Regeneration dynamics of seedling-origin aspen: implications for forest reclamation

by

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Abstract

Resprouting is an important adaptation to aboveground disturbance, whereby plants develop new shoots after loss or death of a portion of their aboveground biomass. Aspen (*Populus tremuloides* Michx.) is a foundational tree species in the boreal forests of North America and is a prolific resprouter, resprouting either through shoots on the lateral roots (suckers) or the stumps (stump sprouts). Aspen is most commonly found as part of a clonal colony, where many aboveground stems make up one genetic individual that is connected through a common root system; consequently most aspen research has focused on the clonal habit of the species. Recently, aspen have been planted as seedlings on reclamation sites and are no longer part of a connected clonal colony. I assessed the response and mechanisms of sprouting in planted aspen root systems in the field and a controlled environment. To explore the response of planted aspen to disturbance, I applied four disturbance treatments on two sites within Edmonton, AB: two cut heights and one root severing treatment in 2015, and a clearcut treatment in 2016. Treatments were applied to a large diameter and a small diameter stand. Following these disturbances I assessed the type (suckers vs. stump sprouts) and amount of regeneration at the tree and the site level; at the tree level, planted aspen produced 5 suckers each (2015) while at the stand level, this average decreased to approximately 4 suckers per initial planted tree (2016). Smaller trees produced more stump sprouts compared to larger trees, and trees cut lower to the ground produced more suckers (up to an average of eight suckers per tree). I also assessed the degree to which suckering is dependant on stored reserves of total non-structural carbohydrates (NSC, comprised of simple sugars and starch) and nitrogen (N). Short root segments were placed in a dark growth chamber and were left to sucker under otherwise optimal growth conditions. The darkness ensured that no new carbon could be assimilated, and suckering was thus solely dependent on stored reserves. A measure of initial NSC content and concentration was determined for the entire root section at the beginning of the experiment. Greater initial NSC and N reserve content resulted in a greater production of total sucker mass and total sucker height, with a trend for the production of more suckers. NSC concentration did not have a significant relationship with total sucker production; however, high initial concentrations of starch were positively and significantly related to the *relative* production of suckers (i.e. once the root size had been controlled for). Overall, this research indicates that root system size and initial reserve status will impact the extent and type of resprouting in aspen, with larger roots producing more suckers, and larger trees producing fewer stump sprouts.

Preface

A version of Chapter 2 of this thesis will be submitted for publication as C.M. King and Simon M. Landhäusser, "Regeneration dynamics of seedling origin aspen (*Populus tremuloides* Michx.)", *New Forests*. I performed data collection, analysis, and preparing the manuscript. S.M.L. assisted with the experimental design and manuscript edits.

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Chapter 1. Introduction

Aspen (*Populus tremuloides* Michx.) has the greatest distribution of any tree species in North America (Little 1971) and is able to grow on a wide range of sites. One of the intriguing characteristics of aspen is its ability to resprout from its extensive root system (Barnes 1966; DeByle & Winokur 1985; Peterson & Peterson 1992). Particularly after aboveground disturbance, aspen can produce new shoots (suckers) from the shallow lateral root system and, when young, they may also produce sprouts from the base of the damaged stems (stump sprouts) (Peterson & Peterson 1992). These multiple new shoots (ramets) that form on the root system of a parent tree (ortet) are genetically identical and can form the beginning of a clonal organism (Barnes 1966; Peterson & Peterson 1992). Aspen is most commonly found as a clone; the size of a clone can be highly variable, and likely ranges in size as a result of the disturbance regimes and overall age of the clone (Brown & DeByle 1987; Peterson & Peterson 1992; Frey et al. 2003). Since the suckering response is the main avenue for regeneration after disturbance, it has garnered significant attention in the study of aspen (Bartos & Meuggler 1981; Peterson & Peterson 1992; DesRochers & Lieffers 2001a; Mulak et al. 2006). However, aspen do also establish and regenerate sexually from seed (Barnes 1966; Peterson & Peterson 1992). In fact aspen is a prolific seed producer, but in the past the establishment of aspen from seed has been considered rare due to the necessity of optimal seedbed and atmospheric conditions during a narrow window of opportunity for germination and during early establishment (Moss 1938; Peterson & Peterson 1992). More recent studies, however, have found that aspen seedlings do establish regularly, particularly after disturbance, and that these seedlings can persist when favourable conditions for germination and establishment are present (Barnes 1966; Fairweather et al. 2014; Krasnow & Stephene 2015; Landhäusser et al. 2010a; Mcdonough 1979; Turner et al. 2003). Further, landscapes in Western North America that were thought to be dominated by a few, extensive clones, have now been shown to contain more genetic diversity and many smaller independent clones than previously thought (Mock et al. 2008; Long & Mock 2012).

The progression from a seedling to an extensive clone in aspen occurs over successive disturbances, such as fire, which come through and renew the stand on multidecadal time scales (Peterson & Peterson 1992). In seedling origin stands the reoccurrence of disturbance might select for aspen genotypes that are able to sucker prolifically, as the ortets that do not sucker will die as the developing ramets are necessary to maintain the root system and produce the next generation of the stand. Genetically controlled factors may also influence which genotypes survive other disturbances on the landscape, e.g. aspen with a high tannin content may be less susceptible to herbivory, and their suckers are more likely to persist (Lindroth et al. 2002; Osier & Lindroth 2001). However, before herbivores are able to exert their selective pressure on these aspen stands, the suckers must first emerge.

In order to develop into a successful clonal stand, there must be sufficient reserves available in the root system to drive the emergence of suckers after a disturbance (Barnes 1966; Clarke et al. 2013; Wachowski et al. 2014; Iwasa & Kubo 1997), and to ensure survival of the parent root system (Landhäusser & Lieffers 2002). Reserves are compounds, such as carbohydrates, lipids and proteins, that the plant can store and then use for future growth and metabolism when the supply of carbon or nutrients falls short of demand (Chapin et al. 1990). The most common carbon reserves are the total non-structural carbohydrates (NSC), which are composed mainly of simple sugars and starch (Loescher et al. 1990; Kozlowski 1992; Hoch 2015). It is generally agreed that resprouting species rely on stored NSC reserves to resprout (Clarke et al. 2013; Moreira et al. 2012; Palacio et al. 2007), and that this is no exception for aspen (Schier & Zasada 1973; Landhäusser & Lieffers 2002; Wachowski et al. 2014). Nitrogen (N) also appears to be an important reserve in respouting species, however our knowledge of the role of N in resprouting is still limited (Chapin et al. 1990; Millard et al. 2007; Moreira et al. 2012; Palacio et al. 2007). Non-structural carbohydrate and N reserves can be discussed in terms of their tissue concentration and their pool size (content), and it is currently unclear if suckering, and resprouting in general, is related more to the concentration or content of these reserves (Canham et al. 1999; Hoch 2015; Ryan 2011).

There is a large body of knowledge on the subjects of aspen regeneration after disturbance, and of NSC reserves as drivers of growth. Despite this, there is a particular dearth of knowledge on seedling origin aspens' response to disturbance, and whether the ability to resprout is driven by the pre-disturbance NSC and N concentration or content. Since seedling origin aspen are more common on the landscape than previously thought, it is valuable to explore how seedling origin aspen respond to disturbance, and to what extent this response is driven by the stored reserves. Because the research into aspens' response to disturbance has largely focused on clonal origin stands (Peterson & Peterson 1992; Bell et al. 1999; Kabzems & Haeussler 2005), my hypotheses on seedling origin aspens' response to disturbance are largely informed by clonal research.

1.1 Clonal Aspen Dynamics

Aspen research has predominantly focused on the clonal growth pattern of the species, and it is worth reviewing the dynamics and variability that can be observed in clonal stands before delving into the lesser-explored aspects of planted aspen seedlings. The physical extent of an aspen clone can vary from a few connected stems up to thousands of stems, with the largest known clone reported to be 43.6 ha (Kemperman & Barnes 1976; Dewoody et al. 2008). The dynamics of clonal aspen can vary geographically, with larger clones (and often more prolific suckering) typically being found in the southwestern parts of its range (e.g. Kemperman & Barnes 1976, clones in Utah), and smaller clones being found in the northern latitudes (Navratil & Chapman 1991; Peterson & Peterson 1992; Barnes 1966). Despite this wide variability of clone size and geography, it can generally be anticipated that aspen will produce suckers and/or stump sprouts after a disturbance (DeByle & Winokur 1985; Peterson & Peterson 1992; Shepperd 1996; Frey et al. 2003), as long as the disturbance to the root system is not too severe (Renkema et al. 2009).

A variety of controlled disturbances can be used to induce stand regeneration in aspen clones, and full stem removal of a clonal stand typically results in successful regeneration through suckering (Schier 1978; Bates et al. 1993; Grewal 1995; Bell et al. 1999; Frey et al. 2003). Indeed, clonal aspen responds best to clearcutting, and retention of some stems may have a negative impact on regeneration (Huffman et al. 1999; Mulak et al. 2006). Aspen may also regenerate successfully following fire (Bartos & Meuggler 1981; Brown & DeByle 1987; Bartos et al. 1991), or following the removal of the forest floor (Stone & Kabzems 2002; Haeussler & Kabzems 2005; Kabzems & Haeussler 2005). The degree of disturbance necessary to induce regeneration of suckers may vary between clones (Shepperd 1986) and the level of regeneration can be variable, ranging from 6000 to 280,000 stems ha^{-1} (Peterson & Peterson 1992).

The suckering of clonal origin aspen stands has been found to depend on a variety of factors, including the season of disturbance (Bell et al. 1999; Mulak et al. 2006), non-structural carbohydrate levels (Shepperd & Smith 1993; Wachowski 2012; Wachowski et al. 2014), soil temperature (Maini & Horton 1966; Fraser et al. 2002) and the density of roots in an area, with more regeneration occurring where root density is high (Peterson & Peterson 1992). Stand density and root system characteristics of clonal origin aspen stands may play an important role in regeneration as well. In 5 to 10 year old clonal stands (15,550 – 61,110 stems ha^{-1}), stem density was found to be significantly and positively related to live root biomass (DesRochers & Lieffers 2001a). Suckering may also be impacted by competition from herbaceous species, as competition can inflict a significant negative effect on the success of aspen by limiting their access to moisture, nutrients, and light (Landhäusser & Lieffers 1998; Frey et al. 2003). Although all of these factors may impact suckering in clonal origin stands, it is unclear how they affect suckering in seedling origin stands.

1.2 Aspen in Reclamation

Although seedling origin aspen may be more common in natural stands than previously thought (Mock et al. 2008; Long & Mock 2012; Fairweather et al. 2014), they are undoubtedly common on reclaimed landscapes. There are many anthropogenic disturbances across aspens' range (Atlas of Canada 2017), and particularly across the boreal forest (Schneider et al. 2003). Surface mining occurring in the boreal forest is a type of major disturbance in which the soil and vegetation layers are removed in order to access subsurface minerals (Macdonald et al. 2012). In Alberta, mining companies are required to establish a self-sustaining, locally common ecosystem as part of the land reclamation process (Alberta Environment 2013). Aspen is a fast growing, early successional species that is locally common in many forest regions of Canada and is often planted on these landscapes (Macdonald et al. 2012). Our knowledge of the suckering and regeneration dynamics of planted aspen is limited, as aspen are most commonly found as part of a larger clonal stand (DeByle & Winokur 1985; Peterson & Peterson 1992). The abundance of planted aspen on reclamation sites necessitates a better understanding of the dynamics of planted aspen in this region.

On reclamation sites it will be imperative to consider that the response of seedling origin aspen to management treatments and disturbances may be different from what we have observed for clonal origin aspen. It is well documented that new suckers will form on aspens' parent root system after a disturbance removes the above ground material in a clonal origin stand (Schier 1972; Peterson & Peterson 1992; Bell et al. 1999; Frey et al. 2003), however, this is less clear for seedling origin stands. Planted aspen did not develop from, and thus are not connected to, a parent root system. After disturbance, the extensive, connected parent root system is the foundation of suckering, as it provides the developing suckers with greater access to water and other reserves that individual smaller root systems may not have access to (Barnes 1966; Kemperman & Barnes 1976; Peterson & Peterson 1992; Miller 1996). Parent root systems are not the only type of connected root system: functional root grafts can connect different clones and allow for the transport of nutrients and water (Desrochers & Lieffers 2001b; Fraser et al. 2006; Jelínková et al. 2009;). However, in the absence of root grafting, planted aspen may lack this extensive root system and may be limited in their ability to produce suckers. It is unclear if the root systems of planted aspen will be substantial enough to sucker prolifically. If there is interest in maintaining aspen stands on reclaimed landscapes, then we must have a clear understanding of how these stands will respond to disturbances.

Canopy closure is an important phase in forest succession, as it may help exclude species that exert a higher competitive pressure on aspen during establishment (Oliver & Larson 1996). However, aspen have often been planted at densities suggested for conifer reforestation (1,500 - 2,000 stems ha^{-1}) on reclamation sites, and some of these sites have not achieved canopy closure. In planted aspen stands these relatively low densities are often compounded by high seedling mortality upon out-planting, and poor seedling performance (Landhäusser et al. 2012a); together, these factors have led to sites that are unable to achieve canopy closure. There is interest in increasing the number of stems on these sites in order to push them onto a trajectory that leads to faster canopy closure; however it may not be as simple as planting more trees. Since aspen is a shade intolerant species (Huffman et al. 1999), being taller than the surrounding vegetation is necessary for it to thrive. Recent research has indicated that aspen seedlings fare better upon out-planting if they have high levels of NSCs, and a high root to shoot ratio (R:S) (Landhäusser et al. 2012b). However, the positive effects of a high R:S are only seen when competition is suppressed during the first few years of growth (Kyle Le, unpublished). Given the apparent intolerance of aspen to competition, it may be beneficial to completely remove the existing trees and induce suckering of the root systems of planted aspen that have already established. Suckers attached to an older, clonal root system can grow rapidly (reaching 2 m in the first growing season), and may be able to escape competition (Peterson & Peterson 1992), as the growth rate of suckers typically exceeds that of seedlings (Heeney et al. 1980). If we are able to induce significant suckering on the root systems of planted aspen, then there may be a way to increase stem density and reach canopy closure more quickly on sites that have previously failed to do so.

We know that aspen will commonly produce suckers and stump sprouts in response to disturbance (Peterson & Peterson 1992; Bell et al. 1999). It may be possible to harness this regeneration strategy to increase the density of trees on low-density sites. An increase in stand density may be achievable through managed disturbance on these sites, as disturbance may induce suckering of planted aspen. Additionally, if we are able to determine relationships between easily measured characteristics of planted aspen and their ability to sucker, then we may begin to develop a robust toolkit to estimate the potential suckering response to managed disturbances. If we can determine the relationship between tree diameter at breast height (DBH) and root system extent (size), then this will give us an idea of the radius around an aspen stem where lateral roots may be present, and where suckers may subsequently emerge. Furthermore, if there is a significant relationship between root system size and the number of suckers produced, we will have an estimate of the potential future stand density. If the relationship between root system size and the number of suckers produced is not clear, then exploring other variables may help to explain patterns of suckering.

1.3 Reserve Dynamics

One of the factors that determines the growth of suckers are stored NSC reserves (Schier & Zasada 1973; Landhäusser & Lieffers 2002; Wachowski et al. 2014). NSCs are composed of starch and water-soluble sugars; stored NSCs are those that are built up in the plant and can be remobilized for future use (Chapin et al. 1990). Nitrogen (N) is another important resource for resprouting, as it may limit growth, independent of carbon status; it is also indicative of protein content, which may drive other plant processes (Cruz et al. 2003; Millard et al. 2007; Palacio et al. 2007: Stevens et al. 2014). N fertilization has also been shown to affect suckering in aspen (Fraser et al. 2002; Frey et al. 2003; Landhäusser et al. 2010b), however the effect of initial N reserves, in concert with the initial NSC reserves, have not been explored for isolated aspen root segments. Studies have indicated that measures of both NSC and N are important in determining how plants allocate resources (e.g. to growth or to increased reserves) (Kobe et al. 2010), and in determining the overall resprouting response (Pate et al. 1990; Palacio et al. 2007; Clarke et al. 2013).

Although it is generally agreed that resprouting relies on NSC reserves (Palacio et al. 2007; Moreira et al. 2012; Clarke et al. 2013; but see Cruz et al. 2003), there are currently conflicting views on whether it is the concentration or content of these reserves that drives resprouting (Canham et al. 1999; Ryan 2011; Hoch 2015). NSC reserves are most commonly discussed as a concentration (a percent dry mass), however some studies also look at the content (total mass) of NSCs (Canham et al. 1999; Ryan 2011; Hoch 2015). The production of aspen suckers is influenced by the initial NSC concentration in the system (Schier & Zasada 1973; Landhäusser & Lieffers 2002). It has been suggested that the NSC concentration is a better measure of the overall carbon status of a plant, as it does not rely on an estimate of plant mass (which is required to calculate NSC content), and since it is a relative measure, it provides a better value for comparison between plants or organs of different sizes (Hoch 2015). However, content and concentration do not always follow the same pattern (Myers & Kitajima 2007; L. Poorter & Kitajima 2007;

Wachowski et al. 2014). It has also been suggested that the total content of NSCs may be a better indicator of the plants ability to build new tissues after disturbance (Canham et al. 1999; Ryan 2011). Additionally, species that resprout often have a greater content (not concentration) of root reserves than species that do not resprout (Pate et al. 1990; Clarke et al. 2016); this difference suggests that NSC content may be a better indicator of the ability of a plant to resprout after disturbance.

If there is interest in determining the extent to which sucker production is driven by the content and/or the concentration of initial NSC and N reserves, then the initial reserve status of a root system should be measured under conditions where new reserves cannot be assimilated. To get a full picture of the NSC dynamics, the measurements of the initial reserves can be separated into the starch and sugar, and NSC and N reserves can be measured in the xylem and phloem tissues separately. NSC and N content is simply the concentration value multiplied by the mass of the tissue: the mass of different tissues in small root segments should be easily attainable, and should provide an accurate measurement of initial reserve mass. Aspen is a species that will sucker prolifically from even small segments of roots, making aspen the model species to understand how initial concentration and content of NSC and N may drive sucker production.

1.4 Research Outline/Objectives

The overall objective of this research was to assess suckering in seedling origin aspen systems. The specific objectives of the study presented in Chapter 2 were to determine how seedling origin aspen respond to above ground disturbance, and whether sucker numbers can be manipulated by using different cutting heights of the stem and by inflicting root damage. In more detail we explored what role stem diameter and individual root system size play in the production of suckers, and whether the number of stump sprouts affected the sucker regeneration of seedling origin aspen. The objective of the study was to provide reclamation managers with an idea of how planted aspen respond to disturbance, and how this response might be manipulated to increase stem density on reclamation sites where aspen have been planted at low densities, but where a higher density is desired.

The objective of the study presented in Chapter 3 was to assess the role of total non-structural carbohydrate and nitrogen concentration and content in sucker initiation and growth. To ensure that our results were driven by stored NSC reserves, rather than by newly assimilated carbon, our entire experiment, from sucker initiation to death, took place in the dark. NSC and N relationships were assessed in the xylem and phloem tissues separately; sugar and starch were also measured separately in order to catch the subtler dynamics of NSC.

Chapter 4 provides a synthesis of the research where the implications of the results of both studies are discussed and future directions are suggested.

Chapter 2. Regeneration dynamics of seedling-origin aspen

2.1 – Introduction

Aspen (*Populus tremuloides* Michx.) is a wide-ranging tree species and is considered a foundational species in many areas of the North American continent (DeByle & Winokur 1985; Peterson & Peterson 1992). As an early successional species, aspen is readily able to occupy sites after disturbance, particularly through its ability to spread and reproduce vegetatively through root sprouts (suckers) (Schier & Smith 1979; Bartos & Meuggler 1981; Peterson & Peterson 1992; Frey et al. 2003). This regeneration strategy, among other properties, has made aspen a focal species for reclamation and restoration projects particularly across its boreal range (Macdonald et al. 2012). Over the past 30 years, aspen seedlings have been planted on boreal forest reclamation sites in western Canada at densities recommended for the re-forestation of conifer stands after forest harvesting (1500-2000 stems ha^{-1}) (Government of Alberta 2013). After 20-30 years these densities have been found to be insufficient to achieve closed forest canopy conditions on some reclamation sites due to mortality and/or poor seedling performance (Landhäusser et al. 2012a). Natural aspen stands of clonal origin have been observed to reach high leaf area index (LAI) levels indicative of canopy closure in as little as four years after disturbance, capturing the site successfully by supressing early successional, ruderal, and competitive herbaceous species (Pollard 1970; 1971; Pinno et al. 2001). Canopy closure is an important phase in the development of forests (Oliver & Larson 1996), and a delay in canopy closure of a decade or more may have implications on the trajectory and functionality of forests. For example, delayed canopy closure provides opportunities for competitive grass and forb species to become established and dominate the site, further impeding the development of a tree canopy (Landhäusser & Lieffers 1998; Frey et al. 2003, Bockstette et al. In Press), impacting soil development (Sorenson et al. 2011), and preventing the development of a diverse forest understory community, characteristic of natural forest stands.

Aspen is a well-studied species, and naturally regenerated stand attributes have been studied in all age classes, from sucker establishment to over-mature or dying forest stands (Shepperd 1986; Bartos et al. 1991; Peterson & Peterson 1992; Frey et al. 2003;

Frey et al. 2004). In the recent past the forest sector, particularly in Canada, have viewed aspen as a competitor to species that are more commercially valuable (Miller 1996), and numerous studies have focussed on how to decrease or inhibit aspen regeneration (Bell et al. 1999; Pitt et al. 2003; Greifenhagen et al. 2005; Pitt & Bell 2005). While these studies have sought to reduce aspen abundance, they have also demonstrated how cutting and the timing of harvest can be used to manipulate regeneration density—via root suckering and stump sprouting—in clonal origin stands (Bell et al. 1999, Mundell et al. 2008). In natural clonal aspen stands, full stem removal has predominantly resulted in significant regeneration through root suckering (Farmer 1962; Schier 1978; Bates et al. 1993; Grewal 1995; Frey et al. 2003) while the retention of some stems may have a negative impact on regeneration (Huffman et al. 1999; Mulak et al. 2006). Removal of the aboveground portion of the stem disrupts the hormonal balance between the root and shoot, which stimulates the development of suckers on the root system (Eliasson 1971; Schier 1972; Schier 1975). In younger stands, the cutting of stems can also produce stump sprouts, which are thought to cause a suppression effect on the production of new root suckers from the parent clonal root system (Sterett & Chappell 1967; Eliasson 1971; Mulak et al. 2006; Wan et al. 2006). Earlier vegetation management research has shown that the ratio of suckers to stump sprouts can be manipulated in young stands by altering the height at which trees are cut, as well as when they are cut (Bates et al. 1993; Bell et al. 1999; Mulak et al. 2006).

The knowledge gleaned from these previous studies may be applicable for reclamation sites where planted aspen was established at low stem densities, but a higher stem density is desired. By inducing suckering through controlled aboveground disturbance, the extended lateral root system of each planted aspen may produce enough root suckers, to significantly increase stem density, and lead to earlier crown closure. Clonal origin aspen stands have extensive parent root systems that are also connected through intra- and inter-clonal grafts (DesRochers & Lieffers 2001a,b; Jelíncová et al. 2009; Snedden 2013). These root connections may persist even after the original parent tree has died off, and may assist in the transfer of resources between clones and ramets (Desrochers & Lieffers 2001b). Since planted (or seedling-origin) aspen are individual root systems and genotypes, it is unclear if these genetically diverse aspen stands will have similar levels of connectedness, and if they will respond to aboveground disturbance in similar patterns to clonal stands that have already endured one or more disturbance cycles in their lifetime (Kemperman 1977; Perala 1978). Studies into aspen root system characteristics have only focused on clonal, rather than seedling origin stands (Strong & La Roi 1983a,b; Desrochers & Lieffers 2001a,b). The depth and size of the root system will have a significant impact on the ability of roots to sucker and the spatial distribution of suckers. Earlier research has shown that aspen roots are concentrated in the top 5 – 20 cm of soil (Strong & La Roi 1983a,b; Snedden 2013) and sucker emergence is unlikely from soil depths greater than 20 cm (Wachowski 2012; Wachowski et al. 2014). Further, Steneker (1976) determined that stand age did not affect suckering potential, however, this was observed in natural clonal origin stands, which most likely had a much older connected clonal root system. Disturbance to the root system –such as severing or scarification of roots – has been found to induce root suckering (Shepperd 1996; Fraser et al. 2004; Kabzems & Haeussler 2005); however, these injuries cannot be too severe (Renkema et al. 2009). If mechanical severing of the existing root system was successful in inducing suckering, it may prove to be a low cost option for increasing aspen stand density in low density stands, eliminating the need to remove the parent trees.

The objective of this study was to determine how seedling origin aspen respond to above ground disturbance, and whether sucker numbers can be manipulated by using different cutting heights of the stem or by inflicting root damage. In more detail we explored what role stem diameter and individual root system size play in the production of suckers, and whether the number of stump sprouts affected the sucker regeneration of seedling origin aspen. We hypothesized that trees cut lower to the ground will have greater sucker numbers, while higher cut trees will have more stump sprouts and fewer suckers. We further predicted that the root system size will have a significant and positive effect on the number of suckers produced, and that larger trees will have larger root systems producing larger number of suckers. Additionally, we anticipate that severed root systems will produce similar sucker numbers to root systems attached to a stump.

2.2. Methods

2.2.1 Study Site

This study was carried out at the Ellerslie Research Station, University of Alberta Edmonton, Alberta (N 53° 24” ; W 113° 32”). The research station is located in the central parkland ecoregion (Natural Regions Committee 2006) on a Malmo silty clay loam (fine textured), which is an Eluviated Black Chernozem developed from a lacustrine parent geological material (Bowser et al. 1962).

Weather data were collected from the South Campus weather station, 9 km due north of the Ellerslie Research Station. Precipitation totalled 145 mm over the growing season (leaf out April 1st until harvest, August 28th, 2015). During the growing season, average maximum daily temperature was 20.6 °C and average minimum daily temperature was 8.1°C. The long-term average maximum and minimum temperatures in this region are 18.8 °C and 6.4 °C respectively. The long-term average amount of precipitation at this site for these months is 289 mm, making the 2015 growing season both warmer and drier than average (Alberta Agriculture and Forestry 2016). Precipitation in the 2016 growing season (April 1st until plot measurements on August 18th, 2016) totalled 365 mm, with an average maximum daily temperature of 20.7 °C and an average minimum daily temperature of 8.79 °C, making 2016 wetter and warmer than average.

Two aspen stands of seedling origin were established in 2003 and 2007. The 12-year-old site (large diameter site, LD) occupied an area of approximately 0.06 ha, was planted at a density of 10,000 stems ha^{-1}, and had trees that ranged in DBH from 2-13 cm in 2015. The 8-year-old site (small diameter site, SD) occupied an area of 0.04 ha and was planted at 29,000 stems ha^{-1}; stem DBH ranged between 2 and 7.5 cm in 2015 (Table 2-1). The difference in tree size was most likely a result of stand planting density; however, because age was also different we cannot separate these effects. Thus tree size, average diameter, and age were considered in combination when comparing trees from the large and small diameter sites (LD or SD respectively) (Table 2-1).

Since the sites were in close proximity to each other and planted on a formerly cultivated field, soil conditions were considered homogenous across the site. Treatments at this site were carried out at the individual tree as well as the site level. Both sites had closed canopies with negligible understory vegetation. However, to control the spread of species that could become competitive (e.g. *Cirsium arvense*) after the selective canopy removal in 2015, a herbicide (Glyphosate, Roundup, Monsanto, St. Louis, MO, USA) was applied once as directed by the product label with a hand sprayer in the early summer of 2015 before any aspen sprouting had occurred.

Only healthy dominant or codominant canopy trees were selected in both sites for this study. Trees with evidence of hypoxylon canker, bark deformations, or evidence of wood boring insects were avoided, as were trees with more than one bole. The DBH of all dominant and co-dominant trees was measured in each site, and these values were used to establish a small, medium and large diameter bracket for each site. A random selection of trees was made for the study based on these brackets; 12 trees each were assigned to the small and large size classes and 16 trees were assigned to the medium size class in each site (80 trees total). The ranges of DBH in each size class were 4.1-5.5 cm (small), 5.5 – 9.5 cm (medium), and 9.5 – 12 cm (large) for the large diameter (LD) and 2.9 – 4 cm (small), 4 – 5.0 cm (medium), and 5.0 – 6.1 cm (large) for the small diameter site (SD).

2.2.2 Treatments

To explore the impact of planting density and disturbance type on suckering and stump sprouting of planted aspen trees, four different disturbance treatments (treatments) were applied on May 29th, 2015 to 40 trees in each site (total of 80 trees). The treatments were: 1) trees with no treatment (Control); 2) trees that had all lateral roots severed to a soil depth of 20 cm (Severed); 3) trees that had the stem removed at ground level (0 cm) (Low Cut); and 4) trees that had the stem removed 25 cm above ground level (High Cut). Treatments 3 and 4 were applied using a handsaw and stems were cut just above the root collar (Low Cut) or 25 cm above the root collar (High Cut). For the Severed treatment, a sharpened, flat-headed spade, followed by a narrow handsaw, was used to sever all roots to a depth of 20 cm in a radius of 10 cm around the bole of each tree. The 20 cm depth was chosen to ensure that all roots with the potential to produce emergent suckers would be severed (Wachowski 2012; Wachowski et al. 2014). Aspen roots are concentrated in the area 5 – 20 cm below the soil surface (Strong & La Roi 1983a, b), so a depth of 20 cm was determined to be sufficient for severing the majority of lateral roots.

To understand the suckering dynamics of the entire site, and to relate total basal area to suckering density, all (approx. 1700) trees were cut in the winter of 2016 at approximately 5 – 10 cm above the ground. The total basal area of each site was determined by measuring the basal diameter of each cut tree at this time. The sites were then left to regenerate over the summer of 2016. Suckering and stump sprouting were assessed in late August 2016 by measuring the sucker and stump sprout density and their heights in four and five 10 m^2 circular plots (1.78 m radius) for the SD and LD sites respectively.

2.2.3 Measurements

One intact lateral root (1-2 cm in diameter) was collected from each control tree at the time of treatment. The root was carefully excavated and traced until it tapered to less than 0.5 cm diameter, or went deeper than 20 cm below soil surface (see above). These root segments were kept moist and brought back to the lab to determine root length, mass, and volume. Coarse root volume was calculated using the water displacement method (Harrington et al. 1994). These measures allowed us to calculate total root surface area according to Equation 1:

$$\boldsymbol{S}lant\ \boldsymbol{H}eight\ = \sqrt{length^2 + (\boldsymbol{Bottom}\ \boldsymbol{R}adius - \boldsymbol{Top}\ \boldsymbol{R}adius)^2}$$

$$Surface\ Area\ = \pi \times (\boldsymbol{BR}\ + \boldsymbol{TR}) \times slant\ height$$

These measures from the control trees were used as the baseline data for the root excavations after the first growing season in August.

After the treatments were applied, the trees were left to regenerate from June 4th to August 13th 2015. To assess suckering of each individual tree, the root systems of all 80 treatment trees were carefully excavated in late August and were evaluated for sucker initiation and development. Each lateral root greater than 0.5 cm in diameter, and originating within the top 20 cm of the soil, was collected individually, and all suckers attached to that root were collected with it. Stump sprouts were collected separately. Roots that were less than 0.5 cm in diameter were collected and later pooled together. During collection, roots were severed at the base of the tree, and traced to at least 0.5 cm in diameter or until the root reached a soil depth below 20 cm. Where suckers were present in the vicinity and general direction of a root, the root was followed until it could be determined that the sucker was or was not from that particular root system. Roots and suckers were stored in bags and kept moist and cool in the field. Roots were brought back to the lab at the end of each day and were stored at 4° C until processing in the lab. Once in the lab, total root length was measured and fine and coarse root volume was estimated via water displacement (see above). Total coarse root dry mass was measured after drying samples to constant weight at 70°C. The relationships between tree diameter and root mass and between root mass and root length from the individual trees were used to estimate an area based root length and root mass of the sites.

2.2.4 Statistical Methods

All data were analyzed using R-Studio (Boston, MA). For parametric analyses, assumptions of normality and homoscedasticity were tested using the Shapiro-Wilks test and Levene's Test. If data did not meet the assumptions, other statistical approaches such as transformation or non-parametrical analyses were applied (see below). Our study was divided into two field seasons. The first section outlines the methods used to parse out relationships between the four treatments and the two sites on sucker production, where the response of the individual tree is of interest. To determine the effect of treatment and site on sucker production of individual trees, a generalized linear model following a Poisson distribution was fitted, as the count data did not meet the assumptions of normality; Pairwise comparisons were determined with the general linear hypothesis testing function with Fisher's least squared difference (LSD test) and an α adjustment with Hommel's method from the multcomp package (Hothorn et al. 2008). The effect of treatment and site on stump sprout production was determined using a two-way ANOVA; Fisher's LSD test with a Bonferroni adjustment was used for the post hoc pairwise comparisons. To determine 1) if the pooled High and Low Cut (Cut) trees produced more suckers than the roots of Severed trees; and 2) if the Cut trees produced more total sprouts (suckers and stump sprouts combined) than the Severed trees, data were log + 1 transformed and two-way ANOVAs with treatment and site as main effects were used. In order to test if the production of stump sprouts inhibited the production of suckers at the individual tree scale, Spearman's Rank Correlation was used to test the correlation between sucker and stump sprout production in the High and Low Cut treatments. Finally, simple linear regressions were used to test the relationships between total sucker and stump sprout production, and between the total root length for the High Cut and Low Cut treatments; sucker and stump sprout values were log + 1 transformed.

In the second field season (below) there was one treatment (clearcutting) applied to two sites (LD and SD): all results are based on mil ha plot measurements within the two sites. To explore differences in total root length and weight between the sites, the non-parametric Kruskal-Wallis test by ranks was used. In 2016, the differences in sucker and stump sprout regeneration between the LD and SD sites were tested using t-tests or Mann-Whitney-Wilcoxon tests; the impact of stump sprouting on suckering was measured with a Spearman's Rank Correlation. Linear regression models were fitted to assess the relationships between stump weight and DBH and tree bole weight and DBH for 10 trees that were completely excavated and weighed (in the SD site). These relationships were used to estimate the bole and stump weights for all trees with a DBH greater than 4.9 cm, which were then used, in combination with the weighed root systems, to compute root to shoot ratios for 42 trees in the SD and LD sites. Non-linear regression models were fitted to assess the relationships between root mass and DBH; root mass and root length; and DBH and root to shoot ratio.

2.3 Results

The suckering response of trees was highly variable between individuals and ranged from 0 – 29 suckers per tree. Of our cut trees, 75% produced at least one sucker and 60% produced at least one stump sprout. Despite this variability, treatments had a significant effect on sucker production ($\chi^2_{3,76} = 417.35$, $p<0.001$), but this response differed between the large diameter (LD) and small diameter (SD) sites (treatment × site interaction $\chi^2_{3,72} = 397.47$, $p < 0.001$). On both the LD and SD sites, the Low Cut treatment produced the greatest number of suckers per tree on both sites ($\bar{x} = 7.5$), while the Control treatment had the fewest (Fig. 2-1). The High Cut and Severed treatments produced similar numbers of suckers but fewer than in the Low Cut treatment; however, the number of suckers in the High Cut and Severed treatments were approximately two times higher in the LD site than in the SD site (Fig. 2-1). Site had a significant effect on the amount of stump sprouts produced; however, cut height had no effect on the production of stump sprouts ($F_{1,36}=0.30$, $p = 0.59$). (Fig. 2-2). The smaller diameter trees on the SD site produced three times more stump sprouts ($\bar{x} =4.5$) than the larger diameter trees on the LD site ($F_{1,36}=6.25$, $p = 0.02$).

To determine if cutting of stems produced more suckers or total sprouts than root severing, sucker and sprout production of individual trees was compared between the Severed and Cut (combined High Cut and Low Cut treatments) treatments in both the LD and SD sites combined. There was no difference in the production of suckers ($F_{1,56}= 0.964$, $p=0.33$) or total sprouts (sum of suckers and stump sprouts) ($F_{1,56}= <0.001$, $p=0.98$) between the two sites. However, based on the initial tree volume, the SD produced approximately 12.2 suckers per m^3 of initial aboveground mass, compared to the LD producing 8.5 suckers per m^3 of aboveground mass. The number of suckers produced in the Cut and Severed treatments was not statistically different ($F_{1,56}= 2.470$, $p=0.12$)(Fig. 2-3A), but showed a trend for greater sucker production in the Cut treatment. The trees in the Cut treatment had an average of 8 sprouts per tree, approximately four times higher than in the Severed treatment ($F_{1,56}= 9.726$, $p=0.003$)(Fig. 2-3B).

Stump sprouts did not have a negative effect on root suckering in 2015. Sucker and stump sprout production were not related with each other in either cut treatment, which may indicate that stump sprouting was not inhibiting the production of root suckers. The relationship between the two sprout types was moderate in strength and marginally insignificant for the Low Cut treatment ($r = 0.43$, $n=40$, $p=0.058$); the relationship was insignificant for the High Cut treatment ($r = 0.33$, $n=40$, $p=0.156$).

The excavation of individual root systems in 2015 allowed us to determine differences in the average root system size between trees on the SD and LD sites. On average, trees in the LD site had greater root mass ($F_{1,78}=17.15$, $p < 0.001$) and greater root length ($F_{1,78}=9.27$, $p = 0.003$) than trees in the SD site (Fig. 2-4). The role of root system size in vegetative reproduction was investigated, and there were no significant relationships between total root length and sucker production ($r^2 = 0.07$, $n=40$, $p=0.079$), nor stump sprout production ($r^2=0.001$, $n=40$, $p=0.82$). Although there were no significant relationships between root characteristics (surface area, length, mass) and sucker production, we found strong relationships between root characteristics and tree size (Table 2-2). The excavated and weighed tree boles and stumps from 10 trees (DBH range 4.9 – 7.5 cm) in the SD site were strongly related to DBH (Table 2-2, relationships 1 and 2); these equations were used to estimate tree bole and stump weights for all trees greater than 4.9 cm DBH ($n = 42$). Estimated stump mass was added to the measured root mass and together used to estimate the root to shoot ratio (R:S) of the individual trees. The measured R:S, based on 10 trees in the SD site, averaged 0.29 (range of 0.22 to 0.39); when evaluating the estimated R:S, there was a strong power relationship between R:S and DBH (Fig. 2-5), and this appears to level off at a R:S of approximately 0.22.

In winter 2016, the basal area of all trees in the LD site (590) and SD site (1137) were measured. The LD site, with a stem density of 10,000 stems ha^{-1}, had a basal area equivalent to 79 m^2 ha^{-1} while the higher density (29,000 stems ha^{-1}) SD site had a basal area of 49 m^2 ha^{-1}. The LD site produced significantly more suckers ($t=3.88$, $n=5$, $p= 0.012$) that were 23% taller than suckers in the SD site ($t_{3057}=7.04$, $p < 0.001$, Table 2-3). The SD site produced significantly more stump sprouts ($t=8.0$, $n=5$, $p < 0.001$), but there was no corresponding increase in stump spout height (t_{756}=1.15, p=0.24, Table 2-3). As in 2015, there was no association between suckering and stump sprouting in the SD or LD sites in 2016 after clearcutting (LD r=0.32, n=4, p=0.59; SD r= -0.49, n=4, p=0.50).

2.4 Discussion

Seedling origin aspen regenerated readily after above ground disturbance through both suckering and stump sprouting; however, the suckering response was highly variable, and ranged from zero suckers to a maximum of 29 suckers per root system, with an average of five suckers per cut tree in 2015. Of our cut trees, 75% produced at least one sucker and 60% produced at least one stump sprout. After the clearcut treatment (2016) the average number of suckers produced decreased to 4 in the LD sites and 1 in the SD site. Although there is no data available on the suckering potential of seedling origin aspen after cutting, our values are very similar to aspen that had established from seed after a fire in Arizona and produced voluntary suckers without aboveground disturbance (Fairweather et al. 2014). In that study 61% seedlings produced suckers, ranging from 1 – 39 suckers per ortet with an average of 5.4 suckers (Fairweather et al. 2014). We cut the whole stand in the second year of our study and found that trees produced similar numbers of suckers per tree, as seen in the individually cut trees the year earlier; however, compared to clonal origin stands, the suckers produced in our study after the whole stand was cut were relatively short, averaging only 55 cm in height. Suckers arising from established clonal stands can reach over 200 cm in the first growing season (Peterson & Peterson 1992). Given that this study was conducted on a rich Chernozemic agricultural soil with no measureable competition pressure and occurred during a wetter than average year (i.e. making water less of a limiting factor during regeneration), the relatively low height growth of suckers suggests that other factors may be driving regeneration and sucker performance in these seedling origin aspen. It is interesting that a significant proportion of individual aspen root systems did not produce any suckers. This response might be related to the large genotypic variability in seedling origin aspen (Fairweather et al. 2014). The expression of genotypic variation has been observed in a multitude of traits in aspen including: carbon allocation to roots and shoots, and root turnover (King et al. 1999); canopy decline and mortality (Schier & Campbell 1980; St Clair et al. 2010); sucker production (Zufa 1971; Schier & Campbell 1980); and concentrations of both phenolic compounds and tannins, which affect both the decomposition rate of leaves, and the degree of herbivory (Osier & Lindroth 2001; Lindroth et al. 2002).

Severing lateral roots from the main stem of established planted aspen trees did produce sucker regeneration on the severed roots. This approach could increase aspen stem density on restoration sites where the cutting of aspen trees to initiate suckering might not be a desirable option. However, in these young stands the sucker density tended to be lower than in the cut trees. Interestingly, the trees with severed lateral root systems remained alive throughout the growing season and the severing did not appear to have any ill effects on the same-year performance of these individuals. However, there were issues related to overall stability of the trees when severing very close to the stem.

Cutting trees at the surface (Low Cut) in our experiment produced more suckers than leaving a 25 cm stump (High Cut); this pattern is similar to observations in clonal origin stands (Bell et al. 1999). However, counter to our results, Bell et al. found that the number of stump sprouts increased with height of cut. In our study the smaller diameter stems produced more stump sprouts than the large diameter stems, which is consistent with clonal research which has found that more stump sprouting can be expected in young or smaller diameter trees (Heeney et al. 1980; DeByle & Winokur 1985; Mulak et al. 2006). It has been hypothesized that the presence of stump sprouts may also prevent the formation of suckers in aspen and other clonal species (Eliasson 1971; Sterett & Chappell 1997; Mulak et al. 2006). Our study showed no evidence of sucker suppression by stump sprouts in 2015 and 2016, as indicated by the lack of correlation between the two measures in both growing seasons. Interestingly, we found that rather than the number of stump sprouts, the stump height had a significant effect on the amount of suckering. Similar results have been observed in smaller aspen seedlings where dormant seedlings that were debuded (i.e. stems were unable to grow new shoots and leaves) or had half of their stems cut off, produced significantly fewer suckers than seedlings that were cut close to the ground (Wan et al. 2006). The authors concluded that the stem that had the largest influence on sucker production, most likely a process driven by plant hormones.

Root system size (mass and length) likely played an important role in the sucker regeneration of planted seedling origin aspen at both the individual tree and stand level. At the stand level, stem density could also affect root system size (through intraspecific competition) and the rate of connectivity (which may relate to the amount of root overlap) (DesRochers & Lieffers 2001b; Jelínková et al. 2009; Snedden 2013). The length of lateral roots of the planted aspen in our study was relatively short (3.8 m LD site and 2.9 m SD); for example, roots of aspen seedlings on sandy soils had lateral roots up to 9 m in length for 8-year-old seedlings (Day 1944). In a different study, 4-year-old aspen planted in a coarse textured soil, but at the same density as the LD site, had a maximum root length of 7.4 m (S. Bockstette, personal communication). In more mature aspen, Strong & La Roi (1983a) noted differences in aspen root forms between fine and coarse textured soils; however, these root systems were clonal and only partially excavated. We measured an average root to shoot ratio (R:S) in the SD site (8 years of growth) of 0.29 and after estimating the stump weight of a larger range of stem diameters for which the root mass was known, the relationship between stem diameter and R:S appears to begin to level out at around 0.22 at DBHs greater than 10 cm (2- 5). This R:S finding is similar to the R:S of 0.22 that was observed after an excavation of healthy and mature 45-year old boreal aspen stands (Strong & La Roi 1983a). Estimating root mass indirectly from aboveground measures is advantageous, due to the well-known challenges of directly estimating root mass; however, our understanding of the above and belowground relationships in seedling origin aspen is still limited and might vary greatly among sites and geographic areas. Rooting system structure of clonal aspen has been studied in some detail (Day 1944; Strong & La Roi 1983a,b; DesRochers & Lieffers 2001a,b; Snedden 2013), but there are still significant gaps in our understanding of rooting dynamics of seedling origin aspen, how they are influenced by edaphic factors, and how they may change with planting density.

Root grafts are commonly observed in clonal aspen root systems (Desrochers & Lieffers 2001b; Jelínková et al. 2009; Snedden 2013), but were generally lacking in our stands. Of the 80 excavated trees with 423 individual roots, only one root graft was observed between two trees, indicating that root systems of planted aspen are isolated even as the stand develops. This is interesting, as root grafts and their role in resource sharing of carbohydrates, nutrients and water are important aspects in the reproduction, growth and stand dynamics of clonal aspen stands (Debyle 1963; Eis 1972; DesRochers & Lieffers 2001a). Functional root grafts and resource sharing have also been observed in high density *Pinus contorta* (seedling origin) stands (Fraser et al. 2006) where functional root grafts commonly occurred in seedlings that were < 80 cm apart (Fraser et al. 2005). Approximately 15 to 25% of 6 and 15 year-old pine trees had grafted roots at that spacing (Fraser et al. 2005). The SD site in our study was planted at a spacing of 50 cm, so it is surprising that the overlapping root systems at this high density did not result in more root connections. The reason for a lack of interconnection is puzzling, but might be due to intraspecific belowground competition of the greater number of individuals (different genoptypes), generally not deemed a significant factor in clonal aspen stand dynamics. The grafting noted in *Pinus contorta* by Fraser et al. (2005) varied with tree age, so adequate time for grafting in our stands might not have elapsed. However, if root systems of planted aspen continue to be independent at the stand level, it might lead to different dynamics in growth and regeneration of seedling origin aspen stands compared to clonal origin stands. The stand dynamics of clonal origin aspen stands are well documented where sucker densities after aboveground disturbance can range from 6000 to 280,000 stems ha^{-1} (Peterson & Peterson 1992). However, these dense stands quickly self-thin to much lower numbers within the first few growing seasons (2-5 years) (Pollard 1971; Perala 1984; Mallik et al. 1997; Kabzems & Haeussler 2005), interestingly our high density planting showed little evidence of self thinning within the first year. The evolving dynamics of our planted stands will likely depend on whether or not roots are competing for resources, and if they are forming intra and inter-clonal root grafts.

The dynamics of post-disturbance regeneration in planted aspen stands is likely dependent on factors that influence the root system size of these stands. If our prediction that the R:S of aspen trees of seedling origin approaches an approximate asymptote of about 0.2 early on is correct, the extent of the root system will likely depend on stem density, the size and age of the individual trees, their ability to develop functional root grafts, and edaphic variables. Additionally, variability in regeneration potential among genotypes will likely affect the spatial distribution of suckers in these stands, as genotypes with poor or no sucker regeneration (25% in our study) will be selected against under a reoccurring aboveground disturbance regime, favouring those genotypes with more prolific sucker production (DeRose et al. 2015). Under these conditions, low-density seedling-origin aspen stands might respond initially with very patchy regeneration compared to natural post-disturbance aspen stands that establish from large extensive clonal root systems that have developed over centuries or millennia.

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Tables

Table 2-1. Average initial site and study tree characteristics (± SD) for the large diameter (LD) and small diameter (SD) site. While 40 individual trees were selected for this study in each site, the site characteristics were based on averages measured on all trees in the entire site. Letters indicate significance differences between study trees in the LD and SD site type ($\alpha < 0.05$)

	Site				Study Trees (n=40)		
Site type	DBH (cm)	Density (stems ha^{-1})	Basal Area (m^2 ha^{-1})	Age (yrs.)	Height (m)	DBH (cm)	Leaf Area (m^2)
LD	6.4 ± 5.4	29,000	79	12	9.9 ± 1.4 **a**	7.1 ± 2.2 **a**	6.8 ± 4.7 **a**
SD	4.4 ± 0.7	10,000	49	8	8.0 ± 0.9 **a**	4.6 ± 0.9 **b**	4.1 ± 2.0 b

Table 2-2. Regression equations and r^2 values for four different relationships used to estimate root to shoot ratios as well as stand level root mass and length. The linear relationships are between 1) measured weight of the stump and tree diameter at breast height (1.3 m, DBH) and 2) measured weight of the tree bole and tree DBH; data for both relationships were measured August 2015 in the small diameter site (29000 stems ha^{-1}, SD) (n=10). The two power relationships are based on the measured root system mass (3) and length (4), collected in August 2015 in both the large diameter (10000 stems ha^{-1}, LD) and SD sites (n=80).

	Relationship	Slope Equation	R^2
1	Linear relationship between stump weight (g) and DBH (cm)	$y = 0.9654\,x - 1503.3$	$r^r = 0.91$
2	Linear relationship between bole weight (g) and DBH (cm)	$y = 2369.6\,x - 9732.7$	$r^r = 0.88$
3	Power relationship between total root mass (excluding stump) and DBH (cm)	$y = 6.3762\,x^{1.9505}$	$r^r = 0.52$
4	Power relationship between total root length and total root mass (excluding stump)	$y = 77.497\,x^{0.3515}$	$r^r = 0.33$

Table 2-3. Height and density of suckering and stump sprouting following clear cutting in April 2016 in both the small diameter (SD, 29000 stems ha^{-1}) and large diameter (LD, 10000 stems ha^{-1}) sites. Standard deviation (±) reported for all 2016 measures, and different letters indicate significant differences within columns ($\alpha < 0.05$) (n = 5 for LD, n = 4 for SD).

Site	Suckers		Stump Sprouts		Estimated Root System Size	
	Regeneration (stems ha^{-1})	Average Height (cm)	Regeneration (stems ha^{-1})	Average Height (cm)	Total Length (m ha^{-1})	Total Weight (kg ha^{-1})
Large Diameter	40380 ± 6264 a	59 ± 44 a	3000 ± 1811 a	67 ± 43 a	81,856	5700
Small Diameter	26025 ± 665 b	48 ± 37 b	15225 ± 2212 b	64 ± 45 a	130,743	3639

Figures

Figure 2-1. Average sucker production in response to site and treatments (Control, High Cut, Low Cut, and Severed). The large diameter (LD) site was planted at 10000 stems ha $^{-1}$ and the small diameter (SD) site was planted at 29000 stems ha $^{-1}$. Error bars represent standard deviation and different letters indicate significant differences ($\alpha < 0.05$) (n = 10).

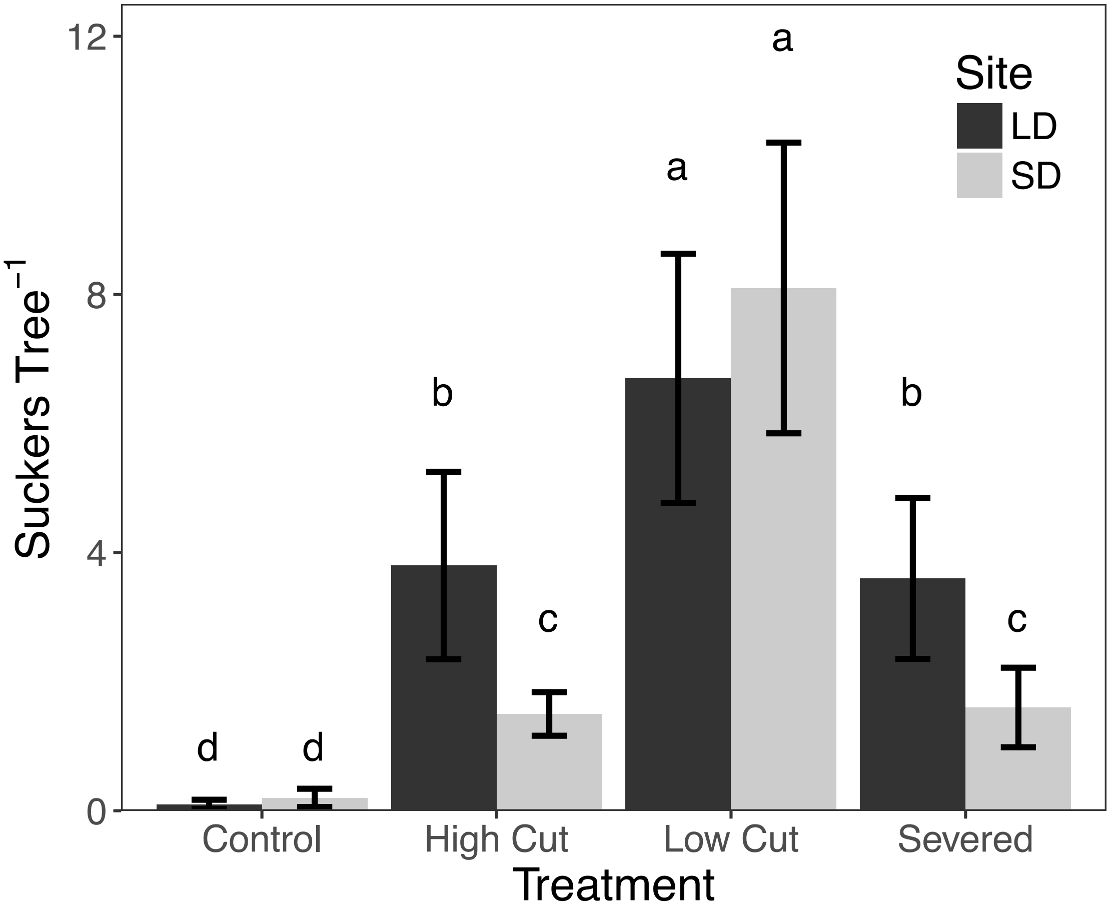


Figure 2-2. Average stump sprout production in response to site and cutting treatment (High Cut and Low Cut only). The large diameter (LD) site was planted at 10000 stems ha $^{-1}$ and the small diameter (SD) site was planted at 29000 stems ha $^{-1}$. Error bars represent standard deviation and different letters indicate significant differences ($\alpha < 0.05$) (n = 10).

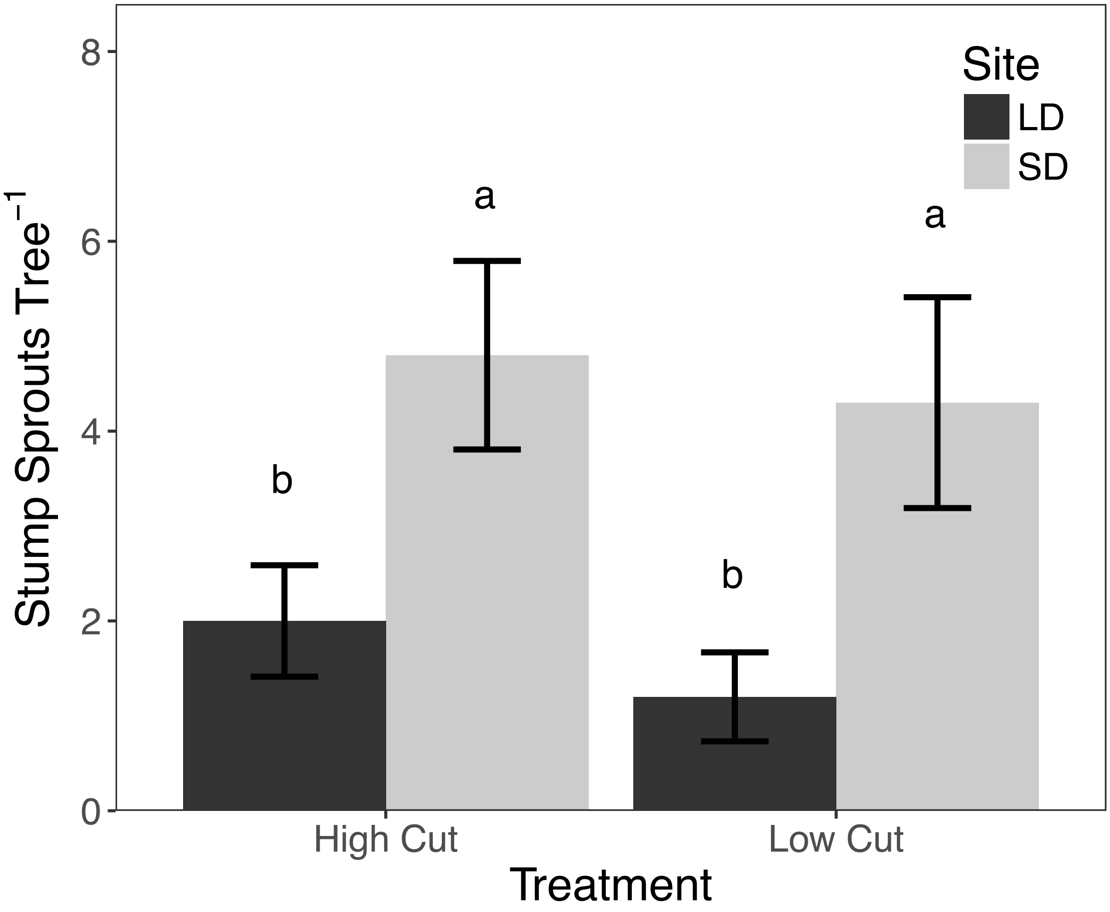


Figure 2-3. Average sucker (A) and sprout (suckers and stump sprouts combined) (B) production per tree. The Cut treatment is the High Cut and Low Cut treatments pooled (see text). Error bars represent standard deviation and different letters indicate significant differences ($\alpha < 0.05$) (n=40 for Cut and n=20 for Severed).

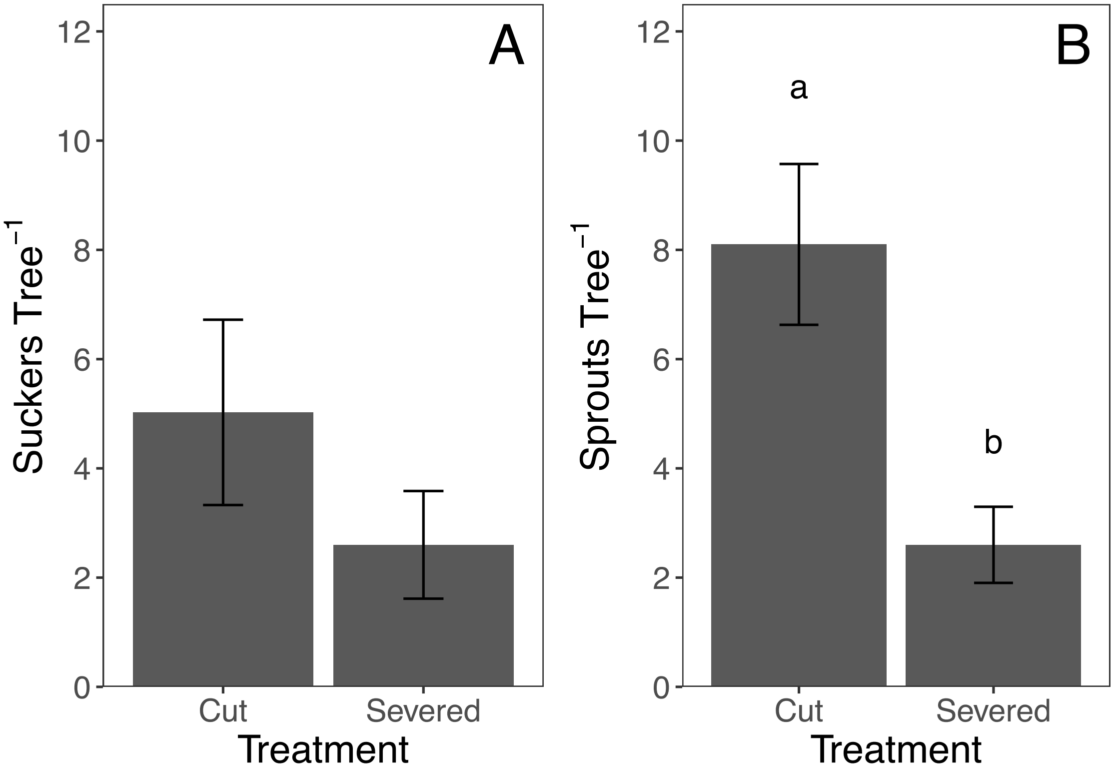


Figure 2-4. Average root weight (A) and average root length (B) per tree in the large diameter (LD) and small diameter (SD) sites. Error bars represent standard deviation, and different letters indicate significant differences (α < 0.05) (n=40).

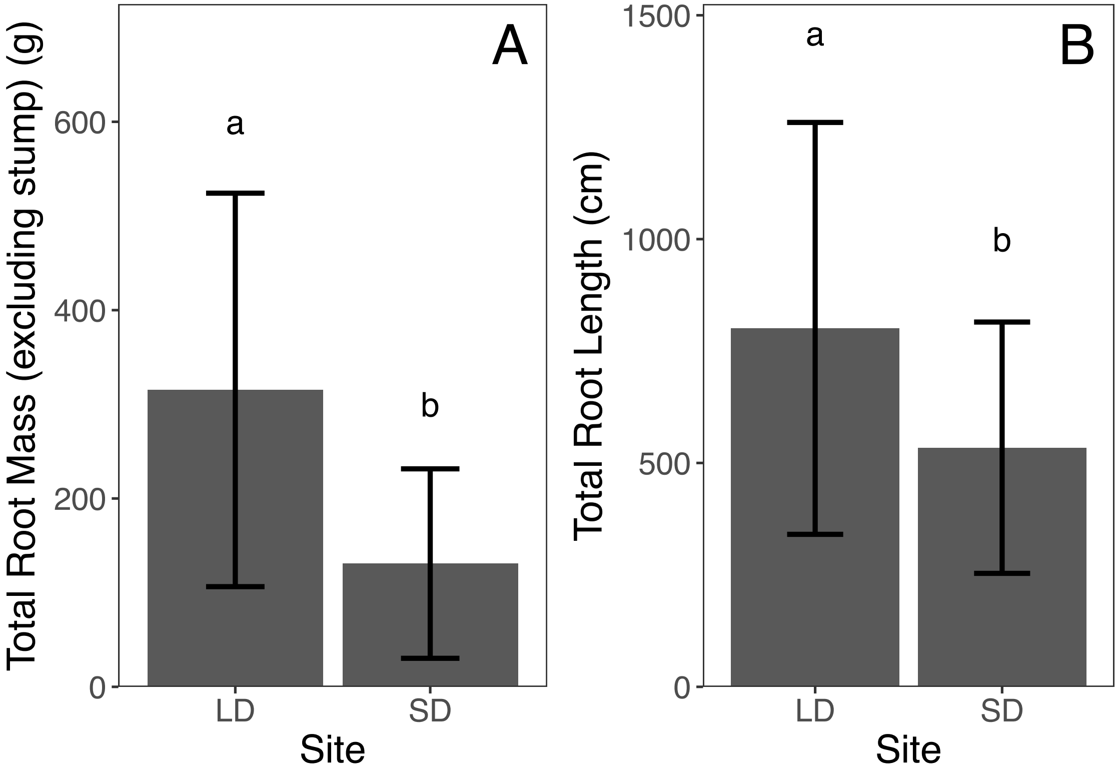
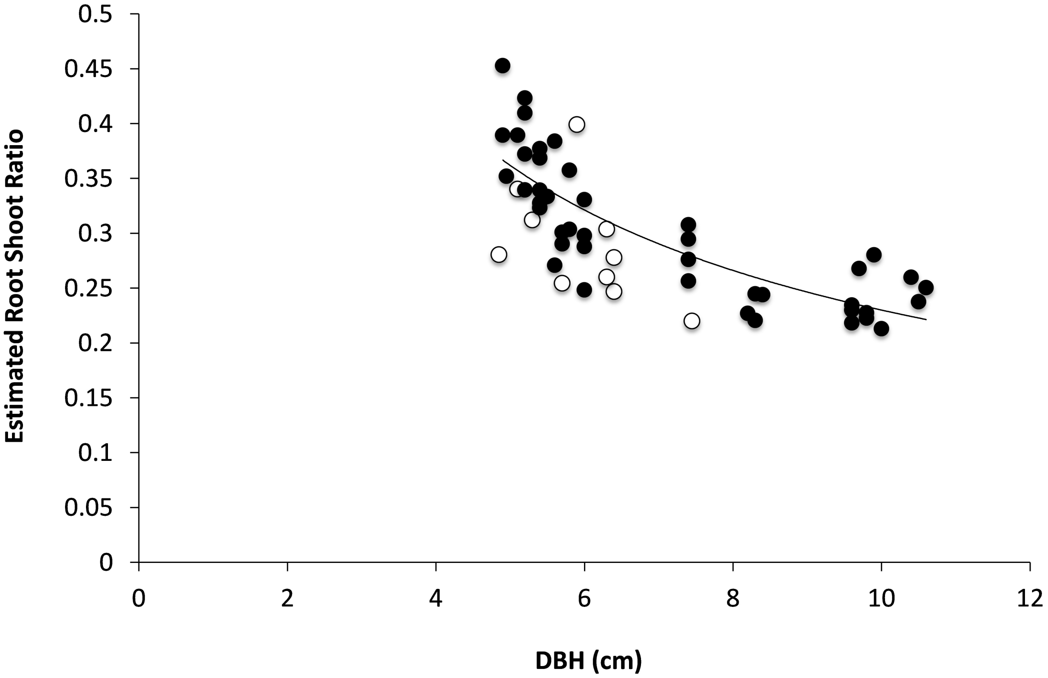


Figure 2-5. Relationship between root to shoot ratio (R:S) and diameter at breast height (DBH). The R:S is based off of measured bole and stump weights for 10 trees in the small diameter site (DBH range 4.8 – 7.5 cm, open circles), and measured root mass from all trees; strong relationships were found between DBH and the bole weight, as well as DBH and the stump weights (Table 2-3). These equations were used to estimate corresponding values for all trees where DBH > 4.9 cm (n = 42). Regression is based on 52 points, 10 measured and 42 estimated (coloured circles), $y=0.9296x^{-0.609}$, $r^2=0.616$, $p<0.001$



“Die not, poor Death, nor yet canst thou kill me.”

-John Donne

(Aspen’s mantra)

Chapter 3. Non-structural carbohydrate and Nitrogen content drive suckering: examining the role of xylem and phloem as storage tissues

3.1 Introduction

Disturbance is an ecologically important yet destructive process that can result in the loss of some (or all) of the aboveground portion of a plant through damage incurred from a variety of disturbances including fire, forest harvesting, insects, disease, and windstorms (Hobbs 2009). While many species will die after the loss of their aboveground biomass, some species are able to tolerate such disturbances because of their ability to resprout. Resprouting is an adaptation commonly used by plants to survive and clonally reproduce in environments that are prone to frequent disturbance (Bellingham et al. 2000; L. Poorter et al. 2010; Clarke et al. 2013). Plants that resprout depend on previously assimilated and stored carbon and nutrient reserves to fuel their resprouting (Palacio et al. 2007; Clarke et al. 2013; Dietze et al. 2014). Because the persistence of resprouters depends upon having substantial reserves, resprouters often have larger carbon reserves than species that do not resprout (Pate et al. 1990; Paula & Ojeda 2009; Clarke et al. 2013; Zeppel et al. 2015).

In many species, the carbon storage pool is mainly composed of non-structural carbohydrates (NSC) (Körner 2003). As storage compounds, NSCs provide plants with a source of carbon that can be remobilized for growth or metabolic processes when the plant's current demands cannot be met by photosynthesis (Chapin et al. 1990), such as following disturbance. Generally, greater NSC storage should increase the resprouting potential of a plant (Paula & Ojeda 2009; Clarke et al. 2013) However it remains unclear whether the relative size (i.e. NSC concentration) or the absolute size (i.e. NSC content; the total mass stored within a system) is a better and more relevant measure of the size of the storage pool, in terms of its ability to maintain plant function during periods of stress and following disturbance (Canham et al. 1999, Ryan 2011, Hoch 2015). It has been suggested that NSC concentration is a better indicator of the overall carbon balance in the plant, as it is a more meaningful comparison when dealing with a different organs and sizes of plants (e.g. a small tree with a high NSC concentration may have the same content as a large tree with a low concentration) (Hoch 2015). In addition, NSC concentration is more easily measured, as the ability to calculate NSC content relies on a measure of the entire biomass, which can be challenging to estimate accurately (Bustan et al. 2011). However, Canham et al. (1999) suggested that both measurements of concentration and content are important, and that the relative importance of each measure will depend on the type of stress. When reserves are required to produce new growth, such as during resprouting after a loss of the carbon assimilating aboveground biomass, the total content may be a better indicator of a plant's ability to resprout, as a greater absolute mass of reserves could be available to produce the new biomass (Canham et al. 1999; Ryan 2011). As NSC content, concentration, or both have been found to correlate with survival under stress and following disturbance (Myers & Kitajima 2007; L. Poorter & Kitajima 2007; L. Poorter et al. 2010), it remains unclear which is a better or appropriate measure.

In addition to how best to evaluate the size of the storage pool, it is also unclear *which* storage pools are the main sources of reserves used for resprouting. First, the xylem and phloem tissues in both the above and belowground organs contain parenchyma, which provide locations for reserve storage (Spicer 2014). Much of the research on carbohydrate storage has thus far focused on xylem storage, as the xylem has a greater content of NSCs—even if it has a lower concentration—because of its large biomass (Loescher et al. 1990; Kozlowski 1992). Alternatively, the role of phloem as a storage tissue has largely been ignored (Hoch et al. 2003; Breda et al. 2006; Spicer 2014), due to the fact that it is considered to primarily function as a transport pathway between sources and sinks (Savage et al. 2016) and that it does not make up a large portion of the trees mass. However, phloem can have substantially higher concentrations of NSCs than the xylem (Shepperd & Smith 1993), and may still be an important source of carbon during remobilization for resprouting. Second, it is not clear whether starch and sugar pools—the two main components of the NSC pool—are equally important sources of remobilized carbon. Starch is purely a storage compound, and for this reason, may be a good indicator of the reserves that are available for use (Loescher et al. 1990; Hoch 2007; Smith & Stitt 2007; Hoch 2015). In contrast, sugars perform other functions in the cell, such as transport, osmotic regulation, and cold tolerance (Graham & Patterson 1982; Ingram & Bartels 1996; Sala et al. 2012; Hoch 2015). Given the diverse roles of sugars, the starch pool may be more indicative of the true storage pool available for resprouting than either sugar or total NSC (Hoch 2015). Starvation studies have often found that starch concentration is depleted to levels near zero while sugars have not decreased as substantially (Mcdowell and Sevanto 2010; Hartmann et al. 2013; Dickman et al. 2015, Wiley et al. unpublished), potentially supporting the idea that not all sugars are available for remobilization.

While research on resprouting dynamics has largely focused on carbon, other nutrients, particularly nitrogen (N), are necessary for resprouting, and their storage may impact recovery from disturbance (Chapin et al. 1990; Millard et al. 2007). Fertilization studies have shown that the addition of N can have a positive effect on the growth of sprouts (Fraser et al. 2002; Frey et al. 2003; Landhäusser et al. 2010b), suggesting that resprouting may be also limited by N storage at the time of disturbance. However, there is little information about how initial N tissue concentration and content relate to subsequent sprout initiation and growth (Clarke et al. 2013). Initial N concentration has been found to be positively related to the initial ability to resprout (Moreira et al. 2012), but the effect of N on the growth of sprouts was not evaluated. In contrast, other studies have found that N content in the resprouting organ does not affect the ability of the plant to resprout, but rather the N content in the soil may be of more importance (Cruz et al. 2003). Species that resprout tend to have greater reserves of NSC than non-resprouting species (Pate et al. 1990; Clarke et al. 2016), however N reserves do not appear to follow this pattern (Palacio et al. 2007). Although some studies have evaluated the effect of N content or concentration on the ability to resprout, there is no consensus on how N reserves affect the amount of resprouting (Cruz et al. 2003; Palacio et al. 2007; Clarke et al. 2013; Clarke et al. 2016).

Populus tremuloides (Michx.) (aspen) is a widely-distributed species that can produce new sprouts (suckers) from adventitious buds that are typically pre-formed on the root system (Farmer 1962; Peterson & Peterson 1992). After removal/death of the aboveground stem, these buds are able to flush, grow prolifically in height, and develop into the next generation of an aspen stand. This response is largely driven by the reserves stored in the root system. Furthermore, aspen root systems with a higher pre-disturbance

NSC concentration have been found to have greater sucker production (by mass) (Schier & Zasada 1973; Landhäusser & Lieffers 2002). Aspen is an ideal species to assess if suckering is driven by total content or relative concentration of NSC and N, as even small segments of roots will sucker prolifically (Maini & Horton 1966). By using aspen root segments, the initial concentration and content of reserves that will fuel suckering can be easily measured; additionally, by collecting roots of different diameter, the initial content can be manipulated, as larger roots should contain a greater NSC and N content. Because the system is spatially small, measurement of the initial reserve concentrations should more accurately represent the initial reserve content (i.e. total mass), and this will enable a more accurate test of how reserve concentrations versus content affect resprouting.

To determine how initial NSC and N reserves relate to root suckering potential we allowed aspen root fragments to resprout in the dark. We assessed resprouting across a range of root diameters to ensure that we captured a wide range of initial NSC and N contents. Further, we addressed whether NSC concentration (a relative measure) and/or content (a total mass) determines the amount of suckering. We also aimed to clarify the roles of initial sugar, starch, and N pools in the xylem and phloem as sources of remobilized carbon and N for sucker growth.

3.2 Methods

3.2.1 Root Collection and Experimental Design

Live aspen roots (~30 cm long, 1 to 3 cm diameter) were hand excavated from a mature aspen stand in September 2015 near Utikuma Lake, Alberta, Canada (56°04' 45"N, 115°28'58"W). Roots were covered with wet paper towels and then plastic wrap to prevent desiccation, transported back to the lab, and stored at 4° C for 30 days. At the start of the experiment, both ends of the root segments (approximately 2 cm) were clipped off for initial non-structural carbohydrate (NSC) analysis and for estimating tissue mass (i.e. xylem and phloem, see below); root length and diameter of both ends of the remaining root segment were measured. Root segments ranged from 20 to 25 cm in length after NSC samples were clipped off and were categorized into three diameter classes: large (21.3 – 34.8 mm diameter; n=17), medium (11.5 – 16.2 mm diameter; n=14), and small (7.6 – 11.2 mm diameter; n=16). Samples for NSC analysis were stored at -20° C until further processing. All fine roots were clipped off the root segments, and root segments were placed into plastic trays (26 × 52 × 6 cm) filled with a 1:2 mixture of perlite and vermiculite (PRO-MIX, Premier Tech Horticulture, Québec, Canada). Each tray contained 3 - 5 root segments (47 root segments in total) (n = 47). Root segments were placed on a 2.5 cm bed of growth medium, covered with an additional 0.5 cm (or enough to cover the entire root surface), and placed in a growth chamber (Conviron; Winnipeg, Canada) kept at a constant 22° C and 65% humidity with no light. Roots were checked daily and kept well watered (approximately every second day). Roots were left to sucker and grow entirely in the dark in order to ensure that only stored rather than newly assimilated carbon was used to produce suckers.

Root segments and their associated suckers were harvested at one of two time periods: 1) when the suckers stopped growing, harvest 1 (n=22) and 2) when all suckers were dead, harvest 2 (n=25). However, initial root characteristics (diameter, NSC and N concentration and content) and sucker characteristics (number of suckers, total sucker mass, and total sucker length) did not differ between harvests (Welch's two sample t-tests: p>0.26), and so the two harvests were pooled for all analyses. To determine when suckers had ceased growth, we measured the height of the five tallest suckers per root every second day; the five tallest suckers were chosen because these tended to grow most vigorously, while the shortest suckers stopped growing early on. Growth was considered to have stopped when the combined new height growth of all five suckers measured was less than 0.1 cm/day. For Harvest 2, sucker death was defined at the point when all suckers on a root segment had at least 1 cm of necrotic tissue at their tip or base. Upon harvesting the length, mass, and number of suckers on each root segment was assessed. To assess total sucker length, the length of all suckers on each root segment were measured to the nearest millimetre and summed together (i.e. total sucker length). To assess total sucker mass, all suckers produced on a root segment were oven-dried at 70° C and then weighed (i.e. total sucker mass). The number of suckers produced by each root was also counted (i.e. total sucker numbers). To account for differences in root sizes, *relative* sucker production was also assessed: sucker production (mass, length, or number) was divided by the total root volume, resulting in a measure of sucker production that was standardized by the root volume (i.e. relative sucker mass, relative sucker length, and relative sucker number).

3.2.2 NSC and Nitrogen Analysis

Root samples for NSC analysis were divided into phloem and xylem. The phloem was sectioned off of the xylem with a razor blade, which was made easy by a clear distinction in colour and texture between the two tissues. Tissues were then oven-dried at 100° C for 1 hour to denature enzymes, and then at 70 ° C for a week. Samples were then ground to pass 40-mesh (0.4 mm) with a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) and analyzed following Chow & Landhäusser (2004). Briefly, samples were extracted with 80% hot ethanol, and the total soluble-sugar content of the extract was determined colorimetrically using a phenol-sulphuric acid assay. Starch content of the remaining pellet was determined by digesting starch to glucose using α-amylase and 5 U amyloglucosidase. The resulting glucose hydrolyzate was measured colorimetrically using a peroxide-glucose oxidase/*o*-dianisidine reagent. Sugar and starch concentrations were calculated as percentages of sample dry weight. Non-structural carbohydrate concentration was determined as the sum of starch and soluble sugars. Total initial nitrogen concentration was determined using the Dumas Combustion

Method with the Costech Model EA 4010 Elemental Analyzer (Costech International Strumatzione, Florence, Italy).

To obtain an estimate of xylem and phloem tissue mass for each root segment at the start of the experiment, one of the clipped ends of each root segment was used to measure the dry weight of phloem and xylem tissue as a proportion of the estimated volume (assuming a cylindrical shape) of the clipped end and multiplied by the estimated volume of the root segment.

Equation 1:

$$Phloem\ OR\ xylem\ mass\ =\ Est.Root\ Segment\ Volume\ \times \frac{Phloem\ OR\ xylem\ mass}{Clipped\ end\ volume}\ (1)$$

Initial total sugar and starch content for each root was then calculated by multiplying the estimated xylem or phloem mass by the measured sugar or starch concentration. Initial non-structural carbohydrate and N content for each tissue was calculated as the sum of sugar and starch content.

3.2.3 Statistical Methods

One-way ANOVAs were used to test for the effect of root size on initial NSC and N reserves and on root sucker production (i.e. number of suckers, total sucker dry mass, and total sucker length). Pairwise comparisons were made using Fisher's Least Significant Difference (LSD) test with a Bonferroni α correction when ANOVAs were significant. Additionally, the effects of root size were assessed as linear regressions, using root volume as the explanatory variable. To understand what aspects of NSC and N reserves influenced root suckering, linear regressions were used to test relationships between measures of sucker production and NSC and N reserve variables (i.e. content vs concentration, phloem vs xylem, and for NSC, starch vs sugar). We also assessed if NSC and N concentrations affected *relative* sucker production (i.e. if differences in root size were controlled for); sucker production (mass, length, or number) was divided by the total root volume, resulting in a measure of sucker production that was standardized by the root volume. All analyses were visually inspected for homogeneity of variance and normality, and data were log-transformed when these assumptions were violated; all NSC and N content data was log transformed to meet assumptions. All data was analyzed using R-Studio (Boston, MA.

3.3 Results

3.3.1 Initial NSC and N Concentration and Content Vary with Root Size

As expected, initial NSC content increased with root size. Total phloem NSC content in large roots was approximately four times greater than in medium roots, and eight times greater than in small roots (data not shown). The difference in xylem NSC content was even more pronounced, with large roots having six and 12 times greater content than medium and small roots, respectively. Large roots had nine times more sugar content and five times more starch content in the phloem than small roots, and 11 times more sugar and seven times more starch in the xylem (Fig. 3-1). The relationships between NSC content and root volume also demonstrate that NSC content increased with root size ($p<0.001$; phloem sugar: $r^2 = 0.92$, xylem sugar: $r^2 = 0.91$; phloem starch: $r^2 = 0.53$; xylem starch: $r^2=0.69$).

Root diameter had mixed effects on initial sugar and starch concentrations (Fig. 3-1,3-2). The initial phloem NSC concentration was similar across all root diameter classes ($F_{2,45}=0.44$, $p=0.65$). When broken down into the sugar and starch components, large roots had significantly higher phloem sugar concentrations than small roots ($F_{2,45}=5.09$, $p=0.01$; Fig. 3-1); however, there were no differences in phloem starch concentration among the three root size classes ($F_{2,45}=1.18$, $p=0.31$). In contrast to the phloem, the initial xylem NSC concentrations in small and medium roots were higher than in large diameter roots ($F_{2,45}=11.5$, $p<0.001$); this was driven by a higher concentration of starch ($F_{2,45}=8.74$, $p<0.001$; Fig. 3-1) found in the smaller diameter roots. The relationships between root size class and NSC concentration were confirmed when root size was treated as a continuous variable (i.e. root volume). While initial xylem sugar concentration did not have a significant relationship with root volume ($r^2 <0.001$, $p=0.98$), both the initial xylem and phloem starch concentrations had a negative relationship with root volume ($r^2 = 0.22$, $p=0.001$ and $r^2 = 0.49$, $p<0.001$ respectively), and the initial phloem sugar concentration had a positive relationship with root volume ($r^2 =0.49$, $p =0.001$).

Large diameter roots also had significantly greater initial N content in both the xylem ($F_{2,44}=125.2$, $p<0.001$) and phloem ($F_{2,44} =77.23$, $p<0.001$) than the medium and small diameter roots (Fig. 3-3A, B). However, N concentration followed the opposite pattern: concentration increased as root diameter decreased in both the xylem ($F_{2,44}$=31.2, p<0.001, Fig 3-4A) and phloem ($F_{2,44}$=7.833, p=0.001, Fig. 3-4B).

3.3.2 Effect of Initial NSC and N Concentration and Content on Total Sucker Production

Root size had an effect on the total length (i.e. sum of all sucker lengths per root; $F_{2,42}$ = 5.34, p= 0.008) and total mass ($F_{2,42}$ = 18.33, p<0.001) of suckers produced on a root segment. Large diameter roots produced significantly greater total sucker mass and total length than small diameter roots. The total sucker number per root segment did not differ between root diameter classes, however, there was a trend for a greater number of suckers on large roots ($F_{2,42}$ = 1.97, p= 0.15; Fig. 3-5). Of the measures of sucker production (total sucker mass, length, and number), only total sucker mass differed among the three root diameter classes (Fig. 3-5). We therefore focussed on total sucker mass to explore relationships between initial root NSC and N reserves and suckering response.

Total sucker mass was strongly related to initial carbon and N reserves. Total sucker mass had strong, positive relationships with initial root NSC content, sugar content, starch content and N content (Table 3-1); however, total sucker mass was not related to the initial concentrations of these same measures (Table 3-1, Appendix A-1, A-2), with the exception of initial xylem nitrogen concentration which was negatively related to total sucker mass (r^2=0.13, n=47, p=0.01). The relationships between sucker production and sugar and starch content were similar when the roots were separated into xylem and phloem tissues (Fig 3-6.). Xylem sugar content had a slightly stronger relationship than phloem sugar content with total sucker mass, whereas phloem and xylem starch content were equally correlated with total sucker mass (Fig 3-6). In both tissues, starch content was a better predictor of total sucker mass than sugar or N content.

Total sucker mass also had a significant, positive relationship with root volume (Table 3-1), but the variation associated with size was not independent of variation in root starch content. Approximately 56% of the variation in total sucker mass was explained by total root starch content (xylem and phloem starch content combined)

(Table 3-1); when the residuals were extracted from this regression and plotted against root volume, no additional variation was explained (r^2 =0.02, n =47, p=0.34). The residuals from total sucker mass versus starch content were also plotted against N content, and again, no significant relationship was found (r^2 =0.002, n=47, p=0.92). Starch content was highly related to both total N content (r^2 =0.78, n=47, p<0.001) and total root volume (r^2 =0.57, n=47, p<0.001). The lack of residual variation explained by root size or N may be due to the fact that they were highly correlated with starch content.

3.3.3 NSC and N Concentrations as Drivers of Relative Sucker Production

Root NSC concentrations were generally not related to any measures of total sucker production; despite this, NSC concentrations were highly related to both *relative* sucker mass and relative sucker length (i.e. total sucker mass or total sucker length per unit root volume). Neither the total sucker number nor the relative sucker number were related to any measures of NSC concentration. As with content, higher initial xylem and phloem NSC concentration resulted in greater relative sucker mass and length, with the relationships for phloem NSC concentration being somewhat weaker (Table 3-2). However, when NSC is separated into the sugar and starch components, only starch shows this positive relationship with relative sucker mass and length (Fig. 3-7, Table 3-2); while sugar concentration had either no relationship (in xylem) or a negative relationship (in phloem) (Fig. 3-7, Table 3-2). There was also a negative correlation between sugar and starch concentrations in the phloem (r= -0.5, n=47, p<0.001), while this relationship did not exist in the xylem (r =-0.23, p=0.12).

Initial phloem and xylem N concentration were also positively related to relative, not total, sucker mass and length (Table 3-3; Appendix A-1). Interestingly, xylem and phloem N concentrations were the only variables significantly related to the relative sucker number (Fig. 3-8). No measures of N concentration were related to the total sucker number.

3.4 DISCUSSION

Larger aspen roots were able to produce more total sucker mass than smaller roots because of their larger carbon and nutrient reserves. Although larger roots did not have a higher initial concentration of non-structural carbohydrates (NSC) or nitrogen (N), they did have a greater initial content of both NSC and N reserves. Non-structural carbohydrate reserves (particularly starch) and N content were both highly correlated with the production of total sucker mass. And, as root size did not explain any additional variation in total sucker mass that was not already associated with root starch content, it appears that reserve content, rather than the root size per se, drives the production of sucker mass and length in aspen roots. Between NSC and N content, which were highly correlated with each other, it is difficult to determine which most limited sucker growth. NSC storage was found to be more important than N storage for determining resprouting in *Quercus crispula* (Kabeya & Sakai 2005), but this may differ by species. However, total sucker mass had a higher correlation with starch content than nitrogen content in our study suggesting that here too it was carbon reserves which largely determined the production of total sucker mass and length

The evaluation of both NSC concentration and content is not as common as the evaluation of concentration on its own (Körner 2003; Clarke et al. 2016), however, studies have found that resprouting can be related to both concentration and content of NSC reserves (Kabeya et al. 2003). Hence it is currently debated whether content (absolute size) or concentration (relative size) provides a better indication of the reserve status of a plant (Canham et al. 1999; Ryan 2011; Hoch 2015). Our results indicate that, with regards to sucker productivity (i.e. the mass and length of suckers) in a species like aspen, the content of NSC reserves was a better measure. This supports the suggestion that following disturbances where survival is dependent on the ability to regrow new tissues, NSC content is a better predictor of survival than NSC concentration (Canham et al. 1999; Myers & Kitajima 2007). Our study did not see an increase in sucker mass or length with increasing concentration, however, studies have found that initial starch concentration is highly related to increases in sucker growth (Landhäusser and Lieffers 2002; Wachowski et al. 2014), and the overall ability to resprout (Kabeya et al. 2003; Kabeya & Sakai 2005). Starch concentrations in our roots were generally high (e.g. ~6%

higher than initial values in Wachowski et al. 2014, using the same method for NSC analysis), and this may indicate that concentration of starch reserves was not limiting production of total sucker length or mass. However, it is possible that if roots had started at a wider range of low and high NSC concentrations within each size class, that concentration might have had an effect on total sucker mass and length, e.g. lower concentration may have resulted in a reduction of suckering. This is supported by our finding that relative sucker production (where differences in root size are accounted for) was positively correlated to higher starch concentration in the xylem and phloem tissue.

Starch content and concentration had the strongest relationships with total sucker mass and relative sucker mass, respectively, indicating that the starch pool—as opposed to sugars—was the main source of carbon for sucker growth. While sugar content was also significantly related to total sucker mass and length, these relationships likely exist because sugar content was highly correlated with starch content (and therefore total NSC content), and not because sugars drive production of total sucker mass**.** This lack of a causal relationship is further supported by the fact that relative sucker production was unrelated to xylem sugar concentration and negatively related to phloem sugar concentration. That production of total sucker mass and length was better correlated with starch is not surprising given that the main role of starch is storage (Loescher et al. 1990; Hoch 2007; Hartmann & Trumbore 2016). Sugars, on the other hand, serve many different purposes in the cell, including signalling, carbon transport, osmotic adjustment, and cold tolerance (Graham & Patterson 1982; Ingram & Bartels 1996; Sala et al. 2012; Hoch 2015). Our roots were collected at the end of the growing season, when starch reserves are being converted to sugars for the winter (Landhäusser & Lieffers 2003). It is therefore possible that the relationships between sugar concentration and relative sucker production were so poor because the sugars in our roots had already been allocated to non-storage roles, particularly in providing protection against freezing damage (Graham & Patterson 1982); although this would not explain why these sugars weren't remobilized when roots were returned to non-freezing conditions in the growth chamber. In support of the hypothesis that not all sugars were available for sucker growth, the root phloem still had a high sugar concentration when suckers died, though starch was largely depleted (data not shown). Some sugars also remained in the xylem at sucker death, but the concentration was substantially lower than in the phloem, suggesting that the availability of sugars for remobilization may be slightly different in the xylem and phloem tissues.

Though wood is considered the main organ for carbohydrate storage, our results indicate that the phloem is an equally, if not more important location of starch reserves in aspen root systems. While xylem and phloem tissues both contain parenchyma cells—the main location for NSC storage (Loescher et al. 1990; Kozlowski 1992; Dietze et al. 2014) - xylem NSC has often been considered the largest storage pool since the xylem in mature trees far outweighs that of the phloem (Loescher et al. 1990). Indeed, xylem NSC concentration was more strongly related to relative sucker production than phloem NSC concentration in our study; however, this fact is somewhat misleading. Phloem starch concentration was just as strongly related to subsequent relative sucker production as xylem starch concentration was; but this correlation was obscured when analyzed as a composite NSC measurement because phloem sugar concentration was negatively correlated with phloem starch. The importance of the phloem is further demonstrated by the larger starch content of this tissue. Roots in our experiment ranged between 0.7 – 3.5 cm diameter, and the phloem tissue accounted for 66 (±7)% of the root mass, (data not shown). By mass, phloem starch content therefore accounted for 78(±7)% of the total starch pool in the root. Therefore, at least in smaller coarse roots, the phloem contains the majority of the starch reserves.

Starch content in the xylem and phloem was clearly an important driver of total sucker mass and total sucker length; however, initial N content was also highly related to both responses, suggesting that carbon may not be the only resource limiting resprouting (Millard et al. 2007; Sala et al. 2012). Because N content (xylem, phloem, or total) did not explain any variation in total sucker mass that was not already explained by starch content, and because total NSC and N concentration and content were highly correlated ($r=0.56$, $r=0.88$, respectively, $p<0.001$), it is difficult to separate the effects of starch and nitrogen reserves on resprouting. The current literature on the role of N in resprouting is restricted to whether or not N is related to the *ability* to resprout (El Omari et al. 2003; Kabeya & Sakai 2005; Moreira et al. 2012); there is little to no information about how N affects the production (measured as number, mass, or length) of those sprouts. Some of the studies that have evaluated the effect of N concentration on the ability to resprout have found mixed results: Kabeya & Sakai (2005) found a negative relationship between N concentration and the ability to resprout, while Moreira et al. (2012) found a positive relationship. Additionally, El Omari et al. (2003), determined that under low N concentrations *Quercus ilex* could not resprout, even with high NSC reserves, suggesting that N does have a role in resprouting. All of the roots in our study did resprout, and since these studies did not report how N related to the growth (in mass or length) of their sprouts, it is difficult to determine what the broader implications of N reserves may be. However, it is likely that both initial NSC and N reserves can limit resprouting, but that this will depend on their relative abundance (H. Poorter et al. 2012; Hoch 2015), Our results lead us to the conclusion that both N and NSC are important reserves for the production of sucker mass and length, and that together measures of N and NSC content will provide a good indication of the ability of a root to produce suckers.

While many aspects of initial NSC and N status appeared to affect sucker mass, the *number* of suckers produced was not well correlated with any of these measures, with the exception of a correlation between initial phloem and xylem N concentrations and relative sucker numbers (i.e. number of sucker per unit root volume). The fact that NSC was not related to any measure of sucker number (relative or total) may indicate that N and NSC have slightly different roles in sucker initiation and growth, and that N may be more involved in sucker initiation. In fertilization treatments of aspen roots, the addition of N (as NH_4NO_3) has been found to significantly increase the mass of suckers produced, but not the number (Fraser et al. 2002). In contrast, in another study, NO_3^- fertilization increased sucker numbers compared to fertilization with NH_4^+ (Landhäusser et al. 2010b); however, both of these studies examined the effects of the addition of N to a system, rather than at how the initial N content or concentration in roots influences the subsequent number of suckers**.** Although our study indicates that N is related to the number of suckers produced, this relationship may not be causal. Higher reserve content and concentration have been found to increase the growth or mass of suckers (Landhäusser and Lieffers 2002, Wachowski et al. 2014), but relationships between reserve content and the number of suckers produced are not as common. Sucker number may be determined more by the number of buds present on the root, and this may be independent of reserves (Vesk & Westoby 2004).

Conclusions

Overall our study indicates that NSC reserve content, rather than NSC concentration, is the better indicator of potential sucker production. Furthermore, starch content appears a better indicator of suckering production (i.e. total sucker mass and length) than sugar or total NSC in both the xylem and the phloem tissues. Phloem is a significant storage location and source of carbon for resprouting, especially in small-diameter root systems where a large percentage of root biomass—and consequently, root starch--is located in the phloem. N storage also appears to affect suckering in aspen systems, however, the respective roles of N and NSC were not easily separated as they tended to be correlated.

Tables

Table 3-1. Total sucker mass in response to measures of initial NSC content and NSC concentration. Total sucker mass was log transformed in all models (n=47).

Total sucker mass relationships with the following variables:	r^2 and direction	p
Log root volume	(+) 0.44	<0.001
Log total initial NSC content	(+) 0.43	<0.001
Total initial NSC concentration	<0.001	0.96
Log initial sugar content	(+) 0.34	<0.001
Initial sugar concentration	0.010	0.51
Log initial starch content	(+) 0.56	<0.001
Initial starch concentration	0.03	0.28
Log initial Nitrogen content	(+) 0.43	<0.001
Initial Nitrogen concentration	0.05	0.12

Table 3-2. Linear relationships for relative sucker growth (length and mass) in relation to initial NSC, initial sugar, and initial starch concentration and content; relative sucker growth is the length or mass of suckers divided by the root volume. Significant relationships are bolded (n=47)

Response variable	Linear Relationships	r^2 and direction	p-value
Relative sucker mass: sucker mass root vol^{-1} (g cm^{-3})	Initial NSC concentration	(+) 0.30	<0.001
	Initial xylem NSC concentration	(+) 0.60	<0.001
	Initial phloem NSC concentration	(+) 0.10	0.02
Relative sucker length: sucker length root vol^{-1} (cm cm^{-3})	Initial NSC concentration	(+) 0.12	0.016
	Initial xylem NSC concentration	(+) 0.36	<0.001
	Initial phloem NSC concentration	0.014	0.41
Relative sucker length: sucker length root vol^{-1} (cm cm^{-3})	Initial xylem starch concentration	(+) 0.25	<0.001
	Initial phloem starch concentration	(+) 0.13	0.01
	Initial xylem sugar concentration	0.05	0.13
	Initial phloem sugar concentration	(-) 0.14	0.01

Table 3-3. Relationships between relative sucker growth measurements and initial nitrogen concentration in the xylem and phloem tissues; relative sucker growth is the length or mass of suckers divided by the root volume. Significant relationships are bolded (n=47).

	Linear Relationship	r^2 and direction	p
Phloem	Relative sucker number (count cm^{-3}) vs initial phloem nitrogen concentration	0.35 (+)	<0.001
	Relative sucker length (cm cm^{-3}) vs initial phloem nitrogen concentration	0.37 (+)	<0.001
	Relative sucker mass (g cm^{-3}) vs initial phloem nitrogen concentration	0.53 (+)	<0.001
Xylem	Relative sucker number (count cm^{-3}) vs initial xylem nitrogen concentration	0.43 (+)	<0.001
	Relative sucker length (cm cm^{-3}) vs initial xylem nitrogen concentration	0.44 (+)	<0.001
	Relative sucker mass (g cm^{-3}) vs initial xylem nitrogen concentration	0.47 (+)	<0.001

Figures

Figure 3-1. Initial NSC content (log transformed) of root phloem and xylem across root diameter classes: small (n = 16), medium (n=14), and large (n=17). Error bars represent standard deviation. Different letters indicate significant differences among means, while N.S. indicates no difference. Bold, uppercase letters are for sugars and lowercase letters are for starch. Differences in phloem are represented by the letters a and b, while differences in xylem are represented by the letters x and y.

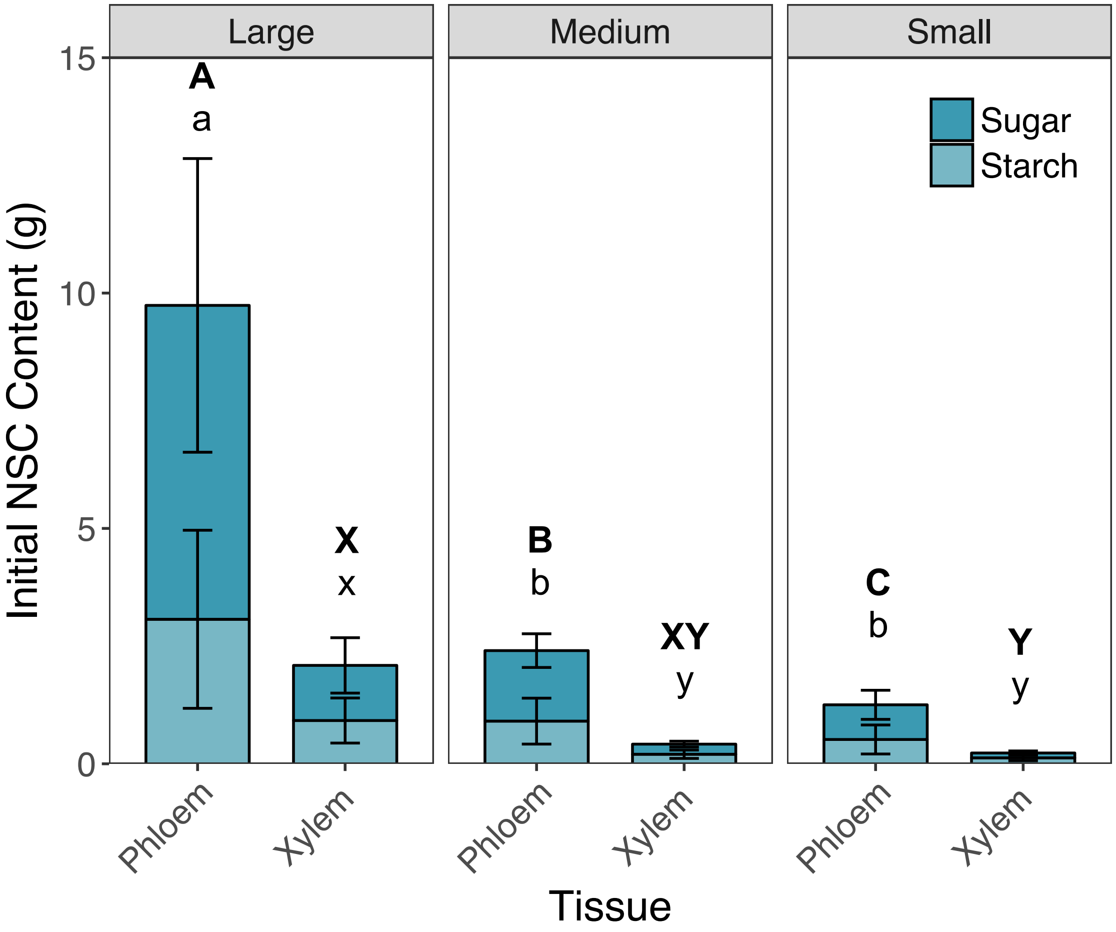


Figure 3-2. Initial NSC concentration of root phloem and xylem across all root diameter classes: small (n = 16), medium (n=14), and large (n=17). Error bars represent standard deviation. Different letters indicate differences among means, while N.S. indicates no difference. Bold, uppercase letters are for sugars and lowercase letters are for starch. Differences in phloem are represented by the letters a and b, while differences in xylem are represented by the letters x and y.

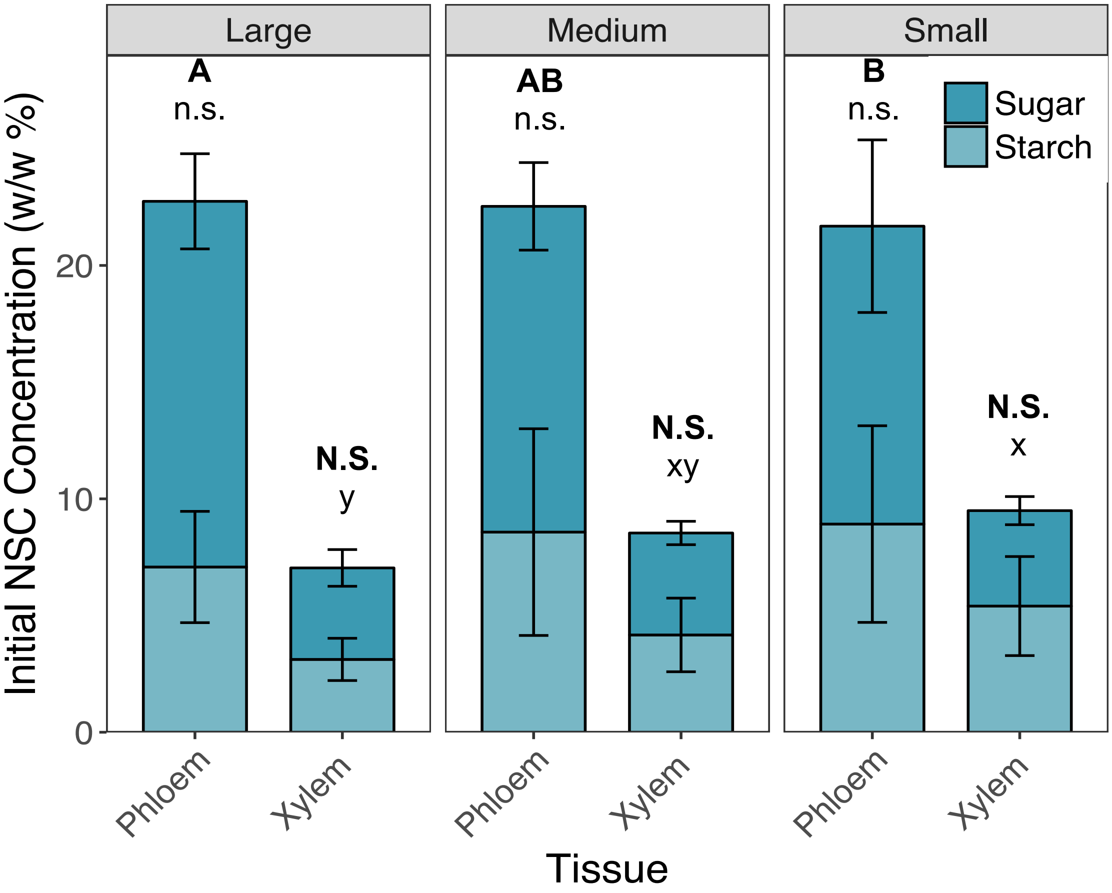


Figure 3-3. Initial nitrogen content of root xylem (A) and phloem (B) tissues across all root diameter classes: small (n = 16), medium (n=14), and large (n=17). Error bars represent standard deviation and different letters indicate significant differences.

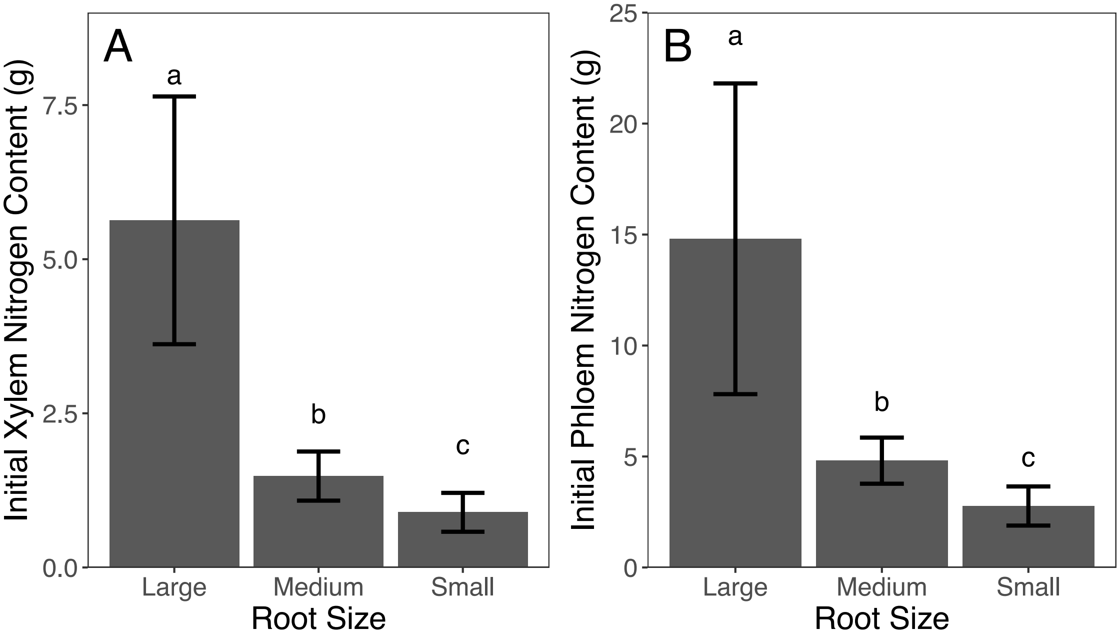


Figure 3-4. Initial nitrogen concentration of root xylem (A) and phloem (B) tissues across all root diameter classes: small (n = 16), medium (n=14), and large (n=17). Error bars represent standard deviation and different letters indicate significant differences.

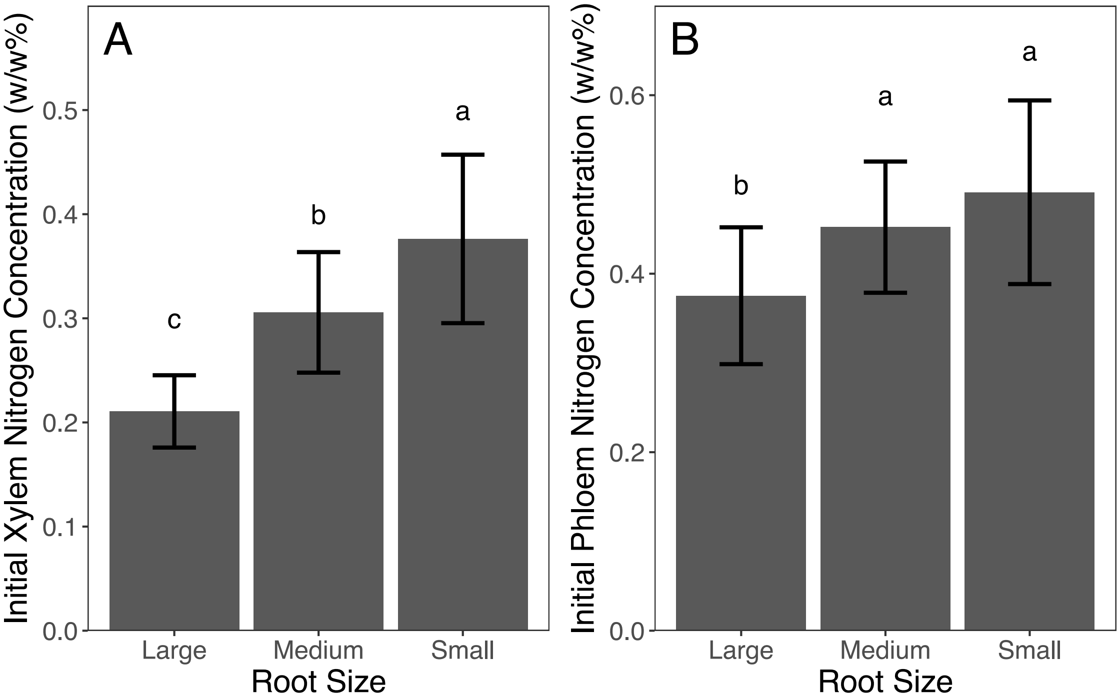


Figure 3-5. Differences in sucker characteristic: total sucker length (A), sucker dry weight (B), and sucker number (C), among root diameter classes. Root size classes are small (n = 16), medium (n=14), and large (n=17). Error bars represent standard deviation and different letters indicate significant differences.

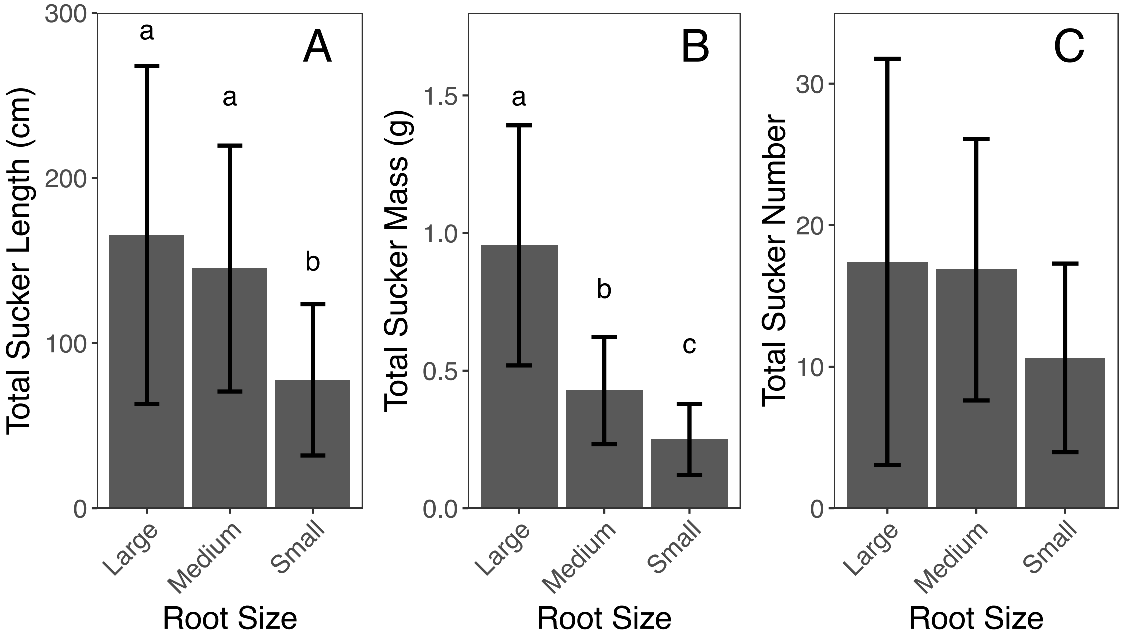


Figure 3-6. Relationships between total sucker mass and the initial content of sugar and starch in the xylem and phloem tissues. In all graphs, both values were log transformed and are presented on log scales (n=47).

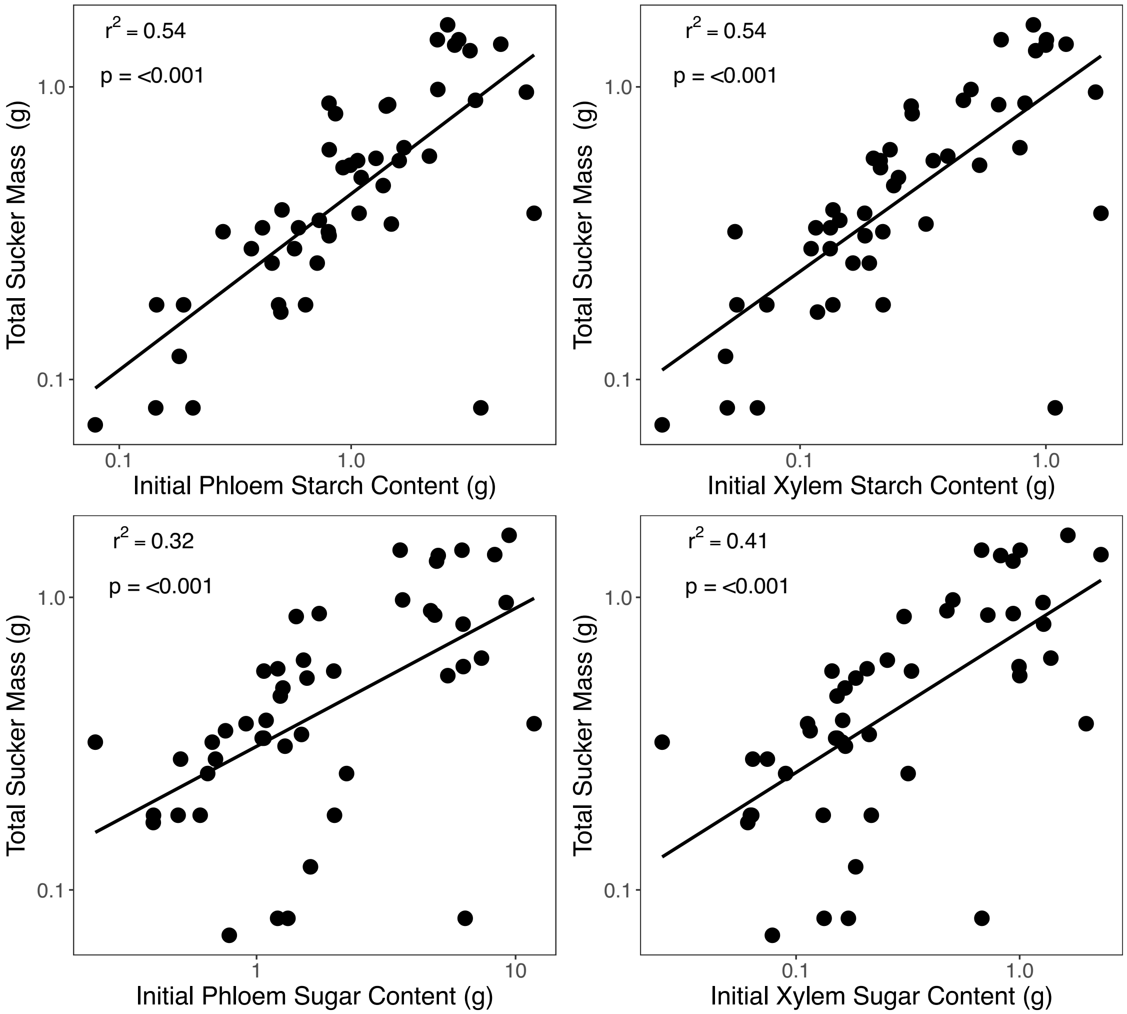


Figure 3-7. Relationships between initial sugar and initial starch concentration and the relative production of sucker mass (sucker mass standardized by the root volume) (n=47)

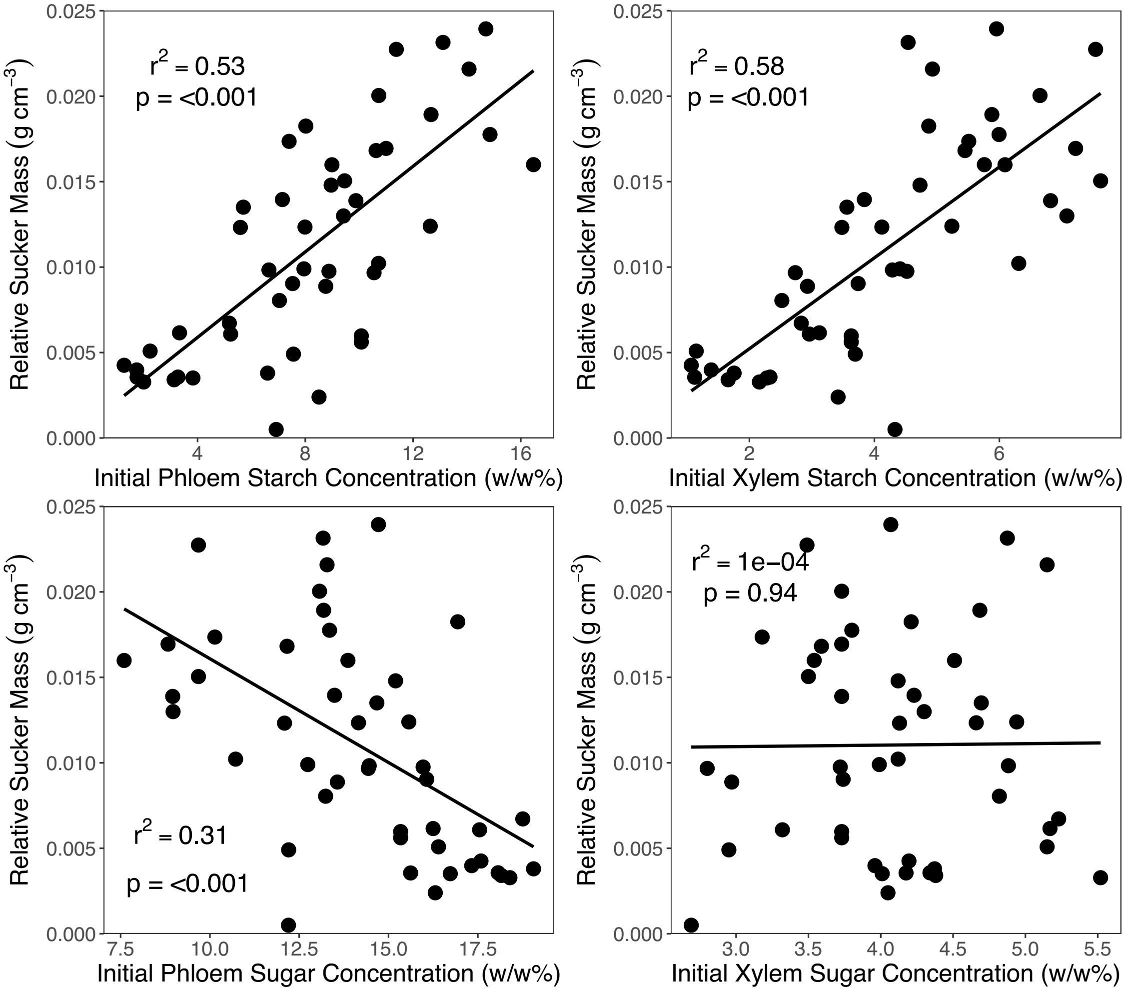
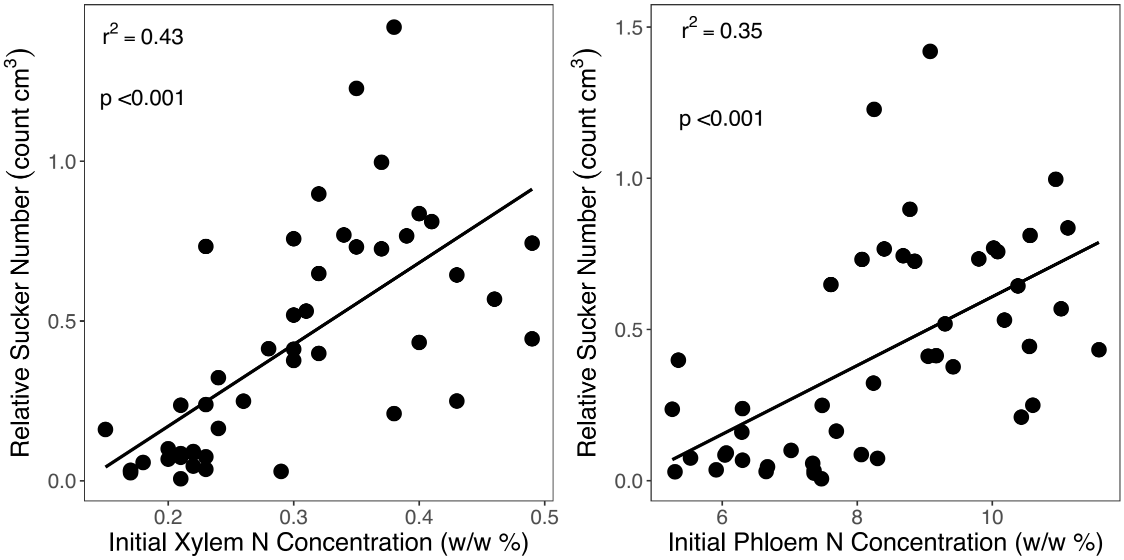


Figure 3-8. Relationship between the relative sucker number (sucker number standardized by root volume) and initial phloem and xylem nitrogen concentration (n=47)



Chapter 4– Synthesis and Discussion

The overall goal of my thesis was to assess suckering in seedling origin aspen systems. The specific goals of my thesis were to determine (1) how planted aspen respond to varied disturbances in a field setting (Chapter 2), and (2) determine the relationship between the ability to resprout and the initial reserve status of the root segment (Chapter 3). Reserves, for our purposes, included both concentration (a relative measure) and content (a total mass) of total non-structural carbohydrates (NSC, composed of simple sugars and starch), and nitrogen (N).

4.1 Research Summary

One of the intended applications of the first study of my thesis was to assess the viability of using managed disturbances (e.g. purposeful cutting treatments, rather than natural disturbances) to induce suckering of aspen root systems, and to assess if these managed disturbances could be used on sites that had been planted with aspen, but where a higher stem density was desired. We anticipated that cutting planted aspen down or severing the lateral roots of these trees would potentially produce enough suckers per root system to increase stand density on low-density sites. The first study of my thesis demonstrated that planted aspen do produce suckers after controlled disturbances. However, the suckering response of the seedling origin aspen was highly variable. Although seedling origin aspen produce an average of five suckers per cut tree (regardless of disturbance type, 2015), the number of suckers produced by any individual tree ranged from zero to 29; however, more importantly, of the 40 trees cut 25% did not produce any suckers. These results answered the question of whether or not seedling origin aspen stands will sucker (they will), however, we also asked: can cutting planted aspen seedlings increase the stand density on low-density sites? The answer to this question is not as straight forward. My research indicates that cutting of seedling origin aspen does not guarantee suckering. If the original stand density was between 1,000 to 2,000 stems ha $^{-1}$, my results indicate that only 750 – 1,500 of those stems would sucker. Following the suckering that I observed, (average of five suckers in 2015, four in 2016), this would translate into a stand density of approximately 4,500 to 9,000 stems ha $^{-1}$. Furthermore, my study focused on the emergence of suckers rather than their persistence and survival; while a density of 4,000 to 9,000 stems ha $^{-1}$ seems acceptable, it is unclear how many of these suckers would survive after the first growing season. Overall, my research indicates that there is a 1 in 4 chance that a planted aspen tree will not produce suckers after being cut down.

The second objective for my thesis was to determine the degree to which the suckering (measured as total and relative sucker length, mass, and number) of aspen is determined by the initial NSC and N concentration and content of roots. Based the idea that reserve content will be the better predictor of the ability to resprout (Ryan 2011; Canham et al. 1999), as the content is the physical mass of reserves that are potentially available for use, I expected that the initial content of NSC and N reserves would be a better predictor of resprouting than the concentration. I also expected that relationships between sucker production and starch would be stronger than for sucker production and sugar. Sugar is noted to have many different functions in the cell, including in cold tolerance and osmotic regulation (Graham & Patterson 1982; Ingram & Bartels 1996; Sala et al. 2012; Hoch 2015), whereas the main function of starch is storage (Loescher et al. 1990; Hoch 2007; Hoch 2015).

My second study (Chapter 3) found that resprouting of aspen root segments is dependent on the content of stored NSC and N reserves, and not the concentration of those reserves. This study found that larger roots had a greater content of reserves than smaller roots. Of the NSC and N reserves measured, starch content in the xylem and phloem tissues had the strongest relationship with measures of sucker production (total sucker mass and length). Additionally, I found that more than half of the starch content is stored in the phloem in roots less than 3.5 cm diameter. As roots get larger, the xylem increases in proportion, and the relative amount of phloem is reduced. However, most roots that sucker are less than 2 cm in diameter (Schier & Campbell 1978; DesRochers & Lieffers 2001b; Wachowski 2012), and at this root diameter the phloem will account for a significant proportion of the root biomass, and therefore a significant store of starch reserves. Moreira et al. (2012) discuss resprouting in three phases: 1) the initial resprouting ability; 2) the vigour and growth of those sprouts; and 3) the survival of those sprouts after the initial event. The study presented in Chapter 2 found that cutting height can be used to influence the amount of resprouting (phase 1), while the study in Chapter

3 found that increasing content of NSC and N reserves increases the mass and length – the vigour - of the sprouts. We did not assess Moreira et al.'s (2012) third phase, where survival is determined. However, if greater content of reserves leads to greater length and mass of suckers, it may follow that suckers that are taller will emerge first, and replenish the NSC reserves in the roots (Landhäusser & Lieffers 2002; Wachowski 2012), potentially leading to better survival of the root overall. Since the larger diameter roots in our study produced greater sucker length than the small diameter roots, it may be that these larger root segments, and possibly larger root systems, will have more emerged suckers, and be able to persist on the landscape more successfully than smaller diameter roots. It is not clear if larger roots, and larger root systems, will produce a greater number of suckers. The total number of suckers produced was not related to any measure of NSC or N concentration or content, although there was a trend for greater sucker production on larger roots. More research is needed to understand how N and NSC interact to produce suckers, and especially to determine which reserve (if any) is more involved in determining the number of suckers produced. Overall, the study presented in Chapter 3 indicated that content of reserves overwhelmingly drives production of total sucker mass and length in aspen, and that large root segments are more likely to have a greater content of reserves

Following my study on reserve dynamics in root segments, it is likely that larger root *systems* will have a greater content of reserves to fuel suckering. If larger root systems are able to remobilize reserves from areas of higher content to areas with sucker buds, then it is likely that larger root systems will have greater production of suckers, and possibly greater numbers of suckers. However, we did not study remobilization of reserves in root systems. The comparison between the Cut and Severed trees in the 2015 filed season of the study presented in Chapter 2 may provide some insight into how reserve are remobilized roots (although we did not measure the reserves of these roots). The Cut and Severed roots both suckered, however, the Cut trees had a trend for greater sucker production, and overall produced a greater numbers of sprouts (suckers and stump sprouts combined). The Severed trees were suckering from individual roots, as opposed to suckering from a larger root system, and may have had access to fewer reserves when compared to the Cut trees. Although it is unclear how far NSCs move across organs after disturbance, it is reasonable to conclude that an intact root system would have a greater mass of reserves when compared to a fragmented root system, or an individual root segment. The remobilization of reserves across roots should be looked into further, in order to give us a landscape understanding of how initial reserve status may impact resprouting after disturbance in the field.

When disturbances occur on sites with planted or seedling origin aspen, having a greater content of NSC and N reserves will likely prove beneficial. If there is interest in increasing the number of stems on a site, then being able to increase the reserves of NSC and N in the plants would be beneficial. To increase the amount of starch in the system, trees could be cut down in the fall when starch reserves tend to be higher (Landhäusser & Lieffers 2002). Additionally, fertilization with nitrate has been shown to increase the number of suckers (Landhäusser et al. 2010b), and may be a pre-treatment option to increase the amount of suckering. From a broader regeneration perspective, our results indicate that having a larger root system may result in greater suckering. The large roots in my second study produced greater total sucker mass and total sucker length: in the field, this greater sucker mass and length might translate into a greater proportion of suckers that emerge successfully. Once suckers have emerged, they will be able to photosynthesize (become a source), and will no longer require the reserves from the parent root (becomes a sink) (Wachowski et al. 2014; Landhäusser & Lieffers 2002). Suckers that are unable to emerge may continue to rely on the reserves from the parent root; this may result in the depletion of reserves, and ultimately death of the parent root (Wachowski 2012).

4.2 Experimental limitations and Future Research:

My thesis helped to elucidate the dynamics of planted aspen and their response to disturbance, as well as some of the variables (such as reserve status) that may influence the success of regeneration. The study presented in Chapter 2 was located on stands that were planted for other studies; as such, we faced limitations from the layout of the planted stands in both field seasons: the two stands were planted 4 years apart, and at different densities. We saw a significant stand by treatment interaction in our results, and this may be due to ontogeny or to the difference in diameter of trees located in each site.

Furthermore, the planting density likely impacted the diameter of the trees, and we cannot separate the density effect from the effect of stand age. It has been noted that stump sprouting is more likely to occur in both younger and smaller trees (Debyle and Winokur 1985; Heeney et al. 1980); our results cannot make any conclusions based on age or diameter due to the layout of the plots, however, our results do support the hypothesis that larger and/or older trees are less likely to sucker. The dynamics between suckering and stump sprouting should be explored further for planted aspen stands of a wider range of ages, and planted at one density (e.g. at 5,000 stems ha^{-1}). Additionally the study presented in Chapter 2 provided an idea of the root to shoot ratio (R:S) of planted stands. It appears that R:S levels off around 0.22, which is comparable to what has been seen for mature, clonal aspen (Strong & La Roi 1983). Although the R:S is comparable to what has been noted for clonal aspen stands, it is based on 42 estimated data points, which were calculated from 10 fully weighed trees. The R:S of planted aspen should be explored further, with a larger range of DBHs and a greater number of samples that have both the above and below ground biomass fully excavated (for belowground) and weighed.

Given that the study presented in Chapter 2 was designed to inform managers of the response of planted aspen to disturbance, we must consider the density of the original stand. My study was conducted at two high-density aspen sites (10,000 stems ha $^{-1}$ and 29,000 stems ha $^{-1}$); it is likely that the root systems of aspen planted at a low density will be different. Each individual lateral root originating form our eight and 12 year old trees was less than 1 m on average, whereas aspen roots on four year old trees on reclamation sites have been found to average 3 m in length (S. Bockstette, personal communication). Since the lateral root is where suckers originate, different lengths of lateral roots may impact the number of suckers produced. Although we did not see a significant relationship between the root length and the number of suckers produced, this relationship was only marginally insignificant ($r^2 = 0.07$, $n=40$, $p=0.079$), and it is worth exploring whether, with a greater sample size, some relationship between total root length and sucker production may emerge. Although the study presented in Chapter 2 did not determine any clear relationships between root system characteristics and the number of suckers produced, the study presented in Chapter 3 did augment our understanding of drivers of suckering in aspen root systems.

In the study presented in Chapter 3, our conclusions were based on resprouting from roots under optimal growing conditions (disregarding the absence of light). The main objective of the study was to understand where reserves were stored, and how the concentration and content of those reserves impacted suckering: we answered those questions and determined that starch content is the main driver of resprouting in aspen, and that a large content of starch is stored in the phloem in aspen roots less than 3.5 cm diameter. However, we also measured N reserves in these roots. Neither NSC nor N reserves appeared to be exceptionally limiting in our roots, and, because reserves were generally high, we could not test whether N or NSC was a better determinant of resprouting in aspen. If we wanted to test the relative importance of NSC and N for resprouting, then we would need to manipulate the reserves in the roots to create combinations of roots with low/low, low/high, high/low, and high/high NSC and N concentrations (see Landhäusser et al. 2012b). With the relatively high concentrations of NSC and N in our roots, we are limited to the conclusion that NSC and N content are both important reserves for determining suckering in aspen.

Although our research increased the understanding of the dynamics of planted aspen and the variability that might be expected from regeneration of planted aspen stands, we are still unsure of the ontogenetic shifts in aspen rooting behaviour. Additionally, the role that starch plays as a driver of suckering, and the role phloem plays as a storage organ has been highlighted, but our study was limited to isolated root segments. It is unclear if the reserves in one distant section of a root can be transferred to the location where those reserves are needed, such as the site of sucker growth. If larger roots are less likely to sucker (e.g. Wachowski 2012), but they have a greater content of reserves, those reserves must be remobilized for use in resprouting. There are numerous areas for further research, but a few starting points are highlighted below:

1. **Evaluate the extent of grafting in planted aspen stands**. This study found that root systems of aspen planted at a high density were not grafted, with the exception of one root. Our sites were relatively young (<13 years), so there is a

possibility that not enough time for grafting has occurred. Root systems of planted aspen stands of different age classes should be excavated to determine if grafting is indeed occurring, and to determine if the grafts are functional. If functional root grafts are occurring, then planted aspen stands may not be as isolated as they appear. Functional root grafts could increase reserve transfer between roots after a disturbance; this access to more reserves may in turn lead to greater suckering for portions of root systems that are connected to other portions that are undisturbed and not trying to sucker on their own.

2. **Explore the relationship between root system size and the number of suckers produced in low-density aspen stands**. It is likely that the root systems of trees in low-density aspen stands will be different from those of high-density stands. If the root systems of trees planted at a low-density aspen are different than those planted at a high-density, the relationship between root system size and the number of suckers produced may change, and this relationship should be established.
3. **Explore root to shoot ratios in planted aspen stands in different age classes**. We found that root to shoot ratio of planted aspen appeared to level off at approximately 0.22. If this value can be confirmed on different sites, and for larger trees, then it will provide a robust estimate of belowground biomass from relatively easy aboveground measurements (such as diameter at breast height, and tree height). An estimate of belowground biomass would provide some idea of the root system extent. In combination with the study proposed above (Bullet 2), this research has the potential to provide an easy estimate of both the root system extent, and the amount of suckers that can be expected on the root systems of planted aspen.
4. **Determine the importance of NSC and N as drivers of suckering.** We saw that both NSC and N content were significantly related to the production of suckers. From our data, we could not conclude which reserve was the main driver; this should be explored further by manipulating plants to have all combinations of high and low concentrations of NSC and N reserves. After high and low plants have been established, they should have their stem removed and

be placed in the dark to sucker, following the same procedure as outlined in Chapter 3 Methods.

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Appendices

Appendix A-1. Linear relationships between total sucker measurements and initial nitrogen concentration; the y variable has been log transformed in all models to meet model assumptions

Response Variable	Linear relationship	R^2 and direction	p
Sucker number	Initial xylem N concentration	0.012	0.46
	Initial phloem N concentration	0.07	0.07
Sucker mass	Initial xylem N concentration	(-) 0.13	**0.01**
	Initial phloem N concentration	0.006	0.60
Sucker length	Initial xylem N concentration	0.02	0.34
	Initial phloem N concentration	0.009	0.52

Appendix A-2. Linear relationships between total sucker measurements and initial starch concentration; sugar results are not shown but have a similar patter to starch. The y variable has been log transformed in all models to meet model assumptions

Response Variable	Linear relationship	R^2 and direction	p
Sucker number	Initial xylem starch concentration	0.006	0.60
	Initial phloem starch concentration	0.04	0.16
Sucker mass	Initial xylem starch concentration	0.006	0.60
	Initial phloem starch concentration	0.07	0.06
Sucker length	Initial xylem starch concentration	0.009	0.53
	Initial phloem starch concentration	0.02	0.35

Appendix A-3. Linear relationships between total sucker measurements and initial nitrogen content; nitrogen content and the y variable have been log transformed in all models to meet model assumptions

Response Variable	Linear relationship	R^2 and direction	p
Sucker number	Initial xylem N content	0.014	0.41
	Initial phloem N content	0.007	0.58
Sucker mass	Initial xylem N content	(+) 0.44	**<0.001**
	Initial phloem N content	(+) 0.40	**<0.001**
Sucker length	Initial xylem N content	(+) 0.11	**0.02**
	Initial phloem N content	0.08	0.052