem that established on their reclamation sites greatly differed from natural forest ecosystems as many of the propagules found in peat were not suitable for establishment in upland sites. These unsatisfying results led to the conclusion that improved forest reclamation methods are needed to establish more natural forests.

To restore natural forests, trials have been conducted that salvage and use the surface soils of upland forests as a topsoil for reclamation (also referred to forest floor-mineral mix (FFM) or as LFH) (Mackenzie 2006; Mackenzie and Naeth 2007; Alberta Environment 2010; Mackenzie and Naeth 2010). This salvaged material consists of the LFH layer and the upper part of the mineral soil (generally A and part of the B horizons). LFH is a thin organic horizon characterized by an accumulation of fresh, intact, identifiable litter (L), fragmented and fermenting litter (F), and humus (H) (Soil Classification Working Group 1998). A main advantage of utilizing forest floor is the abundant source of propagules and microorganisms in this horizon, which provides forest reclamation areas with upland species that are not commercially available (Qi and Scarratt 1998; Mackenzie and Naeth 2010). Further, compared to subsoil or peat-mineral mix, the forest floor-mineral mix (FFM) consist of more available nutrients providing a better growing medium for boreal upland plants (Mackenzie and Naeth 2010).

However, storing the FFM material in stockpiles for several months or years (see above) has shown to decrease propagule viability (Mackenzie and Naeth 2007) and thus might reduce the beneficial effect of the material as a propagule source for reclamation sites. For example, Syncrude conducted a pilot project determining effects of the utilization of forest floor soils and its placement time [direct transfer (August) versus a 4-month storage in a stockpile (placed the following January)] on native species establishment. Shrub and tree species established with average densities of 4,250 stems ha^{-1} from stockpiled material compared to 17,233 stems ha^{-1} after direct transfer. In addition, only reclamation sites that had FFM applied directly after salvage established trembling aspen and balsam poplar with densities from 0 to 2,200 stems ha^{-1} (Alberta Environment 2010). Consequently, the placement of the forest floor material should be scheduled with as little as possible time between salvage and transfer in order to maintain propagule viability. However, as this technique is relatively new to boreal forest reclamation, more research is needed to assess effects of salvage and placement depths as well as use of large operational equipment on revegetation success.

Use of directly transferred soils for natural establishment of native boreal forests

The direct transfer (also referred to as direct placement) of forest floor material describes the process of the immediate relocation of surface soils after its salvage from an area about to be mined, onto an area prepared for reclamation. This method has been successful in restoring forest ecosystems of subtropical, temperate, and arid regions (Iverson and Wali 1981; Koch et al. 1996; Bakker and Berendse 1999; Holmes 2001). For example, in the Australian Jarrah (*Eucalyptus marginata*) forest (bauxite mining) the direct soil transfer is common practice whenever possible (Gardner and Bell 2007). Studies in this area suggest that natural regeneration of understory species on post-mined sites has had increased success since directly transferred topsoils were used for reclamation (Koch et al. 1996; Rokich et al. 2000; Koch 2007). However, Koch and Samsa (2007) reported that the establishment of trees, although always very successful, is mostly accomplished from seeds applied after the direct soil transfer and not from the seed bank of the topsoil. Thus, from these studies no knowledge and experiences about natural tree establishment from stored seeds or through vegetative regeneration is available for the establishment of boreal tree species after a direct soil transfer.

In the boreal forest, mainly in the Athabasca Oil Sands region, recent research has dealt with the effects of direct transfer of forest floor soils on the establishment and diversity of native plant communities on reclamation sites (Mackenzie 2006; Mackenzie and Naeth 2007; Alberta Environment 2010; Mackenzie and Naeth 2010). However, the main focus was placed on assessing understory species response to the direct soil transfer and very little is known about tree establishment on these sites. Reason for this might be the amalgamation of trees under the term “woody species”, which would describe tree AND shrub species together and a separation of those is not possible; thus, there is little specific information on tree performance and establishment (e.g. stems per ha, regeneration method). In the majority of studies at the Athabasca Oil Sands, it was reported that by using the direct soil transfer method the abundance and diversity of herbaceous plants was increased and the establishment was successful, when moisture and nutrient conditions on the reclamation site were similar to the regimes of the donor site. The establishment of woody species, however, was highly variable (Alberta Environment 2010).

It is recognized that the salvage depth and placement thickness of the forest floor are important elements determining success or failure of plant establishment. Salvage depth greatly influences the propagule pool that is transferred onto the reclamation site as propagule abundance declines with increasing forest floor depth (Tacey and Glossop 1980; Iverson and Wali 1982; Grant et al. 1996; Rokich et al. 2000; Mackenzie and Naeth 2010). Choosing a suitable placement thickness seems to be the more challenging task to achieve successful establishment of understory and tree species. For most species that emerge from seeds, it is suggested that burial depth should not exceed 5 cm (Grant et al. 1996; Rokich et al. 2000). However, tree species such as trembling aspen that are known for vegetative regeneration from their root system, can produce suckers from depth of 4 to 15 cm in natural stands (Horton and Maini

1964; Schier 1973; Kemperman 1978; Schier and Campbell 1978; Brown and DeByle 1987; Navratil 1991; Mundell et al. 2007).

At the Athabasca Oil Sands, several studies have been conducted in the past using salvage depth from 8 - 28 cm and placement thicknesses from 5 - 25 cm (Mackenzie 2006; Mackenzie and Naeth 2007; Alberta Environment 2010; Mackenzie and Naeth 2010). For example, Mackenzie (2006) and Mackenzie and Naeth (2007) conducted studies testing among other things the effect of two placement depths (13 and 22 cm) of forest floor material on plant establishment. They observed an increase in numbers of woody plant species with the increase of placement thickness. However, there was no difference in aspen numbers (on average 1,000 stems ha^{-1}) between the two treatments.

The Guidelines for Reclamation to Forest Vegetation in the Athabasca Oil Sands Region (Alberta Environment 2010) propose that salvage depth should not exceed 30 cm from the upper soil profile of donor sites and that this material should be applied to a thickness of at least 10 cm. Further, research suggested that with increased placement thickness (> 10 cm) an increase of woody plant density and canopy cover can be expected (Mackenzie 2006; Mackenzie and Naeth 2007). However, these recommendations are mostly based on the results for herbaceous understory species regeneration. To this date, the planting of nursery-grown tree seedlings is still considered a necessity to restore a tree layer as a part of a functioning forest ecosystem.

Consequently, more research is needed to improve the natural establishment of trees including specific information of tree initiation and performance on reclamation sites; e.g. stems per hectare, method of regeneration: seedling versus sucker, spatial distribution. Particularly the regeneration of trees through root suckering lacks reclamation research, even though it plays a significant role in the reproduction of boreal forest ecosystem. The unique root systems of aspen and poplar could provide a means to establish tree cover quickly on reclamation sites and could reduce the numbers of seedlings that need to be planted. Especially, trembling aspen, which has the ability to vigorously re-sprout from its root system, would make a prime species for reproduction from vegetative propagules in forest land reclamation areas.

1.3 Trembling aspen

Trembling aspen is one of the dominant tree species in Canada's forests and is characterized by a transcontinental distribution. In Alberta aspen [and balsam poplar (*Populus balsamifera* L.] make up 35% of the tree cover (Natural Resources Canada 2001).

Historically, aspen wood was of low demand and was at best ignored by the forestry industry, if not eradicated in favour of more valuable conifer species (Davidson et al. 1988). After years of treating this species as a weed, aspen has gained economical importance and is now used extensively by the pulp and paper industry and for the composite wood industry (Poplar Council of Canada 2008).

Trembling aspen is a broadleaved hardwood species and can be described as a medium to large-sized, fast growing tree. Aspen is known for its ability to adapt to a wide range of site conditions across Canada. As a result of aspen's broad geographic distribution, this species is adapted to a wide range of climates; although conditions found in cool, dry areas tend to be more beneficial than those found in humid and coastal areas (Haeussler and Coates 1986). Stands develop on a great variety of soil conditions ranging from shallow rocky soils to deep loamy sands and wet heavy clays, but optimum growth can be expected on moist, well-drained porous loamy upland soils with nutrient-rich substrates (Krajina et al. 1982; Haeussler and Coates 1986; Perala 1990). Although known as a drought-tolerant species, soil moisture is thought to be one of the most important factors influencing the growth of aspen; it is thought that aspen grow best in mesic soil moisture conditions (Peterson and Peterson 1992).

Due to its dynamic root system, trembling aspen has growth and survival advantages over a range of other tree species in the boreal forest (Graham et al. 1963). The root system is widely spreading in a lateral network with strong vertically-growing roots originating close to the stem base (Maini 1960) serving the tree anchorage, transport and storage functions.

This clonal tree species commonly regenerates through root suckers after natural disturbances such as fire kills or harvest activities remove the above-ground portion (Perala 1990; Peterson and Peterson 1992). Stands originating from suckers are characterized by rapid growth, high nutrient uptake and early crown closure due to leader growth and rapid extension of lateral shoots on suckers. The resulting high density sucker stands rapidly self-thin. Even though aspen are widely distributed across Canada and have the ability to grow in several ecosystems and habitats, this tree species is very shade intolerant, resulting in rapid natural self-pruning, leading to short crowns relative to total tree size (Perala 1990; Peterson and Peterson 1992).

Applicability of trembling aspen in land reclamation

Trembling aspen is a major component of Canada's boreal forest and Parkland ecosystem (Peterson and Peterson 1992). After disturbances such as fire, harvesting, insect and disease outbreak that killed or removed the aboveground portion of the stand, aspen is one of the first species to re-colonize the disturbed site by producing prolific suckers from its vast lateral root system (Maini and Horton 1966; Steneker 1976; Kemperman 1978; Frey et al. 2003). Aspen's adaptation to a wide range of site and environmental conditions, but most importantly its high resiliency (recovery potential) to disturbances makes it to an ideal reclamation species. Reforestation with aspen enhances plant species diversity, has recognized value for wildlife habitat, watershed protection and aesthetics (Poplar Council of Canada 2008). The wide range of options to establish aspen (seeding, planting of nursery-grown seedlings, and vegetative reproduction), its large distribution across North America, and the ability of this species to grow in mixtures with several other boreal tree species (Rowe 1972) makes the use of this species very flexible and easy to in-corporate into reclamation plans and strategies.

Although aspen reproduces naturally mainly through its root system, aspen is a reliable and prolific seed producer (averaging 1.6 million seeds/tree/year) and with its seeds being small and light and attached to a pappus of long silky hairs when released from the capsule, they can wind disperse over long distances (Peterson and Peterson 1992; Miller 1996). The bare soil of mine land or other disturbed sites may provide an excellent opportunity for the natural establishment of aspen from seed (Kaitlin Schott personal communication). However, due to stringent seedbed requirements and climate conditions the natural establishment of aspen from seed can be difficult (Peterson and Peterson 1992; Romme et al. 1997; Greene et al. 2007).

Traditionally, forest reclamation involves the planting of trees. In the boreal forest region nursery-grown seedlings of aspen [along with several other tree species (e.g. spruce, pine)] are planted after an herbaceous vegetation cover is established for erosion control. Slow growth and high mortality rates of planted aspen seedlings is a common issue and can last for up to three years after outplanting (e.g. Steneker 1976; McKay 1997; van den Driessche et al. 2003; Martens et al. 2007). It is proposed that these issues may be caused due to several reasons. Because seedlings root systems are initially small and confined to the planting hole as they do not expand quickly after outplanting, the amount of soil that seedlings can exploit initially may be insufficient resulting in moisture stress and inability to acquire nutrients (Nambiar and Zed 1980; Burdett et al. 1990). However, recent research has been conducted to improve nursery-grown seedling quality with the result of enhanced seedling performance and survival after outplanting at reclamation sites (Martens et al. 2007; Rodriguez Alvarez 2011).

The existing aspen bud bank could also give an opportunity to establish aspen on reclamation sites; however, no information exists on the vegetative regeneration ability and sucker performance after an industrial disturbance such as surface mining. In natural stands, following a surface disturbance such as fire or harvesting, the root system of trembling aspen produces suckers aggressively. These young sucker stands are very dynamic and are characterized by rapid growth and high densities in the first year following major disturbances. Sucker heights of 2.5 m may be reached in the first growing season under favourable conditions (Peterson and Peterson 1992). Davidson et al. (1988) reported that normal sucker growth rates in the first year range from ≤ 30 cm to more than one meter. Stands can establish sucker densities of more than 250,000 stems ha^{-1}, although densities are greatly dependent on the region (Alban et al. 1994). Where several suckers emerge in clumps, self-thinning due to direct intraspecific competition occurs at an early stage, as each clump usually has a dominant stem (Shepperd 1993), which will outgrow the subordinates so that by year 5 only one or two stems remain (Sandberg 1951).

Direct soil transfer and its potential effects on vegetative regeneration

In natural stands suckers originate from the parent root system after the aboveground portion of the tree has been killed. Root characteristics such as root diameter (Kemperman 1978; Schier and Campbell 1978; DesRochers and Lieffers 2001), rooting depth (Strong and LaRoi 1983; Mundell et al. 2007), as well as root carbohydrate reserves (Schier and Zasada 1973; Landhäusser and Lieffers 2002; Frey et al. 2003) of parent roots have been identified as important factors for successful sucker initiation and growth in natural stands.

Most suckers generally originate from roots with diameters of 0.5 - 2 cm (Kemperman 1978; Schier and Campbell 1978; DesRochers and Lieffers 2001); however, it is unclear if this is because roots with larger diameters are not as abundant, or if large roots have poorer ability to produce suckers (Perala 1978).

Most of the aspen root system is located in the upper 5 to 20 cm of the soil profile (Strong and LaRoi 1983; Mundell et al. 2007). In natural stands, roots have been observed to produce successful suckers from roots at a depth of 4 to 15 cm (Horton and Maini 1964; Schier 1973; Kemperman 1978; Schier and Campbell 1978; Brown and DeByle 1987; Navratil 1991; Mundell et al. 2007). It is not clear, however if portions of roots buried greater than these depths have sufficient capacity (e.g. carbohydrate reserves) to produce shoots that will reach the surface.

Harvesting operations and other surface disturbances can significantly affect regeneration in natural boreal aspen stands (Frey et al. 2003; Renkema et al. 2009). The transfer of the root and soil material, where LFH layer, the upper part of the mineral soil and the containing aspen roots get mixed, may cause serious issues influencing sucker establishment. Depending on placement depth of the material, roots may be buried quite deep and as previously mentioned, it is unknown if these roots are able to support suckers that will reach the surface.

Additional factors such as soil compaction, root damage and fragmentation caused by the operational processes (salvage, transport and placement) may seriously affect the successful regeneration of aspen on reclamation sites. The compaction of soil increases bulk density and decreases soil aeration both limiting root growth and root suckering (Maini and Horton 1964; Steneker 1974; Bates et al. 1993).

Reduced suckering and growth from damaged roots caused by heavy machine traffic (Bates et al. 1993; Stone and Elioff 2000; Renkema et al. 2009) could be the result of limited supply of water, nutrients and carbohydrates (Zahner and DeByle 1965; Fraser et al. 2002), and restricted transport of hormonal growth regulators (Frey et al. 2003). Scuffing, crushing, or fragmenting of root systems particularly affect fine roots because of their delicate structure. Fine roots are the primary pathways for water and nutrient uptake (Eissenstat and Caldwell 1988; Pregitzer et al. 1993) and account for a significant portion of ecosystem net primary productivity (Gower et al. 1996; Finér et al. 2007). Although the biomass of fine roots is low compared to coarse roots, their presence (or absence) may play an essential role in successful development of suckers. In addition, wounding of roots may have negative effects as injuries on roots are major entry points for decay causing organisms (Basham 1988; Hiratsuka et al. 1990; Pankuch et al. 2003). Those infections could not only kill parts of the root system, but also spread into the root collar and adventitious roots of developing suckers (Basham 1988).

Critical factors in the boreal forest region such as soil temperature and nutrient conditions (Fraser et al. 2002; Landhäusser et al. 2006) can reduce sucker initiation, while conditions such as competition (Landhäusser and Lieffers 1998), excess litter (Mulak et al. 2006) and slash (Renkema et al. 2009) can also reduce suckering.

Although the regeneration of aspen through root suckering has been well studied in a traditional silvicultural and forest management context (e.g. Frey et al. 2003), the effects of the transfer of a complete parent root system on its suckering ability in the reclamation of boreal forest surface mined sites has not yet been addressed by research.

1.4 Objectives

The overall objective of this research project was to determine if the extensive bud bank of trembling aspen could be used to re-establish forest cover in reclamation areas by transferring a complete parent root system including its surrounding soil from a donor site onto an area prepared for reclamation. Particular emphasis was placed on the determination of the effects of soil depth, root diameter and presence of fine roots on root suckering ability.

In Chapter 2, results of the root transfer study are presented, which are based on data taken over the course of two growing seasons. This study was designed to assess root suckering ability, sucker initiation, performance and mortality responses to two soil depth treatments. Further, environmental conditions including soil temperature, moisture and precipitation were taken in consideration.

Chapter 3 presents results of two controlled studies. The diameter-depth study was conducted to determine the effect of depth of root burial and root diameter on sucker initiation and performance. The fine root study assessed the role of fine root presence on root fragments affecting suckering response. Differences in root carbohydrate reserve levels were measured to determine impact on sucker performance.

Chapter 4 summarizes the most important findings of this research, presents major contributions of this study, provides management implications, and points out future research necessities for improving the establishment of forest ecosystems by transferring aspen roots onto reclamation sites.

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Chapter 2: Transfer of live aspen roots as a reclamation technique for the natural establishment of boreal forests

2.1 Introduction

The increasing demand for non-renewable resources has intensified the exploration and extraction of resources in the circumpolar boreal forest region, which results in large tracts of boreal forest being disturbed by industrial activities (Alberta Chamber of Resources 2011). The boreal forest is a complex and dynamic ecosystem and plant establishment and growth after disturbances is often constrained by the cold and dry climate (Hammond 2009); conditions that make boreal forest reclamation a challenging task.

After mining operations have ended, the disturbed landscape is re-contoured, and overburden is covered by previously salvaged subsoil, which is subsequently capped with a layer of salvaged topsoil. Soil materials (subsoil and topsoil) used in the reclamation of surface mines are generally stored in large stockpiles during active mining operations. A significant disadvantage of this method has been the deterioration of viability of plant propagules contained in this topsoil and the poor natural establishment of native plant species after the capping material had been placed (Koch et al. 1996; Koch and Samsa 2007; Mackenzie and Naeth 2007).

Plant propagules contained in native topsoil are considered a valuable resource that can be used to increase native plant diversity on reclamation sites (e.g. Bowen et al. 2005; Rowland et al. 2009; Alberta Environment 2010). Recent research has focused on the use of forest floor (LFH horizon and upper part of mineral soil) that is salvaged from an area soon to be mined and directly transferred and applied onto the subsoil at the reclamation site (Mackenzie 2006; Mackenzie and Naeth 2007; Mackenzie and Naeth 2010). This has been successful for the establishment of a more diverse plant community of herbaceous forest species after reclamation (Mackenzie 2006; Mackenzie and Naeth 2010, Alberta Environment 2010). Most upland sites in the mixedwood region of the boreal forest also contain a large bud bank on the roots of trembling aspen and balsam poplar (DesRochers and Lieffers 2001). There has been no research investigating the viability of establishing species from the genus *Populus* using its root propagule bank for reclamation purposes.

The effectiveness of the direct transfer of natural soils for forest reclamation purposes is greatly dependent on the salvage and placement depth on the reclamation site (also referred to as application depth). It is known that the abundance of propagules decreases with increasing salvage depth as a result of soil dilution (Grant et al. 1996; Rokich et al. 2000; Mackenzie and Naeth 2010). On the other hand surface soils salvaged too shallow could constrain operations and leave many viable roots and other propagules on the donor site (Tacey and Glossop 1980; Koch et al. 1996; Rokich et al. 2000). For example, in the Australian Jarrah forest, Rokich et al. (2000) found that the salvage of 30 cm of forest floor significantly diluted the propagule pool and subsequent seedling recruitment compared to a 10 cm salvage depth. However, their results suggested that the forest floor material should be applied as thin as operationally feasible (≤ 10 cm). In a study in the Athabasca Oil Sands Region, forest floor was salvaged to a depth of about 20 cm (Mackenzie and Naeth 2010). Plant species richness was higher when the salvaged material was applied at 20 cm thickness compared with a thickness of 10 cm. Thus, the inconsistent results concerning the effect of salvage and placement depth on plant establishment, as well as the lack of knowledge about natural tree establishment from propagules in transferred materials, illustrate that more research is needed to improve this forest reclamation technique.

In Alberta, many mine reclamation sites are located in the boreal mixedwood zone, which is dominated by aspen. Aspen's ability to vegetatively re-sprout from its vast lateral root system (Maini and Horton 1966; Steneker 1976; Kemperman 1978; Frey et al. 2003) provides a propagule bank that has not been explored for forest reclamation of disturbed boreal forest sites. In natural stands, the aspen root system is mainly located in the upper 5 to 20 cm of the soil profile (Strong and LaRoi 1983; Mundell et al. 2007) and it is known that roots located at depths of 4 to 15 cm can successfully produce suckers

(Horton and Maini 1964; Schier 1973; Kemperman 1978; Schier and Campbell 1978; Brown and DeByle 1987; Navratil 1991).

During the salvage, transfer, and placement of surface soils, aspen roots systems will be fragmented, damaged, and placed at varying soil depths. Re-sprouting success from aspen root fragments have been tested extensively in laboratory settings (e.g. Fraser et al. 2004; Stenvall et al. 2009); however, few studies have tested them in field settings. Suckering from root fragments or intact root systems depends on a range of conditions such as soil properties, soil moisture and temperature conditions (Fraser et al. 2002; Stenvall et al. 2005; Frey et al. 2003; Mundell et al. 2007; Landhäusser et al. 2003; Landhäusser et al. 2007).

The objective of this study was to determine the efficacy of forest floor salvage for the vegetative regeneration of aspen from root propagules on reclamation sites, and how salvage and placement depth affect the suckering ability of aspen roots and subsequent sucker growth and mortality. We hypothesize that with greater salvage and placement depth the higher mineral soil content will create better soil contact with root fragments that prevents the desiccation of roots closer to the surface; however, root fragments can be buried deeply at greater placement depth. More shallow salvage and placement would have a soil with less mineral soil content and therefore poorer soil contact; however, root fragments would not become buried as deeply, which may result in greater sucker emergence.

2.2 Methods

2.2.1 Research area

The research area is located on the Genesee Coal Mine lease (Prairie Mines & Royalty Limited) approximately 80 km southwest of Edmonton, Alberta (53°19'N, 114°18'W). Land use prior to mining was dominated by agriculture, both cultivation and grazing, and on less arable areas remnant woodlands dominated the landscape (Dan Kuchmak, personal correspondence 2009).

Ecologically this area lies within the Dry Mixedwood Subregion of the boreal forest and vegetation composition is typical for the transition zone between the Central Parkland and Central Mixedwood subregions (Beckingham and Archibald 1996). Natural forest vegetation is dominated by pure aspen or by mixed stands of aspen and balsam poplar and sometimes with white spruce (*Picea glauca* (Moench) Voss). Topography of this area is level to undulating and comprised of glacial till or lacustrine deposits. Soils in the upland areas are generally Orthic Gray to Dark Gray Luvisolic soils. In the Dry Mixedwood Subregion the summers are warmer, winters are milder and the overall climate is dryer compared to all other subregions within the boreal forest (Natural Regions Committee 2006).

At the Genesee research site precipitation during the growing season between April and September amounted to 505.1 mm in 2010 and to 355.6 mm in 2011. Average temperature between April and September was 11.9°C in 2010 and 12.6°C in 2011. During the winter of 2010/2011 (December to March) the average temperature was -10.4°C and the area received 116.1 mm of precipitation mostly in the form of snow (Patrick Anderson - West Central Airshed Society, personal communication 2011).

2.2.2 Plant material

For the forest floor and aspen root salvage a donor aspen stand (4.6 ha) was selected on the Genesee Mine lease in the summer of 2009. Since the stand had been slated for being mined in the near future, mature trees had been cut approximately 9 years earlier, but were allowed to regenerate via root suckering. A pre-salvage assessment was executed by selecting 20 plots (each 4 m^2) along two transects across the donor stand. In each plot we determined tree density by species, stem diameter of all trees at 1.3 m, and the height of the tallest tree. Within each plot a soil pit was excavated (38 × 38 × 40 cm deep) and all roots bigger than 0.5 cm were collected. In the lab, roots were washed and sorted. Only aspen roots were selected, and their diameter and length were measured to estimate aspen root mass and volume per m^{-3} of soil (Table 2-1). The depth of salvageable forest floor material as estimated by main rooting depth and suitability of mineral soil was about 40 cm. The overstory was comprised of trembling aspen (13,235 stems ha^{-1}) with an average height of 7.0 m and an average stem diameter of 3.2 cm (Table 2-1).

The most abundant and common herbaceous and shrub understory species were *Cornus canadensis* L., *Symphoricarpos albus* (L.), *Calamagrostis canadensis* (Michx.) P. Beauv. var. *canadensis*, *Lonicera involucrata* (Richardson) Banks ex Spreng., *Rosa acicularis* Lindl., and *Prunus virginiana* (Fair 2011). The soil of the stand was classified as a Dark Gray Luvisol with an organic layer (LFH) of approximately 7 cm.

2.2.3 Forest floor salvage and treatments

For this study two salvage and placement depths were targeted; a shallow (approx. 15 cm) and a deep (40 cm) treatment. Based on the effective rooting depth of the trees in the donor stand, a surface soil salvage to 40 cm depth was deemed feasible, as it could be done without including less suitable substrates such as heavy clay (avoiding the Bt horizon). During frozen soil conditions (-16°C) at the end of January 2010, the trees were sheared off and removed using a D-11 Cat with a straight blade positioned just above the forest floor. Shortly after, the soil and root systems were salvaged to either 15 or 40 cm by pushing the materials into windrows using D-11 cats. In the shallow salvage, material could be pushed in one pass into the windrow, while in the deep salvage at least two passes were required to achieve the target salvage depth. While there were minor deviations from the targeted depths, overall the deep salvage treatment was clearly differentiated from the shallow treatment by the larger amount of mineral soil salvaged. To assure randomness in the salvage material from the donor site, the salvage depth was alternated across the site creating 6 windrows. After the salvage was completed, the forest floor material (approximately 14,000 tons in total) was immediately loaded and transported to the reclamation site using Caterpillar 785C dump trucks, where it was spread at the same depth it had been salvaged. Caterpillar bulldozers (D-11 and D-10) were used to spread the donor material directly on top of the overburden material at the required depth. Due to size of the experiment and operational conditions (slope, uneven placement of overburden material placed prior on the reclamation site, and the placement of the forest floor in piles) the spreading of the donor material to the accurate depth proofed to be challenging with the result that there was considerable variation in placement depths within each treatment. To evaluate placement depth, 60 soil pits were established (treatment edges were avoided). In the shallow treatment, placement depth ranged from 9 – 27 cm with an average of 17.2 cm and at the deep treatment, placement depth ranged from 8 – 40 cm with an average of 21.6 cm ($p = 0.017$). It took a total of 16 days to complete the set-up of the experiment. The reclamation site was laid out in a rectangular shape, 300 m wide by 130 m in length. The site was located along a 5 - 12% slope and was divided (blocked) into an upper- and a lower-slope area due to potential differences as a result of slope position. The experiment was set up as a complete randomized block design, with 3 blocks (each 96 × 64 m in size) on the upper-slope and 3 blocks on the lower-slope. In each block the salvaged forest floor material was laid out at the two different salvage depths (shallow or deep), which divided the block into 2 sub-blocks (each 48 × 64 m). All blocks had a 2 m buffer zone between them.

2.2.4 Measurements

Field measurements

Soil temperatures were recorded during the first growing season (April – September 2010) with 31 HOBO data loggers (Onset Computer Corporation, Bourne, Mass.) that were placed in the middle of each sub-block at 5, 15, and 30 cm depths depending on placement depth.

Aspen sucker regeneration sampling was conducted at the end of the first growing season in late August of 2010. Within each sub-block, 32 aspen sucker regeneration plots to a total of 384 plots were established randomly to determine sucker establishment, growth and mortality. In each aspen regeneration plot (4 m²) all emerged suckers were identified as there was some natural aspen regeneration from seed on the reclamation site (Schott unpublished). Sucker heights were measured and their numbers were recorded. In addition, 48 root pits (four 1 m² root pits in each sub-block) were excavated to the top of the overburden layer. The root pits were positioned in a diagonal line (east-south to west-north) down the slope (sub-block) (Figure 2-1). At each root pit, all aspen root fragments bigger than 0.5 cm in diameter were collected. Careful collection of roots fragments was assured by removing the soil in 5 cm thick layers. Depth of root fragment location from the soil surface was measured and the entire root was then excavated. After excavation, root fragments and their suckers were kept chilled in the field until further processing in the lab.

Detailed measurements and sample analyses

In the lab all root fragments and their suckers were carefully washed. Root fragments were then separated into dead and live fragments. Live root fragments were distinguished from dead root fragments by the colour of the outside bark (yellow vs. dark brown) and by the colour of the phloem (white vs. black). Length and diameter at both ends of each root fragment was measured. The amount of damage was determined by percentage of damaged area compared to root fragment surface area and categorized in 10% increments. The number of emerged suckers and non-emerged suckers and their heights were measured. In addition, the number of new roots initiated on these suckers was determined. A small sample from the middle of each root fragment was taken to determine the total non-structural carbohydrate (TNC) root reserves. All roots, suckers and root TNC samples were oven dried at 68°C until constant weight. Dry mass of sucker stems and leaves were determined.

Root tissue samples were ground to pass a 40-mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey). Soluble sugars were extracted from the ground tissue by boiling samples three times in 80% ethanol at 95°C. Phenol-sulfuric acid assay was used to determine colourimetrically total soluble sugar concentrations. The residue was analyzed for starch by enzymatic digestion with a mixture of α-amylase and amyloglucosidase for 20 h, followed by the colourimetric measurement of glucose hydrolyzate with a peroxidase–glucose oxidase-o-dianisidine reagent (Chow and Landhäusser 2004).

In May 2011 the aspen regeneration plots were re-visited to assess sucker winter mortality with a sucker count of flushing suckers. At the end of August 2011, sucker heights were measured and average sucker heights for each plot from 2010 were subtracted from the average 2011 plot height to estimate average sucker growth per plot over the growing season 2011.

2.2.5 Data analysis

The field study was set up as a complete randomized block design with 6 blocks, of which three blocks were located on the upper slope and three blocks were located on the lower slope. Each block consisted of two sub-blocks, which were assigned to one of the two salvage depth treatments (shallow or deep). When analyzing the response variables as a two-way ANOVA it was found that slope position and its interaction with salvage depth did not have a significant effect for any of the measured variables over the experimental period. Therefore, response variables were analyzed as a one-way ANOVA with salvage depth as the main effect. Tested response variables included root numbers, -diameter, -length, -damage, emerged sucker density, their height, number of new roots associated with suckers, sucker leaf dry mass, total sucker dry mass, and the number and height of non-emerged suckers (NES). Sucker density did not meet the assumption of homogeneity of variances and was log transformed. Most response variables did not meet the assumptions of normality (using the Shapiro-Wilk test); therefore, variables were analyzed using both the non-parametric Kruskal-Wallis k-sample test and ANOVA. Since the interpretations were similar between both tests, only the results of the ANOVAs are presented. Since sucker density and height were measured over two growing seasons data were analysed as repeated measures using proc mixed in SAS (SAS 9.2, Cary, North Carolina).

When analyzing the effect of the two treatments on the characteristics of root fragments that produced suckers and on factors that influenced suckering, we only used data of root fragments that produced emerged and non-emerged suckers. Further, when testing the effect of (1) total sucker height on leaf dry mass and (2) sucker height and –dry mass on the number of new roots associated with suckers, we only used data of root fragments that produced emerged suckers for these analyses.

Proportions were used in order to be able to compare suckering responses in association with soil depth, root diameter and root damage. These proportions were calculated by dividing each response variable (e.g. number of dead roots, number of live roots without suckers, number of live roots with suckers) by the total number of roots in each respective category. When testing the effect of root diameter on root suckering ability, root fragments were divided into root diameter classes (class 1: 0.5 – 1 cm; class 2: 1.1 – 2 cm; class 3: 2.1 – 3 cm; etc.) in order to calculate the proportions.

To test the impact of salvage and burial depth on root mortality the proc catmod procedure in SAS was used. A significance level of $\alpha = 0.05$ was used for all analyses.

2.3 Results

Suckering response

At the end of the first growing season (August 2010) 9,355 suckers ha^{-1} had emerged in the deep treatment compared to only 5,026 suckers ha^{-1} in the shallow treatment (Figure 2-2). While in both treatments, sucker density decreased similarly by 13% during the first winter, the decrease in sucker density after the second growing season (August 2011) was significantly higher in the shallow treatment (55%) than in the deep treatment (29%), resulting in a significant time × treatment interaction ($p = 0.048$). Thus, average sucker density after two growing seasons was only 2,253 suckers ha^{-1} in the shallow treatment compared to 6,654 suckers ha^{-1} in the deep treatment ($p < 0.001$; Figure 2-2).

The stocking of aspen regeneration (proportion of $4m^2$ plots that contain at least one sucker) was 65% of the plots containing suckers but was similar between the two treatments. Further, in the stocked plots sucker density per plot was clumpy where sucker densities ranged from a single stem to clumps of up to 22 suckers per plot.

Average height of emerged suckers was affected by the depth treatment ($p = 0.002$). After the first growing season (August 2010) average sucker height was 14.0 cm in the shallow and 18.7 cm in the deep treatment ($p < 0.001$). Although sucker height growth between the two depth treatments was similar with an average of 44.1 cm over the course of the second growing season, suckers in the shallow treatment continued to be shorter with a total height of 56.4 cm compared to 64.5 cm in the deep treatment at the end of the second growing season ($p < 0.001$; Figure 2-3).

Impact of depth treatments on donor root distribution, damage, and mortality

In the more detailed root survey, a total of 346 root fragments were extracted from the 48 root pits. As anticipated, the shallow treatment had double the number of root fragments per volume of soil with an average of 62.9 roots m^{-3} compared to 31 roots m^{-3} in the deep treatment ($p < 0.001$; Table 2-2). Although statistically not significant, root fragments tended to be somewhat longer in the deep treatment compared to the shallow treatment ($p=0.055$; Table 2-2).

Root fragments collected from the root pits in the shallow treatment were located at depths ranging from 0 - 20 cm while root fragments from pits in the deep treatment were located at depths ranging from 0 - 30 cm. In both treatments the number of root fragments decreased with the increase of soil depth ($p < 0.001$); however, the number of root fragments in the shallow treatment was with an average of 90.8 roots m^{-3} at 5 cm soil depth and 55.8 roots m^{-3} at 10 cm soil depth more than double the number of root fragments in the deep treatment with an average of 40.8 roots m^{-3} at 5 cm soil depth and 25.8 roots m^{-3} at 10 cm soil depth ($p < 0.001$; Table 2-3). The number of root fragments of the lower soil depths was not significantly different between the two treatments with an average of 26.3 roots m^{-3} at 15 cm and 6.3 roots m^{-3} at 20 cm soil depth (Table 2-3).

After the first growing season (August 2010) 67% of the excavated roots in the root pits were already dead; however, root mortality in the shallow treatment tended to be somewhat higher (70%) compared to the deep treatment (57%) ($p = 0.079$; Table 2-2). Dead root fragments had neither emerged nor non-emerged suckers (NES) attached.

The amount of surficial damage to the root fragments as a result of the salvage and placement operations was not different between the two depth treatments ($p = 0.522$). At the shallow treatment, on average 32.5% of the root surface of the root fragments were damaged compared to 34.8% surface damage on root fragments of the deep treatment (Table 2-2). The surface damages included abrasions, loss of phloem, cuts, twisted and torn root fragment ends.

Average daily growing season (April to August 2010) soil temperature decreased significantly with soil depth ($p < 0.001$), from 11.8°C at 5 cm to 10.3°C at 15 cm and to 9.3°C at 30 cm depth. In both depth treatments percent soil moisture in 2010 increased with soil depth; however, soil moisture was overall higher in the shallow treatment, which was mostly driven by the upper soil layers (0-10 cm) ($p < 0.005$; Table 2-3). In the shallow treatment, percent soil moisture increased only slightly (21 to 25%) between 5 and 30 cm soil depth, while it increased significantly from 18% to 31% between 5 and 40 cm soil depth in the deep treatment, resulting in a significant depth treatment by soil depth interaction ($p = 0.024$; Table 2-3).

Root fragment characteristics and suckering response

Of all the 346 collected root fragments from the root pits, only 15% had produced suckers (emerged and/or non-emerged suckers (NES)) after the first growing season (Table 2-4). Although the total number of suckering root fragments was not different between the two depth treatments ($p = 0.128$), a lower proportion of the root fragments located in the upper 20 cm of the soil suckered in the shallow treatment (4%) than in the deep treatment (8%) as a result of the differences in root density between the depth treatments (Table 2-3).

Overall, the suckered root fragments collected in the root pits produced a total of 77 emerged and 71 non-emerged suckers across both depth treatments. However, in both treatments no suckering of root fragments occurred from soil depths greater than 20 cm (Figure 2-4). The average soil depth, from which root fragments suckered, was different between the two treatments ($p = 0.007$); in the deep treatment, suckers emerged from root fragments at an average soil depth of 10.4 cm, whereas in the shallow treatment they suckered from an average depth of 7.5 cm (Table 2-5).

As already found in the sucker survey (see above), the number of suckers that emerged above the soil surface ($p = 0.013$) and their total height (including the distance a sucker had to grow through the soil profile) ($p < 0.001$) were higher in the deep treatment. Root fragments in the deep treatment produced on average 1.8 emerged suckers, which grew an average total height of 22.6 cm. Root fragments in the shallow treatment produced on average 1.3 suckers per root and grew an average total height of 15 cm. These differences in sucker height were also reflected in differences in sucker leaf dry mass ($p < 0.001$) and total sucker dry mass ($p = 0.008$; Table 2-5).

Although the average damage on suckered root fragments was not different between depth treatments ($p = 0.303$) with 15% of surficial damage root^{-1} at the shallow treatment and 12% at the deep treatment, the number of emerged suckers in live root fragments decreased with increasing root damage ($p < 0.001$; Figure 2-5). Live root fragments that had suckered had on average 14% surficial damage compared to 24% in live root fragments that did not produce suckers and 40% in dead root fragments ($p < 0.001$).

Volume of the root fragments did not affect the suckering response in both treatments ($p = 0.851$). Root fragments that produced suckers ranged in diameter from 0.5 – 5.3 cm and in length from 8.1 and 132.1 cm and were not affected by the two depth treatments (both $p > 0.1$; Table 2-4). However, the majority of suckers were produced on root fragments with diameters between 1 and 4 cm (Figure 2-6) and ranged from 15.3 to 132.1 cm in length.

As diameter and length varied greatly among root fragments, we expressed the reserve status of root fragments not only as TNC tissue concentration but also as TNC content, which was estimated from the TNC concentration of each root fragment and its estimated volume (based on a cylinder). The amount of TNC content (concentration) in root fragments at the end of the first growing season was affected by the presence or absence of emerged suckers ($p < 0.001$). Live root fragments that produced emerged and non-emerged suckers had 8.9 g (7.9%) TNC root^{-1} ($p = 0.002$ ($p < 0.001$)) while live root fragments without suckers had 3 g (4.7%) TNC root^{-1} (both $p < 0.001$). Root fragments that were dead at the end of the growing season and did not produce any suckers had 1.2 g (1.5%) TNC root^{-1} while root fragments initially had 15.3 g (12.9%) TNC reserves root^{-1} (both $p < 0.001$; Figure 2-7). Similar results were found in matters of starch content and concentration. Dead root fragments had 0.09 g (0.1%) starch root^{-1} while root fragments initially had 0.6 g (0.5%) starch root^{-1} (both $p < 0.001$). Starch content of live root fragments with emerged and non-emerged suckers were 0.4 g root^{-1} ($p = 0.051$) while starch concentration slightly increased by 0.1% root^{-1} ($p = 0.833$) at the end of the growing season compared to the initial starch measurements (Figure 2-7).

Further, it was found that root fragments that were dead at the end of the growing season already had a lower TNC content (14 g root^{-1}; $p = 0.015$) and TNC concentration (11.7% root^{-1}; $p < 0.001$) to start with at the beginning of the growing season compared to root fragments that stayed alive (22.3 g (15.7%) root^{-1}) (Figure 2-8). This was also found for the starch content and concentration. Root fragments that were dead at the end of the growing season had 0.8 g less TNC content ($p = 0.005$) and 0.5% less TNC concentration ($p = 0.006$) than root fragments that stayed alive (1.2 g, 0.9%) (Figure 2-8).

2.4 Discussion

Despite the lower density of donor roots per soil volume, suckering success (e.g. emerged sucker density) was higher in the deep treatment, and with that a greater proportion (almost double) of root fragments suckered in the upper 20 cm compared to the shallow treatment. In addition, root fragments in the deep treatment produced more suckers per fragment, sprouted from somewhat greater depths, grew taller, and had lower mortality rates than suckers from root fragments in the shallow treatment. At the end of two growing seasons 6,654 suckers ha^{-1} still remained at the deep treatment, which could be sufficient to produce a productive aspen forest with crown closure (Alberta Sustainable Resource Development 2011). Thus, the improved suckering in the deep treatment strongly supports our hypotheses that soil-root contact plays a crucial role in the success of root suckering from root fragments. Thus, despite a much lower root density in the top 20 cm of the deep treatment, the greater salvaged soil volume likely resulted in good root-soil contact and provided better protection of these root fragments from early desiccation. The higher mineral to organic soil ratio at the deep treatment might have also ensured better conditions for root survival as a result of better water infiltration and increased moisture storage (Bowen et al. 2005).

This study is the first published experiment that focused specifically on the establishment of aspen from root fragments through suckering in the field. Earlier and somewhat similar studies focused mainly on the impact of forest floor salvage and placement depths on the establishment of forest understory plant communities, and therefore are difficult to compare to our study. However, a few unpublished studies have observed aspen presence on salvage sites and indicated that aspen regeneration could be better when forest sites are salvaged and applied at greater depths (Mackenzie and Naeth 2007; 2008 as cited in Alberta Environment 2010). Sucker numbers in our study were much higher than those reported in the above mentioned studies, which reported an average of 1,500 aspen stems ha^{-1} at the best performing salvage treatments. In addition, the authors of these studies did not distinguish between aspen regeneration from sucker and seed origin. Although sporadic, seed origin aspen density can be significant on reclamation sites, when seed crops from adjacent sites coincide with favorable climatic conditions; on our site it was found to be >5,000 stems ha^{-1} after the first growing season (Schott unpublished).

Regardless of the depth treatment, successful suckering occurred mostly from root fragments ranging in diameter from 1 to 4 cm that were located in the upper 20 cm of the placed soil, while fragment length did not play much of a role. These results are very similar to observations from natural undisturbed aspen root systems where suckers mostly arose from roots with diameters between 0.5 – 2.5 cm (Kemperman 1978; Schier and Campbell 1978; DesRochers and Lieffers 2001) and from soil depths between 4 to 15 cm (Horton and Maini 1964; Schier 1973; Kemperman 1978; Schier and Campbell 1978; Brown and DeByle 1987; Navratil 1991; Mundell et al. 2007). The reason that our suckers grew successfully from slightly deeper soil depth (20 cm) could be the result of a missing LFH layer, where litter or slash can inhibit soil warming at deeper soil depth and/or restrict suckers from reaching the surface (Brown and DeByle 1987; Mulak et al. 2006; Renkema et al. 2009). However more interestingly, root fragments located at greater soil depths failed to initiate suckers. In natural root systems the lack of aspen suckers grown from deeper soil depths has been related to soil temperatures and potentially hormonal control from the complete root system (Maini and Horton 1966; Zasada and Schier 1973; Fraser et al. 2002). However, other studies have also shown that although low soil temperatures affect sucker growth they do not inhibit sucker initiation (Landhäusser et al. 2006). In addition, suckering should have been initiated by light damage to some of the root fragments at deeper soil depths (Landhäusser and Lieffers 1998; King et al. 1999; Landhäusser et al. 2001, 2003). In our study, low soil temperatures at deeper soil depths should not have been a key factor for root fragments failing to initiate suckers from soil depths greater than 20 cm as soils at these depths reached temperatures adequate for suckering (≥ 8°C). Fraser (2002) found that on average 60 degree-days (with an average temperature of 14°C and a base temperature of 8°C) were needed for sucker initiation. Based on her results we found that on the reclamation site the required 60 degree-days were reached on June 21st, 2010 at a soil depth of 30 cm while at a soil depth of 5 cm they were reached on May 20th and on June 13th at a soil depth of 15 cm. In comparison in chapter 3 root fragments initiated suckers at a soil depth of 40 cm; however, here heat sums of 60 degree-days were reached 42 days earlier (May 10th). Thus, by reaching required heat sums relatively late in the growing season, carbohydrate reserves of root fragments of deeper depths might have already been too depleted to allow for the expansion of suckers at the reclamation site.

In our study we found that although the amount of root damage was not different between the depth treatments, there was a strong relationship between suckering ability of root fragments and root damage. Results showed that root damage of up to 40% did not inhibit the initiation of suckers. However, root fragments with higher amounts of damage did not produce any suckers. Similar results were observed in past studies, proposing that light to moderate damage to roots had a positive effect on root suckering likely by affecting the hormonal balance of intact root systems (Farmer 1962; Lavertu et al. 1994; Fraser et al. 2004; Renkema et al. 2009). However, the severe damage and fragmentation of root systems, as it did happen during the forest floor transfer in our study, was found to have detrimental effects on suckering (Fraser et al. 2004; Renkema et al. 2009) by limiting the access to resources through the loss of fine roots and connectivity (Zahner and DeByle 1965) and by creating entry ways for pathogens causing disease (Basham 1988; Wolken et al. 2009).

Given that carbohydrate reserves are known to strongly influence sucker growth and leaf area development (Schier and Zasada 1973; Landhäusser and Lieffers 2002; Frey et al. 2003), we speculate that exhaustion of total non-structural carbohydrate (TNC combined sugar and starch content) reserves could have played a significant role in suckers not emerging above the soil surface. Since only a limited amount of carbohydrates can be stored in root fragments the quick emergence of suckers and establishment of leaf area is crucial to root and sucker survival. Emerged suckers or rather their leaf area appear to somewhat replenish carbohydrate reserves in the parent roots as soon as suckers start to photosynthesize independently, supplying new energy for root maintenance and growth (Lieffers and DesRochers 2001; Fraser et al. 2002; Landhäusser and Lieffers 2002; Landhäusser et al. 2006). This relationship was also found between our root fragments and suckers as roots with emerged suckers attached had a considerably higher TNC content and concentration than root fragments without emerged suckers (Figure 2-7).

Besides soil temperature and carbohydrate reserves some studies suggest that nutrient composition could affect the establishment (Landhäusser et al. 2010) and growth of suckers (Gifford 1967; Frey 2001; Fraser et al. 2002; Landhäusser et al. 2010). Nutrient availability was different between our two depth treatments (data not shown (Fair 2011)). Plant root simulator probes showed that at the deep treatment soil total nitrogen (N) with 26.1 µg 10 cm^{-2} over a three month and nitrate (NO_3-N) with 20.4 µg was higher compared to 14.3 µg total nitrogen and 9.4 µg nitrate in the shallow treatment. Potassium (K) with 44.7 µg and sulphur (S) with 206 µg were lower in the deep treatment when compared to the shallow treatment with 64.7 µg of K and 606.5 µg of S (Fair 2011). Nitrate can enhance aspen sucker initiation while ammonium nitrate (NH_4NO_3) does not influence the initiation of suckers (Landhäusser et al. 2010), but can increase height and root system growth (Fraser et al. 2002). Thus, the higher levels of nitrate may also help explain better suckering success in the deep treatment.

Once emerged, sucker mortality after the first winter was considerably lower in the deep treatment than in the shallow treatment and mortality appeared to mostly affect suckers that had emerged later in the growing season. These late suckers were generally short and had little leaf area, which might have been inadequate to replenish carbohydrate reserves to allow for reflushing in the spring. The overall higher mortality rates observed after the second growing season are likely driven by increased competition from the developing herbaceous vegetation on the reclamation site. Sucker regeneration has been found to be sensitive to competition by herbaceous vegetation such as grasses (Landhäusser and Lieffers 1998). In our study invasive agricultural species like

Alsike clover (*Trifolium hybridum*) flourished on the site and overtopped the smaller suckers, which were prone to being smothered under the heavy leaf cover. Sucker densities were clearly not high enough to shade out and suppress these invasive species and allow for the rapid development of a canopy (Fair 2011). Alternatively, continued spread of disease from wounds on the root fragments may have had an impact on the less vigorous suckers growing in the shallow treatment.

Management implications

Salvage and direct placement appear to be a viable option to transfer aspen root propagules to a reclamation site. In the best treatment (deep salvage) sucker densities of 6,654 sucker stems ha^{-1} remained after the end of the second growing season; however, this density and the sucker growth rates did not provide sufficient canopy initially to control competing vegetation and to help maintain the transferred forest understory species (Fair 2011). However, with 65% of the plots containing one or more suckers, crown closure could be reached in the near future as long as suckers continue to survive and grow.

Salvage depth and therefore improved soil contact of root fragments appears to play a significant role in the success of suckering. Although the deep treatment resulted in higher sucker numbers and better sucker performance at our reclamation site, salvage and placement depth have to be determined for each reclamation project and donor site. Prior to salvage, donor site assessments are necessary to identify soil properties and conditions as well as the rooting depth of the aspen. In cases where soil properties allow for the deeper salvage without an inclusion of unsuitable soil horizons (e.g. heavy clays), it can be proposed that the same donor material may be spread over a larger reclamation area than the donor area, as it appears that a placement depth greater than 20 cm is not essential for the success of aspen regeneration from root propagules. Also, the dilution of the salvaged forest floor material with suitable lower soil horizons appeared to have little effect on the diversity of understory species transferred from the donor site; however, their abundances and growth rates were significantly lower in these more diluted materials (Fair 2011). Placing the forest floor material too shallow; however, (e.g. < 10 cm) may result in an increased risk of desiccation of root fragments due to insufficient root-soil contact (Figure 4).

Although light to moderate damage to root fragments did not inhibit root suckering, severe root damage caused by the salvage and placement did affect root suckering negatively; thus, handling of the material should be minimized. Frozen soil conditions at time of harvest of donor material might have exacerbated root damage and therefore the salvage of donor material in late fall or early spring might be an alternative as suckering potential of the root system is similar (Mundell et al. 2007); however, other variables such as soil compaction might become an issue under the thawed soil conditions (Bates et al. 1993, Mundell et al. 2007). Root diameter was important for the suckering success; as a result, healthy 20 - 60 year old aspen stands that have large lateral root systems are likely prime candidates as root donor stands for reclamation sites. Due to the high mortality rates of root fragments and suckers, clumpy sucker distribution, and much lower sucker density compared to natural stands, the supplemental planting of seedlings is likely still required to speed up the canopy development on these forest reclamation sites. However, the benefits of directly transferring and placing natural plant materials and instantly increasing plant diversity on reclamation sites, clearly outweighs the present operation costs of stockpiling these forest floor materials and losing the viability of the propagules contained within.

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Tables

Table 2-1: Tree and root data (mean ± SD) collected at the donor site prior to salvage (n = 20).

	Stem m^{-2}	Diameter at breast height (cm)	Height (m)	Root density* (roots m^{-3}_{soil})	Root volume (cm^3_{root} m^{-3}_{soil})	Root diameter (cm)
Trembling aspen	1.3 ± 0.8	3.2 ± 0.9	7.0 ± 2.2	178.2 ± 96.7	447.7 ± 341	1.2 ± 0.3

***Aspen root fragments ≥ 5 cm in diameter with length ranging from 4.3 to 45.5 cm.**

Table 2-2: Root response variables (mean ± SE) of the root survey at the reclamation site (August 2010).

Response variables	Treatment		p-values
	shallow	deep	
Root density m^{-3}	62.9 ± 7.1	31.0 ± 3.0	0.001
Root diameter (cm)	1.5 ± 0.1	1.6 ± 0.1	0.647
Root length (cm)	36.2 ± 2.0	41.4 ± 2.4	0.055
Root damage (%)	32.5 ± 2.9	34.8 ± 3.4	0.522
TNC content (g) per root fragment	2.7 ± 0.8	2.6 ± 0.4	0.956
Root mortality (%)*	70 ± 5.0	57 ± 8.0	0.080

***Root mortality: Percentage of root fragments that were dead by the time they were excavated (August 2010). Roots were classified dead when the outside bark was dark brown and the phloem black.**

Table 2-3: Root distribution in 5 cm soil depth increments and soil moisture in 10 cm soil depth increments for the deep and shallow depth treatments (mean ± SE; n = 6).

Soil depth (cm)	Root density m^{-3}		p-values	Soil moisture (%)		p-values
	shallow	deep		shallow	deep	
0 - 5	90.8 ± 11.5 a	40.8 ± 8.0 a	0.001	21.4 ± 1.1 a	18.4 ± 0.6 c	0.005
6 - 10	55.8 ± 16.3 b	25.8 ± 9.7 b	0.001			
11 - 15	29.2 ± 8.7 c	23.3 ± 6.3 b	0.722	24.0 ± 1.2 b	23.8 ± 0.7 b	0.843
16 - 20	6.7 ± 3.1 d	5.8 ± 1.5 c	0.533			
21 - 25	no sample	4.2 ± 1.5 c	n/a	25.0 ± 2.5 ab	23.7 ± 1.1 b	0.522
26 - 30	no sample	5.8 ± 3.0 c	n/a			
31 - 35	no sample	no sample	n/a	no sample	31.3 ± 3.1 a	n/a
36 - 40	no sample	no sample	n/a			

Note: Within-response variables values sharing the same letter are not significantly different (α = 0.05) (LSD means comparison test).

Table 2-4: Characteristics of root fragments with emerged and/or non-emerged suckers (mean ± SE) collected in soil pits after the first growing season (August 2010) in the shallow and deep depth treatments (n = 6).

Response variables	Treatment		p-values
	shallow	deep	
Number of suckered root fragments[1] (m^{-3})	9.3 ± 1.0	8.1 ± 0.7	0.128
Diameter of suckered root fragments (cm)	2.2 ± 0.3	1.7 ± 0.2	0.197
Length of suckered root fragments (cm)	42.0 ± 2.6	49.4 ± 5.5	0.088
Percent damaged area of suckered root fragment (%)	14.8 ± 3.9	11.7 ± 2.8	0.303
TNC content of suckered root fragment (g)	8.3 ± 4.4	7.0 ± 1.9	0.991
Number of non-emerged suckers per root fragment	1.0 ± 0.2	1.9 ± 0.7	0.148
Height of non-emerged suckers (cm)	4.0 ± 0.6	3.6 ± 0.7	0.075

[1] **Number of root fragments > 0.5 cm in diameter that produced emerged and non-emerged suckers.**

Table 2-5: Characteristics of emerged suckers initiated on root fragments (mean ± SE) collected in the root pits and associated with the two depth treatments after the first growing season (August 2010) (n = 6).

Response variables	Treatment		p-values
	shallow	deep	
Root suckering depth (cm)	7.5 ± 1.3	10.4 ± 1.7	0.007
Number of emerged suckers m^{-2}	0.2 ± 0.03	0.5 ± 0.1	0.013
Number of emerged suckers per root fragment	1.3 ± 0.2	1.8 ± 0.3	0.040
Total emerged sucker height[1] (cm)	15.0 ± 1.1	22.6 ± 1.3	0.001
Surface emerged sucker height[2] (cm)	8.9 ± 1.3	12.5 ± 0.9	0.020
Leaf dry mass per emerged sucker (g)	0.2 ± 0.04	0.4 ± 0.04	0.001
Total emerged sucker dry mass[3] (g)	0.6 ± 0.1	1.4 ± 0.3	0.008
Number of new roots per emerged sucker[4]	2.3 ± 0.9	2.2 ± 0.9	0.866

[1] Sucker height measurement from root fragment to sucker terminal bud.
[2] Sucker height measurement from soil surface to sucker terminal bud.
[3] Dry mass of the entire emerged sucker including below ground stem.
[4] Number of new roots associated with emerged suckers.

Figures

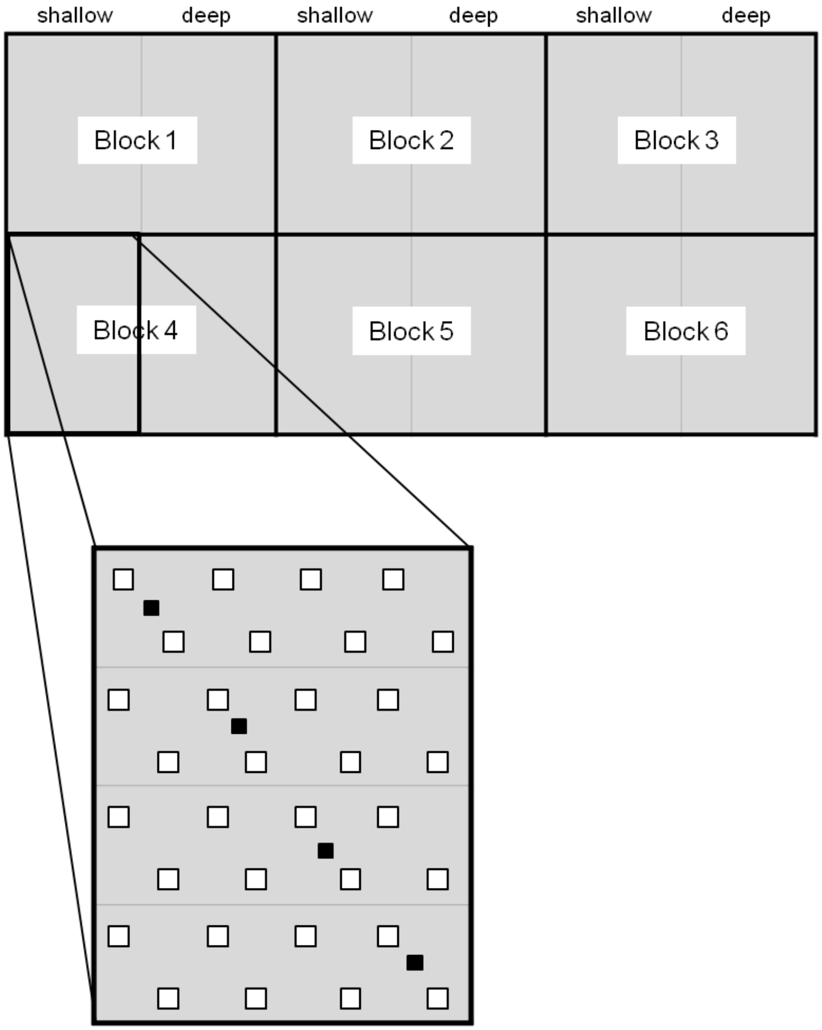

Figure 2-1: Experimental set-up of the reclamation site with 6 blocks. In each block the forest floor - mineral mix was laid out at the two different salvage depths (shallow or deep), which divided the block into 2 sub-blocks. Within each sub-block, 32 permanent aspen regeneration plots (each 4 m^2; white squares) and four 1 m^2 root pits (black squares) were established.

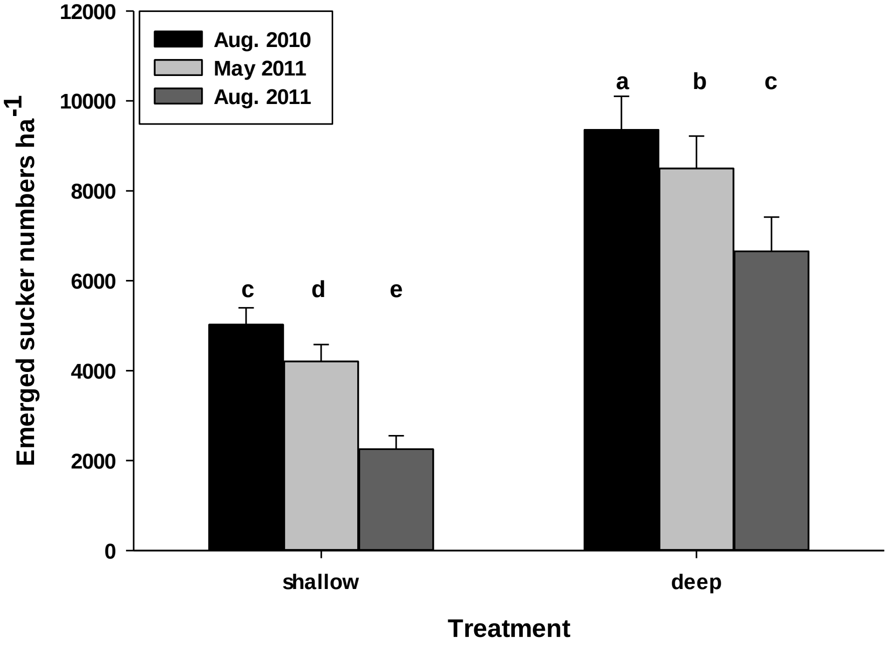

Figure 2-2: Density of emerged suckers from the sucker survey between August 2010 and August 2011 on the reclamation site in response to the two depth treatments. Error bars indicate one standard error of the mean (n = 6).

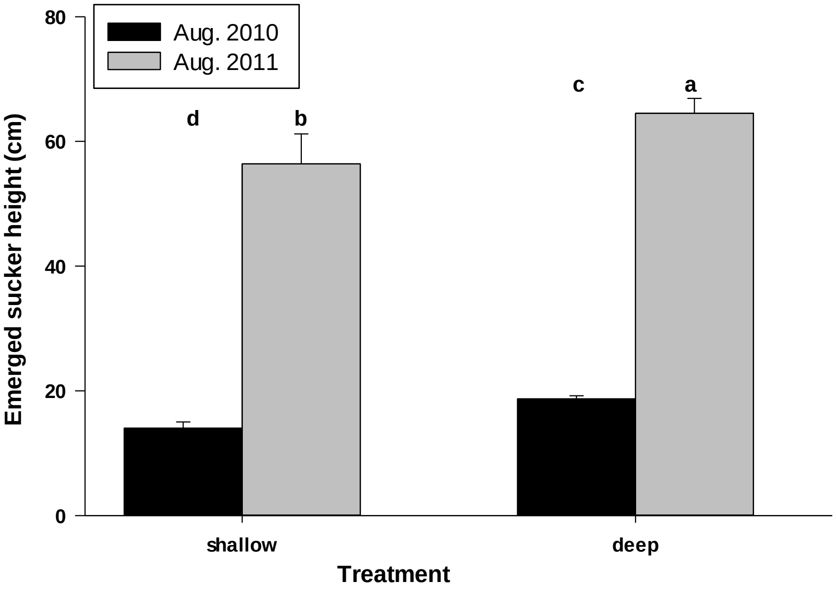

Figure 2-3: Height of emerged suckers from the sucker survey for the shallow and deep depth treatments at the end of the first (Aug. 2010) and second growing season (Aug. 2011). Error bars indicate one standard error of the mean.

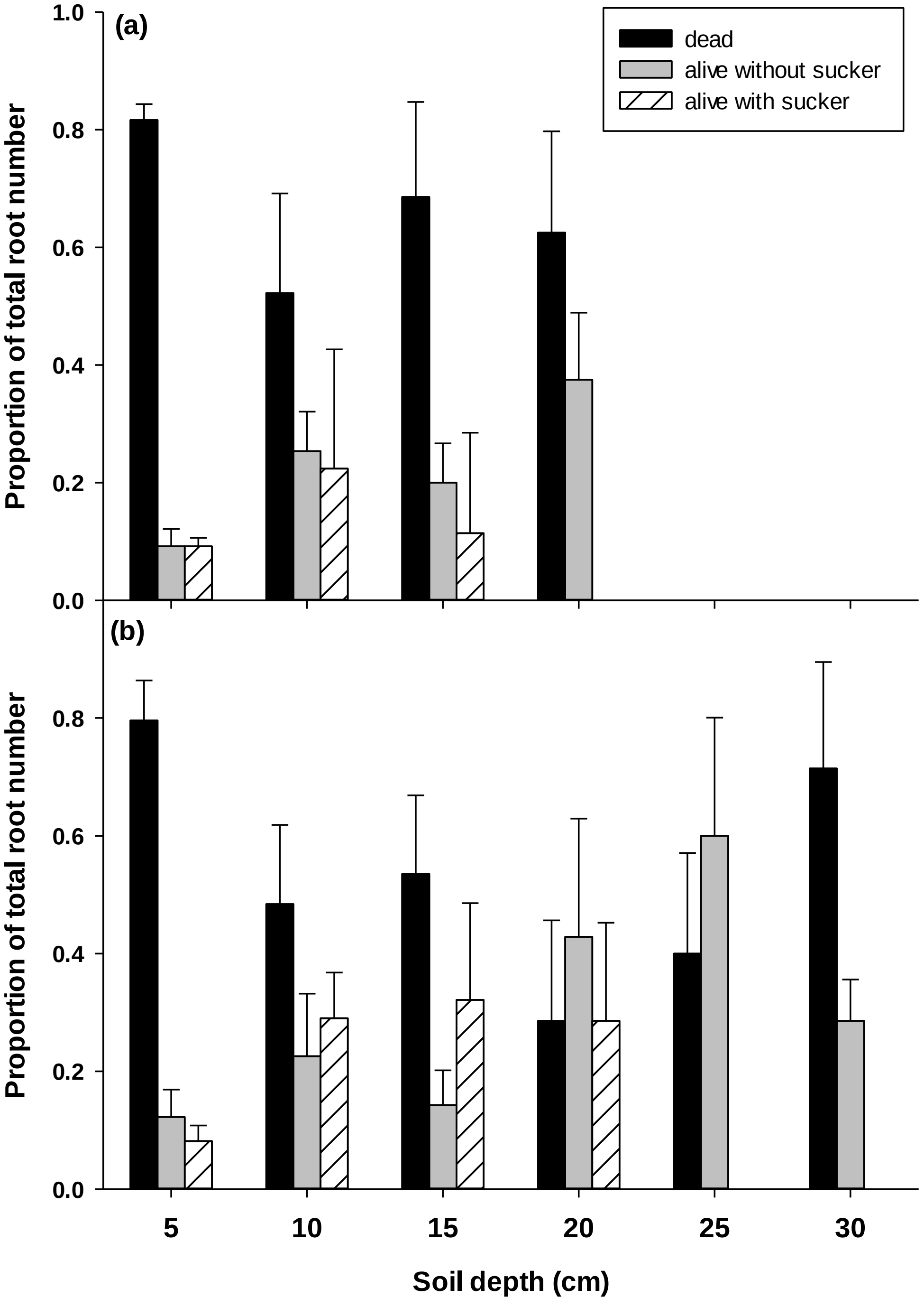

Figure 2-4: Distribution of dead and alive root fragments in the shallow (a) and deep (b) depth treatment after one growing season. Data are presented as a proportion of live root fragments that had emerged and/or non-emerged suckers (alive with sucker), live root fragments that had no suckers (alive without sucker), and dead root fragments (dead) vs. the total number of root fragments found in each respective soil layer (5 cm). Error bars indicate one standard error of the mean (n = 6).

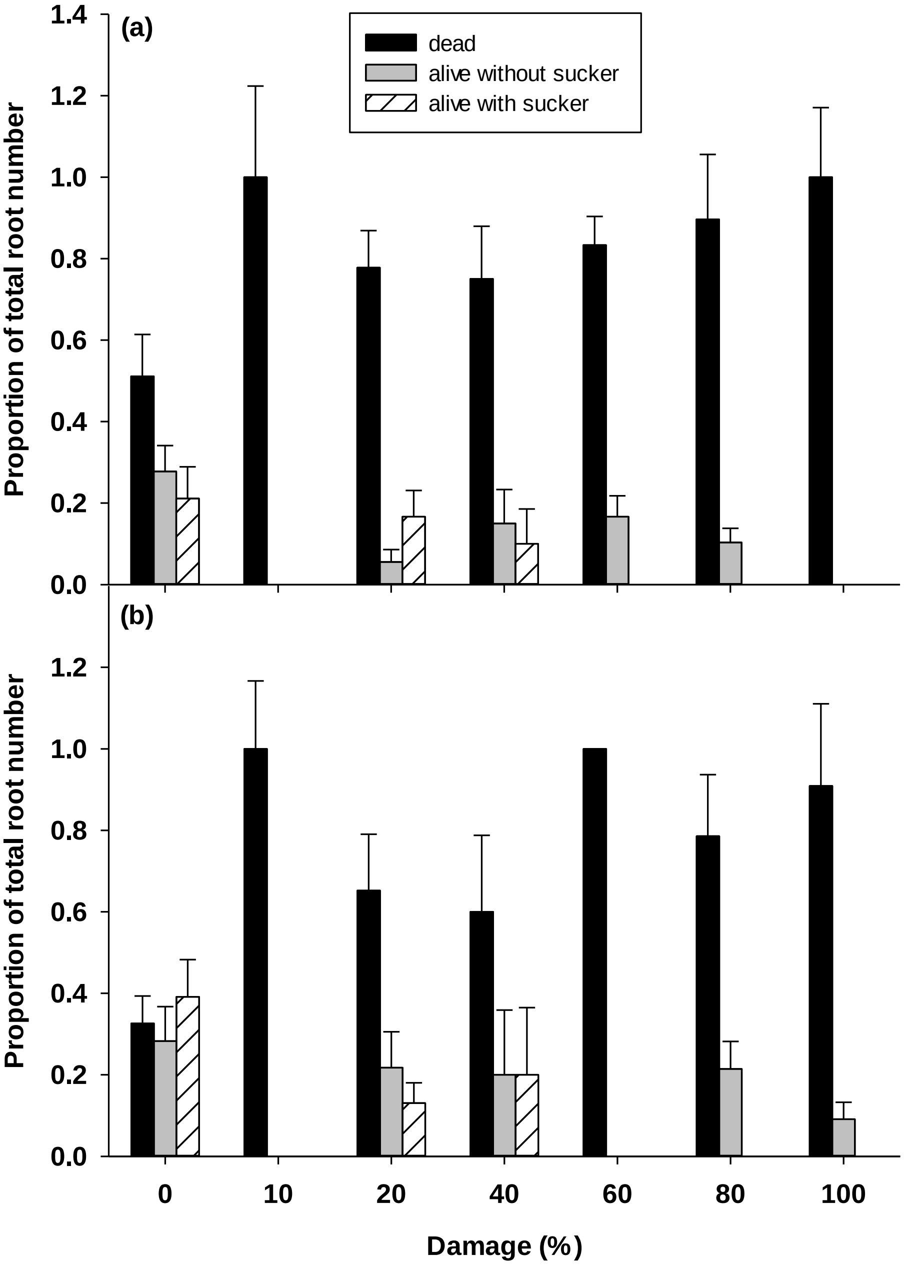

Figure 2-5: Distribution of dead and alive root fragments in the shallow (a) and deep (b) depth treatment after one growing season. Data are presented as a proportion of live root fragments that had emerged and/or non-emerged suckers (alive with sucker), live root fragments that had no suckers (alive without sucker), and dead root fragments (dead) vs. the total number of root fragments found in each respective damage category. Error bars indicate one standard error of the mean (n = 6).

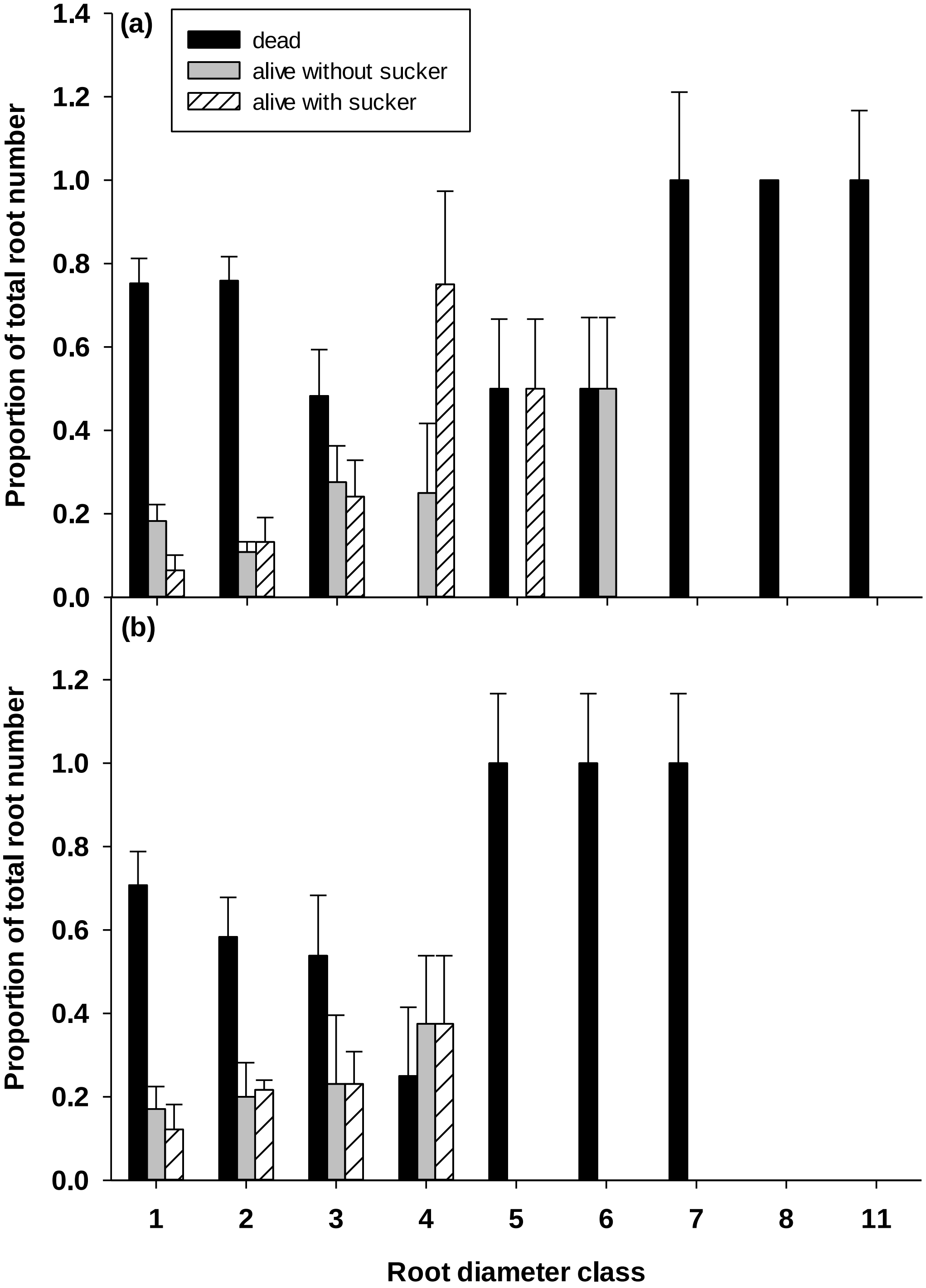

Figure 2-6: Distribution of dead and alive root fragments in the shallow (a) and deep (b) depth treatment after one growing season. Data are presented as a proportion of live root fragments that had emerged and/or non-emerged suckers (alive with sucker), live root fragments that had no suckers (alive without sucker), and dead root fragments (dead) vs. the total number of root fragments found in each respective diameter class (n = 6). Diameter class 1: 0.5 – 1 cm; class 2: 1.1 – 2 cm; class 3: 2.1 – 3 cm etc. Error bars indicate one standard error of the mean.

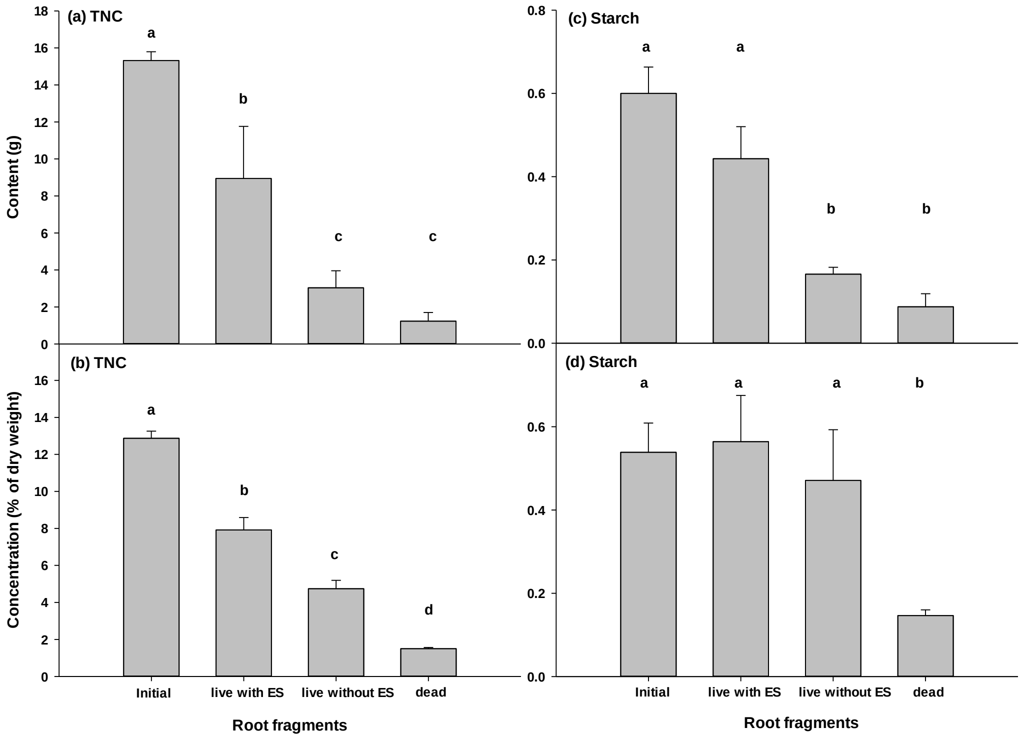

Figure 2-7: TNC content (a) and concentration (b) and starch content (c) and concentration (d) of root fragments at the beginning of the growing season (initial), and of live root fragments that produced emerged suckers (live with ES), live root fragments without emerged or non-emerged suckers (live without ES), and dead root fragments without suckers (dead) at the end of the growing season (n = 6). Live root fragments that produced emerged suckers also could have non-emerged suckers. Data set includes root fragments of the shallow and deep treatment. Error bars indicate one standard error of the mean.

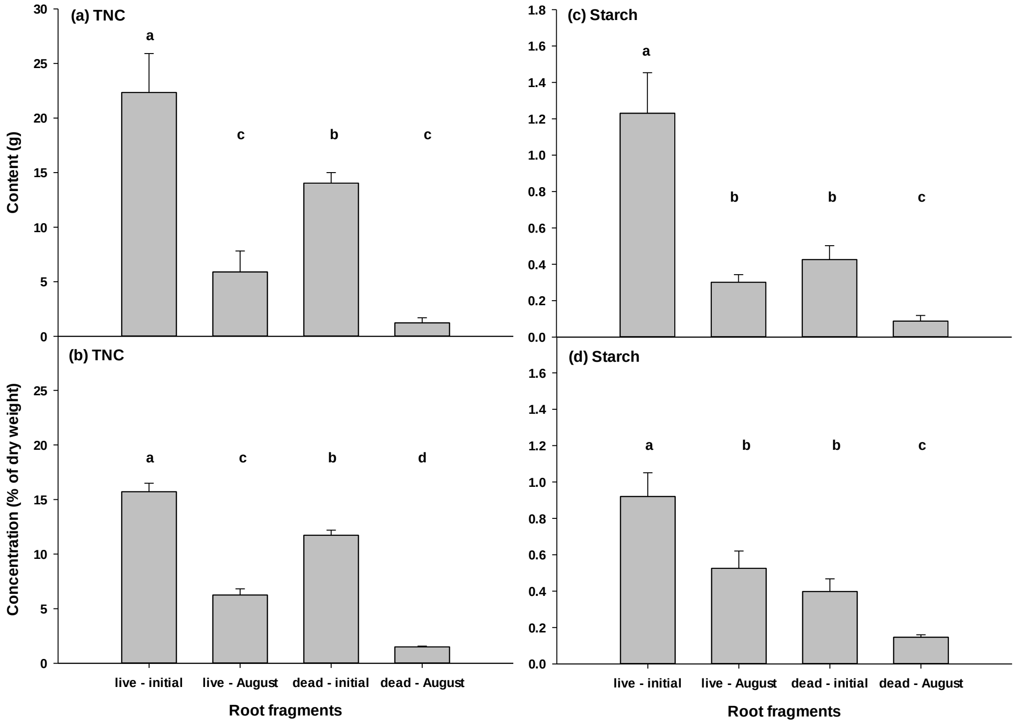

Figure 2-8: TNC content (a) and concentration (b) and starch content (c) and concentration (d) of live root fragments at the beginning (live-initial) and at the end of the growing season (live-August) and of dead root fragments at the beginning (dead-initial) and at the end of the growing season (dead-August) (n = 6). Data set includes root fragments of the shallow and the deep treatment. Error bars indicate one standard error of the mean.

Chapter 3: Effects of soil depth, root diameter and fine root growth on root suckering ability of aspen

3.1 Introduction

Trembling aspen (*Populus tremuloides* Michx.) is known to regenerate aggressively from its root system through suckering after a disturbance such as fire or logging kills or removes the above ground portion of the clone (Farmer 1962; DesRochers and Lieffers 2001; Frey et al. 2003). When the aboveground portion of the stand is removed, the rapid re-establishment of new suckers and leaf area is of significant importance to the survival of parent roots, as the suckers supply carbohydrates for root maintenance and growth (Lieffers and DesRochers 2001; Fraser et al. 2003; Landhäusser and Lieffers 2002; Landhäusser et al. 2006). Suckers initiate from preformed or adventitious shoot primordia formed on roots (Schier 1973), relying on the parent root system for the initial growth demands such as water, nutrients and carbohydrate reserves (Zahner and Debyle 1965; Schier and Zasada 1973; Landhäusser and Lieffers 2002; Frey et al. 2003). In other words not only are the young suckers dependent on the original root system, the root system is also dependent on suckers for survival (DesRochers and Lieffers 2001).

Important factors such as rooting depth (Strong and LaRoi 1983; Mundell et al. 2007) and root diameter (Kemperman 1978; Schier and Campbell 1978; DesRochers and Lieffers 2001) as well as root carbohydrate reserves (Schier and Zasada 1973; Landhäusser and Lieffers 2002; Frey et al. 2003) are known to influence sucker initiation and growth in natural regenerating stands. Aspen generally has a shallow lateral root system where most of the roots are located in the upper 5 to 20 cm of the soil profile (Strong and LaRoi 1983; Mundell et al. 2007) and due to the more favourable conditions in these upper soil layers (e.g. warm soil temperature, aeration, high water-holding capacities, and high concentration of nutrients) most suckers originate from roots that are located at depths of 4 to 15 cm (Horton and Maini 1964; Schier 1973;

Kemperman 1978; Schier and Campbell 1978; Brown and DeByle 1987; Navratil 1991).

Besides rooting depth and root diameter, root carbohydrate reserves are considered a key factor in successful suckering. Although carbohydrate reserves appear not to influence the initiation of suckers, they are important for early sucker growth and leaf area development (Schier and Zasada 1973; Landhäusser and Lieffers 2002; Frey et al. 2003). The amount of carbohydrate reserves stored in a root fragment does increase with root size (Dirr and Heuser 1987; Nguyen et al. 1990; Ede et al. 1997); consequently, it could be assumed that the production and growth of suckers should be more successful on larger roots. However, it is known that in intact root systems most suckers originate from roots ranging in diameter from 0.5 – 2.5 cm (Kemperman 1978; Schier and Campbell 1978; DesRochers and Lieffers 2001). Limited carbohydrate reserves may explain reduced suckering on roots smaller that 0.5 cm (Steneker and Walters 1971); however, the reasons why suckering is less observed on roots with larger diameters are unclear. It has been speculated that since large diameter roots are not as abundant as smaller diameter roots (Perala 1978), suckering from large diameter roots is not as often observed. However, larger roots may also have less ability to produce suckers because their tissue is mature and less active (Mahlstede and Haber 1957; Farmer 1962; Perala 1978; Hackett 1988). Stenvall et al. (2006) suspected that this is also the reason for reduced fine root development and growth on root fragments of larger diameter. The ability to grow new fine roots is essential to the survival of aspen roots and the initiated suckers, as fine roots are the primary pathway for water and nutrient uptake to support the growing suckers (Eissenstat and Caldwell 1988; Pregitzer et al. 1993). This is particularly relevant in fragmented root systems, where few or no fine roots are attached to the root fragments. Harvesting operations and other surface disturbances can significantly damage and fragment aspen root systems affecting regeneration in natural boreal aspen stands (Frey et al. 2003; Renkema et al. 2009).

Two experiments were carried out to investigate some of the underlying factors influencing the regeneration potential of aspen from root fragments. The two controlled field studies explored the impact of (1) the depth of root burial and root diameter, and (2) the presence of fine roots on buried root fragments on root carbohydrate reserve content, sucker initiation, and performance.

3.2 Methods

3.2.1 Research site

The research site was located at the Crop Diversification Centre North on the north-east boundary of the city of Edmonton, Alberta (53°38'N, 113°21'W). A level area (3 × 12 m) in a field was used for the experiment. The soil was deep and well drained and the soil texture was a silty loam. Precipitation during the study between May and August of 2010 amounted to 174.1 mm and no extended drought period was observed. Mean temperature over the four months was 14°C (Environment Canada 2010) and average soil temperatures were 16.9°C at soil depth 5 cm, 16.1°C at soil depth 20 cm, and 15.3°C at soil depth 40 cm.

3.2.2 Plant material

The root material used in this study was collected in February 2010 during the forest floor salvage operations of the 9-year old aspen stand at the Genesee Coal Mine, Alberta (53°19'N, 114°18'W) (see chapter 2). After the above ground portion of the aspen stand had been sheared off and pushed aside, soil containing the root system was salvaged. From the salvage piles 30 root fragments were chosen in each of three diameter size classes (Class 1: 1-2 cm, Class 2: 2-3 cm; Class 3: 3-4 cm) for a total of 90 roots. Straight and undamaged root fragments each with a minimum length of 60 cm were selected. The first two diameter classes were chosen based on the knowledge that most suckers originate from roots of diameters between 0.5 and 2.5 cm (Kemperman 1978; Schier and Campbell 1978; DesRochers and Lieffers 2001). Roots with larger diameters (Class 3) were chosen to evaluate if higher carbohydrate reserves could enable suckers originating from roots at deeper burial depth to reach the surface.

To test the influence of fine root (>1 mm) presence on root fragments on fragment survival and suckering success, only visibly undamaged root fragments (0.6 – 1.5 cm in diameter, 18 – 60 cm long) that had some fine roots still attached were collected in the winter during the root salvage study (see chapter 2). All collected roots were kept frozen and brought back to the lab wrapped in plastic, and were stored at -5°C in a chest freezer until the end of April 2010.

3.2.3 Treatments

For the root diameter and soil depth study and prior to planting, root fragments were re-cut on both ends to remove potential pathogens that may have developed during storage on the cut ends. A sample of each root fragment (1 cm in length) was also taken to determine initial root total non-structural carbohydrate (TNC) reserves prior to planting. All root fragments were trimmed to a total length of 50 cm. For this study any fine roots attached to the root fragments were removed to ensure equal growing conditions. Root diameter was measured at both ends of each fragment to estimate root fragment volume. For planting, root fragments were buried horizontally at 3 different soil depths (5, 20 and 40 cm) at the end of April. The experiment was designed as a complete block design with ten blocks, each consisting of three plots, which were randomly assigned to one of the three soil depths and each containing one root fragment of each of the three different diameter size classes (class 1: 1-2 cm; class 2: 2.1-3 cm; class 3: 3.1-4 cm). A HOBO soil temperature data logger (Onset Computer Corporation, Bourne, Mass.) was also placed at each soil depth (total of 3 data loggers) to record soil temperatures over the four summer months.

For the fine root study, collected root fragments were thawed under moist conditions and some excess soil was removed. Root fragments with similar diameter and length, as well as similar number and length of attached fine roots, were selected and grouped into 10 pairs. The number of attached fine roots per root fragment ranged from 8 to 19 fine roots. Prior to burial, one root of each pair had all fine roots removed and a sample of each root fragment (1 cm) was taken to determine initial TNC reserves. The experiment was set up as a paired design with ten pairs of root fragments; each pair comprising of one root fragment with fine roots attached and one root fragment without fine roots. Each pair was buried horizontally at a depth of 10 cm and placed parallel to each other 20 cm apart. A soil temperature data logger was also placed at the same location.

Over the course of the growing season (May 1, 2010 to August 24, 2010) the plots were visited 3 times in order to remove weeds and monitor sucker emergence.

3.2.4 Measurements

At the end of August, root fragments were carefully excavated and kept in a cooler until brought back to the lab. In the lab all roots and suckers were carefully washed and then separated into dead and live root fragments. Live root fragments were distinguished from dead root fragments by the colour of the outside bark (yellow vs. dark brown) and by the colour of the phloem (white vs. black). To determine sucker initiation and establishment, the number of emerged suckers, non-emerged suckers (NES), their heights and the number of new fine roots initiated on the suckers were measured and recorded. From the middle of each root fragment a sample was taken for total non-structural carbohydrate (TNC) reserves. All roots and suckers, as well as the TNC samples were oven dried at 68°C until constant weight. Dry mass of sucker stems and leaves were determined.

For TNC analyses, samples were ground to pass a 40-mesh screen using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey). Soluble sugars were extracted from ground tissue by boiling samples three times in 80 % ethanol at 95 °C. Phenol-sulfuric acid assay was used to determine colourimetrically total soluble sugar concentrations. The residue was analyzed for starch by enzymatic digestion with a mixture of α-amylase and amyloglucosidase for 20 h, followed by the colourimetric measurement of glucose hydrolyzate with a peroxidase–glucose oxidase-o-dianisidine reagent (Chow and Landhäusser 2004).

3.2.5 Data analysis

The root diameter and soil depth experiment was set up as a complete block design with ten blocks consisting of three plots, which were randomly assigned to one of the three depths and containing one root fragment each of the three different diameter size classes. Response variables of emerged suckers were analyzed as a randomized 2 x 3 factorial design with two soil depths (5 and 20 cm) and three diameter size classes, as there were no emerged suckers at the 40 cm burial treatment. However, data of the non-emerged sucker variables were analysed as a randomized 3 ×3 factorial design with three soil depths and three diameter size classes as non-emerged suckers were present at all three depths and root diameter size classes.

The model tested was

$Y = \mu + A + B + AB + e$

where Y is the mean of the different response variables, μ is the overall mean, A is the effect of the treatment burial depth, B is the effect of the treatment root diameter size class, AB is the effect of the interaction between the two treatments and e is the random error. Since the two-way ANOVA did not show a significant interaction of soil depth and root diameter size, only the main effects are presented.

The design of the fine root study was a paired design with ten replicate pairs of root fragments with and without attached fine roots. This study was analyzed as a paired one-way ANOVA. Tested variables included number of emerged suckers, number and length of fine roots associated with new suckers, leaf dry mass, total sucker dry mass and the number of non- emerged suckers, their heights and dry mass.

All the response variables related to the whole root data set did not meet the assumption of normality (using the Shapiro-Wilk test) but most did meet the assumption of homogeneity of variances. Number of new roots, length of new roots, and number of non-emerged suckers did not meet the assumption of homogeneity of variances and therefore were log transformed. The variables were analyzed using both the non-parametric Kruskal-Wallis k-sample test and ANOVA. Since the interpretations were similar between both tests, only the results of the ANOVAs are presented.

Emerged sucker numbers and their height growth over the course of the growing season (3 measurements) were analyzed using the repeated measures ANOVA. For this analysis only the data from root fragments buried at 5 and 20 cm depth were used, as suckers emerged at these two depths only. A reduced data set, which included only those root fragments that produced emerged suckers was used in the analyses of differences in emerged sucker numbers per root fragment between treatments and of relationships between (1) sucker leaf dry mass and the number of new fine roots associated with emerged suckers, (2) the leaf mass and TNC content in the root fragment, and (3) initial May starch content and total sucker dry mass.

The results of the TNC analysis (root sugar and starch content and concentration) were analyzed as a one-way ANOVA. The TNC data set included root fragments of all three diameter size classes, soil depth 5 and 20 cm, and root fragments without fine roots attached of the fine root study.

Since root mortality data were categorical, the influence of soil depth, root diameter size, roots with fine roots, roots without fine roots and presence of suckers on root fragment mortality was analyzed using the proc catmod procedure in SAS. A significance level of $\alpha = 0.05$ was used for all analyses.

3.3 Results

Average daily growing season (May to August 2010) soil temperatures decreased slightly with soil depth from 16.8°C at 5 cm to 16.1°C at 20 cm, and to 15.3°C at 40 cm depth with the soil temperature at 5 cm depth being higher than at 40 cm depth ($p = 0.034$). Fraser (2002) found that 60 degree-days (with an average temperature of 14°C and a base temperature of 8°C) were needed for sucker initiation. On our research site these required heat sums were reached on May 19th at a soil depth of 5 cm, on May 20th at a soil depth of 20 cm, and on May 29th, 2010 at a soil depth of 40 cm.

Since the two-way ANOVA did not show a significant interaction of soil depth and root diameter size, only the main effects are presented. Of the 90 root fragments that were planted in the root diameter and soil depth study, 27 (30%) root fragments produced emerged and/or non-emerged suckers (NES). The number of emerged and non-emerged suckers combined with an average of 1.7 suckers root^{-1} was not different among the soil depth treatments ($p = 0.407$; Table 3-1) or the root diameter size treatments ($p = 0.378$; Table 3-2). Of the 20 root fragments planted for the fine root study, 9 (45%) root fragments produced emerged and/or non-emerged suckers. Total number of suckers was different between fine root treatments ($p = 0.032$) as root fragments without fine roots had three times more suckers (1.8 suckers root^{-1}) than root fragments with fine roots attached (0.6 suckers root^{-1}; Table 3-3).

Interestingly, at the 40 cm depth none of the suckers emerged above the soil surface, while at depths of 5 and 20 cm root fragments produced on average 0.55 emerged suckers root^{-1} ($p = 0.011$; Table 3-1). There were no differences in number of emerged suckers from the root fragments with different diameters ($p = 0.301$, with an average of 0.3 emerged suckers root^{-1}). The number of emerged suckers, with an average of 0.75 emerged suckers root^{-1}, was also not different between the fine root treatments ($p = 0.545$; Table 3-3).

Of the 27 suckered root fragments of the diameter and depth study, 18 root fragments produced emerged suckers (at depths 5 and 20 cm). Sucker numbers were higher at a burial depth of 5 cm (2.7 emerged suckers root^{-1}) compared with 20 cm (1.3 emerged suckers root^{-1}) ($p = 0.026$). The suckers from the shallow root fragments reached the surface about 10 days earlier than suckers from deep root fragments ($p = 0.008$; Figure 3-1a). The extension growth (including the distance these suckers had to grow through the soil profile) of these emerged suckers was influenced by soil depth ($p = 0.029$) and by root fragment diameter ($p = 0.001$). Shoot extension of emerged suckers grown from a soil depth of 20 cm was greater (39.8 cm) than from a depth of 5 cm (23.0 cm); however, once the suckers reached the soil surface the above-ground portion was not different between the soil depths (18.9 cm; $p = 0.822$; Figure 3-1b).

As a result emerged suckers of root fragments at a depth of 5 and 20 cm had also similar leaf dry mass with 0.7 g root^{-1} in August ($p = 0.471$; Table 3-1). Root diameter class 2 produced the tallest emerged suckers with 28.3 cm compared to root fragments of diameter class 1 and 3 with an average of 10.8 cm ($p = 0.002$). Accordingly, emerged suckers from root fragments of diameter class 2 (0.7 g root^{-1}) had more leaf dry mass per root fragment than root fragments of diameter class 1 and 3 (both 0.2 g root^{-1} ($p = 0.003$; Table 3-2).

New roots were only produced on the belowground portions of the stem of emerged suckers (adventitious roots). The number of new roots on emerged suckers was not different between depth treatments with an average of 3.1 new roots root^{-1} ($p = 0.646$); however, new roots were longer, at a depth of 5 cm, with an average of 58.7 cm compared to 12.2 cm at a depth of 20 cm ($p = 0.003$). Emerged suckers on root fragments with larger diameter (class 2 and 3) had an average of 3.0 new roots sucker^{-1} compared to 0.3 new roots sucker^{-1} generated from root fragments of diameter class 1 ($p < 0.005$; Table 3-2). Length of new roots increased with the increase of root diameter ($p < 0.005$), where new roots attached to suckers on root fragments of diameter class 1 were 4.7 cm long compared to a length of 28.3 cm on suckers of root fragment class 2 and 147.1 cm on suckers of root fragment class 3. There was no relationship between the amount of leaf dry mass and the number of new fine roots ($p = 0.982$).

Root fragments at a soil depth of 20 cm produced an average of 2.0 NES root^{-1} compared to 0.6 NES root^{-1} from root fragments at a soil depth of 5 cm ($p = 0.014$) and root fragments at a soil depth of 40 cm (1.3 NES root^{-1}, $p = 0.197$; Table 3-1). Root fragments of diameter class 3 produced 2.1 NES root^{-1} compared to 0.6 NES root^{-1} on root fragments of diameter class 1 ($p = 0.011$). Non-emerged sucker numbers on root fragments of diameter class 2 with 1.3 NES root^{-1} were not different from NES numbers of root fragments of diameter class 1 ($p = 0.123$) and NES numbers of root fragments of diameter class 3 ($p =

0.181; Table 3-2). Only root fragments without fine roots attached had non-emerged suckers at the time of excavation (0.9 NES root^{-1}) ($p = 0.043$; Table 3-3).

Non-emerged sucker height was impacted by soil depth ($p = 0.005$), but not by root fragment diameter ($p = 0.396$). At a soil depth of 40 cm NES were on average 8.5 cm tall and only one NES expanded to 20 cm in length, while at a soil depth of 20 cm NES were 5.3 cm and 2.2 cm at a depth of 5 cm. Accordingly, NES dry mass was lower (0.06 g) at soil depth 5 cm than at 20 cm with 0.4 g and soil depth 40 cm with 0.2 g ($p = 0.044$; Table 3-1).

Root fragment mortality was higher at 5 and 40 cm burial depth (80%) compared to 53% at a soil depth of 20 cm ($p = 0.045$; Table 3-1); however, diameter of fragments did not play a role in their mortality ($p = 0.608$; Table 3-2). Interestingly, some of the root fragments that were considered dead at the end of the growing season had produced a few emerged suckers over the growing season that were 15.9 cm (above-ground height) tall and most of these had produced new roots (0.2 new roots sucker^{-1}). Whether or not fine roots were attached to root fragments had little impact on fragment mortality (55%) ($p = 0.185$; Table 3-3). None of the other response variables were different between fine root treatments (Table 3-3).

As diameters varied among root fragments, we expressed the reserve status of root fragments not only as TNC tissue concentration, but also as TNC content. TNC content was estimated by multiplying the TNC concentration of each root fragment with its estimated volume (based on a cylinder). At the beginning of the experiment root fragments had 15.3 g (12.9%) TNC root^{-1}. At the end of the experiment, live root fragments that produced emerged suckers had 8.5 g ($p = 0.005$) and 8.7% TNC root^{-1} ($p = 0.001$), while live root fragments without emerged suckers had 5.4 g (4.5%) TNC root^{-1} ($p < 0.002$) and dead root fragments had 2.9 g (2.4%) TNC root^{-1} (both $p < 0.001$; Figure 3-2). Interestingly, live root fragments with emerged suckers had higher tissue starch concentration (1.7%) compared to the initial measurement (0.5%) ($p = 0.003$; Figure 3-2). There was no difference in TNC and starch content and concentration between live root fragments and dead root fragments that had no emerged suckers (all $p > 0.121$; Figure 3-2).

When comparing the initial TNC content of root fragments that were found to be dead by the end of the experiment in August (final) with the root fragments that were found to be alive, it became apparent that dead root fragments had initially a lower TNC content and concentrations (both $p < 0.01$; Figure 3-3). Initial TNC content and concentration of root fragments that survived was on average 22.3 g and 15.7% while initial TNC content and concentration of root fragments that had died at the end of the experiment was on average 14.0 g and 11.7%. Accordingly, initial starch content and concentration were also much lower in root fragments that were dead at the end of the experiment with 0.4 g root^{-1} (0.4%) than it was in live root fragments with 1.2 g root^{-1} (0.9%) (both $p < 0.001$; Figure 3-3). At the end of the experiment dead root fragments had lost on average 74% of their initial TNC content and concentration (both $p < 0.001$), whereas live root fragments had only lost 49% of their initial TNC content ($p = 0.003$) and concentration ($p < 0.001$; Figure 3-3). Similar results were found for the differences between initial starch content and concentration and final starch content and concentration of live and dead root fragments (Figure 3-3).

There was no significant linear relationship between the difference in initial and final TNC concentration (e.g. the loss of TNC conentration from May to August) and leaf dry mass in dead ($p = 0.251$) or live root fragments ($p = 0.102$) of the diameter and depth study. However, when visualizing this data we noticed an exponetial trend in TNC difference (%) and leaf dry mass of live root fragments. After log-transforming the data, we found a positive linear relationship between TNC difference and leaf dry mass of live root fragments ($p = 0.014$), but not of dead root fragments ($p = 0.754$; Figure 3-4). There was no significant effect of TNC differences on leaf dry mass ($p = 0.474$) of root fragments of the fine root study.

3.4 Discussion

Soil depth had a significant impact on the ability of suckers to emerge above the soil surface. Suckers from root fragments buried at a depth of 40 cm were unsuccessful in reaching the soil surface, indicating that the reserves in the root fragments were not sufficient to allow suckers to grow from a depth greater than 20 cm. Several studies reported that suckers typically emerge from roots located within 8 cm of the soil surface (Schier and Campbell 1978, Brown and DeByle 1987, Navratil 1991). As soils of our study site were of excellent quality and we had no competing vegetation or organic soil layers, which could have inhibited soil warming at deeper soil depth and/or restrict suckers from reaching the surface in natural stands (Brown and DeByle 1987; Mulak et al. 2006; Renkema et al. 2009), suckers in this study arose only successfully from a burial depth of 20 cm or less. Very similar responses were also found at the root transfer study, where suckers emerged only from roots that were buried at depths less than 20 cm (see chapter 2). The initiation of suckers on root fragments, however, was not affected by soil depth, root diameter size (within our tested range), and the presence of fine roots. This is not surprising, as it is known that the initiation of suckers is mainly driven by the absence of apical dominance, which is primarily mediated by growth regulators such as auxin and cytokinin (Farmer 1962; Eliasson 1971; Schier 1972; Steneker 1974).

At a depth of 5 cm, root fragments produced more emerged suckers (2.7 emerged suckers root^{-1}) than root fragments at a depth of 20 cm (1.3 emerged suckers root^{-1}). Since root carbohydrate reserves influence the growth and performance of suckers (Schier and Zasada 1973, Landhäusser and Lieffers 2002), we assume that the shorter distance to the soil surface enabled more suckers at the shallower depths to reach the surface before apical dominance started to influence sucker initiation (Farmer 1962; Eliasson 1971; Schier 1972; Steneker 1974; Wan et al. 2006). Similar to natural conditions, where suckers will replenish carbohydrate reserves of the parent root system (DesRochers and Lieffers 2001, Landhäusser and Lieffers 2002), root fragments of our study were provided with TNC reserves by the emerged suckers. In turn, limited root reserves and the longer distance needed to reach the surface may have been the reason why root fragments buried at a depth of 20 cm were able to support only one dominant stem until it started photosynthesizing. This assumption is also supported by the fact that burial depth had a significant effect on emerged total sucker height as root fragments buried at depth 20 produced the tallest suckers, but their subsequent height growth above the surface was not different between soil depth treatments.

Root diameter size did not influence emerged sucker numbers, which conforms to the results of several other studies. It is reported by Schier (1973), Peterson and Peterson (1992), DesRochers and Lieffers (2001) that suckers mostly sprout from lateral roots with diameters between 0.5 – 2.5 cm. Further, diameter size of small root cuttings (2 – 10 mm in diameter) of hybrid aspen clones (*P. tremula* L. × *P. tremuloides* Michx.) (Stenvall et al. 2006) and aspen (Starr 1971; Schier 1978) did not affect suckering efficiency. Similar results were also found in chapter 2 where root fragments ranging from 1 to 4 cm in diameter produced most suckers.

Non-emerged suckers (NES) were found on root fragments at all soil depths and root diameter classes. Although no suckers emerged from root fragments buried at 40 cm, suckers did not grow taller than 20 cm below ground, indicating that there are restrictions that come into effect if suckers do not reach the surface in time. However, it is not clear whether the root fragments had sufficient carbohydrate reserves available to support the growth of the NES, plus maintain root respiration over a second growing season (Kozlowski 1992; DesRochers et al. 2002). Even if suckers may have arisen from a depth of 40 cm above the soil, it would have been late in the growing season. This would give the sucker not sufficient time for height growth, leaf area development and the subsequent replenishment of root carbohydrate reserves for the next growing season.

The presence of fine roots on root fragments appears not to play a positive role in root suckering; indeed, attached fine roots appear to be potentially a liability, as overall sucker numbers (emerged and non-emerged suckers) were three times higher in root fragments that had all their fine roots removed (1.8 suckers root^{-1}) compared to root fragments with fine roots attached (0.6 suckers root^{-1}). However, there was no difference between treatments of the fine root study concerning emerged sucker numbers, emerged sucker height, and leaf dry mass. This was not anticipated, as it is known that fine roots play an important role in the supply of trees with soil resources, as they are the main pathways for water and nutrient uptake (Charlton 1996). We propose that the amount of initial carbohydrate reserves stored in the root fragments is likely of greater importance at the early stages of sucker development than the existence of fine roots. Attached fine roots could also be a sink of reserves, as these roots might likely increase the amount of respiration of the root fragment (DesRochers et al. 2002). Further, growth regulators (hormones) produced from wounded fine roots may also have inhibited sucker initiation (Frey et al. 2003).

The emerging suckers appear to be well connected to the root fragment, which might be used as a reserve storage organ, as emerged suckers appeared to supply reserves (starch) to the fragment late in the growing season. As a result root fragments that produced emerged suckers had higher carbohydrate reserves compared to root fragments without suckers. Accordingly, we found that an increase of sucker leaf mass also resulted in higher reserve levels in root fragments. As there was no statistical difference of TNC reserves in live root fragments without emerged suckers and dead root fragments without suckers, we believe that these live root fragments will die in the near future.

We found that growth of new fine roots was only associated with the presence of emerged suckers. No new root growth was detected on the root fragment with or without the old fine roots attached. It is thought that the initiation of new roots may be controlled by the successful emergence of suckers. Eliasson (1968) proposed that the supply of carbohydrates from sucker leaves promotes the start of root growth. Further, Stenvall et al. (2006) found that the initiation of new roots on root fragments (diameter of 0.15 – 1.0 cm and 3 cm in length) took twice as long as the initiation of suckers. They also found that root fragments with the best suckering efficiency produced the highest number of new roots.

In summary, burial depth did not influence root sucker initiation, but the ability of suckers to reach the soil surface; therefore, suckers originating from root fragments buried deeper than 20 cm are unlikely to emerge above the soil. The amount of initial carbohydrate reserves stored in root fragments rather than the presence of fine roots, play an essential role in sucker performance and root fragment survival. The importance of sufficient carbohydrate supply for sucker growth was underlined by the fact that root fragments, that were dead by the end of the growing season, had already initial lower carbohydrate reserves to start with (in May) compared to the root fragments that stayed alive over the growing season. More research is needed to investigate the relationships between root diameter size and sucker height growth. Further, as root carbohydrate levels of this study were quite low and varied between root fragments, it may be interesting to explore how the selection of a clone with initially higher levels of carbohydrates affects root suckering ability and sucker performance. It may also be advisable to consult initial carbohydrate levels prior burial and only bury those roots that have similar reserve levels to ensure equal conditions.

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Tables

Table 3-1: Impact of burial depth on suckering response and mortality after one growing season (mean ± SE; n = 10). Within-response variable values sharing the same letter are not significantly different (α = 0.05; LSD means comparison test).

Response variable	Soil depth (cm)		
	5	20	40
Total number of suckers per root fragment[1]	1.2 ± 0.5 a	2.5 ± 0.8 a	1.3 ± 0.9 a
Number of emerged suckers per root fragment	0.6 ± 0.2 a	0.5 ± 0.1 a	0 b
Total emerged sucker height[2] (cm)	23.0 ± 1.7 b	39.8 ± 5.4 a	N/A
Surface emerged sucker height[3] (cm)	18.0 ± 1.7 a	19.8 ± 5.4 a	N/A
Leaf dry mass per root fragment (g)	0.5 ± 0.2 a	0.8 ± 0.3 a	N/A
Emerged sucker dry mass per root fragment[4] (g)	0.4 ± 0.1 a	0.8 ± 0.3 a	N/A
Number of new roots per emerged sucker[5]	5.0 ± 3.3 a	1.2 ± 0.7 a	N/A
Length of new roots (cm)	58.7 ± 23.4 a	12.2 ± 3.2 b	N/A
Number of non-emerged suckers per root fragment	0.6 ± 0.2 b	2.0 ± 0.7 a	1.3 ± 0.9 ab
Non-emerged sucker height (cm)	2.2 ± 0.3 b	5.3 ± 0.6 a	8.5 ± 1.4 a
Non-emerged sucker dry mass per root fragment (g)	0.06 ± 0.03 b	0.4 ± 0.1 a	0.2 ± 0.1 a
Root fragment mortality[6] (%)	80 ± 7.0 a	50 ± 7.0 b	80 ± 7.0 a

[1] Number of emerged and non-emerged suckers combined.

[2] Sucker height measurement from root fragment to sucker terminal bud.

[3] Sucker height measurement from soil surface to sucker terminal bud.

[4] Dry mass of the entire emerged sucker including below ground stem.

[5] Number of new roots initiated on the below ground stem of emerged suckers.

[6] Root fragments were classified as dead when the outside bark was dark brown and the phloem black. These roots, however, could still have live emerged suckers attached.

Table 3-2: Impact of root fragment diameter (class 1: 1-2 cm; class 2: 2.1-3 cm; class 3: 3.1-4 cm) on suckering response and mortality after one growing season (mean ± SE; n = 10). Within-response variable values sharing the same letter are not significantly different (α = 0.05; LSD means comparison test).

Response variable	Root diameter class (cm)		
	1 - 2	2.1 - 3	3.1 - 4
Total number of suckers per root fragment[1]	0.9 ± 0.4 a	1.7 ± 0.6 a	2.4 ± 1.1 a
Number of emerged suckers per root fragment	0.3 ± 0.1 a	0.4 ± 0.2 a	0.3 ± 0.1 a
Total emerged sucker height[2] (cm)	24.2 ± 1.1 b	42.3 ± 3.4 a	26.9 ± 2.7 b
Surface emerged sucker height[3] (cm)	9.1 ± 1.7 b	28.3 ± 2.9 a	12.5 ± 2.1 b
Leaf dry mass per root fragment (g)	0.2 ± 0.01 b	1.4 ± 0.2 a	0.3 ± 0.04 b
Emerged sucker dry mass per root fragment[4] (g)	0.2 ± 0.1 b	0.7 ± 0.3 a	0.2 ± 0.1 b
Number of new roots per emerged sucker[5]	0.3 ± 0.2 b	3.8 ± 2.5 a	2.1 ± 1.5 a
Length of new roots (cm)	4.7 ± 1.0 c	28.3 ± 9.1 b	147.1 ± 54.9 a
Number of non-emerged suckers per root fragment	0.6 ± 0.3 b	1.3 ± 0.4 ab	2.1 ± 1.0 a
Non-emerged sucker height (cm)	5.1 ± 0.9 a	4.4 ± 0.5 a	6.9 ± 1.4 a
Non-emerged sucker dry mass per root fragment (g)	0.1 ± 0.03 a	0.2 ± 0.07 a	0.3 ± 0.1 a
Root fragment mortality[6] (%)	70 ± 9.0 a	70 ± 10.0 a	80 ± 7.0 a

[1] **Number of emerged and non-emerged suckers combined.**

[2] **Sucker height measurement from root fragment to sucker terminal bud.**

[3] **Sucker height measurement from soil surface to sucker terminal bud.**

[4] **Dry mass of the entire emerged sucker including below ground stem.**

[5] **Number of new roots initiated on the belowground stem of emerged suckers.**

[6] **Root fragments were classified as dead when the outside bark was dark brown and the phloem black. These roots, however, could still have live emerged suckers attached.**

Table 3-3: Role of fine roots in the suckering response and mortality of root fragments after one growing season (mean ±SE; n = 10). Within-response variable values sharing the same letter are not significantly different (α = 0.05; LSD means comparison test).

Response variables	Treatment	
	With fine roots	No fine roots
Total number of suckers per root fragment[1]	0.6 ± 0.3 b	1.8 ± 0.6 a
Number of emerged suckers per root fragment	0.6 ± 0.3 a	0.9 ± 0.3 a
Total emerged sucker height[2] (cm)	41.1 ± 9.4 a	37.1 ± 5.7 a
Surface emerged sucker height[3] (cm)	31.1 ± 9.4 a	27.1 ± 5.7 a
Leaf dry mass per root fragment (g)	2.0 ± 0.6 a	1.6 ± 0.4 a
Emerged sucker dry mass per root fragment[4] (g)	4.2 ± 1.4 a	3.3 ± 0.8 a
Number of new roots per emerged sucker[5]	1.5 ± 1.5 b	9.6 ± 4.2 a
Length of new roots (cm)	9.6 ± 5.3 b	108.3 ± 44.8 a
Number of non-emerged suckers per root fragment	0 b	0.9 ± 0.5 a
Non-emerged sucker height (cm)	N/A	3.6 ± 0.5 a
Non-emerged sucker dry mass per root fragment (g)	N/A	0.6 ± 0.02 a
Root fragment mortality[6] (%)	70 ± 15.0 a	40 ± 16.0 a

[1] **Number of emerged and non-emerged suckers combined.**
[2] **Sucker height measurement from root fragment to sucker terminal bud.**
[3] **Sucker height measurement from soil surface to sucker terminal bud.**
[4] **Dry mass of the entire emerged sucker including below ground stem.**
[5] **Number of new roots initiated on the belowground stem of emerged suckers.**
[6] **Root fragments were classified as dead when the outside bark was dark brown and the phloem black. These roots, however, could still have live emerged suckers attached.**

Figures

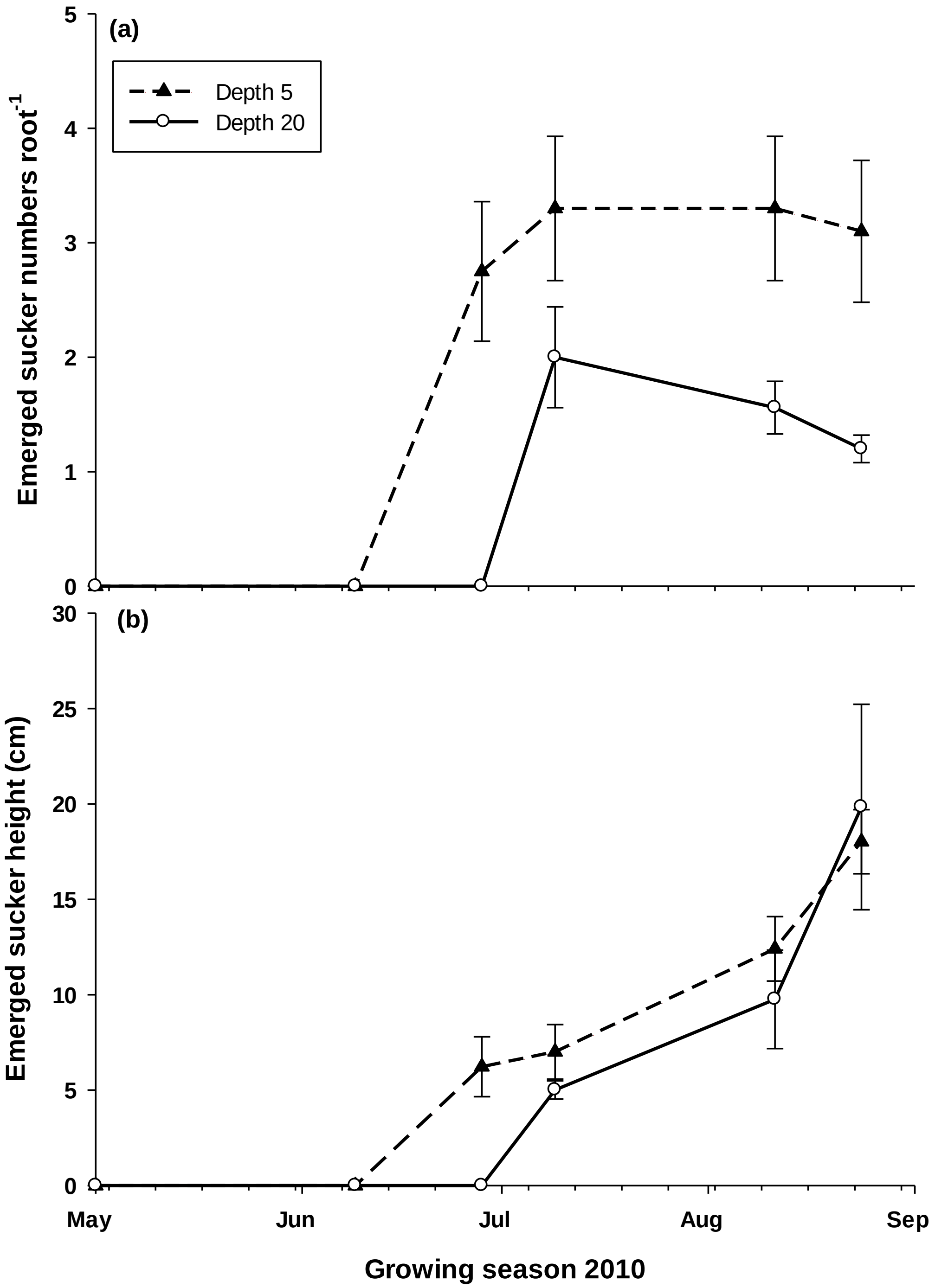

Figure 3-1: Emerged sucker numbers (a) and surface heights (b) of root fragments of the soil depths 5 and 20 cm over the course of the growing season 2010. Error bars indicate one standard error of the mean (n = 10).

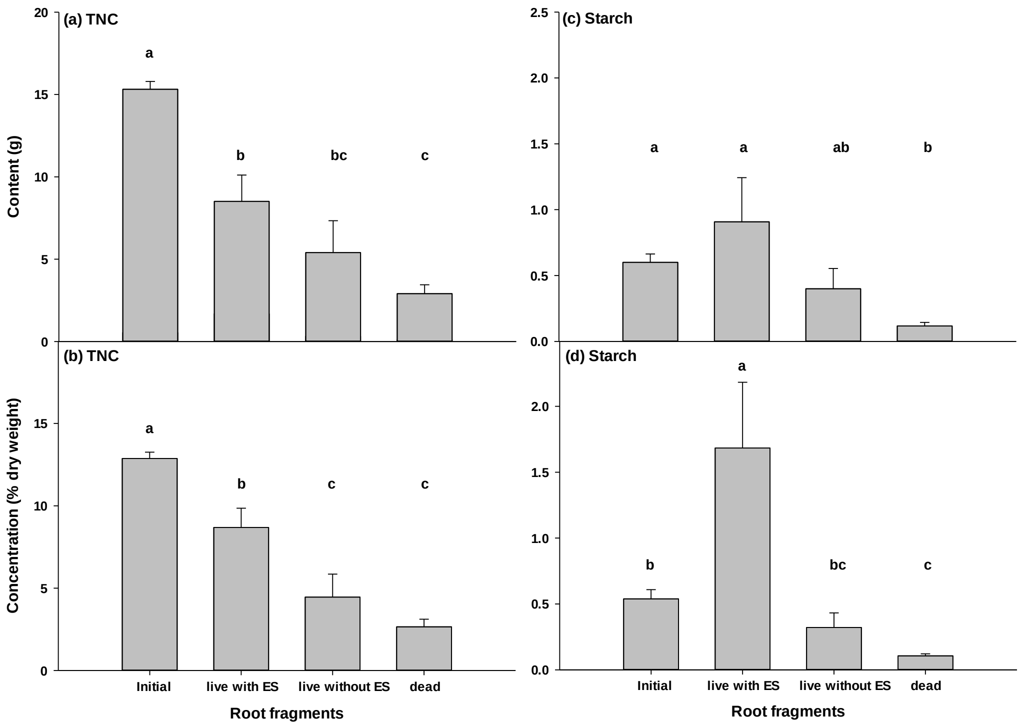

Figure 3-2: TNC content (a) and concentration (b) and starch content (c) and concentration (d) of root fragments prior to burial (initial) and at the end of the first growing season (root fragments that produced emerged suckers (live with ES), live root fragments that did not produce emerged suckers (live without ES), and dead root fragments without suckers (dead) (n = 10). Data set for initial measurements includes all root fragments used in the diameter and depth study and fine root study. Data set of post experiment measurements only includes root fragments from both experiments buried at a depth ≤ 20 cm. Error bars indicate one standard error of the mean.

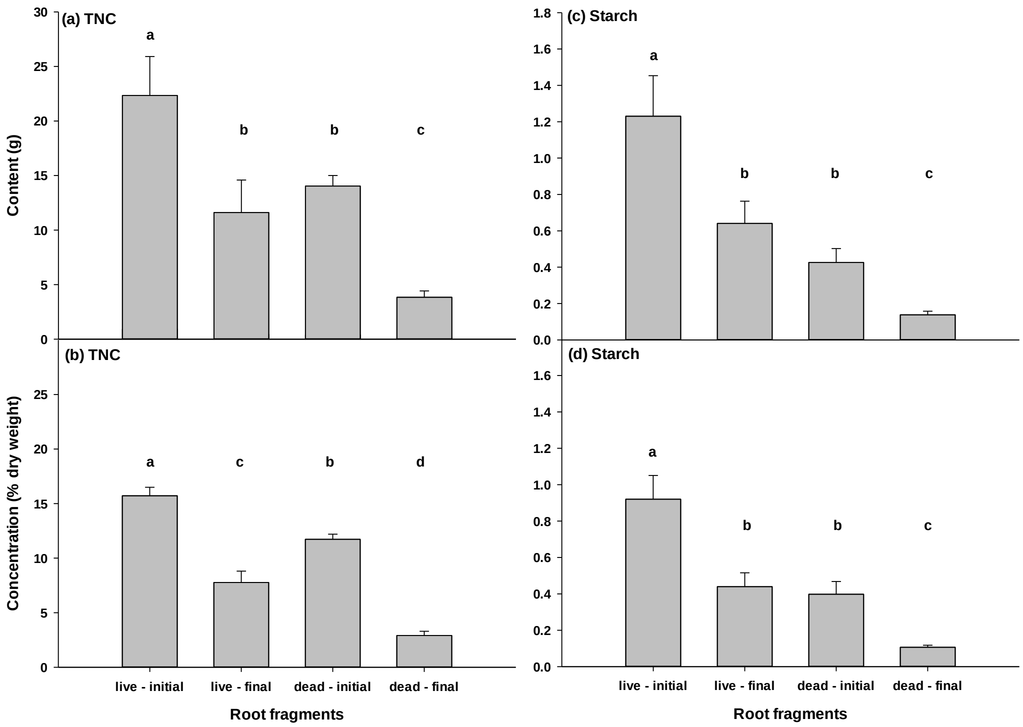

Figure 3-3: TNC content (a) and concentration (b) and starch content (c) and concentration (d) of alive root fragments at the beginning of the experiment (live-initial) and at the end of the experiment (live-final) and of dead root fragments at the beginning (dead-initial) and at the end of the experiment (dead-final) (n = 10). Initial measurements were taken in late April and final measurements in August. Data set for initial measurements includes all root fragments of the diameter and depth study and fine root study, regardless of emerged, non-emerged or no suckers. Data set of post experiment measurements only includes root fragments from both experiments buried at a depth ≤ 20 cm. Error bars indicate one standard error of the mean.

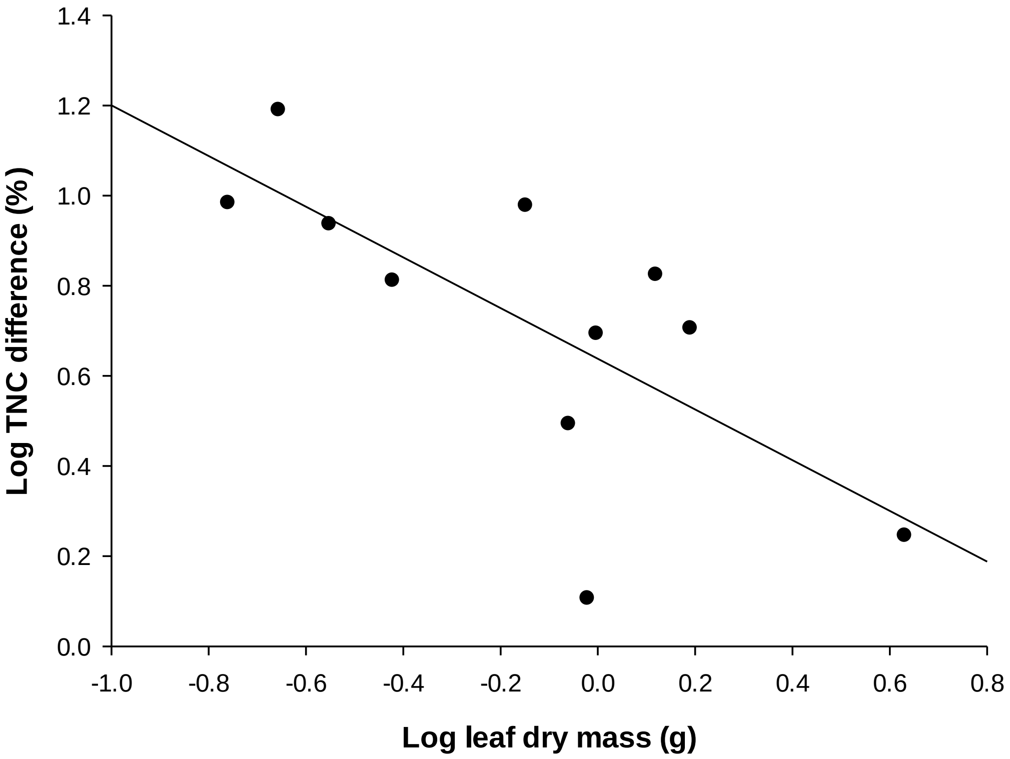

Figure 3-4: Sucker leaf mass (log transformed) in relation to the reduction of TNC reserves (%) (log transformed) from initial reserves of all live root fragments of the diameter and depth treatments (y = 0.64x – 0.57; r^2 = 0.51; p = 0.014) (n=10).

Chapter 4: Summary and Implications

4.1 Research summary

The main objectives of this research project were to determine the efficacy of forest floor salvage for the vegetative regeneration of aspen from root propagules on reclamation sites, and to gain a better understanding of the importance of root propagule characteristics and placement. We tested the effect of two salvage and placement depths [shallow (15 cm) and deep (40 cm)] on the suckering ability of aspen roots and the subsequent sucker growth and mortality. Despite the lower donor root density, the deep salvage and placement of donor material (deep treatment) had three times higher sucker numbers compared to the shallow treatment after two growing seasons. In addition, root fragments in the deep treatment produced more suckers per fragment, sprouted from somewhat greater depths, grew taller, and had lower mortality rates than suckers from root fragments in the shallow treatment. These results indicate that mineral soil-donor root contact plays a crucial role in the success of root suckering from root fragments. Lower root fragment numbers within the deep treatment combined with the higher mineral soil volume resulted in good root-soil contact and likely provided better protection of roots from exposure and subsequent desiccation. Further, root suckering ability was controlled by the depth the root fragments were buried in the soil. Best suckering results were found on roots buried between 5 and 20 cm regardless of depth treatment. Interestingly, in the field study root fragments did not produce any suckers when buried deeper than 20 cm in the soil, while that was not the case in the more controlled study; here, root fragments buried at a depth of 40 cm initiated suckers. In the controlled studies we tested the effect of root fragment diameter, root carbohydrate reserves, and the presence or absence of fine roots on the root fragments on root suckering. Within our tested range, soil depth, root diameter, and the existence of fine roots did not affect sucker initiation after one growing season. However, suckers initiated from root fragments buried at a depth of 40 cm did not emerge above the soil surface. Soils of the controlled study site were of very good quality compared to the soils at the reclamation site, which may explain why root fragments of the controlled studies were able to produce suckers at deeper depths. Further, soils in the controlled study site reached sufficient soil temperatures [60 growing degree-days with an average temperature of 14°C and a base temperature of 8°C (Fraser 2002)] that allowed for sucker initiation and growth earlier in the season than soils at the reclamation site. Here, sufficient soil temperatures at deeper depths were reached 42 days later than at the controlled study site. Thus, by reaching required heat sums relatively late in the growing season, carbohydrate reserves of root fragments at deeper depths might have already been too depleted to allow for the expansion of suckers at the reclamation site. Indeed, the amount of carbohydrates initially stored in the root fragment showed to be strongly linked to root and sucker survival, as root fragments that stayed alive had an initially higher carbohydrate reserve levels than root fragments that were dead by the end of the growing season. Since only a limited amount of carbohydrates can be stored in root fragments, the quick emergence of suckers and the establishment of leaf area are crucial to root and sucker survival. We found that root fragments with emerged suckers attached had considerably higher carbohydrate contents than roots without emerged suckers. This lead us to speculate that emerged suckers or rather their leaf area appear to replenish carbohydrate reserves in the parent root fragment as soon as suckers start to photosynthesize independently, storing new energy for root fragment maintenance and subsequent sucker growth.

Strictly based on the initial carbohydrate reserve levels of roots, salvage of our donor material in January appeared to be beneficial to the suckering success, as it is thought that carbohydrate reserves are higher during late fall and winter months compared to levels in spring after leaf flush and early summer (Mundell et al. 2007; Stenvall et al. 2009). However, due to the fact that this reclamation technique has not been extensively tested yet, more research has to be conducted to determine best season for salvage considering additional factors (e.g. frozen ground vs. unfrozen ground) and its consequences to the reclamation success (see also 4.3).

After transferring root systems from a natural stand onto the reclamation site, root fragments will produce suckers; however, root suckering was not as vigorous as we expected. More than half (67%) of the roots died after the transfer and did not produce any suckers. In the deep salvage and placement treatment, however, initial sucker densities from the remaining live root fragments were high enough to potentially produce a closed forest stand. Cleary, the outcome of this study cannot be compared with the suckering response found in natural stands, where sucker densities can reach more than 250,000 stems ha^{-1} (Alban et al. 1994). These higher sucker densities in natural stands are likely achieved because of its intact root system allowing access to and allocation of much higher amounts of carbohydrate reserves and water necessary for prolific sucker growth. Further, these sucker densities are usually achieved after the above ground portion of the stand has been completely removed by fire or harvesting operations. Commonly, these disturbances cause considerably less root damage compared to the procedures of our root transfer and therefore do not impede root suckering

However, one has to keep in mind that the forest floor transfer technique is not applicable to all reclamation projects or ecosystem types. For example, old aspen stands that are in the dieback and breakup phase with little regeneration (> 65 years, depending on region, Frey et al. 2004) or very young stands (< 10 years) may not have suitable lateral root systems (e.g. low abundance of roots capable to produce suckers) that are able to produce a new aspen stand following a forest floor transfer. Thus, donor site pre-assessments are likely important to determine the suitability of a potential donor site for this kind of reclamation technique.

Taking all these factors into consideration, this experiment was a first step in identifying conditions and prerequisites needed for root suckering as a forest land reclamation technique, as this method has the potential to at minimum supplement forest cover development within the first years. Obviously, future research is needed and long-term monitoring is a key element to evaluate the efficiency of this technique in the establishment of more natural forest ecosystems on reclamation sites.

4.2 Major contributions of this study

The regeneration of trembling aspen through root suckering has been well studied and is mostly well understood in a traditional silvicultural context (e.g. Frey et al. 2003). However, due to the significance of this species for the reclamation of boreal forest ecosystems (see chapter 1.3) an advanced understanding was and is still needed in order to take full advantage of aspen's regeneration abilities. Our study demonstrated that aspen have the ability to regenerate vegetatively following a transfer of the entire root system including its surrounding soil onto a reclamation site. Although root suckering was not as prolific as we had hoped, sucker numbers of over 6,500 suckers ha^{-1} at the end of the second growing season produced better numbers than has been reported in the few available studies. However, none of these studies concentrated on the effect of transfer on the vegetative regeneration of trembling aspen. Even though studies have mentioned the presence of aspen following a forest floor transfer, it is unknown if these aspen were of seed or sucker origin (Mackenzie 2006; Mackenzie and Naeth 2007; Mackenzie and Naeth 2008 unpublished, as cited in Alberta Environment 2010). The focus of these studies was primarily on the establishment of forest understory; thus, salvage and placement depths were chosen to accommodate development of understory species from the seed bank in the soil, which were not necessarily beneficial to the vegetative regeneration of aspen.

Our study is the first published experiment that focused specifically on the establishment of aspen from root fragments. By focusing on suckering in the field and under controlled conditions, the results enabled us to identify and understand the physical and physiological variables of roots (e.g. amount of damage, carbohydrate reserves), as well as site conditions (e.g. soil depth, soil temperature) that are important for assessing whether, and which factors impact aspen sucker regeneration from root fragments. Besides testing the effect of root diameter size and burial depth of root fragments, another focus of this study was laid on the importance of carbohydrate reserve levels of root fragments for sucker growth. Unlike in natural aspen stands with undisturbed root systems, where developing suckers have access to higher amounts of resources such as water, nutrients and carbohydrate reserves, root fragments can only store a limited amount of reserves and have few fine roots to access water and nutrients. We found that the initial carbohydrate levels in these root fragments were strongly linked to the suckering success and subsequent root fragment survival. The knowledge gained from this study enables us to predict the possibilities of root survival, which can be applied to assist in the selection of a suitable donor stand and consequently may increase the reclamation success following a forest floor transfer.

Further, this study represents realistic operational-scale mine reclamation conditions as related to equipment size, volume of transferred material, and size of donor and reclamation area. Past research has often been based on small-scale experiments, where difficulties occurred operating large equipment and causing considerable damage to soil and propagule sources (Koch et al. 1996; Mackenzie and Naeth 2010). Koch et al. (1996) reported that salvage to their target depth of 5 cm on sites of only 1 ha was seriously challenged by operating large machinery. Tacey and Glossop (1980) considered the use of smaller equipment such as scrapers, but even then they encountered difficulties in accuracy of salvage operations. Spreading of salvaged material evenly at 10 cm depth onto a reclamation site was operationally not feasible in a study conducted by Mackenzie and Naeth (2010) resulting in several bare patches of mineral soil that did not received any material. Although our study encountered issues of uneven distribution, methods to improve the forest floor transfer reclamation techniques can be suggested (see 4.4). However, this study showed that the direct transfer of forest floor has potential for aspen establishment on reclamation sites and could be implemented in the restoration of ecosystem processes in reclamation of surface mines in the boreal forest region.

4.3 Application for reclamation

Results clearly show that the salvage of forest floor to a depth of 40 cm was beneficial for suckering from root fragments, likely due to sufficient mineral soil-root contact. However, the salvage depth will have to be determined individually for each donor site, although it appears a higher mineral soil content will result in betters sucker establishment and performance from root fragments. Prior to salvage, donor site assessments are necessary to determine soil properties and conditions, as well as the main rooting depth of the aspen. In cases where soil properties allow for the salvage to deeper depth without the inclusion of unsuitable soil horizons (e.g. Bt or C), it can be proposed that the forest floor-mineral mix (FFM) could be placed over a larger reclamation area, as it appears that a placement depth deeper than 20 cm is not necessary for the success of aspen regeneration from root propagules to occur.

Due to the lack of research it is unclear yet, when salvage and placement operations should be conducted. Based on root carbohydrate reserve levels and its importance to the suckering success and root survival, best results may be achieved by transferring the FFM material during the summer months after height growth of the trees has ceased (Landhäusser and Lieffers 2003; Mundell et al. 2007). However, due to the cold soil conditions in winter, root respiration is reduced and less carbohydrate reserves are needed for root maintenance (Landhäusser et al. 2001). Frozen ground reduces soil compaction by heavy equipment (Bates et al. 1993), but will likely damage roots more as they are frozen and brittle. Salvage during summer, however, may reduce damage on roots due to unfrozen ground, but spreading the material in unfrozen conditions may lead to higher soil compaction increasing bulk density and decreasing soil aeration both limiting root growth and root suckering (Maini and Horton 1964; Steneker 1974; Bates et al. 1993). Also, salvage in summer would give suckers less time for height growth and the establishment of sufficient leaf area to replenish carbohydrate reserves before winter, which could lead to higher mortality rates of suckers and roots in the following growing season. Regardless of timing of salvage and placement, it is important to notice that root damage caused by the salvage and placement negatively affected root suckering; thus, machine traffic and handling of the material should be kept to a minimum.

The salvage of FFM material to deeper depths is thought to be beneficial for successful regeneration of aspen from root fragments as it ensures sufficient root-soil contact and may limit the exposure of roots and subsequent desiccation reducing sucker growth. However, as already mentioned above, assessment of the donor site prior to salvage is important to determine depth of unsuitable soil horizons. The placement depth of the FFM material at the reclamation site might not need to exceed 20 cm, as aspen roots located at deeper depths were found not to produce suckers or the suckers produced will not make it above the soil surface. However, a placement depth too shallow (e.g. ≤ 10 cm) may operationally not be feasible and also lead to reduced sucker development, likely through exposure.

Direct transfer appears to be a viable method to establish aspen on reclamation sites early in the first year. However, at this point in time planting of nursery-grown seedlings can likely not be replaced by this operation, due to the high mortality rates of root fragments and suckers, the non-uniformity of sucker distribution, and the much lower sucker numbers compared to natural stands. More research into methods to reduce root damage and improve root suckering and growing conditions on reclamation sites may change this. In any way, the benefit of directly transferring natural plant materials and instantly increasing plant diversity on a reclamation sites, clearly outweighs the current practice of stockpiling these materials and thereby losing the viability of the propagules contained within.

4.4 Future research

The ongoing loss and the fragmentation of productive forestland by human activities is probably one of the most significant land management challenges within Alberta’s forested areas. The direct transfer of forest floor as a reclamation technique may reduce the impact and loss of this valuable resource and help in the restoration of ecological processes in reclaimed post-mined lands, necessary for the establishment of functional boreal forest ecosystems.

Since this study was the first experiment that focused on the vegetative regeneration ability of aspen from root fragments following a forest floor transfer, there is an obvious need for further research into this technique. As we found that initial carbohydrate reserve levels of roots were strongly linked to sucker growth and root survival, we propose that future research should investigate if utilizing a donor stand with higher carbohydrate reserves will improve the suckering success and reduce mortality rates of roots. Root carbohydrate reserve levels fluctuate over the course of the year (Landhäusser and Lieffers 2003; Stenvall et al. 2009) and it may be interesting to test the effect of transfer at different times of year, which would also clarify how other factors such as unfrozen soil affect the reclamation success.

Further, we see a need to improve methods of operation in order to reduce damage to roots during salvage and placement, as it also affected suckering success at our reclamation site. In our study the FFM material was handled quite intensely; instead of dumping each truck load in large piles and then spreading those with bulldozers and creating smooth surfaces, it may be worth investigating if loose dumping and/or dumping the material while slowly moving the truck forward would be viable options. In this way soil handling, compaction of soil, damage and fragmentation of roots should be reduced and a heterogeneous soil surface could create micro-sites enhancing plant species recruitment from seed dispersed by wind or wildlife (Kay 1993; Landhäusser et al. 2010). Although this technique would likely increase the variability of capping thickness, it might be outweighed by the improved plant performance due to the reduced physical impact on the donor material. If regulatory requirements do not allow for this variability, smaller machinery with wider tracks could be used for spreading the materials to more even depths.

As forest floor materials are often a limited resource close to mine sites, we propose that future research should test the effect of deep salvage depths (after consideration of soil properties) and thinner placement depths. Based on our results, placement depth for aspen root suckering to occur does not need to exceed 20 cm, as root fragments did not produce surface suckers when buried at deeper depths. However, placing soil much shallower than 15 cm is likely of no benefit, particularly during dry conditions, as FFM materials could dry out and would not provide enough depth for developing roots to access deeper soil moisture.

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