Resource partitioning, allocation, and remobilization in trembling aspen saplings

by

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Abstract

The long-term survival of trees is highly dependent on their ability to acquire, utilize and store resources to endure local environmental conditions and stresses. Although external uptake and internal cycling of essential resources in deciduous trees is largely regulated by seasonal phenology, resource use and allocation may change under stress. This thesis focused on how trembling aspen (*Populus tremuloides* Michx.), a deciduous tree species widely distributed across boreal and temperate regions of North America, may share and/or store resources between and within organs when subjected to stress. To explore the remobilization of resources between source and sink organs during spring bud flush and after leaf area recovery following complete defoliation, I used aspen saplings which were isotopically labeled with C and N and then grafted. I also observed changes in non-structural carbohydrate (NSC) and N concentrations in the stems and roots to explore potential constraints on remobilization during leaf area recovery. In a second study, I used a split-pot experiment that subjected saplings to either heterogeneous (a root system that was partially exposed to drought conditions) or homogeneous (the whole root system droughted or well-watered) soil moisture conditions. I measured aboveground and belowground reserve and mass allocation in response to these treatments.

In the first study I found that C and N were remobilized from both stem and root reserve pools during spring budflush and following defoliation to support leaf growth. Leaves rapidly shifted their dependence on remobilized reserves as they matured, and distance from the source (root system) affected the reliance on C reserve remobilization, an effect that was even greater following defoliation. I found evidence of the supply-driven nature of N, as stem N reserve levels decreased following budburst and the distance effect on remobilization disappeared after

defoliation. It remains unclear however, whether C and/or N limitation contributed to the incomplete recovery of leaf mass following defoliation.

In the second study I found that under heterogeneous soil moisture conditions, saplings maintained gas exchange and aboveground growth similar to well-watered saplings while leaf and fine root shedding were observed in the homogeneous full drought treatment. For saplings subjected to the heterogeneous soil moisture conditions, the portion of the root system that was water limited had no root dieback and increased NSC reserve concentrations, while the portion that was not resource limited added new roots (30% increase). My results suggest differential allocation of mass or reserves between above- and belowground organs, but also within a root system when resource availability is spatially variable. While the mechanisms and processes involved in these allocation patterns are not clear, these responses could be interpreted as adaptations and acclimations to preserve the functionality of the entire sapling and suggest that different portions of plant organs might respond autonomously to local conditions.

Preface

This thesis is an original work by Ashley T. Hart.

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Chapter 1: General Introduction

1.1 Resource uptake, movement and storage in trees: carbon, nutrients and water

Forests are complex natural systems which span approximately 30% of global terrestrial area, and offer a multitude of ecological, economical and societal services (Bonan, 2008). Trees are the dominant component of forested ecosystems, providing a foundation for biodiversity and ecological function. In addition to providing oxygen, soil stability and habitat, trees have an important role in global biogeochemical cycles and climate dynamics (Bonan, 2008). As it is currently unknown how forested ecosystems may respond to expected changes in climate and other environmental drivers (IPCC, 2013), determining how trees function and perform on the landscape is especially valuable.

Due to the immobility and longevity of trees, their long-term survival is highly dependent on their ability to acquire, utilize and store resources to endure changing environmental conditions and stochastic and chronic stress specific to locale. There is a significant body of literature focused on resource use in trees, particularly carbon (C), nutrients and water, as they are vital for maintaining growth and function. Trees assimilate C via photosynthesis to build the foundation of macromolecules required for growth, maintenance, defense and reproduction. These complex macromolecules are also dependent on primary macronutrients, including nitrogen, phosphorus and potassium, which are assimilated by the roots (Campbell, 2004). Nitrogen (N) is of particular importance, as it can be highly limiting in many ecosystems, including the boreal forest, due to its slow mineralization rate from organic matter (Näsholm et al., 1998). Yet, it is required for protein, nucleic acid and hormone synthesis (Campbell, 2004). N is also directly linked to photosynthetic capacity, as both chlorophyll and Rubsico are comprised of large portions of N (Evans, 1989). The

acquisition and use of both C and N are additionally highly dependent on sufficient access to water. Water is central to physiological processes including photosynthesis, growth, biochemical reactions and the transport of nutrients and hormones throughout the tree (Campbell, 2004). Root uptake of water is dependent on the gradient in water potential established between the soil-plant-atmosphere continuum, with changes in access to soil water supply and humidity affecting tension in the water column and thus internal cycling. Different environmental stressors often impact trees by reducing the availability of C, nutrients and/or water. A tree's ability to cope with these challenging conditions can in part be due to their capacity to acquire these resources, for example, root access to nutrient-rich soil patches, but also due to the storage and allocation of these resources within and among organs of the tree. These shifts in internal allocation, for example, increased root to shoot ratio to enable root proliferation in favorable soil patches, are key determinants in plant performance under stress.

Since the acquisition of resources occurs at different locations in a tree, determining how trees may share or store internal resources among or within compartments (leaves, stem and roots) is necessary for the understanding of resource use and allocation, and how allocation varies in time (phenology) and in response to environmental stress. Although organs of a tree are highly integrated, they may act (semi-)autonomously depending on localized resource conditions (Watson and Casper, 1984), affecting their function as a source or sink. Sources are typically tissues or organs that act as net resource exporters, while sinks are tissues or organs that consume more resources than they produce, thus acting as net importers (Wardlaw, 1990; Kozlowski, 1992). Available resources are exported from source organs and transported towards specific sinks, often based on the organ's resource accumulation and allocation capacity (Wardlaw, 1990; Kozlowski, 1992). The strength of a sink may be determined by the sink size and activity, referring to the

number of cells and their activity, which is regulated by their potential maximum rate of resource acquisition, utilization and internal storage (Wardlaw, 1990; Kozlowski, 1992). The degree to which an organ acts as a source or sink may vary over time and may transition its role depending on the current environmental demands on a tree.

Depending on the conditions, resources may be allocated internally to different pools: 1) direct translocation for growth, defense, reproduction 2) utilized for ATP generation during respiration (in the case of carbon) or export to symbionts, or 3) storage in perennial tissues, with potential for future remobilization (Chapin et al., 1990) (Figure 1-1). Storage pools are of particular interest, as resources are partitioned into these pools can be remobilized for future use (Chapin et al., 1990). There are three different modes of storage in plants: 1) accumulation, a product of resource supply exceeding demand 2) reserve formation, the allocation of resources for storage compound synthesis as a trade-off to allocation for immediate use, and 3) recycling, the remobilization of molecules that are not functioning primarily as storage molecules, usually prior

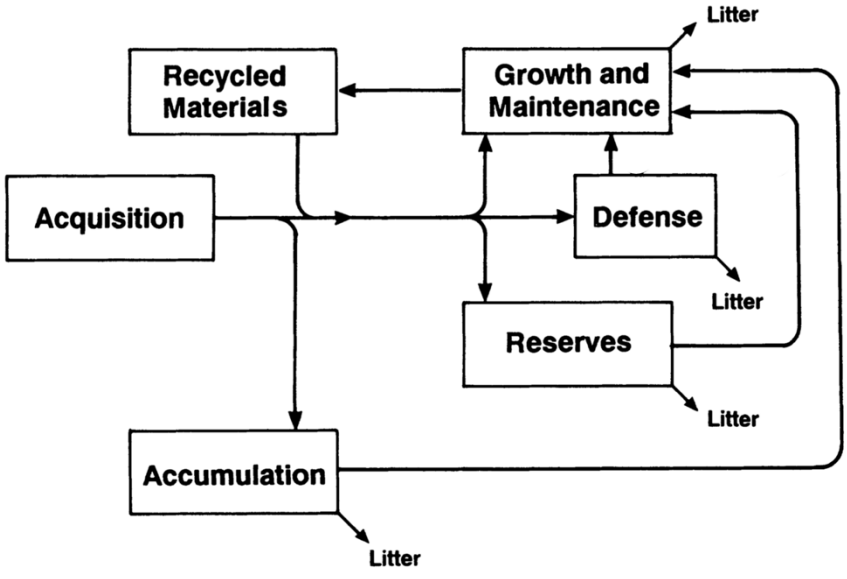

Figure 1-1. Allocation of resources to internal pools. Modified from Chapin et al., 1990.

to abscission of the tissue, and allocated for future use (Chapin et al., 1990) (Figure 1-1). Although reserve formation is in direct competition with immediate use of resources for growth, it is a valuable form of storage for perennial plants as these resources may be remobilized to provide a buffer during changing conditions. The size of storage pools within a tree has been correlated with survival in several cases of disturbance (Millard and Grelet, 2010; Sala et al., 2012; Wiley, 2020), emphasizing the important role of reserves in recovery.

Stored resources are used during periods of asynchrony in supply and demand, including transitions during different phenological stages and during stress mitigation (Chapin et al., 1990). By exploring patterns of resource allocation and remobilization within and among specific organs, we may be able to identify potential mechanisms regulating or constraining the use of stored resources. There is some evidence for factors that can limit remobilization pathways, such as constraints in vascular connections or branch autonomy (Watson and Casper, 1984; Lacointe, 2004; Spicer et al., 2014). However, it can be difficult to determine the specific organ from which remobilized resources are derived or the target organ (or even the portion of an organ) to which newly acquired or remobilized resources are ultimately allocated. Thus, the mechanisms controlling the ability to share (remobilize) resources within or among organs of a tree needs further investigation, especially under seasonality and stress, where trees may be exposed to heterogeneous distribution of resources (soil moisture, nutrients) and/or carbon-limiting environmental conditions.

1.2 Seasonality and stress as drivers of resource allocation and remobilization

Trees are subjected to changing environmental conditions, whether they are the recurring and predictable patterns of seasonality or the increasingly variable and unpredictable conditions of

disturbance under a globally changing climate (IPCC, 2013), which has led to the evolution of a variety of morphological and physiological adaptations and acclimatory responses in trees (Brunner et al., 2015; Hartmann and Trumbore, 2016). Seasonal patterns and fluctuations in resource use have been well studied in various trees, particularly in deciduous species (Millard and Grelet, 2010; Martinez-Vilalta, 2016). The allocation and remobilization of essential resources, including C and N, is in large part seasonally driven by plant phenology. In late autumn, resources are known to accumulate as reserves in perennial organs prior to dormancy (Loescher et al., 1990; Millard, 1996). In the spring, reserve levels decrease due to the asynchrony between demand for growth and the ability to acquire new photosynthates or soil N (Malaguti et al., 2001; Millard, 2006; Keel and Schädel, 2010). Following early leaf development, the canopy increases photosynthetic capacity and the roots increase uptake of soil resources to meet nutrient demands, permitting the gradual buildup of reserves during the summer and early autumn (Landhäusser and Lieffers, 2003; Da Silva et al., 2014).

Although external uptake and internal cycling of essential resources in deciduous trees is regulated by seasonal phenology, under stressful environmental conditions, resource use and allocation may change to support tree growth and survival. For example, partial or severe defoliation of the canopy by insects or pathogens is a common disturbance that can restrict photosynthesis in deciduous species (Reichenbacker et al., 1996; Millard et al., 2001; Palacio et al., 2008), affecting source-sink dynamics and remobilization patterns. To cope with severe defoliation early in the growing season, trees must re-grow their leaf area. Re-foliation may be reliant on reserve mobilization (Nakajima, 2018), increased C assimilation of any remaining leaves (Pinkard and Beadle, 1998) and/or increased root uptake of nutrients (Kosola et al., 2001). The extent of foliage recovery after disturbance varies among species (Nakajima, 2018), but the factors

limiting re-foliation capacity, such as the amount of stored reserves (specifically carbohydrates and nutrients) or photosynthetic efficiency of re-flush leaves, remains poorly understood.

Another common disturbance that impacts resource acquisition and allocation in trees is drought. The predicted increase in frequency of drought events (IPCC, 2013) may not only create temporal heterogeneity in water availability but also spatial heterogeneity in soil moisture (Vereecken et al., 2014; Metz and Tielbörger, 2016). There is a developing body of literature on drought-induced mortality in trees, with catastrophic hydraulic failure due to severe embolism in the xylem leading to desiccation, and carbon starvation due to reduced carbon assimilation followed by depletion of reserves, as two potentially interacting drivers (McDowell et al., 2008; Sala et al., 2010; Galvez et al., 2011; Anderegg et al., 2012). Additionally, reduced water and carbon availability may weaken defenses, leading to increased susceptibility to biotic attack (Anderegg et al., 2015; Wiley et al., 2016). Thus, a trees ability to adjust its resource allocation to cope with these predicted changes in water accessibility may determine its growth and survival and species maintenance on the landscape (Hesse et al., 2021). Variations in root architecture and root distribution (Jackson et al., 1996), in addition to physiological adjustments (Prieto et al., 2012; Brunner et al., 2015) may enable trees to successfully exploit the soil for resources. And while we have learned a lot about plant responses to drought from controlled experiments with seedlings, one shortcoming of potted drought studies is the relatively homogeneous application of soil moisture, which may not accurately capture the natural heterogeneity of soil moisture distribution. Accurate predictions of forest responses to global change on larger spatial and temporal scales will require a better understanding of how trees allocate and utilize critical resources under more realistic drought conditions. We, therefore, need more experiments to assess how trees allocate

resources aboveground and belowground in response to drought conditions that are spatially heterogeneous.

1.3 Trembling aspen

Trembling aspen (*Populus tremuloides* Michx.) is a deciduous tree species widely distributed across boreal and temperate regions of North America. *Populus* species have been used extensively to study ecological and physiological responses to changing environmental conditions and stress due to their easy propagation and economic and ecological importance. Trembling aspen is characterized by its large, interconnected clonal root system which enables regeneration by root suckering after disturbance (Peterson and Peterson, 1992; DesRochers and Lieffers, 2001; Frey et al., 2004; Wiley et al., 2019). Because the aspen root system must balance investment in growth for exploration of surrounding soil resources with the accumulation of reserves for future utilization in regeneration, the regulation of resource allocation and remobilization in the roots is likely critical to clonal survival in this species.

Previous research has suggested that trembling aspen is a highly relevant species to examine the consequences of defoliation and drought stress on resource allocation and remobilization. Trembling aspen forests across northwestern Canada and the southwestern US experienced widespread mortality and die-back, termed as Sudden Aspen Decline (SAD) following a severe drought in 2001-2002 (Bonsal and Wheaton, 2005; Allen et al., 2010). While tolerance to both severe and moderate drought has been previously studied in aspen (Frey et al., 2004; Hogg et al., 2008; Galvez et al., 2011; Anderegg and Callaway, 2012;), the sensitivity of growth and reserve allocation responses in aspen exposed to localized drought or partial root zone drying is not well studied. Increased frequency and intensity of drought conditions, along with

rising temperatures, may have implications for insect outbreak dynamics as well (Ayres and Lombardero, 2000). Trembling aspen forests are susceptible to severe defoliation by the forest tent caterpillar (*Malacasoma disstria*), the large aspen tortrix (*Choristoneura conflictana*) and Bruce spanworm (*Operophtera bruceata*). In trembling aspen, partial, complete or recurring defoliations have been shown to contribute to increased cavitation risk (Galvez and Tyree, 2009; Hillabrand et al., 2018), increased vulnerability to pests and pathogens (Anderegg and Callaway, 2012), increased risk of carbon starvation (Landhäusser and Lieffers, 2012), and ultimately tree decline and dieback (Hogg et al., 2002). Given the outcomes of defoliation stress, understanding how aspen allocates and remobilizes resources, along with potential constraints on remobilization pathways is critical.

1.4 Research Outline/Objectives

The overall objective of this research was to investigate how trembling aspen allocates and remobilizes resources in response to various environmental stresses. This thesis focused on how individual trees may share and/or store resources among and within organs when subjected to changing or limiting conditions. A particular emphasis was placed on exploring processes that occur in belowground organs.

The objective of the study presented in Chapter 2 was to explore C and N remobilization in trembling aspen during early spring leaf development and re-flush after complete defoliation. I used grafted aspen saplings which had been exposed to isotopically labeled C and N to determine where resources were remobilized from during spring budflush and following defoliation, the potential effects of distance between source and sink organs, and how timing of leaf synthesis affected the use of remobilized reserves. I also measured changes in non-structural carbohydrate

concentration and N concentration in the stem and roots to quantify the amount of net remobilization from different storage pools. I discuss these results in the context of potential constraints on remobilization during leaf growth and canopy recovery.

The objective of the study presented in Chapter 3 was to assess how spatially heterogeneous soil moisture conditions affect morphological and physiological responses in trembling aspen. I used a split-pot experiment to determine how soil moisture availability affects aboveground and belowground reserve and mass allocation. I measured growth and non-structural carbohydrate reserves among and within organs in response to variation in soil moisture, to better understand how plants cope with water stress.

Chapter 4 provides a synthesis of the research conducted and outlines potential implications of the results. Research limitations and future directions are proposed.

Chapter 2: Tracing the fate of carbon and nitrogen reserves – exploring remobilization during spring leaf expansion and leaf area recovery following defoliation

2.1 Introduction

The remobilization of stored resources is essential for supporting plant growth, development and survival. In climates with marked seasons, deciduous woody plants build up carbon (C) and nitrogen (N) reserves near the end of the growing season to support cold hardening and basic maintenance during dormancy and to support initial leaf, root and stem growth at the start of the growing season when demand exceeds supply from current photosynthesis and root uptake (Loescher et al., 1990; Millard, 1996; Hoch et al., 2003). The amount and location of stored C and N may also enable survival and recovery following disturbances. Reserve remobilization is commonly observed under drought (Geßler et al., 2004; Silla and Escudero, 2006; Galiano et al., 2011; Dai et al., 2018), and following defoliation (Kosola et al., 2001; Millard et al., 2001; Li et al., 2002; Wiley et al., 2013; Nakajima, 2018). In woody plants, C is most commonly stored as non-structural carbohydrates (NSC) – soluble sugars and starch – throughout the whole plant (Dietze et al., 2014). Nitrogen, an essential macronutrient, accumulates in metabolically active proteins such as Rubisco (Cooke and Weih, 2005; Millard et al., 2007) and then is stored prior to dormancy in vegetative storage proteins (Staswick, 1994; Stepien et al., 1994; Cooke and Weih, 2005) or as free amino acids (Cheng et al., 2004; Visozo et al., 2008) found in the bark, wood and roots (Cooke and Weih, 2005; Millard and Grelet, 2010). Despite their importance, we still have a poor understanding of how the remobilization of stored C and N is regulated in woody plants and what factors may constrain the remobilization, transport and use of reserves throughout the plant

and under different conditions; this limits our ability to predict growth, stress response and survival in woody plants.

For C reserves, recent work has highlighted that stored C should not be considered a single, well-mixed pool that is equally available to any organ or function (Dietze et al., 2014; Hartmann et al., 2018; Hart et al., 2021). Thus, we need a better understanding of which storage pools supply remobilized reserves in different organs. The growth of tissues like leaves is a critical process that often relies on the remobilization of reserves, but it is unclear if stored C utilized for growth is remobilized only locally or whether it is readily supplied from other, more distant locations, or organs. First, reserve sharing between organs may be limited due to competing growth demands within the tree. For example, while bud break may act as a major sink for NSC in early spring, root growth may also occur at the very beginning of the growing season (Sword et al., 1996; Joslin et al., 2001; Gaudinski et al., 2009), potentially creating internal competition for NSC (Reich et al., 1980). Additionally, long distance C remobilization could be limited by stressors, morphological constraints, or by regulatory pathways that inhibit transport or provoke organ autonomy (Watson and Casper, 1984; Lacointe, 2004; Hartmann et al., 2018). Such constraints on reserve sharing have been proposed to occur under drought (Sevanto et al., 2014; Adams et al., 2017), during light deprivation (Wiley et al., 2017), and following bark beetle attack (Wiley et al., 2016) based on a pattern of only localized NSC remobilization or depletion. Similarly, branches within a tree often display C autonomy (Watson and Casper, 1984; Hoch, 2005), and locally stored NSC has been shown to be sufficient to support early leaf and shoot growth (Landhäusser, 2011). However, there is also evidence that C may be remobilized from more distant locations within the tree. Lacointe et al. (2004) found evidence of a lack of branch autonomy in the spring, concluding that *Juglans regia* produced new shoots using C originating from distant branches. Additionally,

using radiographs of ^{14}C-labeled pecan saplings that were grafted together with unlabeled sapling stems or roots, Lockwood and Sparks (1978) found that while initial new leaf growth relied primarily on local (i.e. stem-labeled) C, C *was* remobilized from the roots as shoot elongation progressed. These studies suggest that remobilized C may be regularly shared between organs when buds and leaves are net C importers.

For N reserves, it is similarly unclear whether remobilized N tends to be more locally versus distantly derived (Millard et al., 2006). Root uptake of ammonium or nitrate can be limited in early spring if soil temperatures and soil mineralization rates are low, and thus initial leaf development can rely heavily on the remobilization of stored N (Sauter et al., 1989; Millard, 1996; Bollmark et al., 1999; Grassi et al., 2003). In evergreen trees, which store substantial N in their foliage, remobilized N supporting new leaf growth may be mostly transported over relatively short distances from old leaves (Millard et al., 2001; Warren et al., 2003). But for deciduous species, N supporting leaf growth may be remobilized over the entire length of the tree from both stems and roots (Millard et al., 2001, 2006). There is evidence of long-distance N translocation, as foliar-absorbed N in *Prunus persica* var. *nectarina* was translocated by the phloem to the roots compared to short-distance alternatives in aboveground organs, for leaf growth the following spring (Tagliavini et al., 1998). However, the effect of remobilization distance and potential constraints on the accessibility of stored N during periods of new growth has been understudied.

Reserves may also be remobilized to support growth following disturbances like defoliation, but it is unclear whether leaf flush after defoliation differs from spring flush in both its reliance on stored reserves and whether long distance remobilization from other organs occurs. There is evidence that during initial budflush, local reserves (branch/stem) may be plentiful and

capable of supplying sufficient remobilized C and/or N for leaf growth (Millard, 2006; Landhäusser, 2011). However, this initial use of remobilized C and N for spring leaf flush then lowers reserve levels (Landhäusser and Lieffers, 2003; Millard and Grelet, 2010), and if trees then experience defoliation, branches may lose autonomy and may need to import C from more distant, remaining reserves (Hoch, 2005; Carbone et al., 2013). In contrast, while defoliation could also lead to a shift to reliance on more distant N reserves, trees can still acquire new N from the soil which could help supplement reduced N reserves. Finally, the amount and source of remobilized C or N for canopy regrowth may depend on whether the resource in question is in limiting supply or not. There is some evidence that, in fruit trees, N reserves limit canopy growth in the spring, not stored carbohydrates (Cheng and Fuchigami, 2002). However, lower sprout regrowth after cutting during times of the year when C reserves are low suggests that canopy recovery is C-limited (Kays and Canham, 1991; Landhäusser and Lieffers, 2002). Whichever nutrient limits canopy recovery, limitation by one resource likely affects the remobilization of the other resource (Chapin and Slack, 1979; Tuomi et al., 1990). Therefore, because deciduous trees use both C and N reserves during regrowth after defoliation, and either could potentially limit this growth, a better understanding of what limits leaf regrowth is needed to understand the interdependency of these key resources and their remobilization.

To investigate C and N remobilization during early spring leaf development and re-flush after defoliation, I selected a deciduous tree species, trembling aspen (*Populus tremuloides* Michx.). Trembling aspen is widely distributed across North America, growing in regions with distinct seasonality. Trembling aspen is susceptible to defoliation attacks by the forest tent caterpillar (*Malacasoma disstria*), the large aspen tortrix (*Choristoneura conflictana*) and Bruce spanworm (*Operophtera bruceata*), with damage occurring between late April to late June. While

many studies have explored the net remobilization of C and N during spring flush and following defoliation by monitoring changes in NSC and N concentration, stable isotopic labeling offers the potential for more precision in capturing important fluxes out of storage pools. Additionally, by combining isotopic labeling with grafting, it is further possible to capture the remobilization of resources *between* organs, allowing us to identify the source(s) of remobilized resources used for new growth and potential spatial constraints on their utilization. I therefore used grafted aspen saplings which were pulse-labeled with isotopic C and N to characterize patterns of C and N remobilization during spring leaf development and following defoliation. I addressed the following questions during leaf development: (1) to which locations in the sapling are stem and root C and N remobilized during budflush, (2) what sources of stored C and N are utilized for the development of pre-formed and neo-formed leaf growth during spring flush and for leaf recovery following defoliation – and does their reliance on these reserves differ, and (3) does proximity (distance) from the reserve source affect the use of remobilized reserves? Then in order to better understand potential constraints on remobilization following defoliation, I tested (4) whether the remaining NSC and N concentrations after defoliation were correlated with percent leaf mass recovery to explore whether C or N availability limited recovery or remobilization of reserves. I hypothesized that, in saplings, both stem and root stored C and N would contribute to spring early leaf growth and leaf recovery following defoliation, with net remobilization occurring in only one direction, from belowground to aboveground. I also predicted that distance from reserve locations would impact remobilization, with expanding leaves farther from the roots utilizing less root-derived C and N reserves than leaves developing closer to the source. Furthermore, I hypothesized that early, pre-formed leaves would be more reliant on stored C reserves compared to more recently formed leaves due to increasing availability of newly assimilated C over time. Finally, I hypothesized that

the abundance of NSC storage would regulate the degree of leaf recovery and affect the remobilization of N following defoliation because defoliation may initially limit C assimilation and aspen root systems may preferentially maintain C reserves for future use instead of immediate re-flush.

2.2 Materials and Methods

2.2.1 General overview of the experimental timeline

Two-year old aspen saplings grown from open-pollinated seed sources of Central Alberta (Smoky Lake Forest Nursery, AB, Canada) were potted in June of 2017 and grown outside at the University of Alberta (Edmonton, AB, Canada) (Figure 2-1). In August of 2017, the saplings were distributed evenly into two groups based on size, with half of the saplings pulse labeled with $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$ while the remaining half of the saplings were not pulse labeled (Figure 2-1). In April of 2018 while dormant, all saplings were moved into a growth chamber simulating early spring conditions, where they were grafted with either the stem pulse labeled (stem origin sapling) or the root system pulse labeled (root origin sapling) (Figure 2-1). Timing of budburst and timing of pre-formed leaves unfurling was recorded individually for each sapling during April of 2018 (Figure 2-1). A subset of saplings (including both stem origin and root origin saplings) were harvested 18 days after the pre-formed leaves unfurled to assess early leaf expansion in May of 2018 (Figure 2-1). The remaining subset of saplings (including both stem origin and root origin saplings) were defoliated 18 days after the pre-formed leaves unfurled and kept in the growth chamber for an additional 3 weeks to re-flush, then were harvested in June of 2018 (Figure 2-1).

2.2.2 Plant material and pulse labeling with $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$

One hundred and five two-year old nursery grown containerized trembling aspen saplings (container size 6 cm diameter and 15 cm deep) from open-pollinated seed sources collected in Central Alberta (Smoky Lake Nursery, AB, Canada) were planted in 1 L pots containing a 2:1:1 mixture of peat (PRO-MIX, Premier Tech Horticulture, Quebec, Canada), vermiculite (Specialty Vermiculite Canada Corp., Alberta, Canada) and clay (Turface Athletics MVP, USA) in June 2017. Saplings were grown outside at the University of Alberta (Edmonton, AB, Canada) for 42 weeks (June 1, 2017 – March 26, 2018). The saplings received 50 mL of 1g L^{-1} 10-52-10 NPK fertilizer (Agrium Inc, Alberta, Canada) once a week for the first two weeks of growth followed by 50 mL of 2 g L^{-1} 15-30-15 NPK fertilizer (Agrium, Inc., Alberta, Canada) once a week for eleven weeks. In mid-July 2017, 50 mL of 5 mL L-1 paclobutrazol plant growth regulator (Bonzi ®, Syngenta Canada Inc., Ontario, Canada) was applied to the soil of each potted sapling to promote early bud set and maximize the uptake of C and N to storage pools during the remainder of the growing season (Landhäusser et al., 2012; Schott et al., 2013).

In mid-August 2017, pulse-labeling with $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$ was used to label storage pools. First, saplings were measured for height and root collar diameter (RCD) and evenly distributed into two groups. One group of 60 saplings was not pulse-labeled (*ie*. unlabeled saplings); the other group of 45 saplings was pulse-labeled with both $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$ (*ie.* labeled saplings) (Norris et al., 2012; Karst et al., 2016). Over the course of two consecutive days with zero cloud cover, 45 saplings were pulse-label with $^{13}CO_2$. The canopy of each of these saplings was covered with a transparent plastic bag (40 x 20 cm^2) equipped with a small patch of reinforced tape. The bag was sealed to the base of each stem to prevent loss of injected $^{13}CO_2$. Using a syringe and needle, 50 mL of 99% $^{13}CO_2$ (Sigma-Aldrich, Oakville, Canada) was injected

into the bag through the reinforced tape patch and the puncture hole was immediately covered with an additional layer of reinforced tape. The transparent bags enclosed the saplings canopies for two hours, allowing assimilation of the $^{13}CO_2$, and then were removed. To pulse-label with $^{15}NH_4{}^{15}NO_3$, 100 mL of 60 atom% $^{15}NH_4{}^{15}NO_3$ 0.19 g L^{-1} solution (Sigma-Aldrich, Oakville, Canada) was applied to the soil of each potted sapling. In late October 2017, saplings were insulated with a thick layer of straw to prevent root damage from soil temperatures that were well below freezing (< 5˚C). In late March 2018, 25 labeled saplings and 25 unlabeled saplings were re-measured for lower stem diameter and paired together to ensure accurate fit with a similarly sized sapling for stem grafting. While frozen outside, all but 26 sapling stems were cut 6 cm from the root collar, with the cut surface treated with a 10% bleach solution to prevent bacterial and fungal growth. The remaining length of stems were wrapped at the base with moist paper towel and stored in plastic bags in a -10 ˚C freezer. The pots with the 6 cm segment of stem and root system in addition to 26 saplings that remained fully intact, were moved into a growth chamber (Conviron, Winnipeg, Canada) set to a constant 10°C with a 12-hour light/12-hour dark cycle, average relative humidity at 47% and PAR of 500 $\mu molm^{-2}$ $s^{-1}$of for 6 days to allow soil and saplings to thaw. The growth chamber was then set to simulate spring conditions, at 20°C during the day and 16°C at night with a 16-hour light/8-hour dark, average relative humidity at 47% and PAR of 500 $\mu molm^{-2}$ s^{-1} for the remainder of the experiment. Saplings were watered regularly and received 50 mL of 1 g L^{-1} 15-30-15 NPK fertilizer every 4 days until harvested. In May of 2018, a subset of saplings was defoliated by clipping off all of the leaves. The defoliated saplings remained in the growth chamber for an additional three weeks until June of 2018. During this individual 3-week time period, all saplings produced new re-flush leaves.

2.2.3 Grafting

In April of 2018, sixty-five aspen saplings were either grafted to themselves (15 saplings) or to their pre-matched pair (50 saplings total). To graft the saplings, the 6 cm segment of stem attached to the root system (root stock) as well as the pre-selected corresponding stem segment previously stored in the freezer (scion), were trimmed at the base and an omega-shaped incision was cut into both stem segments using a grafting tool (Lee Valley, Canada). The two stem segments were positioned to fit closely together to promote vascular cambium contact, then bound with rubber grafting bands and sealed with Parafilm. Three types of grafted saplings were constructed to trace the origin of labeled carbon and nitrogen: (1) to calculate background isotopic signal, unlabeled saplings were grafted to themselves to create an unlabeled scion attached to an unlabeled rootstock (unlabeled self-grafted control treatment); (2) to detect carbon and nitrogen remobilization originating from the stem (stem origin treatment), a $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$ pulse-labeled scion was attached to an unlabeled rootstock; (3) to detect carbon and nitrogen remobilization originating from the roots (root origin treatment), an unlabeled scion was attached to a $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$ pulse-labeled rootstock. Grafting was deemed successful if the sapling was capable of bud burst and development of a healthy canopy without stem dieback. The success of the graft resulted in 14 saplings in the unlabeled control treatment, 12 saplings in the stem origin treatment, and 13 saplings in the root origin treatment, which were used in the remainder of the experiment. Twenty-six saplings were left ungrafted to assess any potential effects the graft may have had on growth and development which could affect remobilization patterns.

2.2.4 Harvest

Aspen saplings were harvested at three timepoints: 1) pre-bud burst at time of grafting 2) early leaf expansion and 3) post-defoliation (Figure 2-1). For post-bud burst harvests, grafted saplings were monitored individually then harvested when they had reached a similar phenological stage to ensure more accurate comparisons.

The pre-bud burst harvest was used to assess initial concentrations of non-structural carbohydrates and nitrogen. Thus, one week after saplings were exposed to spring conditions in the growth chamber, fourteen saplings – seven pulse-labeled with $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$ and seven unlabeled – were harvested. Both root and stem samples were collected.

The grafted saplings were harvested following early leaf expansion and defoliation recovery. The specific timing of budburst and leaf roll out, marked by the complete unfurling of the first four pre-formed leaves from within the bud, was recorded for each sapling. Once the first four leaves were flat, each sapling was monitored for continued leaf production and shoot elongation for 18 days. A subset of saplings, each at their individual 18-day timepoints, was harvested to assess storage (non-structural carbohydrate and nitrogen concentrations) and remobilization (location of detected isotope) following early leaf expansion; unlabeled self-grafted control saplings (n=7), stem origin saplings (n=6) and root origin saplings (n=7). Saplings were divided into roots and aboveground tissues, with the latter being further subdivided in the following manner. The stems of saplings were divided into four sections: (1) the grafted region encompassing the base 6 cm of the stem, (2) the lower stem consisting of the stem segment below the first branch, (3) the mid stem and (4) the upper stem sections consisting of the two halves of the stem segment above the lowest branch (Figure 2-2). Leaf samples for isotope analysis were divided based on distance from the root system as well as timing of synthesis in relation to bud

flush: (1) lower early leaves consisting of up to 6 sets of the first emerged four leaves sampled on branches *closest* to the root system, (2) upper early leaves consisting of up to 6 sets of the first emerged four leaves sampled on branches *furthest* from the root system, and (3*)* upper late leaves consisting of up to 6 sets of the seventh and subsequently produced leaves on the same branches sampled for upper early leaves (Figure 2-2). New shoot growth and all remaining leaf tissue were collected separately. Twelve ungrafted saplings were also harvested using the same method as described above.

The remaining grafted saplings that were defoliated at the 18-day timepoint – unlabeled, self-grafted control saplings (n=8), stem origin saplings and root origin saplings (n=6 each) were harvested 3 weeks later. Root and stem tissue were harvested in the same manner as the 18-day timepoint harvest. Newly produced re-flush leaves were collected and divided into two groups: (1) lower re-flush leaves and, (2) upper re-flush leaves, taken from the branches that corresponded to the mid stem and upper stem sections (Figure 2-2). At that time fourteen ungrafted saplings were also harvested using the same method as described above.

For all harvested saplings, soil was carefully removed from the root systems. All tissue samples were dried for 1 hour at 100°C to denature enzymes, followed by 72 hours at 70°C. All dried material was weighed, and samples were ground to a 40-mesh (0.4) using a Thomas mini Wiley mill (Thomas Scientific, Inc., Swedesboro, NJ, USA) for subsequent non-structural carbohydrate analysis. A portion of the samples were further ground to a homogenous powder using a TissueLyser bead mill (QIAGEN).

2.2.5 Carbon and Nitrogen Analyses

Non-structural carbohydrates (NSC) were analyzed following protocols S1, S2, S5 and S6 (Landhäusser et al., 2018). In brief, total soluble sugars were extracted in 80% hot ethanol followed by a phenol-sulfuric assay to determine their concentration colorimetrically by measuring the absorbance at 490 nm with a spectrophotometer. To determine starch concentration, the remaining pellet was digested with α-amylase (Sigma cat. no. A4551) and amyloglucosidase (Sigma cat. no. ROAMYGL). The resulting glucose hydrolysate was then also measured colorimetrically at 525 nm with an assay utilizing a peroxide-glucose oxidase/o-dianisidine reagent and sulfuric acid. Absorbance values were used to calculate sugar and starch concentrations expressed as percent of sample dry weight.

Aliquots of approximately 2.00-2.50 mg of the finely ground tissue were packaged into 4 × 6 mm tin capsules (Costech Analytical, Fischer Scientific) with two replicates per sample in preparation for isotopic analysis. To measure isotopic enrichment, tissue samples were sent to the Institute of Biochemical Plant Pathology (Helmholtz Zentrum München, Germany) to measure isotopic enrichment. An Isotope Ratio Mass Spectrometer (delta V Advantage, Thermo Fisher, Dreieich, Germany) coupled to an Elemental Analyzer (Euro EA, Eurovector, Milano, Italy) was used to determine ^{13}C and ^{15}N abundances and total C and N concentration. An autosampler introduced the samples into the combustion column, heated to 1000°C for quantitative oxidation of the samples. The combustion products were passed into a reduction reactor filled with metallic copper at 650°C. Following removal of water with magnesium perchlorate, CO_2 and N_2 were separated on a packed column. These gases were introduced to the ion source of the IRMS using the ConFlow Interface, accelerated and then separated in a magnetic field depending on their

masses. A lab standard (acetanilide) was used in every sequence and isotope linearity of the system using different weights of standards was determined. The lab standards were calibrated using several suitable international isotope standards (International Atomic Energy Agency: IAEA; Vienna), which were also used for the final correction of ^{13}C and ^{15}N.

The atom percent (AP) of ^{13}C was calculated for root, stem and leaf samples for both unlabeled self-grafted control treatment saplings – to determine the background isotopic signal – and enriched tissue samples from the stem origin and root origin treatments

$$AP = \frac{100 \times AR \times \left(\delta^{13}C/_{1000} + 1\right)}{1 + AR \times \left(\delta^{13}C/_{1000} + 1\right)}$$

Where $\delta^{13}C$ was calculated as $[(R_s/R_{ref})-1] \times 1000$, where R_s and R_{ref} is the ratio of $^{13}C/^{12}C$ for the sample and the reference standard, Vienna Pee Dee Belemnite (VPBD), respectively. AR is the absolute ratio of $^{13}C/^{12}C$ of the reference standard, VPBD.

The ^{13}C atom percent excess (APE), i.e., the percentage of ^{13}C atoms ($AP_{labeled}$) in excess of background ^{13}C levels ($AP_{background}$), was then calculated for each individual tissue type as:

$$APE = AP_{labeled} - AP_{background}$$

The above calculations for atom percent (AP) and atom percent excess (APE) were repeated for ^{15}N. $\delta^{15}N$ was calculated as $[(R_s/R_{ref})-1] \times 1000$ where R_s and R_{ref} is the ratio of $^{15}N/^{14}N$ for the sample and the reference standard, atmospheric N_2 (Mariotti 1983), respectively. AR is the ratio of $^{15}N/^{14}N$ of the reference standard, atmospheric N_2 (Mariotti 1983).

2.2.6 Statistical Analysis

All data were analyzed using R statistical software v3.5.1 (R Development Core Team, 2018). Assumptions of normality and homoscedasticity were tested using the Shapiro-Wilks test and Levene's test for parametrical analyses. If these assumptions were not met, outlier data points were removed for specific tissue samples from four individual saplings, and transformations were applied (log transformations for stem and root nitrogen concentration and root sugar concentration, while reciprocal transformation was used for stem starch concentration). One-sided t-tests were used for 1) leaf samples and root samples of stem origin saplings and 2) leaf samples and stem samples of root origin saplings, to detect the presence of excess ^{13}C and ^{15}N (APE), significantly more than zero. To account for these multiple comparisons, p-values were adjusted using the Benjamini-Hochberg correction (Appendix A-1). To assess the effects of distance and timing on carbon and/or nitrogen APE, linear mixed effects models with individual sapling as a random factor were used. The emmeans package (Lenth, 2019) was used for the post-hoc tests of these models. One-way ANOVA was used to test for differences in non-structural carbohydrate concentrations and total nitrogen concentration in stem and root tissue among timepoints, followed by pairwise post-hoc tests using the emmeans package with a Benjamini-Hochberg p-value adjustment. Pearson correlation was used to test the linear correlation of two variables with percent leaf mass recovery in defoliated saplings: root starch concentration and root total nitrogen concentration. Differences among treatments and among tissue types were considered statistically significant at $\alpha = 0.05$.

2.3 Results

To assess any effects of the graft on the sapling health and growth 18 days after early leaf expansion and three weeks after defoliation, aboveground and belowground parameters were compared between the grafted saplings and the ungrafted saplings. At 18 days after early leaf expansion, grafted saplings did not differ in height, root collar diameter, aboveground mass, stem mass, leaf mass, root mass or root volume from ungrafted saplings (Appendix A-2). Further, 3 weeks after defoliation, height, root collar diameter, aboveground mass, stem mass, re-flush leaf mass, leaf mass recovery, root mass and root volume of grafted saplings were not different from ungrafted saplings (Appendix A-3). The lack of differences in growth parameters between grafted and ungrafted saplings at both time points suggests that there was no detectable effect of the graft on overall sapling health and productivity.

2.3.1 Early spring leaf development

In stem labeled saplings (stem origin treatment), at 18 days after early leaf expansion, stem-remobilized C was detected in both belowground and aboveground tissues (Figure 2-3a). Labeled C originating from the stem was present, although relatively minimally, in the roots of aspen following early leaf expansion ($p = 0.047$; Figure 2-3a). Although I did not detect a measurable increase in root volume between pre-bud burst and 18 days after early leaf expansion (36.0 cm^3 vs 40.7 cm^3, respectively; $p = 0.4$), I did observe new root growth (root tips) at this time point.

C used to support spring leaf flush and development was remobilized from both the stem and roots of aspen saplings. In all three leaf types harvested: lower early leaves, upper early leaves and upper late leaves, labeled C originating from the stem was present ($p < 0.01$; Figure 2-3a).

However, the upper *early* leaves had a higher percentage of stem-labeled carbon (atom percent excess, APE) present than the upper *late* leaves ($p < 0.01$; Figure 2-3a). In root-labeled saplings (root origin treatment), at 18 days after early leaf expansion, root-remobilized C was detected in aboveground tissues (Figure 2-3b). There was a marginally significant amount of labeled C originating from the roots in the lower and upper segments of the stem ($p = 0.058$ and $p = 0.059$ respectively; Figure 2-3b). Root-remobilized C was detected in all three leaf types: lower early leaves, upper early leaves, and upper late leaves ($p < 0.01$, $p < 0.01$, and $p = 0.049$ respectively; Figure 2-3b). However, the upper *early* leaves had a higher percentage of root-labeled C (APE) compared to upper *late* leaves ($p = 0.04$; Figure 2-3b). Furthermore, in root origin saplings, *lower* early leaves had a greater ^{13}C APE than *upper* early leaves which were further removed from the roots ($p = 0.02$; Figure 2-3b).

Changes in NSC concentrations suggest that stem starch and root sugar pools are the likely sources of C used to fuel initial leaf development in early spring. Stem soluble sugar concentrations did not significantly differ between pre-bud burst (11.2 %) and 18 days after early leaf expansion (9.7 %) (Figure 2-4). Stem starch concentrations, however, decreased significantly from 2.9 % at pre-bud burst to 1.7 % following early leaf expansion ($p < 0.01$; Figure 2-4). Root soluble sugar concentrations were 13.2 % at pre-bud burst and then declined by half to 6.5 % following early leaf expansion; root starch concentrations remained unchanged between pre-bud burst and 18 days after early leaf expansion, at approximately 9 % (Figure 2-4).

N was remobilized from both the stem and root system during spring leaf development. In stem-origin saplings at 18 days after early leaf expansion, labeled N was present in both belowground ($p = 0.01$) and aboveground tissues ($p < 0.01$) although at a considerably lower APE

in the roots (Figure 2-5a). All three leaf types – lower early, upper early and upper late – contained labeled N originating from the stem ($p < 0.01$; Figure 2-5a). However, stem-origin saplings' upper *late* leaves had a lower ^{15}N APE than upper *early* leaves ($p < 0.01$; Figure 2-5a), indicating a reduced reliance on stem N reserves over time. In root-origin saplings, at 18 days after early leaf expansion, labeled N was present in all aboveground tissues (Figure 2-5b). Both the lower stem and upper stem contained root-remobilized N ($p < 0.01$; Figure 2-5b). All three leaf types sampled also had root-remobilized N present ($p < 0.01$; Figure 2-5b). Root-origin saplings' upper *early* leaves did not significantly differ in ^{15}N APE from upper *late* leaves, indicating a similar reliance on root-stored N over time (Figure 2-5b). However, leaves produced furthest from the root system (i.e. *upper* early leaves) contained significantly less root-labeled N than leaves closer to the root system (i.e. *lower* early leaves) ($p = 0.01$; Figure 2-5b).

N concentration decreased in stems only during initial leaf development. Stem total N concentration was 0.77 % at pre-bud burst and decreased to 0.54 % at 18 days after early leaf expansion ($p < 0.01$; Figure 2-6). Root total N concentration did not significantly change between pre-bud burst (1.27 %) and 18 days after early leaf expansion (1.19 %) (Figure 2-6). The N concentration of upper early leaves (2.1 ± 0.1 %) and upper late leaves (2.0 ± 0.1 %) did not differ (data not shown).

2.3.2 Leaf area recovery after defoliation

Carbon used for leaf area recovery following defoliation originated from both stem and root reserves in aspen saplings, however there was a source-distance effect. Three weeks following defoliation, stem origin saplings had labeled C present in new leaves, but not in the roots (Figure 2-3a). At three weeks following defoliation, root systems had a volume of 47.0 cm^3, which was

not statistically different than the root volume of saplings at initial leaf development (40.7 cm^3; $p = 0.3$). Although I did observe the presence of new roots, I cannot determine if this growth was produced prior to defoliation, however there was no visual indication of root loss after defoliation. Both lower and upper re-flush leaves had labeled C originating from the stem present ($p < 0.01$ and $p = 0.04$, respectively; Figure 2-3a). In root origin saplings, labeled C was not detected in all aboveground tissues after defoliation (Figure 2-3b). Although the lower stem had a ^{13}C APE marginally significantly different from zero ($p=0.055$), the lower re-flush leaves had a significant amount of labeled C originating from the roots present 3 weeks post defoliation ($p = 0.049$; Figure 2-3b). The ^{13}C APE of the upper stem segment and the upper re-flush leaves of root origin saplings did not differ from zero ($p = 0.09$ and $p = 0.19$, respectively; Figure 2-3b), suggesting minimal import of remobilized root C reserves to these tissues. However, the ^{13}C APE of lower re-flush leaves did not differ from the ^{13}C APE of upper re-flush leaves ($p = 0.35$; Figure 2-3b).

Stem sugar, stem starch and root starch were all remobilized following defoliation stress. Three weeks following defoliation, stem soluble sugars had decreased to 7.2 % ($p < 0.01$; Figure 2-4). Stem starch concentrations also decreased from early leaf expansion to 1.1 % following defoliation ($p < 0.01$; Figure 2-4). Root soluble sugar concentrations remained at 6.5 % after following defoliation, but root starch concentrations significantly decreased, though still remained relatively high at nearly 5 % ($p < 0.01$) (Figure 2-4).

In response to defoliation, N was again remobilized from both the stem and root system, but labeled N was not found in all tissues (Figure 2-5). Labeled N originating from the stem remained present in the roots after defoliation stress ($p < 0.01$; Figure 2-5a). Both lower re-flush leaves and upper re-flush leaves contained stem-origin ^{15}N three weeks post defoliation ($p < 0.01$

for both; Figure 2-5a). In root origin saplings, labeled N was also not found consistently throughout all aboveground tissue types after defoliation stress (Figure 2-5b). Root-remobilized N was found in the lower stem segment ($p < 0.01$) but not the upper stem segment (Figure 2-5b). Both lower re-flush leaves and upper re-flush leaves contained labeled N originating from the roots ($p < 0.01$ for both; Figure 2-5b).

Following defoliation, both stem and root N concentrations decreased. In the stem, N concentration decreased from 0.54 % at initial leaf development to 0.43 % after defoliation ($p < 0.04$; Figure 2-6). In the roots, N concentration decreased from 1.19 % at 18 days after early leaf expansion to 0.90 % following defoliation ($p < 0.01$; Figure 2-6).

2.3.3 Relationship between leaf area recovery and carbohydrate and nitrogen storage

Saplings recovered 31.3 % of leaf mass on average during the three weeks following defoliation (Appendix A-3). Despite the potential availability of root starch (average: 5 %), there was no correlation between percentage of recovered leaf mass and the remaining root starch concentration ($R = -0.044$; $p = 0.85$; Figure 2-7a). However, a higher percent leaf mass recovery following defoliation was associated with a higher N concentration remaining in the root system ($R = 0.62$; $p < 0.05$; Figure 2-7b). There was no negative correlation between root starch concentration and root N concentration during re-foliation ($R = -0.11$, $p = 0.6$), which might be expected if N availability limited leaf recovery and thus the remobilization of root starch to fuel regrowth. Atom percent excess of labeled C in re-flush leaves of root-origin saplings also did not have a significant correlation with N concentration within the roots ($R = 0.74$; $p = 0.15$; data not shown), however it suggests a trend of more root-labeled C remobilized to the re-flush leaves when more root N was available. N concentrations were also compared in leaves harvested at 18 days

after early leaf expansion and 3 weeks following defoliation; leaves produced at initial spring flush had significantly lower N concentrations of (2.0 ± 0.1 %) than re-flush leaves produced after defoliation (2.8 ± 0.2%; $p < 0.01$).

2.4 Discussion

While many studies have shown that spring growth relies on the remobilization of reserves, this study is one of the first to identify the specific source organs – both the stem and the roots, from which remobilized C and N originates to support new leaf growth in aspen saplings. While remobilization of root-derived C and N provides evidence that saplings are capable of long-distance remobilization, root C reserves were not shared equally with the whole stem. Leaves developing closer to the source of stored reserves (i.e. the root system) utilized more root-derived C than leaves developing further from the source; and this distance effect on root C remobilization appeared even greater following defoliation, as the upper stem segment and upper re-flush leaves had no significant import of root remobilized C. These patterns are likely explained by sink topology. Sink organs within a plant will compete when the supply of a resource is limited, and it is thought that a sink hierarchy exists with priority given to sinks closest to the resource source along the transport pathway (Zimmermann, 1971; Minchin et al., 1993; Lacointe, 2000). As a result, this allocation priority may lead to root C shortages when C supply from the canopy is low (Landhäusser and Lieffers, 2012). This hierarchy has usually been described under conditions when recent photosynthate is the main C source, but the results presented in this study support the idea of such an allocation hierarchy during reserve remobilization as well. Thus, when root reserves are the main C source, the shortage may be in the opposite direction with the upper segments of the tree suffering disproportionally. This distance effect on remobilization explains

the observations during carbon starvation experiments using light deprivation where aspen stems experience gradual dieback from the top down (Wiley et al., 2017; Wiley et al., 2019). This dieback of distal tissues is commonly observed in stressed trees, and the results of this study suggest that this could be due to lower allocation priority based on increased distance from the source with most distal tissues suffering most acutely when C supply is lowest, such as following defoliation.

Distance between the root system and leaves also affected the allocation of N remobilized during spring flush, with *lower* early leaves containing more root-labeled N than the *upper* early leaves; but unlike C, this distance effect for N disappeared after defoliation. There was a significant decrease in root N concentration after defoliation, suggesting that root N reserves did contribute to growth of lower and upper re-flush leaves. However, the disappearance of the distance effect after defoliation could result if the closest N reserves had been preferentially used up during spring leaf flush. Bazot et al. (2016) applied labeled N to the previous years' foliage of mature *Quercus petraea* to determine the contribution of N reserves to spring leaf growth, concluding that closely stored and easily accessible forms of N were likely remobilized first. The disappearance of the distance effect after defoliation could also indicate that N supply was sufficient for leaf re-growth. Declines in stem N concentration but not root N concentration after spring flush suggests that N supply was not greatly reduced after initial leaf flush, with newly assimilated N replacing most N remobilized from the root system, as saplings continued to receive fertilizer during this time period. There is further evidence that trees do rely on newly assimilated N in the spring, as Frak et al. (2002) found that walnut saplings used both N remobilization and N uptake from the soil simultaneously during spring growth while other tree species relied more heavily on soil uptake (Millard and Proe, 1991; Millard et al., 2001). Following defoliation, demand for N may have also been lower as leaf mass recovery was only 31.3 %. Both lower and upper re-flush leaves had higher

total N concentrations than leaves produced during initial spring flush. The higher N concentration in the re-flush leaves may be due to thinner and smaller leaves in comparison to the leaves collected during spring leaf development or may be evidence of the supply-driven nature of N, as the size of the N storage pool determines the amount of remobilization (Millard and Grelet, 2010). Similarly, studies which use winter browsing to investigate spring N remobilization suggest that the remaining buds which flush have higher levels of remobilized N because there are fewer buds requiring the same N supply (Lehtilä et al., 2000; Millard et al., 2001; Millett et al., 2005).

In addition to differences in remobilization between organs, aspen saplings relied on different NSC pools (sugars vs starch) *within* organs during spring flush and after defoliation. During spring initial leaf development, the reductions in root sugar but not starch concentrations between pre-bud burst and early leaf expansion suggests that it was root sugars that were remobilized to support leaf synthesis (and potentially stem growth). Furthermore, the significant decrease in stem starch but not sugar concentrations suggests starch in the stem was converted to sugar and remobilized to fuel early spring growth. In contrast, the reductions in root starch but not root sugar concentrations following defoliation suggests that some root starch was converted to sugar to support leaf area recovery. It is likely that not all root starch was remobilized for leaf re-growth, as root starch concentrations remained at 5 % following defoliation. Additionally, stem starch and stem sugar concentrations decreased after defoliation, indicating that both were remobilized for leaf re-growth.

Surprisingly, the hypothesis of only unilateral remobilization from roots to aboveground organs following budflush was not supported, as the aspen saplings remobilized both C and N bi-directionally. Remobilized C and N from the root system was detected in the leaves and stem

tissues. Multiple hypotheses exist for transport pathway of reserves from belowground to aboveground organs during budbreak, as primarily through the xylem (Loescher et al., 1990; Alves et al., 2007) or the phloem (Münch, 1930; Lacointe and Minchin, 2008). However, Tixier et al. (2017) provides evidence using a girdling study to suggest that both xylem and phloem contribute to spring reserve remobilization, with the xylem transporting C reserves and the phloem maintaining water recirculation via Münch flow. The presence of root-labeled C and N in the stem segments could indicate net import for the formation of earlywood in the spring (Kagawa et al., 2006) or perhaps only the transient presence of labeled C and N as these resources are transported via the stem to the developing canopy. The remobilization of both C and N from the stem to the root system was also detected during spring flush and may have supported early root growth for initial soil resource uptake. In contrast, Lockwood and Sparks (1978) used radiographs of ^{14}C-labeled pecan saplings and did not detect remobilization of C from the stem to the roots. However, I found that the remobilization of both C and N from the stem to the roots was minimal, an indication of either a weaker sink strength or lower allocation priority for root growth. Alternatively, the small amount of stem-origin C and N in the roots may simply result from random molecule diffusion and may not necessarily indicate a *ne*t import of remobilized C and/or N from the stem. It is also possible that within the first 18 days of early leaf development, unilateral remobilization occurred as a stepwise process. Reserves may have first been remobilized basipetally from the stem to support active root meristems, then soon after budflush began, acropetally from the roots. Following defoliation, stem-labeled C was not detected in the root system, suggesting that under C-limiting stress, stem reserves in saplings may not be remobilized to belowground organs.

During spring bud flush, pre-formed leaves utilized more stem and root stored C and stem-stored N than neo-formed leaves and re-flush leaves. Unlike the Lockwood and Sparks (1978) study, C was remobilized from the roots to support both the initial new leaf growth (lower and upper *early* leaves) and the neo-formed leaves (upper *late* leaves), suggesting a reliance on both stem and root reserves as shoot elongation progressed. However, the decreased presence of stem and root labeled C in the neo-formed leaves compared to pre-formed leaves indicates an increased reliance on import of new photosynthate as opposed to use of older reserves. This rapid transition from carbon sink to carbon source (Turgeon, 1989) during leaf maturation reduced the dependence on remobilized C originating from elsewhere in the sapling, with similar remobilization patterns observed in the leaves of mature deciduous trees (Keel and Schädel, 2010). The extent to which remobilized C is mixed with newly assimilated C during early leaf development is known to vary among species and with growing conditions (Keel et al., 2006). Aspen branches can become autonomous within a few days of bud flush, before the leaves even mature to their final size, as the first few leaves are quickly able to export new photosynthates thus minimizing the need to remobilize C from more distant sources (Marchi et al., 2005; Landhäusser, 2011). The amount of C remobilized to re-flush leaves was lower in comparison to initial spring leaf development (lower and upper *early* leaves) and more similar to the amount remobilized for upper *late* leaves. Although, there is an assumption that the labeled portions of the reserve pool were equally accessible for remobilization and well mixed throughout the entire storage pool, this result may suggest that the smaller re-flush of leaves produced after defoliation were supplied with less remobilized C initially and were therefore more reliant on recently assimilated C to support their growth. During spring flush, the pre-formed leaves (upper *early* leave*s*) and the neo-formed leaves (upper *late* leaves) used root stored N similarly regardless of timing of leaf development. However,

the upper *late* leaves had lower amounts of remobilized N from the stem than the upper *early* leaves. Since the upper *early* leaves and upper *late* leaves had a similar total N concentration, this likely indicates that the stem N reserve levels were declining during leaf development. The upper *late* leaves were likely more reliant on the recently assimilated N in the root system as previously discussed. This evidence would support the conclusion drawn by Millard and Grelet (2010) suggesting that N remobilization relies on the size of the storage pool, not necessarily on the demand for growth.

An average of 31.3 % of the leaf mass was recovered three weeks following defoliation, with the potential for continued leaf growth for the remainder of the growing season. However, studies have found that canopy size may stay reduced later in the growing season following defoliation (Schäfer et al., 2010; Wiley et al., 2013; Schmid et al., 2017; Nakajima, 2018) and it is unclear whether this incomplete recovery is driven by C or N limitation, or neither. In this study, growth of the new re-flush leaves resulted in significant declines in both root starch and root N concentrations, suggesting that a shortage of either is possible. Firstly, there is evidence that the aspen saplings were *not* limited by C, as a substantial amount of starch (5 %) was remaining in the root system three weeks after defoliation. Though, it is possible that instead of remobilizing available root reserves for more complete leaf area recovery, aspen actively maintains starch in the root system, potentially as an adaptation to further herbivory or disturbance. Piper and Fajardo (2014) suggest the storage of C and N in perennial organs of deciduous trees is an adaptation to tolerate complete and recurring defoliation events. Furthermore, prioritizing NSC storage in the root system over leaf re-growth may be highly advantageous for aspen, as they are a species capable of regeneration via root suckering post disturbance (Peterson and Peterson, 1992; DesRochers and Lieffers, 2001; Frey et al., 2004; Wiley et al., 2019). Secondly, there is some

evidence that aspen saplings may have been limited by N. There was a significant decrease in N concentration in perennial organs following defoliation which may be evidence of re-growth being N dependent, as noted, but not observed by Palacio et al. (2008) and Piper et al. (2015). If N was limiting leaf recovery, this may explain why there was a positive correlation between remaining root total N concentration and percentage of recovered leaf mass, while there was no correlation between root starch concentration and percentage of recovered leaf mass; saplings with more available N could regrow a larger portion of their leaf area while using current photosynthates rather than root reserves. However, there is also evidence suggesting that leaf area recovery was *not* limited by N. New re-flush leaves had higher N concentrations compared to the leaves grown during the initial spring flush. In other deciduous species, defoliation may have no significant effect on leaf N concentration (Ovaska et al., 1993; Volin et al., 2002), increase leaf N concentrations as there are fewer buds requiring the same N supply (Lehtilä et al., 2000; Millard et al., 2001; Millett et al., 2005) or even decrease leaf N concentrations due to an accumulation of phenolics during stress response (Tuomi et al., 1990; Kaitaniemi et al., 1998). Furthermore, the distance effect disappeared for N following defoliation, instead of becoming more pronounced, suggesting that utilization of N was not constrained. Additionally, there is some evidence that the aspen saplings may have been C limited. Re-flush leaves appeared to receive proportionally less labeled C from the stem and root system compared to leaves produced early during spring flush. This reduced C import may have made re-flush leaf growth more dependent on current photosynthates, potentially explaining the smaller size of leaves produced during recovery. Anderegg and Callaway (2012) noted that following initial defoliation of mature aspen clones, root starch concentrations declined for re-foliation and resulted in fewer and smaller leaves. The authors suggest that the reduction in leaf area may have been due to the preferential storage of

NSC, potential N limitation, or a slower rate of leaf growth (Anderegg and Callaway, 2012). However, there appears to be some inconsistencies which hinder the ability to tease apart C and/or N limitation in leaf area recovery for the aspen saplings used in this experiment. Further studies manipulating C and N supply or reserve amounts to help identify whether leaf area recovery was driven by C or N limitation, or neither are needed.

Finally, extrapolating the remobilization patterns that were observed in saplings to large mature trees presents a challenge, as distance between organs may be greater and sink strength (a reflection of canopy size) may differ (Minchin and Lacointe, 2005; Hartmann et al., 2018). The storage capacity of the stem wood tissue of a mature tree is considerably larger than the small stems of tree saplings, which are often equated to young branches of mature trees (Hartmann et al., 2018). Based on the biomass available for storage pools and the sink hierarchy within a mature tree, C and N remobilization to the canopy in larger trees may originate from closer sources (e.g. large branches within the crown) and not necessarily from the root system. Furthermore, the composition of NSC pools in mature trees is likely more complex than that of saplings, characterized by mixing of newly acquired NSC with much older NSC from previous years (Keel et al., 2007; Trumbore et al., 2015). The accessibility of these older pools of NSC relative to the newer pools of NSC in mature trees likely affects the extent of remobilization (Sala et al., 2012), however there is evidence that older stored carbon is a positive contributor to leaf growth in mature trees (Keel et al., 2006). Furthermore, as a tree matures, the reliance on remobilized N may increase as storage pools accumulate (Miller, 1986). This may suggest a more significant role of root N reserves in mature aspen, especially under N-limiting conditions.

The ability of deciduous trees to remobilize stem and root reserves during conditions of asynchrony between supply and demand is highly adaptive. However, this study suggests that distance from source plays an important role in determining C remobilization and allocation during bud burst, and even more so following defoliation in saplings. This implies that distal tissues are more susceptible to dieback under stress when reserves are the main source of C. The effect of distance from the source, however, disappeared for N following defoliation, suggesting that root N was remobilized equally to distant and nearer leaves, perhaps because it was not strongly limiting as soil N was available for continued uptake. Bilateral remobilization of C and N between roots and shoots during spring flush was detected, while following defoliation, C remobilization only occurred in one direction from belowground to aboveground. Percentage of recovered leaf mass following defoliation was 31.3 % and it remains unclear if C and/or N limitation contributed to the incomplete recovery. While remaining N concentration in roots correlated with higher leaf mass recovery, the higher N concentrations of re-flush leaves suggests more than adequate access to soil N. Concomitantly, root starch reserves remained plentiful following defoliation, yet the lower levels of labeled C in the re-flush leaves suggests lower C import and a greater reliance on current photosynthates. Further studies investigating C and N remobilization and potential limitations in the transport of reserves are needed, as they are essential for predicting future tree growth and survival.

2.5 Figures

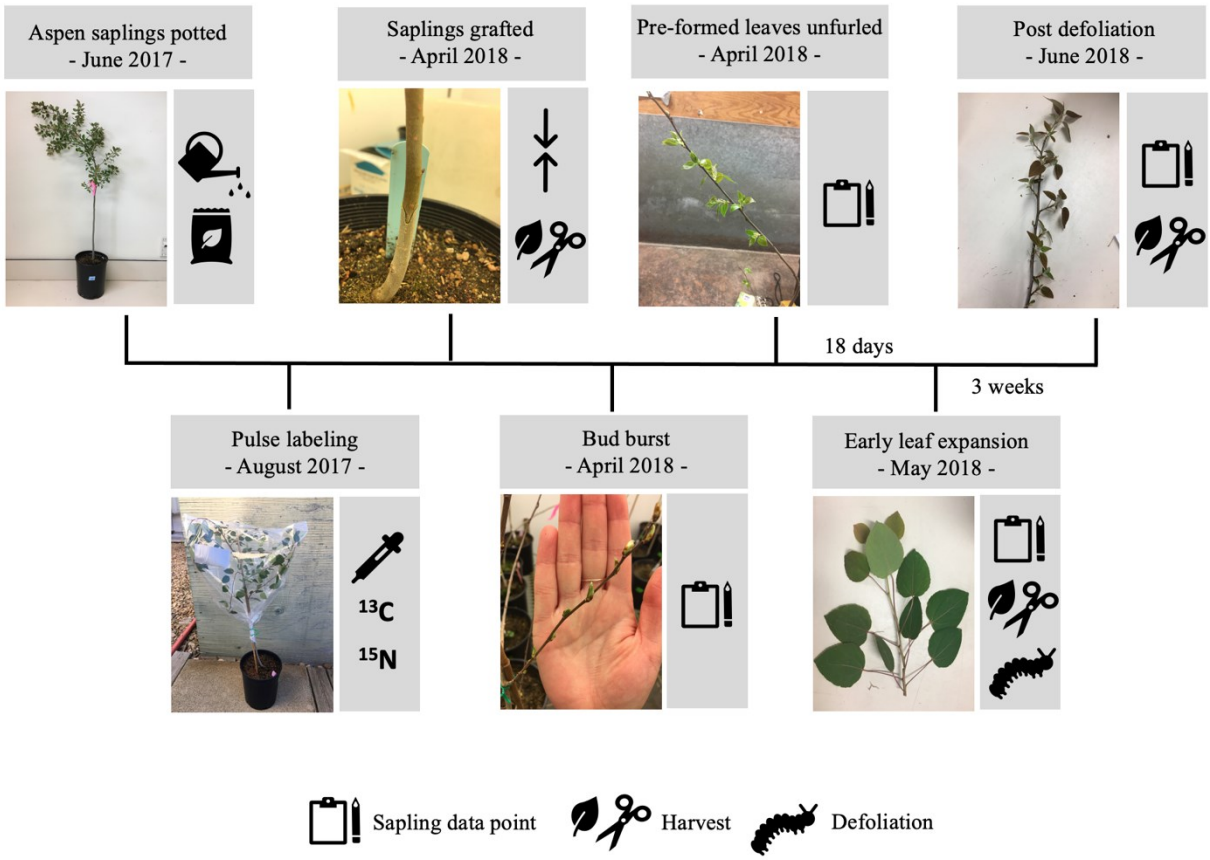

Figure 2-1. Experimental timeline. Two-year old aspen saplings were potted in June of 2017 and grown outside. In August of 2017, half of the saplings were pulse labeled with $^{13}CO_2$ and $^{15}NH_4{}^{15}NO_3$. In April of 2018 while dormant, all saplings were moved into a growth chamber simulating early spring conditions, where a subset were harvested for pre-bud burst assessment, and the remainder were grafted so that labeled carbon and nitrogen originated from either the stem or root system. A subset of saplings (including both stem-origin and root-origin labeled saplings) were harvested 18 days after the pre-formed leaves unfurled in May of 2018. The remaining subset of saplings were defoliated and allowed to re-flush for an additional 3 weeks, then were harvested in June of 2018.

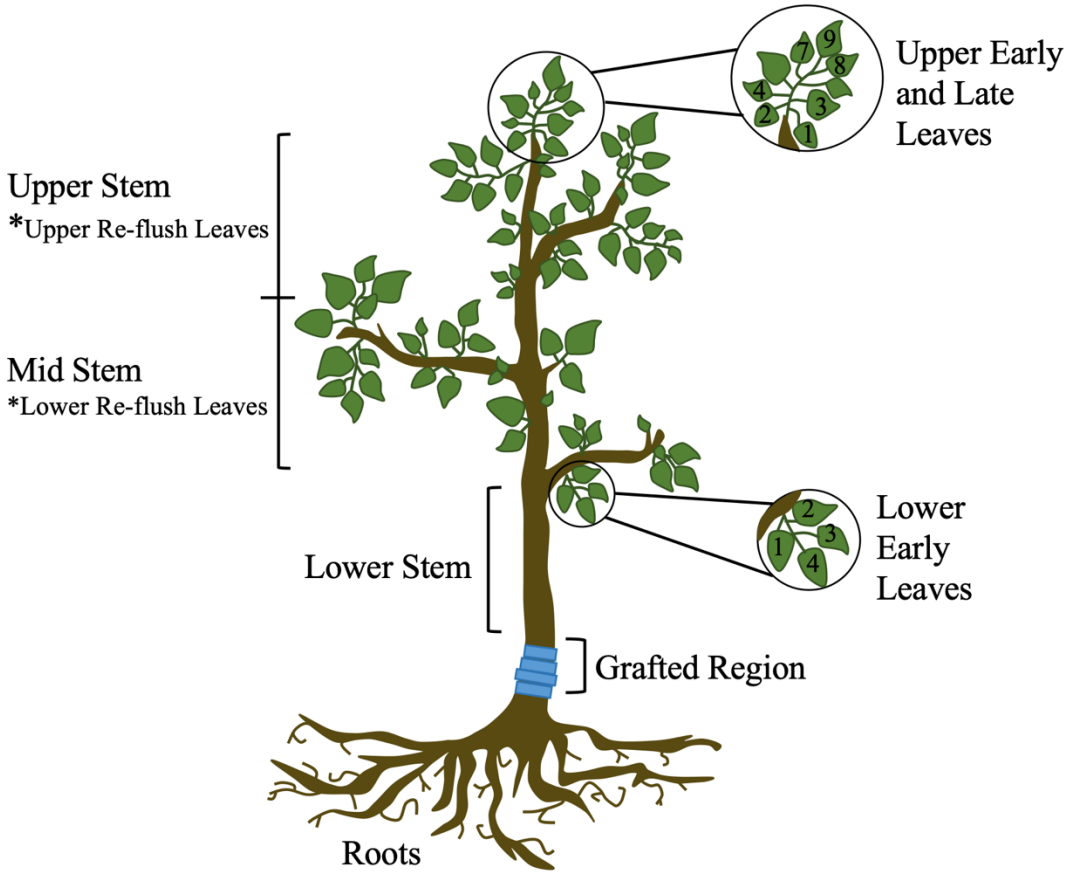

Figure 2-2. Sapling harvest schematic. At 18-days after early leaf expansion, saplings were separated into root, stem and leaf samples. The stem was divided into 4 regions: the grafted region (the base 6 cm of stem), the lower stem (up to the first branch), the mid stem and upper stem (the remaining section split into two halves). Leaves were collected based on position (lower vs upper) and timing (early: leaves 1-4; late: leaves 7 and subsequent). Three weeks following defoliation, root and stem samples were collected in addition to lower re-flush leaves (collected from the mid stem region) and upper re-flush leaves (collected from the upper stem region).

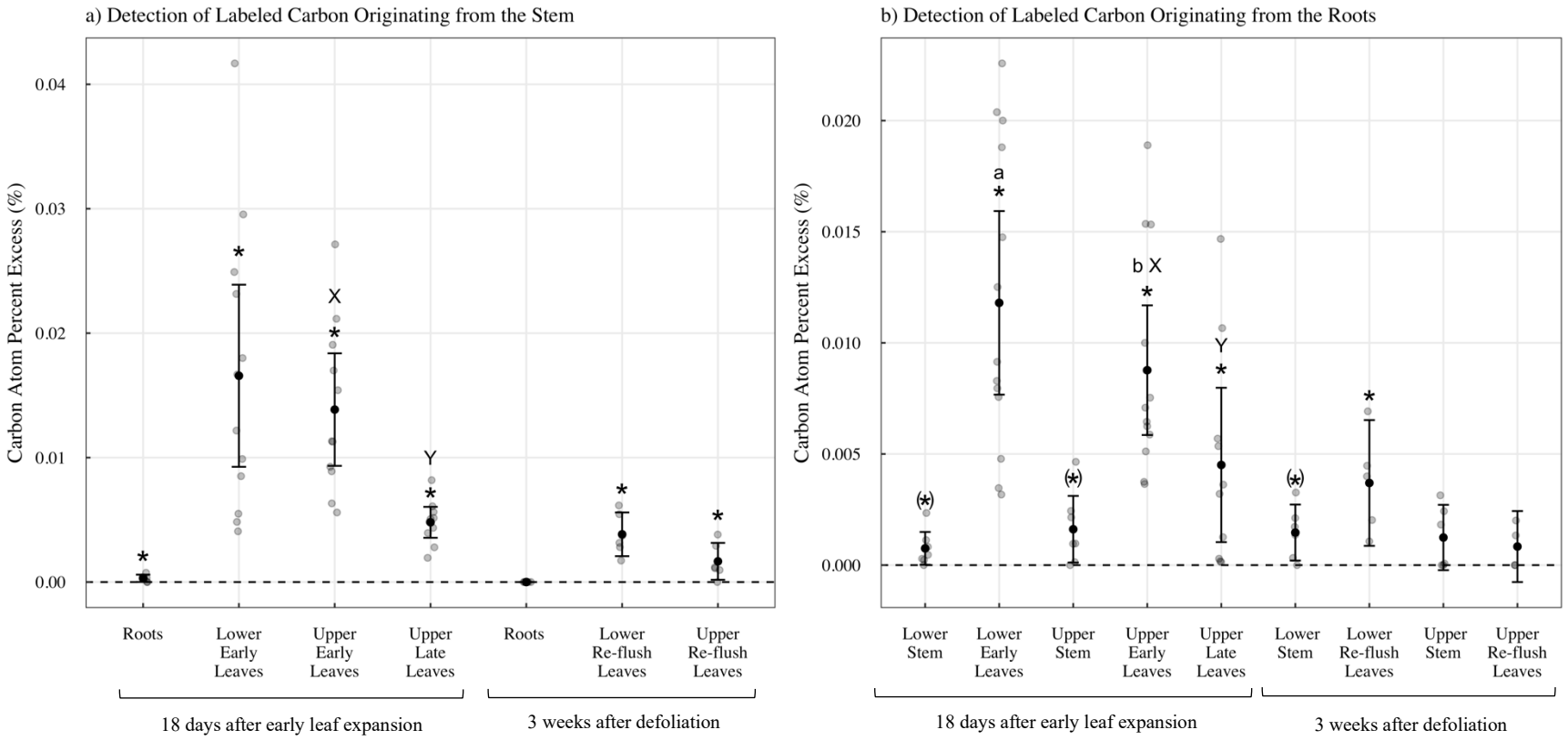

Figure 2-3. Mean (± 95% confidence interval) carbon atom percent excess (%) overlapping raw data points (a) for stem origin saplings, detected in tissue types (roots, lower early leaves, upper early leaves and upper late leaves) harvested 18 days after early leaf expansion and detected in tissue types (roots, lower re-flush leaves and upper re-flush leaves) harvested 3 weeks after defoliation, and (b) for root origin saplings, detected in tissue types (lower stem, lower early leaves, upper stem, upper early leaves and upper late leaves) harvested 18 days after early leaf expansion and detected in tissue types (lower stem, lower re-flush

leaves, upper stem and upper re-flush leaves) harvested 3 weeks after defoliation. * indicates a statistical difference from zero for $p < 0.05$ and (*) indicates a marginally significant difference at $p < 0.06$, with p-values adjusted using the Benjamini-Hochberg method, while lowercase letters (distance effect) and uppercase letters (timing effect) indicate statistical differences at $p < 0.05$ between tissue types ($\alpha < 0.05$).

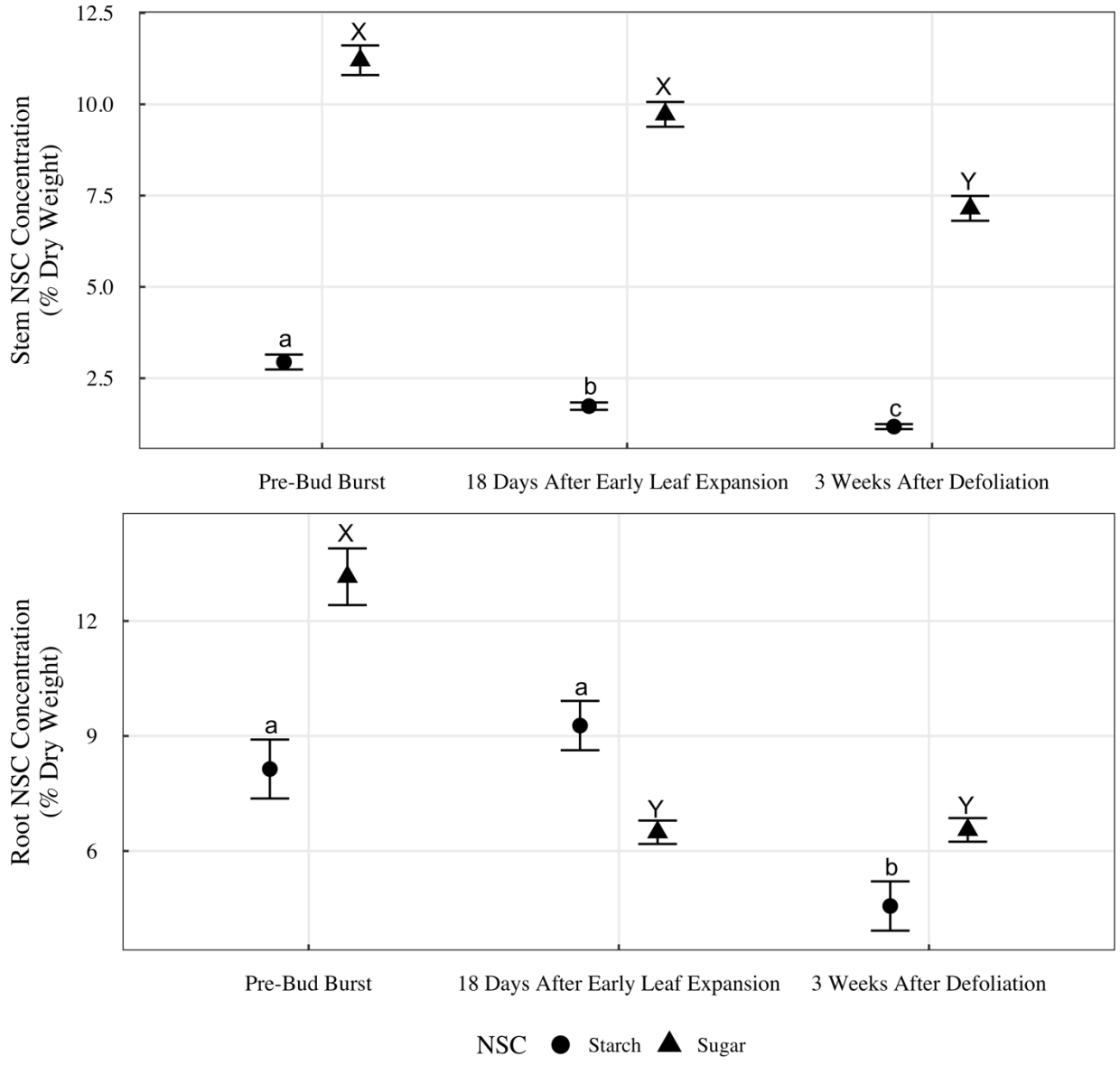

Figure 2-4. Estimated marginal means (± 1 standard error) of (top) stem NSC concentration (% dry weight) and (bottom) root NSC concentration (% dry weight) for saplings harvested at three timepoints (pre-bud burst, 18 days after early leaf expansion and 3 weeks after defoliation) (circle represents starch concentration and triangle represents sugar concentration). Letters indicate statistical differences among timepoints using post-hoc comparisons with a Benjamini-Hochberg adjustment ($\alpha < 0.05$).

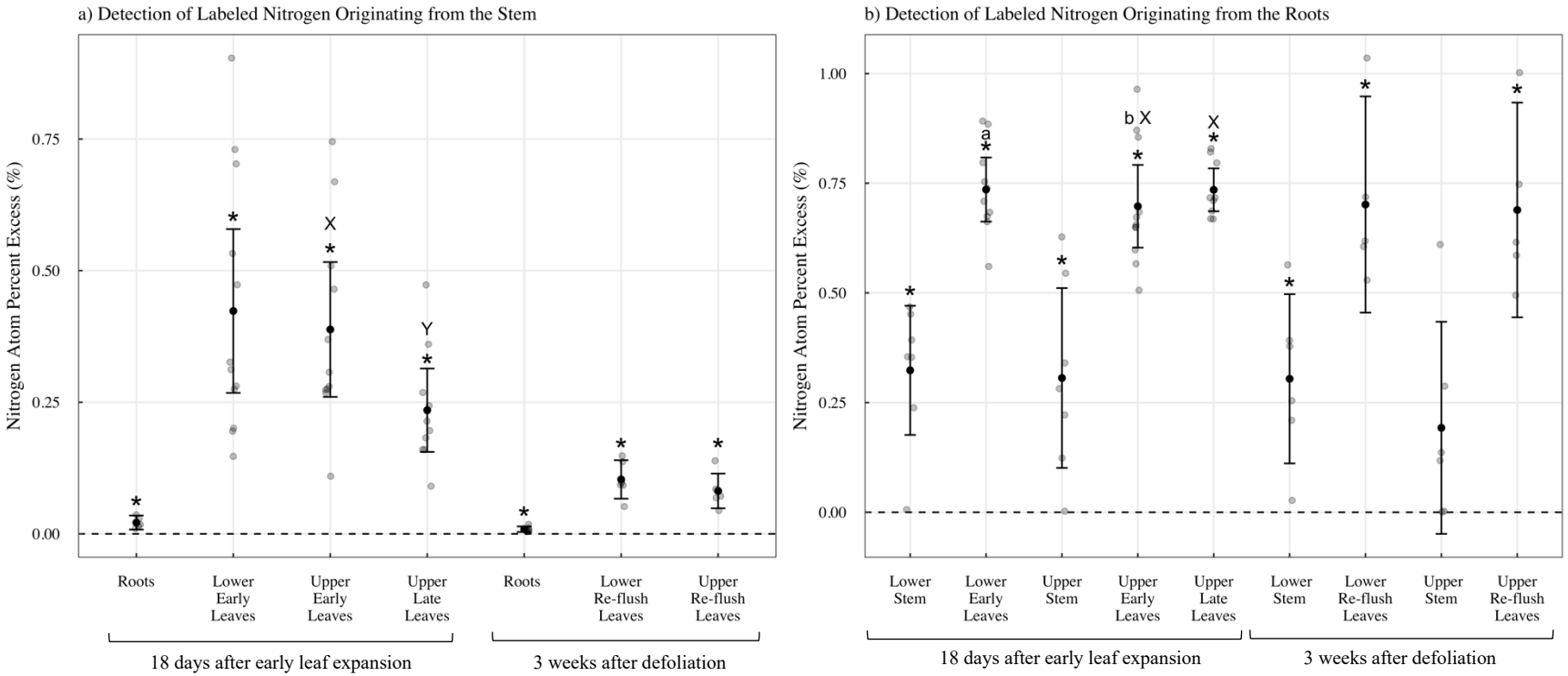

Figure 2-5. Mean (± 95% confidence interval) nitrogen atom percent excess (%) overlapping raw data points (a) for stem origin saplings, detected in tissue types (roots, lower early leaves, upper early leaves and upper late leaves) harvested 18 days after early leaf expansion and detected in tissue types (roots, lower re-flush leaves and upper re-flush leaves) harvested 3 weeks after defoliation, and (b) for root origin saplings, detected in tissue types (lower stem, lower early leaves, upper stem, upper early leaves and upper late leaves) harvested 18 days after early leaf expansion and detected in tissue types (lower stem, lower re-flush

leaves, upper stem and upper re-flush leaves) harvested 3 weeks after defoliation. * indicates a statistical difference from zero, with p-values adjusted using the Benjamini-Hochberg method, while lowercase letters (distance effect) and uppercase letters (timing effect) indicate statistical differences at $p < 0.05$ between tissue types ($\alpha < 0.05$).

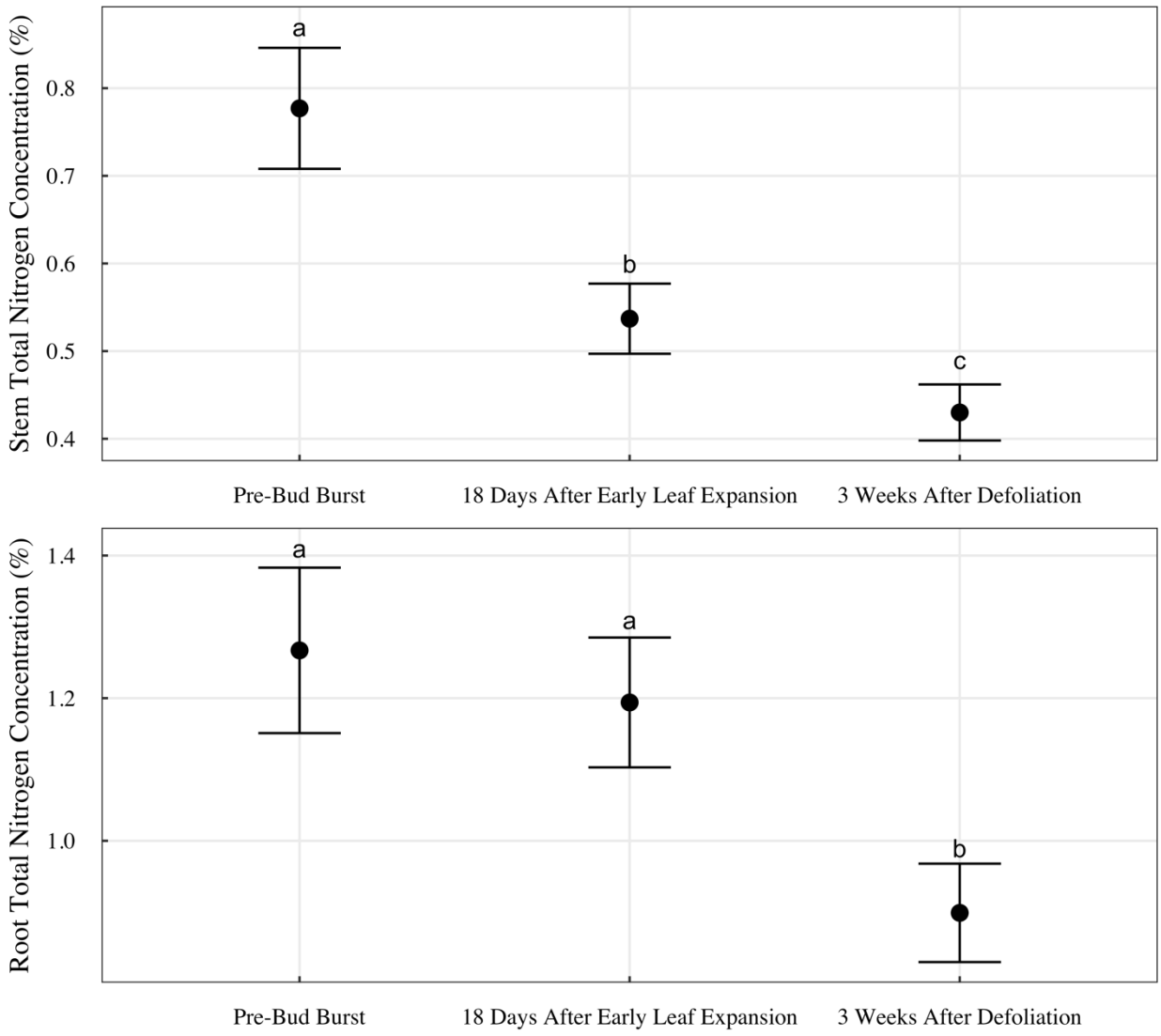

Figure 2-6. Estimated marginal means (± 1 standard error) of (top) stem total nitrogen concentration (%) and (bottom) root total nitrogen concentration (%) for saplings harvested at three timepoints (pre-bud burst, 18 days after early leaf expansion and 3 weeks after defoliation). Letters indicate statistical differences among timepoints using post-hoc comparisons with a Benjamini-Hochberg adjustment ($\alpha < 0.05$).

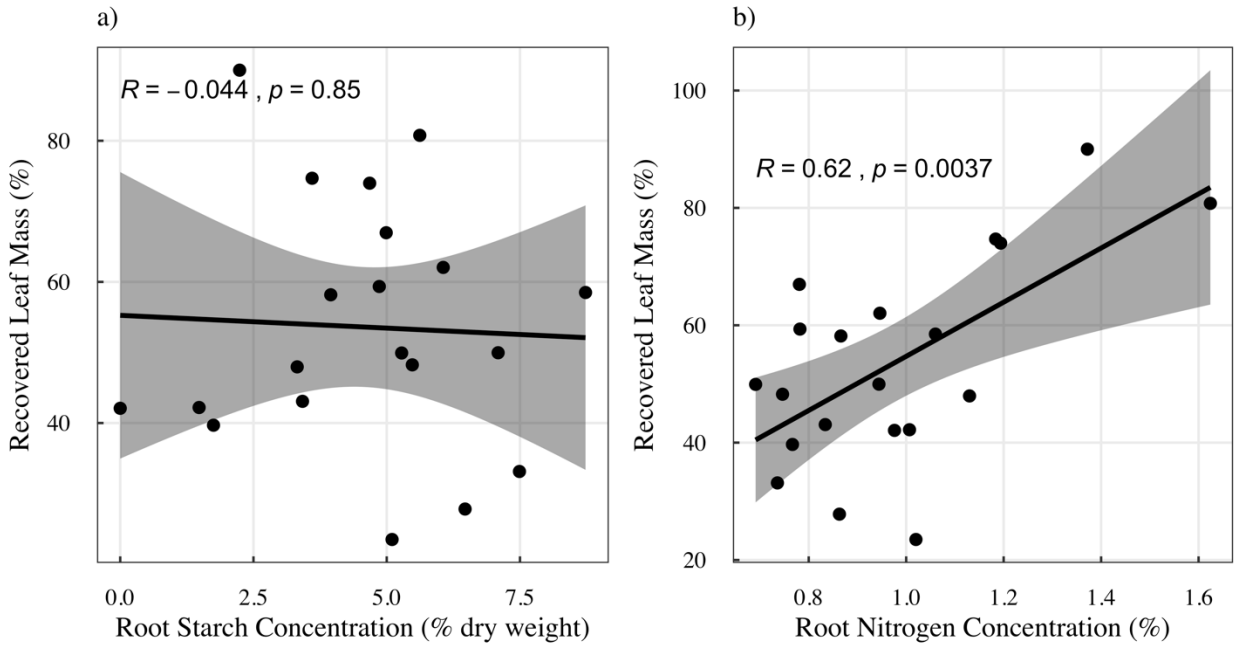

Figure 2-7. Correlation between percentage of recovered leaf mass and a) root starch concentration (% dry weight) and b) root total nitrogen concentration (%) at 3 weeks following defoliation.

Chapter 3: Splitting the difference – heterogeneity of soil moisture availability affects aboveground and belowground reserve and mass allocation in trembling aspen

3.1 Abstract

When exploring the impact of resource availability on perennial plants, artificial treatments often apply conditions homogeneously across space and time, even though this rarely reflects conditions in natural systems. To investigate the effects of spatially heterogeneous soil moisture on morphological and physiological responses, trembling aspen (*Populus tremuloides*) saplings were used in a split-pot experiment. Following the division of the root systems, saplings were established for a full year and then subjected to either heterogeneous (portion of the root system exposed to non-lethal drought) or homogeneous (whole root system exposed to non-lethal drought or well-watered) treatments. Above- and belowground growth and non-structural carbohydrate reserves (soluble sugars and starch) were measured to determine how allocation of reserves and mass between and within organs changed in response to variation in soil moisture availability.

In contrast to saplings in the homogeneous drought treatment, which experienced reduced shoot growth, leaf abscission and fine root loss, saplings exposed to the heterogeneous conditions maintained similar aboveground growth and increased root system allocation compared to well-watered saplings. Interestingly under heterogeneous soil moisture conditions, the portion of the root system that was resource limited had no root dieback and increased carbon reserve concentrations, while the portion of the root system that was not resource limited added new roots (30% increase). Overall, saplings subjected to the heterogeneous soil moisture regime over-compensated belowground, both in mass and non-structural carbohydrate reserves. These results indicate that the differential allocation of mass or reserves between above- and belowground

organs, but also within the root system can occur. While the mechanisms and processes involved in these patterns are not clear, these responses could be interpreted as adaptations and acclimations to preserve the integrity of the entire sapling and suggests that different portions of plant organs might respond autonomously to local conditions. This study provides further appreciation of the complexity of the mechanisms by which plants manage heterogeneous conditions and offers evidence that spatial and temporal variability of resource availability, particularly belowground, needs to be accounted for when extrapolating and modelling stress responses at larger temporal and spatial scales.

3.2 Introduction

As the climate changes, the stochasticity of precipitation events is predicted to increase, and droughts are expected to become more intense and more frequent (IPCC 2013). These changes have the potential to produce novel soil moisture conditions for many species (Harte and Shaw, 1995; Fridley et al., 2011; Metz and Tielbörger, 2016). Root systems of long-lived plants, such as trees will likely need to acclimate both morphologically and physiologically to these changing soil moisture conditions to ensure long-term survival. Controlled drought studies of potted plants have provided valuable insights into how species may respond to a drier future; however, these studies have several drawbacks. One issue is that the soil medium of pot-grown plants tends to be unrealistically homogenous, yet spatial and temporal heterogeneity in moisture is an inherent characteristic of soil ecosystems (Loranty et al., 2008; Guswa 2012; Vereecken et al., 2014). Water availability varies both horizontally and vertically throughout a soil profile and is driven by topographical variability (*e.g.* hillslopes), soil pedogenesis and associated differences in soil properties (Chamran et al., 2002; Tromp-van Meerveld and McDonnell, 2005), vegetation cover

and climate dynamics (Berry et al., 2006; Legates et al., 2010; Seneviratne et al., 2010). Therefore, as emphasized by Hutchings and John (2004), controlled studies investigating plant responses to heterogeneous distributions of environmental resources, whether that be moisture, space, light, or nutrients, are necessary to strengthen our understanding of plant growth and behaviour, especially for the prediction of species responses and forest dynamics under future climate scenarios.

There has been considerable exploration of how trees and seedlings respond morphologically and physiologically to drought conditions (Breda et al., 2006; Brunner et al., 2015). However, knowledge of plant responses to hetero- vs. homogeneous soil moisture conditions is lacking, in particular how plants may alter the allocation of resources to maintain plant functionality and potentially survival in response to spatial variation. Recognizing how perennial plants balance the allocation of remobilized and newly acquired carbon between and within above- and belowground organs (Bloom et al., 1985; Chapin et al., 1990; Eissenstat, 1997) to structural components (Poorter and Nagel, 2000; Poorter et al., 2012), such as cellulose, hemicellulose and lignin, and/or to non-structural components (Magel et al., 2000; Dietze et al., 2014) such as soluble sugars, starch and secondary compounds, is critical to our understanding of plant stress responses. Based on studies simulating drought conditions that are spatially homogeneous, plants are known to respond to increasing water stress by reducing shoot growth, shedding leaves, reducing stomatal conductance and accumulating solutes in aboveground tissues to maintain turgor and limit xylem cavitation and desiccation (Rood et al., 2000; Arango-Velez et al., 2011; Galvez et al., 2011; Claeys and Inzé, 2013; Buckley et al., 2017). Belowground, as soil water potential decreases and the rhizosphere progressively dries, common responses include structural root growth and/or accumulation of solutes in root tissues to maintain a more negative water potential than the surrounding soil (Meier and Leuschner, 2008; Markesteijn and Poorter,

2009; Galvez et al., 2013). Yet when assessing belowground responses, we must consider that under natural conditions, soil moisture availability is often heterogeneous, and since root systems are capable of exploring and proliferating into favorable patches of soil resources (Drew, 1975; Hutchings and de Kroon, 1994), there is also the potential for distinct morphological and physiological adaptations within a root system depending on the conditions experienced by different parts of a root system (Gersani and Sachs, 1992). Thus, characterizing how carbon is allocated within perennial plants that are subjected to more natural heterogeneous soil moisture conditions could provide more accurate insights into drought avoidance and tolerance mechanisms, as well as how those impact our understanding of hydraulic failure and/or carbon starvation responses (McDowell, 2008; Sala et al., 2010; Pinheiro and Chaves, 2011; Adams et al., 2017).

To investigate how newly assimilated and remobilized carbon may be allocated within both aboveground and belowground organs in response to spatially variable soil moisture conditions, we selected a widely distributed tree species, trembling aspen (*Populus tremuloides* Michx.). Trembling aspen is well-known for its clonal root system, which is essential for its regeneration (root suckering) after disturbance (Peterson and Peterson, 1992; DesRochers and Lieffers, 2001; Frey et al., 2004; Wiley et al., 2019). The clonal root system of aspen is large and consists of interconnected lateral roots which can span across large gradients of soil moisture availability (Day, 1944; Snedden, 2013). While responses to both severe and moderate drought have been previously studied in aspen seedlings and large trees (Braatne et al., 1992; Galvez et al., 2011; Anderegg, 2012; Hogg et al., 2008; Frey et al., 2004), no studies have determined how aspen's drought response – particularly allocation patterns within different portions of a root system – is modulated by the heterogeneity of soil water availability.

The objective of this study was to characterize the morphological and physiological response of aspen saplings that had all or portions of their root systems exposed to progressive, non-lethal drought conditions. Specifically, we assessed the influence of hetero- and homogeneous soil moisture conditions on the aboveground and belowground allocation of non-structural carbohydrate (NSC) components (soluble sugars and starch) and of other mass components (non-NSC, mostly structural) using a split-pot experiment. We hypothesized that saplings subjected to heterogeneous soil moisture conditions would compensate for the partial stress by preferentially increasing carbon (i.e. structural mass and non-structural carbohydrates) allocation towards the root system, accompanied by a decrease in aboveground growth. We also hypothesized that under heterogeneous soil moisture conditions, a sapling would allocate relatively more carbon to the drought exposed portion of the root system compared to a root system that was exposed to a homogeneous drought, as under these soil moisture conditions carbon acquisition and investment into growth would be greatly reduced. Furthermore, we hypothesized that the portion of the root system exposed to non-limiting soil moisture conditions in the heterogeneous treatment would respond similarly to a root system that was exposed to homogeneous non-limiting conditions.

3.3 Material and Methods

3.3.1 Split-pot design

A split-pot apparatus was used to spatially split the root systems of each tree sapling to allow for portions of a common root system to be independently exposed to different soil moisture conditions (Gowing et al., 1990; Fort et al., 1997; Sakuratani et al., 1999; Hirota et al., 2004) (Figure 3-1). Split-pots were constructed using two square Kordlock pots (10 × 10 × 14 cm tall) stapled together. A square section of rubber liner (Pond Building Series, reinforced PVC pond

liner, plant compatible) was glued and sealed with waterproof caulking over the joining portion of the two pots to prevent any water transfer along the edges of the pot. Reinforced tape was wrapped around the two joined pots and a 2.5 cm foam block was glued between the two pots to increase rigidity of the split-pot apparatus. To monitor soil moisture conditions, one half of the split-pot had a matric potential sensor (dielectric water potential sensors MPS2, Decagon Devices, Inc., Pullman, WA, USA) installed through a hole in the pot wall and was sealed into position with waterproof caulking. A piece of very fine mesh was placed at the bottom of each pot to prevent soil loss during watering. A sifted mineral agricultural topsoil with a sandy-loam texture was used as a growing medium. Each pot was then filled with the same weight of sifted soil and compacted to the same soil volume in the pot. A soil water potential response curve was created for the soil at the same bulk density as found in the split-pots, using the pressure extractor method to assess hydraulic properties to ensure a better control of drought conditions (Reynolds and Clarke Topp, 2008) (Appendix; Figure A-1).

Fifty one-year old nursery grown containerized trembling aspen saplings (6 cm diameter and 15 cm deep) grown from open-pollinated seed sources of Central Alberta (Smoky Lake Forest Nursery, AB, Canada) were used. During planting, the existing root system of each sapling was carefully split by first removing some of the growing medium. Care was then taken to equally divide the root system to accommodate the split-pot design. Separation of the root system was accomplished by dividing the total number of major lateral roots in half and planting them in each pot with the root collar of the sapling sitting on the pot joint (Figure 3-1). The presence and position of a large root within the split-pot was recorded for each sapling in all treatments to assure similar root distribution across treatments. A small piece of burlap was wrapped around the root collar to cover the exposed section of the root system sitting on the joint between the pots, preventing

desiccation and root death during early establishment of the saplings. Saplings were watered regularly and fertilized once using 10-52-10 N-P-K fertilizer (Agrium, Inc., Calgary, AB, Canada). The saplings were kept outside at the University of Alberta (Edmonton, AB, Canada) for 20 weeks (July 4th, 2016 – November 21st, 2016) to fully establish and allow the root system to occupy both pots and produce a healthy crown.

To prevent root damage from soil temperatures that were well below freezing (< -5˚C), the saplings were moved to a dark growth chamber in November 2016. The chamber was set at a constant temperature of -1°C for a period of six weeks to allow saplings to accumulate additional chilling hours. Saplings were watered regularly with a small volume of water during that time, approximately 20 mL weekly, to prevent soil desiccation. After the six-week dormant period, saplings were exposed to progressively higher air temperatures and increased light conditions over a period of seven weeks (Appendix; Table A-4). In that period, temperature increased to a maximum of 18°C during the day and 16°C at night with 12 hours of light (500 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR)) which simulated spring conditions (Appendix; Table A-4). Relative humidity in the chamber was maintained at 60% throughout the period. During the seven-week spring period, saplings were watered daily, fertilized weekly with 50 mL of 1 g L^{-1} 15-30-15 N-P-K fertilizer (Agrium, Inc., Calgary, AB, Canada) and pots were rotated weekly to minimize spatial variability. Of the 50 saplings grown initially, only 29 saplings were considered healthy (*i.e.* successfully flushed and produced new large leaves and elongated new shoots) and were used for the remainder of the experiment.

3.3.2 Experimental period and application of watering treatments

The experimental period (4 weeks) started at the beginning of March 2017, during which the growth chamber conditions were set to 20°C both day and night with a 17-hour light/7-hour dark cycle, a relative humidity at 60% and PAR of 500 μmol m^{-2} s^{-1}. Initial measurements of height and root collar diameter were taken on all saplings generating four groups with similar sapling size distributions (total n = 29). Six saplings were harvested at the beginning of the experimental period (Initial). The remaining saplings were separated into treatment groups based on three soil moisture regimes: eight saplings were assigned to have both pots watered to field capacity (homogeneous well-watered treatment: WW); another set of eight saplings had one pot watered to field capacity (wet pot) while the other pot underwent a progressive dry-down (dry pot) (heterogeneous soil moisture: localized drought treatment: LD); and the remaining seven saplings had both pots undergoing a progressive drought (homogeneous full drought treatment: FD) (Figure 3-1). For all saplings, soil water potential was recorded every 15 minutes in one of the wet pots in the WW treatment, one of the dry pots in the FD treatment, and in the dry pot of the LD treatment, using the installed soil water potential sensors connected to EM50 dataloggers (Decagon Devices Inc. Pullman, WA, USA). For the first week of the experimental period, saplings received water (only), but then for the remainder of the experiment, water that included a 2 g L^{-1} solution of 15-30-15 N-P-K fertilizer (Agrium, Inc., Calgary, AB, Canada). Saplings were moved weekly to different positions on the growth chamber benches to reduce any effects of spatial variability in the ambient conditions.

For the WW treatment and the wet pot of the LD treatment, at the start of each daytime period, each pot was watered to saturation and then allowed to drain freely reaching field capacity.

For the dry pot of the LD treatment and the FD treatment, a progressive drought was applied. To apply the drought in the LD treatment, the initial starting weight at field capacity for the entire split-pot was determined at the start of the experimental period. During the experimental period, the wet portion of the split-pot was always re-watered to field capacity (watered to saturation and allowed to completely drain) at the beginning of the day, then the entire split-pot was weighed, and the difference to the initial (previous) weight was attributed to the water loss from the dry pot only. The dry pot was then watered with half of the water loss amount, based on the weight lost, thus contributing to a gradual decrease in soil water potential over a period of 4 weeks (Appendix; Figure A-2). For the last two weeks of the experimental period, the soil water potential in the dry pots was maintained between -700 kPa to -1200 kPa, with the pots receiving only small water additions (<5 g) during the last four days (Appendix; Figure A-2). Soil water potentials were maintained within this range to avoid catastrophic drought-induced cavitation, as previous research has demonstrated that an average xylem pressure below -1200 kPa can produce a loss of hydraulic conductivity greater than 50% in aspen (Plavcova and Hacke, 2012; Fichot et al., 2015; Schreiber et al., 2016). A similar watering strategy was applied to the FD treatment; however, here both sides of the split-pot were subjected to the same progressive drought, with both pots receiving water in the amount replacing only half the water lost from the previous day and then maintained at the same range of water potential to achieve a similar drought intensity at the pot-level compared to the LD dry pot (Appendix; Figure A-2).

3.3.3 Measurements

To monitor physiological responses, net assimilation and stomatal conductance were measured with a LI-6400XT portable photosynthesis system (LiCor, Inc., Lincoln, NE, USA) once

a week throughout the experimental period on three saplings (two leaves each) randomly selected within each treatment. Leaf chamber light was set at 800 µmol m^{-2} s^{-1}, CO_2 was set at 400ppm, incoming relative humidity at 60% and leaf temperature at 20°C to mimic the conditions in the growth chamber. Net assimilation and stomatal conductance were measured 2 hours after the beginning of the daytime period.

At the end of the experimental period, the final height and root collar diameter (RCD) were measured on all saplings at harvest. Saplings were separated into leaves, stem (old and new primary growth) and the two portions of the split root system. Projected leaf area was measured for each sapling in the LD and the WW treatments using a LI-3100 area meter (LiCor, Inc., Lincoln, NE, USA). Leaf area could not be measured for the FD saplings, as dead leaves in the FD treatment were too brittle to measure. The portions of the root system were extracted separately from each side of the split-plot and kept separated. After careful removal of all soil, fresh root volume, which more closely represents the root surface area (estimate of water uptake potential), was measured for each portion of the split root system via the water displacement method (Harrington et al., 1994). All collected tissues from each sapling were dried for 1 hour at 100°C to denature enzymes, followed by 48 hours at 70°C to constant weight. Dried root samples were separated into coarse roots (diameter > 1 mm) and fine roots (diameter < 1 mm). All dried material was weighed, and samples were ground to a 40-mesh (0.4 mm) using a Thomas mini Wiley mill (Thomas Scientific, Inc., Swedesboro, NJ, USA) for subsequent non-structural carbohydrate analysis.

Non-structural carbohydrates (NSC) were analyzed following the protocol described in Landhäusser et al. (2018). In brief, total soluble sugars were extracted in 80% hot ethanol followed

by a phenol-sulfuric assay to determine their concentration colorimetrically by measuring the absorbance at 490 nm with a spectrophotometer. To determine starch concentration, the remaining pellet was digested with α-amylase (Sigma cat. no. A4551) and amyloglucosidase (Sigma cat. no. ROAMYGL). A peroxide-glucose oxidase/o-dianisidine reagent was then added to the resulting glucose hydrolysate. After incubation, concentrated sulfuric acid was added before measuring absorbance at 525 nm. Absorbance values were used to calculate sugar and starch concentrations by comparison with standard curves and expressed as percent of sample dry weight.

3.3.4 Calculations and statistical analyses

The following calculations were used to compare the effects of heterogeneous soil moisture versus uniform soil moisture on the allocation of structural and non-structural (soluble sugars and starch) components between aboveground and belowground tissues and within the root system (*i.e.* between the split-pots). Treatment effects on growth were assessed using sapling height, root collar diameter (RCD) and biomass. Height and RCD growth during the experimental period were calculated by subtracting the initial height and RCD measured on each treated sapling at the beginning of the experiment from the final height and RCD of that same sapling. To evaluate changes in leaf and stem mass that occurred during the 4-week experimental period (*i.e.* new leaf and new stem growth), the average of initial measurements, taken from the six destructively sampled saplings at the beginning of the experimental period, were subtracted from the individual final treatment measurements of leaf mass (which included dead leaves in the FD treatment) and the mass of primary stem growth. Specific leaf area was calculated by dividing total leaf area by total leaf dry mass. To determine mass allocation in saplings, the ratio of leaf, stem or root mass to total sapling mass was calculated for each sapling. Leaf, stem and root non-structural

carbohydrate (NSC) mass (pools) were estimated by multiplying the total sugar and starch concentrations by the total dry mass of each sampled organ. Further, the remaining mass (hereafter called structural mass) that was not related to reserve mass of each organ was estimated by subtracting the respective NSC mass from the total dry mass of each organ. These measures were estimated to evaluate any differences in leaf structural mass, stem structural mass and root structural mass in response to our treatments. In addition, root structural density was calculated as a ratio of the structural mass of the entire root system (fine and coarse roots combined) over the measured root volume (fine and coarse roots combined), to explore potential changes in the morphological composition of the root system.

All data were analyzed using R statistical software v3.5.1 (R Development Core Team, 2018). Assumptions of normality and homoscedasticity were tested using the Shapiro-Wilks test and Levene's test for parametric analyses. If these assumptions were not met, removal of outliers and transformations were applied. The soil water potential data was fit with a logistic non-linear model using the nlme package (Pinheiro et al. 2016) to show the gradual dry down of soil within the split-pots (Appendix; Figure A-2). No differences in root measures between the two sides of the split-pots were found in either well-watered or full-drought treatments using t-tests (data not shown; $p > 0.1$). Thus, in subsequent analyses, the two sides of the split-pots in these treatments are considered equivalent. Two statistical analyses were applied to the data. First, to test for differences among the initial harvest and three treatments for aboveground and combined belowground measures, one-way ANOVA was used followed by pairwise post-hoc tests with a Benjamini-Hochberg adjustment using the emmeans package (Lenth, 2019). Second, to understand how the localized drought (LD) treatment impacted *within*-root system response, a linear mixed model was used with pot-type (LD-dry, LD-wet, FD, WW) as a fixed factor.

Individual sapling was included as a random factor to account for the fact that the same individual was repeatedly measured (2 pots per sapling). The initial presence/absence of a large root within a pot was also included as a random factor to account for the fact that a large root could impact pot-level variables like final root mass, volume, etc. The post-hoc tests for these models were restricted to the following planned comparisons: 1) dry pots versus the wet pots of the LD treatment, 2) dry pots of the LD treatment versus the FD treatment pots, and 3) wet pots of the LD treatment compared to the WW treatment pots. The Benjamini-Hochberg adjustment was used with the emmeans package. Differences among treatments or between sides of the split-pot were considered statistically significant at $\alpha = 0.1$. Estimated marginal means and standard errors are reported in the Results and Discussion sections.

3.4 Results

3.4.1 Growth and mass allocation

Overall, saplings exposed to the localized drought (LD) had various aboveground measures that were similar in comparison to the well-watered (WW) saplings but were greater than those measures in saplings of the full drought (FD) treatment. Although average height growth did not differ among the three treatments ($p > 0.18$; Table 3-1), the LD and the WW saplings had overall larger root collar diameters, with over three times more RCD growth compared to the FD saplings ($p < 0.01$; Table 3-1). Total aboveground dry mass was approximately 30% greater in the LD and the WW treatments compared to the FD treatment, for which no significant increase in aboveground mass occurred ($p < 0.01$; Table 3-1). Saplings in the LD and the WW treatments produced 1.74 g and 2.34 g, respectively, of new leaf mass during the four-week experimental period, while saplings in the FD treatment did not produce any new leaves ($p = 0.03$; Table 3-1).

Saplings in the FD treatment also experienced partial browning of pre-existing leaves and significant leaf abscission prior to harvest. Specific leaf area was similar between the LD and the WW saplings (Table 3-1). During the experimental period, stem growth of the WW saplings was 1.27 g, which was similar to the stem growth of the LD saplings (0.87 g) and greater than the stem growth of the FD saplings (0.36 g; $p < 0.01$) (Table 3-1). The allocation of mass to leaves (25%) and stems (30%) was statistically similar among the three soil moisture treatments (Table 3-1), although the allocation to leaves in the FD treatment is likely lower if only live leaf mass had been considered. During the period when the soil water potential was maintained between -700 kPa to -1200 kPa, there were no differences in the net assimilation rate and stomatal conductance between the LD saplings (7.74 ± 1.23 μmol_{CO_2} m^{-2} s^{-1} and 0.196 ± 0.029 $mmol_{H_2O}$ m^{-2} s^{-1}, respectively) and the WW saplings (8.62 ± 1.23 μmol_{CO_2} m^{-2} s^{-1} and 0.243 ± 0.029 $mmol_{H_2O}$ m^{-2} s^{-1}, respectively), whereas the FD saplings had significantly lower net assimilation and stomatal conductance compared to the other two treatments (-1.50 ± 1.73 μmol_{CO_2} m^{-2} s^{-1} and 0.019 ± 0.041 $mmol_{H_2O}$ m^{-2} s^{-1}, respectively; both $p < 0.01$).

Localized drought saplings had a total root dry mass (both pots combined) of 15 g, similar to the 12.4 g of WW saplings, and nearly 50% more compared to the FD saplings (10.1 g, $p = 0.01$) (Table 3-1). Full drought saplings had a total root dry mass that was similar to the initial saplings (Table 3-1). However, when comparing total root volume which relates to root surface area and its potential for water uptake, FD saplings also had a total root volume of 37.2 cm^3 at harvest, which was significantly lower than the LD and the WW saplings (63.1 cm^3 and 58.3 cm^3 respectively; $p = 0.01$ and $p = 0.02$, respectively), but also significantly lower than the average initial root volume of 59.1 cm^3 ($p = 0.03$) (Table 3-1). Root structural density of the root system, a measure that indicates potential changes in root system morphology, was overall higher in all

three experimental treatments after the four-week experimental period compared to the start of the study (0.141 g cm^{-3}; $p < 0.01$). However, while the root systems of the LD and WW saplings had similar root structural density at the end of the experiment (0.203 g cm^{-3} and 0.186 g cm^{-3}, respectively), root structural density in FD saplings was higher than both treatments (0.247 g/cm^3; both $p < 0.01$) (Table 3-1). Overall, the LD saplings and the FD saplings allocated a greater amount of mass towards the root system (45%) compared to the WW saplings (38%) ($p = 0.07$; Table 3-1). This suggests that the WW saplings allocated more mass to the aboveground variables, such as height growth, stem and leaf mass which all tended to be greater in the WW saplings, but we could not detect significant statistical differences between the LD and the WW saplings ($p = 0.2$, $p = 0.1$ and $p = 0.1$, respectively).

A closer analysis of belowground measurements between the dry and the wet root system portions in the split-pots of the LD saplings revealed distinct patterns of allocation. Under the localized drought conditions, saplings allocated more mass to the roots within the wet pot (8.33 g) than to the roots within the dry pot (6.70 g; $p = 0.01$; Figure 3-2A). This greater root mass in the wet pot can be attributed to an increase in fine root production compared to the dry pot ($p < 0.01$; Figure 3-2C). The portion of the root system contained in the wet pot of the LD treatment also had a greater mass (8.33 g), comprised of significantly more fine roots ($p = 0.02$; Figure 3-2C) than either section of the root systems in the WW treatment (6.19 g; $p = 0.03$) (Figure 3-2A). The root system portion contained in the dry pot of the LD treatment was greater in mass, with a significant increase in fine roots ($p = 0.09$; Figure 3-2C), compared to either root system portion in the FD treatment (5.06 g; $p = 0.08$; Figure 3-2A). When comparing root system volumes, which relate to root surface area and potential for water uptake, across the split-pots, the root system portion in the wet pot of the LD treatment had a greater volume (37.51 cm^3) than the root portion in the dry

pot (25.63 cm^3; $p < 0.01$; Figure 3-2D). However, the root volume in the wet pot of the LD treatment was greater than the volume of the root portions measured in the WW treatment (29.14 cm^3; $p = 0.08$; Figure 3-2D). Although there was a trend for the dry portion of the root system in the LD treatment to have a greater volume (25.63 cm^3) than in the FD treatment (18.6 cm^3) the difference was not statistically significant ($p = 0.12$; Figure 3-2D).

3.4.2 Non-structural carbohydrate concentrations

At the end of the 4-week period, there were only a few differences in the starch concentrations in the aboveground tissues among the three watering treatments, while stark differences existed in the root NSC concentrations in response to the different soil moisture conditions. Aboveground, there were no differences in total NSC (sum of starch and soluble sugars) and sugar concentrations in leaf tissue among the three watering treatments, and these concentrations did not differ from the initial measurement (Table 3-2). However, the FD saplings had lower leaf starch concentrations (0.20%) compared to the LD and the WW saplings (0.78% and 0.66%, respectively; $p < 0.01$ and $p = 0.01$, respectively; Table 3-2). In comparison to the initial measurement, stem NSC concentrations did not change in LD saplings (10.28% versus 9.66%, respectively), WW saplings (8.27%) or FD saplings (8.52%) (Table 3-2). Among treatments, the LD saplings had a similar stem starch concentration (1.31%) compared to WW saplings (0.73%; $p = 0.12$), yet a significantly greater starch concentration compared to the FD saplings (0.05%; $p < 0.01$) (Table 3-2). Only the LD saplings increased stem starch concentrations over the initial measurement of 0.38% ($p = 0.01$; Table 3-2). Stem sugar concentrations prior to the start of the experiment were 9.87% which decreased slightly during the experimental period (p

< 0.06), but no differences were detected in soluble sugar concentrations in the stems among the three watering treatments (Table 3-2).

Belowground, the total NSC concentrations found across the entire root system (both pots combined) did not differ from the initial measurements or among the three watering treatments (Table 3-2). However, when the NSC concentrations of the root systems were compared between the split-pots, the roots in the dry pot of the LD treatment had higher NSC concentrations (18.35%) than the roots in the wet pot of the LD treatment (13.03%; $p < 0.01$; Figure 3-3). Furthermore, the roots in the dry pot of the LD treatment had higher NSC concentrations than the roots in the FD treatment (12.82%; $p = 0.03$) (Figure 3-3). In contrast, NSC concentrations of the roots in the wet pot of the LD treatment (13.03%) did not differ from those in the WW treatment (12.83%) (Figure 3-3). When broken down into soluble sugar and starch, roots in the dry pot of the LD treatment had higher soluble sugar (4.85%) and starch (13.44%) concentrations than the roots in the wet pot, with 3.51% and 9.49%, respectively ($p < 0.01$ and $p = 0.05$, respectively; Figure 3-3). The roots in the wet pot of the LD treatment had soluble sugar and starch concentrations similar to the roots in the WW treatment. The roots in the FD treatment had higher soluble sugar (9.54%) but lower starch (3.11%) concentrations compared to the roots in the dry pot of the LD treatment (both $p < 0.01$; Figure 3-3).

3.4.3 Allocation to structural mass and non-structural carbohydrate pools

The non-structural carbohydrate (NSC) concentrations in the leaves, stem and roots of each individual sapling were used to estimate the structural mass and the NSC pool sizes and their relative allocation (% of total) in response to the soil moisture treatments. Of the total structural pool, the LD saplings allocated 24.2% to leaves, 32.1% to the stem, and 43.7% to roots (Table 3-

3). There were no differences in the relative allocation of structural mass to leaves among the three soil moisture treatments (Table 3-3). Well-watered saplings allocated slightly more structural mass to the stem in comparison to the LD saplings ($p = 0.09$; Table 3-3). However, while the relative allocation of structural mass to the root system was similar for the LD and the FD saplings, the WW saplings allocated less structural mass to the root system (37.2%) compared to both treatments (both $p = 0.03$; Table 3-3). Of the total NSC pool (sum of soluble sugars and starch), the LD saplings allocated 25.3% to leaves, 22.3% to the stem, and 52.4% to roots (Table 3-3). The relative allocation of NSC to leaves, stems and the root systems were similar among the three soil moisture treatments (Table 3-3).

Differences in the structural mass within the two portions of the split root system in the LD saplings were driven by the localized soil moisture conditions. Roots in the dry pot had less structural mass than the roots contained within the wet pot ($p < 0.01$) but did not differ from the amount of structural mass of a root system portion found in the FD treatment (Figure 3-4A). In contrast, the roots in the wet pot of the LD treatment had 30% more structural mass compared to the roots in the WW treatment ($p = 0.01$; Figure 3-4A). LD saplings had similar NSC mass in their dry and wet pots, however the dry portion of the root system had over double the NSC mass compared to either half of the root systems of the FD saplings ($p = 0.06$; Figure 3-4B). The NSC mass of roots in the wet pot of the LD treatment was not significantly different from either half of the root system in the WW treatment ($p = 0.4$; Figure 3-4B).

3.5 Discussion

Our study demonstrates that the root systems of aspen saplings subjected to heterogeneous water availability (LD treatment) responded unlike saplings with root systems that were exposed

to homogeneous soil moisture conditions (full drought (FD) or well-watered (WW)). Based on the heterogeneous conditions in the LD treatment, saplings responded quickly by partitioning structural mass and non-structural carbohydrates functionally across and within organs. As expected, saplings exposed to either the full or the localized water limitation increased overall allocation towards the root system (45%) compared to saplings growing in non-limiting conditions (38%). However, the saplings exposed to the full drought experienced reduced gas exchange, terminated aboveground growth, shed leaves and lost root volume. In contrast, the saplings exposed to the LD treatment maintained gas exchange and aboveground growth similar to the WW saplings, avoided root loss in the dry soil, while increasing root structural mass and volume in the wet soil. The different responses of the roots in the dry versus wet soil under the localized drought treatment suggests the potential for some autonomy within root systems to adaptively adjust allocation depending on the soil conditions individual roots are exposed to.

While the responses of aspen exposed to either homogeneous or heterogeneous drought appear to be consistent with the concept of functional equilibrium of biomass allocation or optimal partitioning theory (Brouwer, 1963; Thornley, 1972; Iwasa and Roughgarden, 1984; Bloom et al., 1985), where plants preferentially allocate biomass to the organ responsible for the uptake of the limiting resource (Poorter et al., 2012), the manner by which the saplings in both drought treatments arrived there is very distinct. In the LD saplings, the proportional increase in root mass was the result of a differential allocation towards the root system, while the increase seen in saplings in the FD treatment was mostly the result of a differential mortality of organs. Further, the saplings exposed to the heterogeneous water availability responded with significant increases in leaf, stem and root mass, but attained the higher root mass ratio (also root to shoot ratio) by allocating more carbon to root growth relative to shoot growth. In contrast, the increase in the root

mass ratio of the FD saplings was the result of terminated primary growth and a greater net tissue loss in above- versus belowground parts; this increase would have been even larger if we had discounted the abscised leaves in this treatment. Leaf and branch shedding have been hypothesized as key drought adaptations in *Populus* species, as it decreases transpiration loss through leaf area (Rood et al., 2000; Galvez et al., 2011). The loss in root mass in the FD saplings was more difficult to discern, as the root mass of the FD saplings was similar to the initial root mass, it would appear that the root system of these saplings was maintained during the drought conditions. However, this observation is not supported by the reduction in root volume of the FD saplings from the initial volume, indicating that significant root death occurred (Table 3-1). It appears most likely that fine root mass was shed in these root systems (Figure 3-2C), while coarse roots remained viable and alive (Figure 3-2B). This is further supported by an increase in structural root density we observed in the FD treatment (Table 3-1), which might also indicate that only roots with higher densities were maintained. In grasses it had been observed that roots with higher density tend to have longer life spans than roots with lower density which are more likely to die (Ryser, 1996). Similar fine root deaths have been observed in other studies exploring drought and carbon limitation in aspen (Galvez et al., 2011, 2013; Wiley et al., 2017). Our observation of fine root and leaf abscission also supports the hypothesis that the more distal parts of plants whose role is primarily resource acquisition are potentially more expendable and/or more prone to damage under stress compared to other parts, such as larger diameter roots, stems and branches, which required significant investments over time and have additional crucial functions such as transport and storage within a plant (Kozlowski, 1973; Zimmerman, 1983; Tyree et al., 1993; Sperry and Ikeda, 1997; Landhäusser and Lieffers, 2012; Wiley et al., 2017). This may be especially important when considering a tree species (here aspen) with a root system that is adapted for long-distance resource

acquisition (Snedden, 2013), as well as for the storage of non-structural carbohydrates as reserves for post-disturbance regeneration (Wiley et al., 2019). Similar adaptations in root allocation have also been observed in other species growing in environments prone to water limitation and fire disturbance (Bell et al., 1996; Hoffman et al., 2004; Tomlinson et al., 2012).

The continued carbon acquisition in saplings under localized drought combined with an increased investment of carbon into the growth and maintenance of the root system allowed these plants to quickly adapt and generate new leaf mass and leaf area similar to the WW saplings, suggesting continued investment in leaf area development and its maintenance even under locally reduced water availability. Our results suggest that when a plant experiences drought but has access to areas in the soil that are less or non-limiting in moisture availability, the plant should be able to compensate. If the plant is able to use the portion of the root system that experiences the non-limiting conditions, it could potentially maintain the rest of the root system and continue to support aboveground growth and prevent leaf abscission. Interestingly, the LD saplings had overall similar total aboveground mass compared to the WW saplings, but they had less stem structural mass, suggesting that the WW saplings might have prioritized stem growth over other potential carbon allocation strategies. Instead, LD saplings increased stem starch concentrations from initial, which suggests a reserve storage strategy to potentially increase chances of rapid growth when conditions improve or for re-filling of xylem vessels when drought conditions worsen and cavitation occurs (Brodribb et al., 2010; Sala et al., 2012; Trifilò et al., 2017; Trugman et al., 2018). There were no differences in the overall NSC concentration of the combined root system among the three treatments. However, the FD saplings had two times higher soluble sugar concentration in the roots (9.54% dry weight) compared to the root system portion in the dry soil of the LD treatment (4.85% dry weight), which suggests a significant osmotic adjustment to improve the

acquisition of water from the soil and avoid water loss back to the soil. This response is expected as it is well established that under drought stress, a higher solute concentration in roots will reduce their water potential and therefore increase the passive movement of water from the soil into the roots, in an attempt to relieve plant stress (Chaves, 1991; Gebre et al., 1998; Arndt et al., 2001; Kozlowski and Pallard, 2002; Galvez et al., 2011). Interestingly, while the localized drought conditions also led to an increase in soluble sugar concentration in the drought exposed portion of the root system, this increase was over 30% (4.85% dry weight) relative to the wet roots. This differential response of sugar concentration between the roots in the full and the localized drought might suggest a different strategy for how these plants cope with low soil water availability affecting only a portion of a root system (see below).

The fact that there was a striking difference between the degree of sugar accumulation between a full droughted root system (FD) and a partially droughted root system (LD) indicates that homo- or heterogeneous water limitations trigger different allocation responses within a root system. When faced with heterogeneous soil moisture availability, a root system showed very distinct patterns of structural and reserve mass allocation. Under the localized drought conditions, structural mass was allocated towards the portion of the root system that experienced the non-limiting condition and led to overall higher root mass and root volume. The increased allocation to root growth in the wetter soil likely allowed the saplings to compensate for the reduced water uptake in the part of the root system that experienced the low moisture availability, allowing the plant to maintain gas exchange and growth rates aboveground that were similar to those in saplings experiencing no water limitation. Additionally, the portion of the root system that experienced the limiting conditions likely preserved some of its functionality, as it maintained its mass and volume. We speculate that the preservation of the drying portion of the root system may have been favored

in comparison to complete abscission, as the sapling had already invested in its establishment and the roots may be able to assist with future resource capture if the soil becomes rewetted. The benefits of maintaining a drying portion of a root system in comparison to complete abscission have been considered in other species subjected to heterogenous soil moisture conditions (Kosola and Eissenstat, 1994; Fort et al., 1997).

Since saplings in the LD treatment had access to water from the wet pot and the root system proliferated in these conditions, the demand for water supply from the dry portion of the root system was low, resulting in a reduced for need for osmotic adjustment via sugar accumulation (see above). However, the dry portion of the root system in a LD sapling accumulated significantly more starch than the wet portion of the root system, which might indicate a preferential allocation towards storage of reserves in the drier portion of the root system. These reserves would be available for future translocation or remobilization for growth, reproduction and/or other physiological processes such as osmotic adjustments in case drought conditions persist or worsen. This increase in NSC concentration in the dry portion of the root system could be driven by several possible mechanisms and processes within a plant. As mentioned previously, the lower soil water potential in the drought-exposed roots could have induced an active solute buildup for osmotic adjustment to allow for improved water uptake or the adjustment was more passive, where a lower turgor in the dry portion of the root system may have limited its growth, leading to an accumulation in NSCs due to reduced growth demand (Körner, 2003). Alternatively, the heterogeneous water availability could also have created a steeper gradient in water potentials across the entire root system, allowing for lateral water redistribution within the root system, increasing the hydration and with that the maintenance of functionality in the drought-exposed portion of the root system. Similar responses have been observed in other studies and species (Burgess and Bleby, 2006;

Bleby et al., 2010; Prieto et al., 2012). By hydrating the roots experiencing water limitations from the portion of the root system that experienced less or non-limiting conditions, the risk of root cavitation and desiccation of the drought-exposed roots was likely reduced and these roots were more likely to maintain contact with the soil, enabling continued resource acquisition and other functional interactions such as mycorrhizae (Bleby et al., 2010).

This study highlights the importance of considering spatial heterogeneity of belowground resources when explaining above- and belowground responses of trees to stress. This is particularly important when studying mature trees that have extensive root systems. These trees most likely experience considerable vertical and lateral moisture gradients in the rooting space and within a root system. Since our application of the drought treatment was applied at the pot-level to assure that the drought at the root level was comparable, we recognize that the drought effects at the whole plant level were different among our treatments. Exploring these relationships and responses on these root systems in greater detail is further complicated by the generally poor accessibility of whole root systems (Hartmann et al., 2018). However, the results of our study demonstrate an adaptability and a multi-faceted response of root systems of a perennial species exposed to heterogeneous soil moisture environments. Depending on the soil moisture conditions, the root systems we studied exhibited plasticity in carbon allocation between structural mass and NSC, with differences in allocation between and within organs. The aspen saplings appeared to optimize functionality of the root system during water limiting conditions that affected only a portion of the root system by increasing root volume where water was locally available and preferentially accumulating additional NSC where root growth was limited. Our study highlights a need for exploring other potential measures of carbon allocation under stress, such as measures of total carbon and nitrogen. Short-term responses, like those noted here, will likely have impacts on how

a plant will react to subsequent changes in stress conditions and might play a role in the adaptation and/or acclimation processes that have been observed in perennial plants exposed to stress over the short- and long-term (Rachmilevitch et al., 2008; Pomiès et al., 2017). In our short-term study, aspen saplings responded relatively rapidly to moisture stress by enhancing the functionality of the entire root system through adaptation (increase in root system size (LD) or leaf and root loss (FD)) and acclimation processes (accumulation of reserves), which can be considered beneficial even under prolonged drought conditions, as roots are a critical organ in aspen not only for resource uptake, but also for maintaining its resilience (*i.e.* vegetative regeneration) to disturbances.

3.6 Tables

Table 3-1. Estimated marginal means (±1 standard error) of aboveground and belowground variables: height growth (cm), root collar diameter (RCD) growth (mm), total sapling mass (g), total aboveground mass (g), new stem growth (g), leaf mass (g), new leaf growth (g), specific leaf area ($cm^2\ g^{-1}$), total root mass (g), total coarse root mass (g), total fine root mass (g), total root volume (cm^3), structural root density ($g\ cm^{-3}$), leaf mass ratio (%), stem mass ratio (%) and root mass ratio (%) prior to (INITIAL) and after three watering treatments (FD: full drought, LD: localized drought, WW: well-watered). Letters represent statistical difference (n = 29; $\alpha = 0.1$) among treatments using post-hoc comparisons with a Benjamini-Hochberg adjustment. NA: not applicable.

	INITIAL	FD	LD	WW
Height growth (cm)	NA	3.3 (1.3) **a**	4.1 (1.5) **a**	8.8 (2.6) **a**
RCD growth (mm)	NA	0.72 (0.26) **b**	2.50 (0.24) **a**	2.62 (0.24) **a**
Total sapling mass (g)	21.8 (2.1) **b**	22.6 (2.0) **b**	33.5 (1.9) **a**	32.1 (1.9) **a**
Total aboveground mass (g)	12.2 (1.2) **b**	12.5 (1.1) **b**	18.4 (1.1) **a**	19.7 (1.1) **a**
New stem growth (g)	NA	0.36 (0.20) **b**	0.87 (0.18) **ab**	1.27 (0.18) **a**
Leaf mass (g)	6.29 (0.58) **b**	5.40 (0.56) **b**	8.03 (0.53) **a**	8.63 (0.53) **a**

New leaf growth (g)	NA	0.27 (0.46) **b**	1.74 (0.43) **a**	2.34 (0.43) **a**
Specific leaf area (cm^2 g^{-1})	162.9 (6.4) **a**	NA	125.5 (4.7) **b**	123.7 (4.7) **b**
Total root mass (g)	9.58 (1.25) **b**	10.13 (1.16) **b**	15.03 (1.08) **a**	12.37 (1.08) **ab**
Total coarse root mass (g)	3.94 (0.65) **c**	5.66 (0.61) **bc**	8.03 (0.57) **a**	7.04 (0.57) **ab**
Total fine root mass (g)	5.64 (0.67) **ab**	4.47 (0.62) **b**	7.00 (0.58) **a**	5.33 (0.58) **ab**
Total root volume (cm^3)	59.1 (6.1) **a**	37.2 (5.7) **b**	63.1 (5.3) **a**	58.3 (5.3) **a**
Structural root density (g cm^{-3})	0.141 (0.01) **c**	0.247 (0.01) **a**	0.203 (0.009) **b**	0.186 (0.009) **b**
Leaf mass ratio (%)	29 (2) **a**	24 (2) **a**	24 (2) **a**	28 (2) **a**
Stem mass ratio (%)	26 (2) **b**	31 (2) **a**	31 (2) **a**	34 (2) **a**
Root mass ratio (%)	44 (2) **ab**	45 (2) **a**	45 (2) **a**	38 (2) **b**

Table 3-2. Estimated marginal means (±1 standard error) of starch and soluble sugar concentration (% dry weight) for leaf and stem tissue, and of total non-structural carbohydrate (NSC) concentration (% dry weight) of leaf, stem and root (both pots combined) tissue, prior to (INITIAL) and after the three watering treatments (FD: full drought, LD: localized drought, WW: well-watered). Letters represent statistical difference (n = 29; α = 0.1) among treatments using post-hoc comparisons with a Benjamini-Hochberg adjustment.

Organ		INITIAL	FD	LD	WW
Leaf	**Starch**	0.75 (0.15) **a**	0.20 (0.10) **b**	0.78 (0.13) **a**	0.66 (0.12) **a**
	Sugar	13.78 (0.58) **a**	13.53 (0.54) **a**	12.56 (0.50) **a**	12.69 (0.50) **a**
	NSC	14.62 (0.64) **a**	13.75 (0.63) **a**	13.37 (0.60) **a**	13.36 (0.60) **a**
Stem	**Starch**	0.38 (0.18) **b**	0.05 (0.12) **b**	1.31 (0.26) **a**	0.73 (0.19) **ab**
	Sugar	9.87 (0.55) **a**	8.29 (0.43) **b**	8.19 (0.39) **b**	7.34 (0.35) **b**
	NSC	10.28 (0.61) **a**	8.52 (0.62) **a**	9.66 (0.58) **a**	8.27 (0.58) **a**
Root	**NSC**	12.95 (1.64) **a**	12.85 (1.58) **a**	15.49 (1.48) **a**	12.90 (1.48) **a**

Table 3-3. Estimated marginal means (±1 standard error) of the relative allocation (% of total sapling pool type) of structural and non-structural carbohydrate (NSC) pools for leaves, stem and roots prior to (INITIAL) and after the three watering regimes (FD: full drought), LD: localized drought, WW: well-watered). Letters represent statistical difference (n = 29; α = 0.1) among treatments using post-hoc comparisons with a Benjamini-Hochberg adjustment.

Organ	Pool Type (%)	Initial	FD	LD	WW
Leaf	**Structural**	28.4 (1.7) **a**	23.1 (1.7) **a**	24.2 (1.6) **a**	26.9 (1.6) **a**
	NSC	33.8 (3.7) **a**	29.3 (3.7) **a**	25.3 (3.5) **a**	33.2 (3.5) **a**
Stem	**Structural**	27.5 (1.4) **c**	32.9 (1.2) **ab**	32.1 (1.1) **b**	35.7 (1.2) **a**
	NSC	21.2 (3.6) **a**	21.6 (3.3) **a**	22.3 (3.1) **a**	24.5 (3.1) **a**
Root	**Structural**	44.1 (2.0) **a**	44.0 (2.0) **a**	43.7 (1.8) **a**	37.2 (1.8) **b**
	NSC	44.3 (4.4) **a**	47.6 (4.5) **a**	52.4 (4.2) **a**	41.4 (4.2) **a**

3.7 Figures

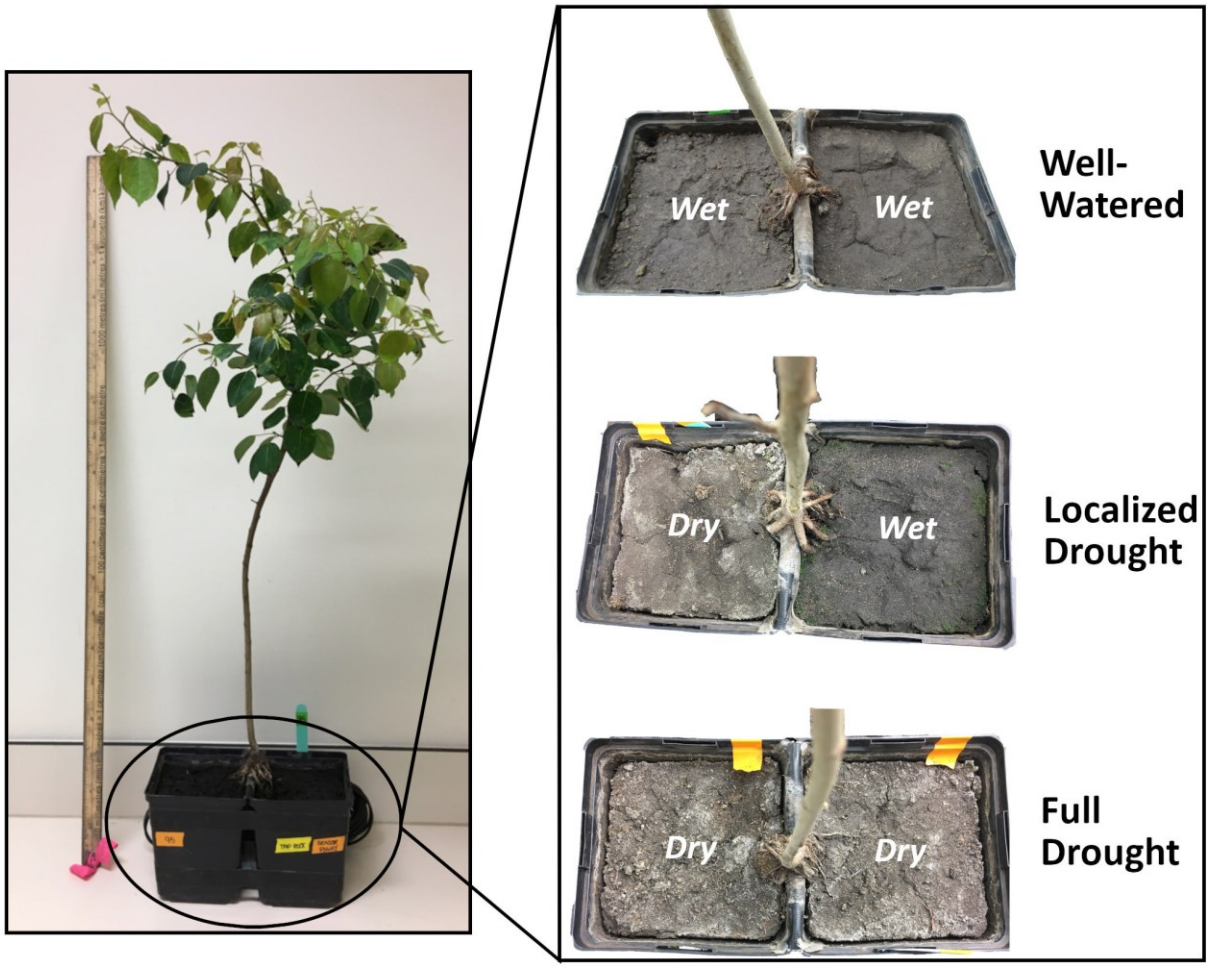

Figure 3-1. Aspen sapling grown with spatially separated root system using a split-pot design. Aspen saplings were subjected to one of three watering treatments (well-watered (WW), localized drought (LD) or full drought (FD)) characterized by differences in soil moisture availability.

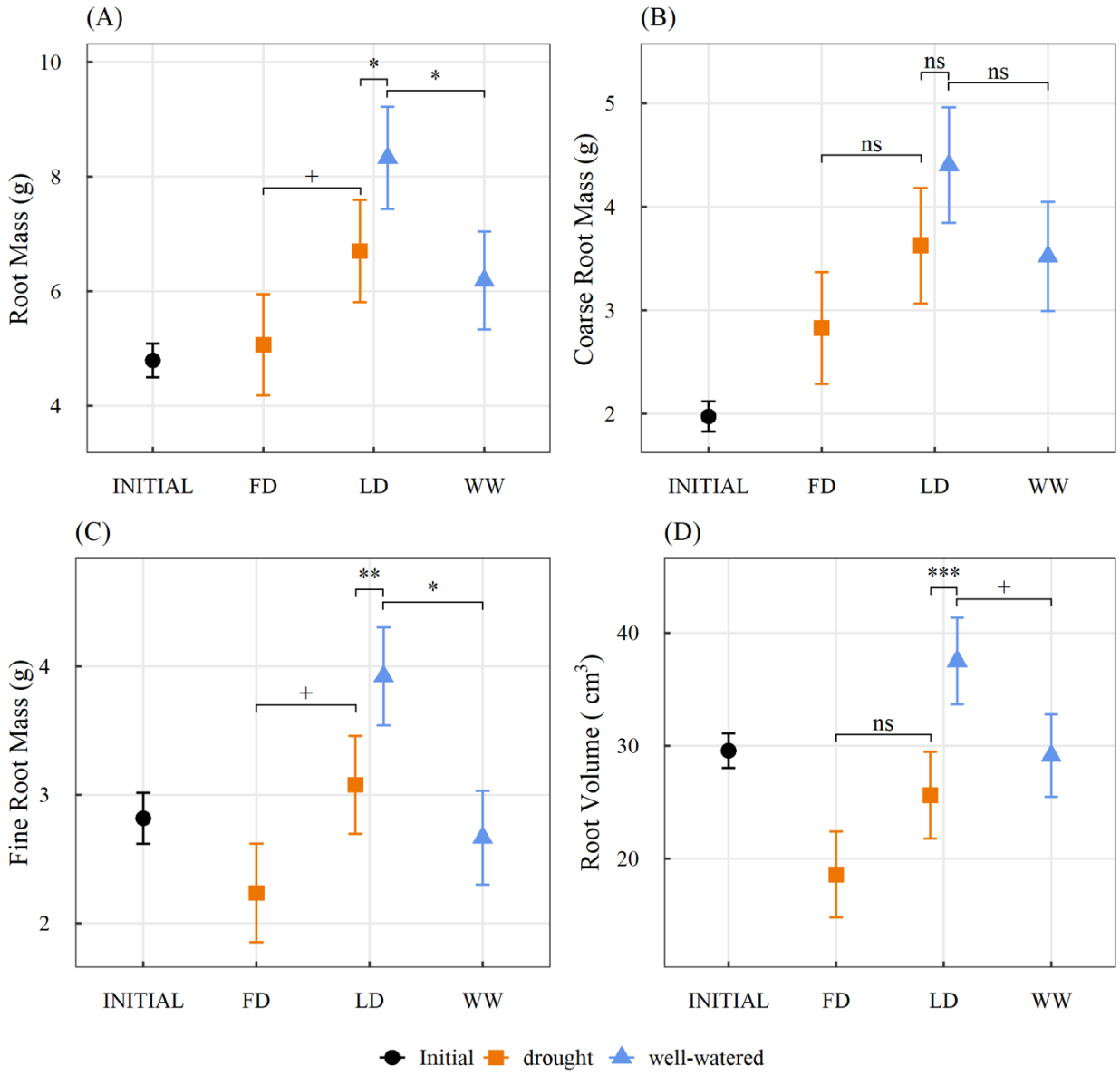

Figure 3-2. Estimated marginal means (±1 standard error) of (A) root mass (g), (B) coarse root mass (g), (C) fine root mass (g) and (D) root volume (cm^3) within the split root system of saplings, prior to (INITIAL) and after the three watering treatments (FD: full drought, LD: localized drought, WW: well-watered) (orange: dry, blue: well-watered). Statistical differences between the three planned comparisons (FD vs LD-dry, LD-dry vs LD-wet, WW vs LD-wet) are indicated by + for $p < 0.1$, * for $p < 0.05$, ** for $p < 0.01$, * for $p < 0.001$ and**

ns for no significance (n = 29; α = 0.1) using pairwise comparisons with a Benjamini-Hochberg adjustment.

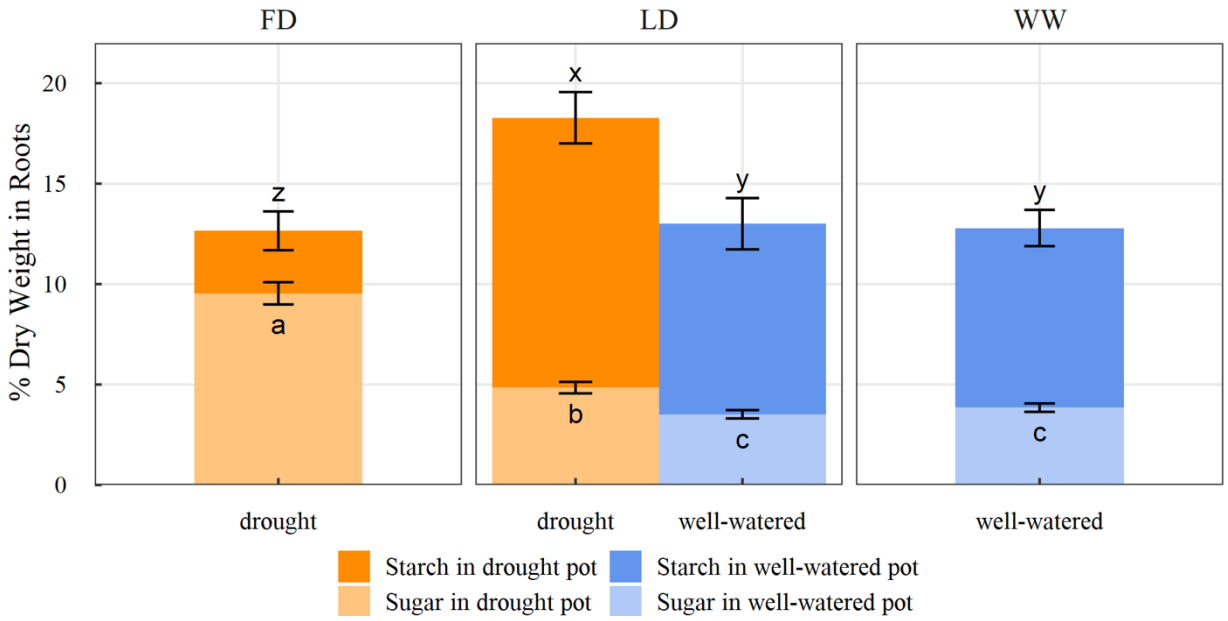

Figure 3-3. Estimated marginal means (±1 standard error) of root starch and soluble sugar concentration (% of dry weight) within the split root systems of saplings subjected to three watering treatments (FD: full drought, LD: localized drought, WW: well-watered), (light orange: sugar concentration of roots in drought pot, dark orange: starch concentration of roots in drought pot, light blue: sugar concentration of roots in well-watered pot, dark blue: starch concentration of roots in well-watered pot). Letters indicate statistical differences among treatments and pot watering regimes (n = 29; α = 0.1) using pairwise comparisons with a Benjamini-Hochberg adjustment.

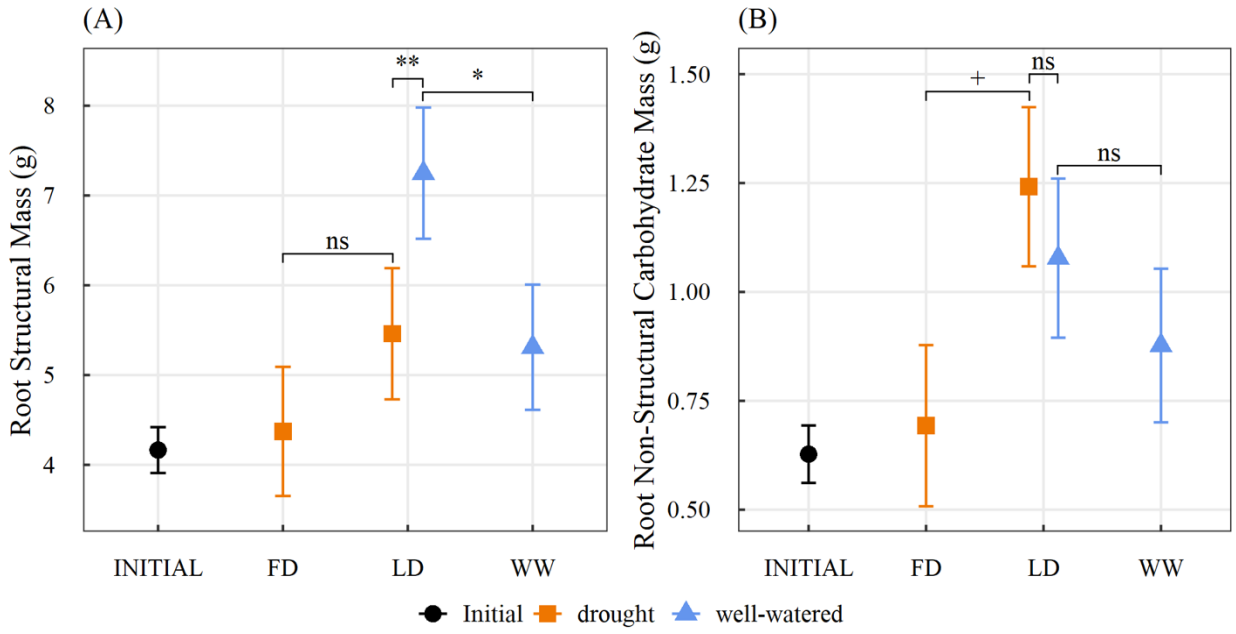

Figure 3-4. Estimated marginal mean (±1 standard error) of (A) root structural mass (g) and (B) root non-structural carbohydrate mass (g) within the split root system for saplings prior to (INITIAL) and after the three watering treatments (FD: full drought, LD: localized drought, WW: well-watered) (orange: dry, blue: well-watered). Statistical differences between the three planned comparisons (FD vs LD-dry, LD-dry vs LD-wet, WW vs LD-wet) are indicated by + for $p < 0.1$, * for $p < 0.05$, ** for $p < 0.01$ and ns for no significance ($n = 29$; $\alpha = 0.1$) using pairwise comparisons with a Benjamini-Hochberg adjustment.

Chapter 4: Synthesis and Discussion

The overarching goal of my thesis was to assess resource partitioning, allocation and remobilization under changing environmental conditions in trembling aspen saplings. The specific goals of my thesis were to (1) explore patterns and constraints on the remobilization of carbon (C) and nitrogen (N) during spring leaf development and following complete defoliation (Chapter 2), and (2) determine how trembling aspen saplings allocate mass and reserves to aboveground and belowground organs when subjected to heterogeneous soil moisture availability (Chapter 3). These studies required exploring allocation and movement of resources between and within organs, which was achieved in the first study (Chapter 2) by using C and N isotopic labeling with grafted saplings to trace resource remobilization during spring flush and following defoliation, and in the second study (Chapter 3) by using a split-pot to subject a shared root system to spatially heterogeneous soil moisture conditions.

4.1 Research Summary

The first study (Chapter 2) explored the remobilization of C and N during spring leaf development and following defoliation. The ability to access and utilize stored reserves during periods of asynchrony between supply and demand, such as during bud break or C-limiting stress, is critical for tree growth and survival. It is well established that reserves can be remobilized from perennial organs to support growth (Millard et al., 2001; Grassi et al., 2003; Lacointe et al., 2004; Kagawa et al., 2006; Visozo et al., 2008; Bazot et al., 2016). However, this study contributed to a knowledge gap in the literature, which was identifying the specific sources of stored reserves, the stem and/or roots, from which the remobilized C and N originates, in addition to characterizing potential constraints on remobilization, during new leaf growth.

This study showed that leaf growth (both during spring flush and recovery following defoliation) was dependent on C and N stored reserves from both stem and roots, with remobilization affected by distance, as less resources were imported further from the source (root system). The observed distance effect during spring leaf development suggests an allocation priority along the transport pathway, a hierarchy typically used to describe the allocation of recently acquired photosynthate (Zimmermann, 1971; Minchin et al., 1993; Lacointe, 2000; Landhäusser and Lieffers, 2012). The distance effect on root C remobilization appeared even greater following defoliation with no significant amount of labeled C present in the upper stem and re-flush leaves. This intensified C distance effect indicates that reserves were likely consumed during spring flush, resulting in less remobilization after defoliation, with distant low priority sinks impacted more strongly. This result likely explains the commonly observed pattern of top-down dieback in trees under C-limiting stress (Wiley et al., 2017; Wiley et al., 2019). In contrast, the N distance effect disappeared following defoliation, suggesting that demand for N may have been reduced and/or root acquisition of N was sufficient to support leaf area re-growth.

Dependence on stored reserves, and specifically which pool of NSC was remobilized (soluble sugars or starch), also differed over time. Pre-formed leaves utilized more stored C and N reserves than neo-formed leaves or re-flush leaves. This provides further evidence that during budflush, leaves transition from a net C sink to a net C source, with less reliance on reserves from the stem and roots as leaves mature (Lacointe et al., 2004; Marchi et al., 2005; Landhäusser, 2011). Less labeled C was detected in the smaller re-flush leaves, suggesting less remobilization of stored reserves and more dependence on current photosynthate production following defoliation stress. Pools of NSC remobilized within organs also differed between spring flush and following defoliation. Based on changes in NSC concentrations in the root systems, root sugar was likely

remobilized to support early spring leaf synthesis (and potentially stem growth), whereas stored root starch was likely converted to sugar to support leaf area recovery after defoliation. For N, both pre-formed leaves and neo-formed leaves imported similar amounts of root stored N, regardless of timing of leaf synthesis. However, pre-formed leaves contained more stem stored N than neo-formed leaves, contributing to a decline in the stem N storage pool available for leaf growth following defoliation. These results support the idea that N remobilization is controlled by the size of the storage pool (i.e. the N supply), not necessarily by the demand for growth (Millard and Grelet, 2010). In contrast to C, the smaller re-flush leaves had greater N concentrations than the leaves produced in early spring, which may be further evidence that the size of the N storage pool determines the amount of remobilization. Similarly, studies which use winter browsing to investigate spring N remobilization suggest that the remaining buds which flush have higher levels of remobilized N because there are fewer buds requiring the same N supply (Lehtilä et al., 2000; Millard et al. 2001; Millett et al., 2005).

Percent leaf recovery was 31 % three weeks following defoliation, with potential for continued new leaf growth for the remainder of the growing season. However, studies have found that canopy size may stay reduced later in the growing season following defoliation (Schäfer et al., 2010; Wiley et al., 2013; Schmid et al., 2017; Nakajima, 2018), thus it remains unclear whether C or N limitation or neither contributes to this incomplete recovery. In my study, the evidence for N limitation was inconclusive as N concentration in the roots correlated with higher leaf mass recovery, yet the N concentrations of re-flush leaves was higher than spring flush leaves, suggesting that roots had sufficient access to soil N. In terms of C limitation, ample levels of starch were detected in the root systems, however, re-flush leaves appeared to import less C and rely on current photosynthates. A better understanding of factors controlling recovery following

defoliation, as canopy size is often a measure of productivity (NPP), is required. My study highlights the need for continued work on resource storage and remobilization patterns in trees under **different** environmental stressors, as it is critical for predicting tree responses to a globally changing climate.

The second study (Chapter 3) described the effects of spatially heterogeneous soil moisture conditions on aboveground and belowground mass and reserve allocation compared to homogeneous soil moisture conditions (full drought (FD) and well-watered (WW) treatments). Few studies investigating resource availability apply spatially heterogeneous conditions, instead resources are typically homogeneously applied in controlled growth chamber experiments (Hutchings and John, 2004). Thus, the results of my study may more accurately mirror the responses to natural conditions of topographical variation or changing climate. My study contributes to the extensive body of literature focused on adaptation and acclimation processes which perennial plants use in response to short-term and long-term stress (Kozlowski and Pallard, 2002; Dietze et al., 2014; Brunner et al., 2015; Hartmann et al., 2018).

In this study (Chapter 3), saplings subjected to localized drought (LD) over a short-term, partitioned structural mass and non-structural carbohydrates within and between organs in a manner that likely optimized functionality. Aboveground, saplings in the LD treatment maintained similar growth and gas exchange to the WW saplings. In contrast, FD saplings experienced leaf and branch shedding in response to drought (Rood et al., 2000; Galvez et al., 2011). This suggests that even under locally reduced water availability, aspen is capable of compensating to support aboveground function and prevent tissue abscission. Belowground, patterns of mass and reserve allocation presented the most novel results of this study. Under both drought treatments (full or

localized), overall allocation towards the root system was greater (45%) than under well-watered conditions (38%). This result aligned with the optimal partitioning theory, where biomass is preferentially allocated to the organ required for uptake of the limiting resource (Brouwer, 1963; Thornley, 1972; Iwasa and Roughgarden, 1984; Bloom et al., 1985). However, root systems responded differently when exposed to the homogeneous drought compared to the heterogeneous drought. Under full drought, saplings showed a significant loss of root volume, an indicator of fine root death. This result, in addition to the loss of aboveground tissue under full drought, highlighted the expendability of more distal and potentially less crucial organs under stress (Kozlowski, 1973; Zimmerman, 1983; Sperry and Ikeda, 1997; Wiley et al., 2017). Saplings subjected to full drought accumulated significant concentrations of sugar, likely for osmotic adjustment (Chaves, 1991; Arndt et al., 2001; Kozlowski and Pallard, 2002; Galvez et al., 2011). Under localized drought, distinct responses were observed in the portion of the root system in the dry pot compared to the portion of the root system in the wet pot. In the wet pot, there was an increase in structural mass allocation, with root mass and root volume measurements exceeding that of a sapling in the WW treatment. This increased allocation of structural mass likely permitted continued water uptake to support aboveground function and potentially assisted in the re-hydration of the drought-exposed portion of the root system through lateral hydraulic redistribution (Burgess and Bleby, 2006; Bleby et al., 2010; Prieto et al., 2012). In the dry pot, there was a significant accumulation of starch, indicating a preferential allocation towards storage of reserves. These reserves in the drying portion of the root system could be used for future growth, reproduction or osmotic adjustment if drought conditions persisted or worsened. This study emphasized aspens' plasticity in mass and reserve allocation under conditions of spatially heterogeneous soil moisture availability.

Characterizing the morphological and physiological mechanisms which enable aspen to persist on the landscape is crucial to our understanding of tree stress tolerance.

4.2 Experimental Limitations and Future Research

This research was able to identify key patterns of resource partitioning, allocation and remobilization that occur within and between organs of trembling aspen in response to changing environmental conditions. However, I encountered several limitations in the experimental designs for both research studies. A central limitation of both studies is the extrapolation of results from potted saplings to mature trees grown in natural environments. However, the methods selected (grafting and split-pot design) to address the research objectives would not have been as feasible on larger trees. In the first study (Chapter 2), I selected older saplings to isotopically label in the growing season prior to the application of experimental treatments. In the second study (Chapter 3), I split the root systems an entire growing season prior to the application of the experimental treatments to allow adequate establishment of the root system portions. Use of older saplings in controlled experiments would require significant pre-planning.

In the study presented in Chapter 2, both the pulse-labeling and grafting limited the interpretation of the results. Firstly, the conclusions were based on a single application of isotopic C and N prior to the end of the growing season. This single pulse-labeling event only labeled a very small portion of the reserve pool prior to dormancy (especially for C), likely affecting the amount of reserve remobilization that was detectable using isotopes. Furthermore, the label may not have been evenly mixed across the whole storage pool. A potential solution would be to pulse-label multiple times prior to the end of the growing season. Furthermore, it would be valuable to use foliar labeling of N prior to dormancy. Foliar N represents a large pool of N that is recycled

before leaf abscission to perennial storage organs (Millard and Grelet, 2010). This application method would also prevent any residual ^{15}N from remaining in the soil in subsequent growing seasons. The original experimental design included a destructive harvest of a sample of trees at the pre-bud burst time point to assess the amount of C and N reserves (and label) present in the stem and root tissue prior to leaf flush. I had anticipated using these preliminary values to determine the initial C and N labeled reserves present in saplings to calculate percentages of label remobilized from storage organs at 18 days after initial leaf development and 3 weeks following defoliation. However, due to the variability in the amount of label present within each sapling, I could not obtain an accurate estimate of initial label present. The final limitation to the experimental design that may have affected the interpretation of the results would be the effect and contribution of the graft on reserve use. I attributed the changes in the NSC and N concentrations between pre-bud burst and 18 days following early leaf expansion, and then 3 weeks following defoliation to remobilization of reserves for spring growth and leaf area recovery, however there is potential that a portion of these stored resources were used to repair the significant wound created from grafting. However, I did have a subset of saplings that were not grafted and subjected to the same treatments and timepoints as the grafted saplings. If these tissue samples from the ungrafted saplings were analyzed for NSC and N concentrations, I could determine if the graft repair affected the amount or patterns of C or N remobilization.

In the drought study presented in Chapter 3, the conclusions were based on measurements of growth, dry weight and non-structural carbohydrate concentrations over a short drought period. Although I monitored soil water potential, I had no measurements of root, stem or leaf water potential as the watering regimes (full drought, localized drought and well-watered) were applied. Measurements of tissue water potential may have provided additional insight into the extent of the

drought conditions applied and assisted with comparisons between watering treatments. Furthermore, the saplings were subjected to a progressive drought over a 4-week experimental period, thus limiting the study to the assessment of short-term stress responses only.

Although this research contributed to our current understanding of resource partitioning, allocation and remobilization under conditions of temporal and spatial heterogeneity in trembling aspen saplings, it also highlighted new research opportunities.

1. **Explore the effects of repeated and incomplete defoliation over multiple growing seasons.** In Chapter 2, only short-term C and N remobilization in response to a single defoliation event early in the growing season was assessed. The effects of repeated incomplete defoliations over multiple growing seasons should also be considered, as the capacity for remobilization may change if storage pools deplete and do not recover (Landhäusser and Lieffers, 2012).
2. **Investigate N remobilization in more detail.** Understanding constraints on N uptake from the soil which potentially impact remobilization capacity would be valuable to understanding limitations to tissue re-growth following disturbance. Maxwell et al. (2020) investigated the impact of competition by soil microorganisms during *Quercus petraea* N uptake in the spring, further contributing to our understanding of limitations in N acquisition and subsequent N movement within trees. Furthermore, a specific measure of how much N was accessible from nearby proteins and transient storage pools for leaf growth was lacking. A direct measure of N storage in bark proteins would provide a clearer estimation of reserve remobilization and be more equivalent to the measurement C storage in the study.

3. **Explore the effects of repeated localized drought over multiple growing seasons.** As previously mentioned, the experimental period was only 4-weeks. It would be valuable to determine whether the preferential allocation of mass and reserves observed during this temporary localized drought is advantageous over an extended period or in future growing seasons with drought events. Such a study would allow for the analysis of accumulated effects over time which provide more useful insight into aspen's acclimations and adaptations to moderate or localized drought.
4. **Investigate lateral hydraulic redistribution.** There is evidence that connected root systems are capable of sharing acquired resources amongst the network of clones (Hutchings and Wijesinghe, 1997; Fraser et al., 2006). Over a clonal network, would aspen be capable of laterally redistributed water if a portion of the root system had access to high soil moisture availability? In Chapter 3, differences in soil and root water potential across the split root system may have enabled passive water movement from the wet soil of one pot, through the root system to the drying portion of roots. Although MPS2 soil water potential sensors were installed in the dry pot of the localized drought treatment, it is questionable whether these sensors would have been precise enough to detect small changes in soil and root water potential driven by hydraulic redistribution. An alternative method would be the application of deuterium water to the wet pot of the localized drought treatment, with soil collection in the dry pot to detect any movement of water through the root system.
5. **Explore C remobilization under drought using grafted saplings.** Chapter 2 is one of the first studies to identify the specific storage pools remobilized for the formation of leaves during spring flush and under defoliation stress, as stem and root storage pools are not often

differentiated, and remobilization of reserves is detected primarily through changes in resource concentration in organs. Peltier et al. (2020) emphasizes the importance of investigating NSC pool responses within and between organs under moisture stress in a variety of species. Use of isotopically labeled saplings which are grafted then subjected to drought conditions would likely generate many interesting results which could contribute to our understanding of resource remobilization under drought.

References

Adams, H. D., Zeppel, M. J. B., Anderegg, W. R. L., Hartmann, H., Landhäusser, S. M., Tissue, D. T., et al. (2017). A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology and Evolution* 1, 1285–1291. doi: 10.1038/s41559-017-0248-x

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J., Allard, G., Running, S.W., Semerci, A. and Cobb, N. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259, 660–684. doi: 10.1016/j.foreco.2009.09.001

Alves, G., Decourteix, M., Fleurat-Lessard, P., Sakr, S., Bonhomme, M., Améglio, T., et al. (2007). Spatial activity and expression of plasma membrane H+-ATPase in stem xylem of walnut during dormancy and growth resumption. *Tree Physiology* 27, 1471–1480. doi: 10.1093/treephys/27.10.1471

Anderegg, W. R. L. (2012). Complex aspen forest carbon and root dynamics during drought. *Climatic Change* 111, 983–991. doi: 10.1007/s10584-012-0421-9

Anderegg, W. R. L., Berry, J. A. Smith, D. D., Sperry, J.S., Anderegg, L. D. L., and Field, C. B. (2012). The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings of the National Academy of Sciences* 109, 233–237. doi: 10.1073/pnas.1107891109

Anderegg, W. R. L., and Callaway, E. S. (2012). Infestation and hydraulic consequences of induced carbon starvation. *Plant Physiology* 159, 1866–1874. doi: 10.1104/pp.112.198424

Anderegg, W. R. L., Hicke, J. A., Fisher, R. A., Allen, C. D., Aukema, J., Bentz, B., et al. (2015). Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* 208, 674–683. doi: 10.1111/nph.13477

Arango-Velez, A., Zwiazek, J. J., Thomas, B. R., and Tyree, M. T. (2011). Stomatal factors and vulnerability of stem xylem to cavitation in poplars. *Physiologia Plantarum* 143, 154–165. doi: 10.1111/j.1399-3054.2011.01489.x

Arndt, S. K., Clifford, S. C., Wanek, W., Jones, H. G., and Popp, M. (2001). Physiological and morphological adaptations of the fruit tree *Ziziphys rotundifolia* in response to progressive drought stress. *Tree Physiology* 21, 705–715. doi: 10.1093/treephys/21.11.705

Ayres, M. P. and Lombardero, M. J. (2000). Assessing the consequences of global change for forest disturbances for herbivores and pathogens. *The Total Science of the Environment* 262, 263–286. doi: 10.1016/S0048-9697(00)00528-3

Baret, M., and DesRochers, A. (2011). Root connections can trigger physiological responses to defoliation in nondefoliated aspen suckers. *Botany* 89, 753–761. doi: 10.1139/B11-062

Bazot, S., Fresneau, C., Damesin, C., and Barthes, L. (2016). Contribution of previous year's leaf N and soil N uptake to current year's leaf growth in sessile oak. *Biogeosciences* 13, 3475–3484. doi: 10.5194/bg-13-3475-2016

Bell, T. L., Pate, J. S., and Dixon, K. W. (1996). Relationship between fire response, morphology, root anatomy and starch distribution in south-west Australian Epacridaceae. *Annals of Botany* 77, 357–364. doi: 10.1006/anbo.1996.0043

Berry, S. L., Farquhar, G. D., and Roderick, M. L. (2006). "Co-evolution of climate, soil and vegetation," in *Encyclopedia of Hydrological Sciences*. Eds M.G. Anderson and J.J. McDonnell (New York, USA: John Wiley and Sons). doi: 10.1002/0470848944.hsa011

Bleby, T. M., McElrone, A. J., and Jackson, R. B. (2010). Water uptake and hydraulic redistribution across large woody root systems to 20 m depth. *Plant, Cell and Environment* 33, 2132–2148. doi: 10.1111/j.1365-3040.2010.02212.x

Bloom, A. J., Chapin, F. S., and Mooney, H. A. (1985). Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics* 16, 363–392. doi: 10.1146/annurev.es.16.110185.002051

Bollmark, L., Sennerby-Forsse, L., and Ericsson, T. (1999). Seasonal dynamics and effects of nitrogen supply rate on nitrogen and carbohydrate reserves in cutting-derived *Salix viminalis* plants. *Canadian Journal of Forest Research* 29, 85–94. doi: 10.1139/cjfr-29-1-85

Bonan, G. B. (2008). Forests and climate change: forcings, feedbacks, and the climate change benefits of forests. *Science* 320, 1444–1449. doi: 10.1126/science.1155121

Bonsal, B., and Wheaton, E. (2005). Atmospheric curcualtion comparisons between the 2001 and 2002 and the 1961 and 1988 Canadian prairie droughts. *Atmos. Ocean* 43, 163-172. doi:

10.3137/ao.430204

Braatne, J. H., Hinckley, T. M., and Stettler, R. F. (1992) Influence of soil water on the physiological and morphological components of plant water balance in *Populus trichocarpa*, *Populus deltoides* and their F_1 hybrids. *Tree Physiology* 11, 325–339. doi: 10.1093/treephys/11.4.325

Breda, N., Huc, R., Granier, A., and Dreyer, E. (2006). Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annuals in Forest Science* 63, 625–644. doi: 10.1051/forest:2006042

Brodribb, T. J., Bowman, D. J. M. S., Delzon, N. S., and Burlett, R. (2010). Xylem function and growth rates interact to determine recovery rates after exposure to extreme water deficit. *New Phytologist* 188: 533–542. doi: 10.1111/j.1469-8137.2010.03393.x

Brouwer, R. (1963). Some aspects of the equilibrium between overground and underground plant parts. Jaarboek IBS, 31–39.

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., and Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in Plant Science* 6: 547. doi: 10.3389/fpls.2015.00547

Buckley, T. N., Sack, L., and Farquhar, G. D. (2017). Optimal plant water economy. *Plant, Cell and Environment* 40, 881–896. doi: 10.1111/pce.12823

Burgess, S. S. O., and Bleby, T. M. (2006). Redistribution of soil water by lateral roots mediated by stem tissues. *Journal of Experimental Botany* 57, 3283–3291. doi: 10.1093/jxb/erl085

Campbell, N. A., Williamson, B., and Heyden, R. J. (2006). Biology: exploring life. Pearson Prentice Hall.

Carbone, M. S., Czimczik, C. I., Keenan, T. F., Murakami, P. F., Pederson, N., Schaberg, P. G., et al. (2013). Age, allocation and availability of non-structural carbon in mature red maple trees. *New Phytologist* 200**,** 1145–1155. doi: 10.1111/nph.12448

Chamran, F., Gessler, P. E., and Chadwick, O. A. (2002). Spatially explicit treatment of soil-water dynamics along a semiarid catenna. *Soil Science Society of America Journal* 66, 1571–1582. doi: 10.2136/sssaj2002.1571

Chapin, F. S., Schulze, E., and Mooney, H. A. (1990). The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* 21, 423–447. doi: 10.1146/annurev.es.21.110190.002231

Chapin, F. S., and Slack, M. (1979). Effect of defoliation upon root growth, phosphate absorption and respiration in nutrient-limited tundra graminoids. *Oecologia* 42, 67–79.

Chaves, M. M. (1991). Effects of water deficit on carbon assimilation. *Journal of Experimental Botany* 42, 1–16. doi: 10.1093/jxb/42.1.1

Cheng, L., and Fuchigami, L. H. (2002). Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiology* 22, 1297–1303. doi: 10.1093/treephys/22.18.1297

Cheng, L., Ma, F., and Ranwala, D. (2004). Nitrogen storage and its interaction with carbohydrates of young apple trees in response to nitrogen supply. *Tree Physiology* 24, 91–98. doi: 10.1093/treephys/24.1.91

Claeys, H., and Inzé, D. (2013). The agony of choice: how plants balance growth and survival under water-limiting conditions. *Plant Physiology* 162: 1768–1779. doi: 10.1104/pp.113.220921

Cooke, J. E. K., and Weih, M. (2005). Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. *New Phytologist* 167, 19–29. doi: 10.1111/j.1469-813

Da Sliva, D., Qin, L., DeBuse, C., and DeJong, T. M. (2014). Measuring and modelling seasonal patterns of carbohydrate storage and mobilization in the trunks and root crowns of peach trees. *Annals of Botany* 114, 643–652. doi: 10.1093/aob/mcu033

Dai, Y. X., Wang, L., and Wan, X. C. (2018). Relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality caused by drought. *AOB Plants* 10. doi: 10.1093/aobpla/plx069

Day, M. W. (1944). The root system of aspen. *American Midland Naturalis*t 32, 502–509.

DesRochers, A., and Lieffers, V. J. (2001). Root biomass of regenerating aspen (*Populus tremuloides*) stands of different densities in Alberta. *Canadian Journal of Forest Research,* 31, 1012–1018. doi: 10.1139/cjfr-31-6-1012

Dietze, M. C., Sala, A., Carbone, M. S., Czimczik, C. I., Mantooth, J. A., Richardson, A. D., et al. (2014). Nonstructural carbon in woody plants. *Annual Review of Plant Biology* 65, 667–687. doi: 10.1146/annurev-arplant-050213-040054

Drew, M. C. (1975). Comparison of the effects of a localised supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytologist* 75, 479–490. doi: 10.1111/j.1469-8137.1975.tb01409.x

Eissenstat, D. M. (1997). "Trade-offs in root form and function," in *Ecology in Agriculture*. Ed. L. E. Sackson (San Diego, USA: Academic Press), 173–199.

Evans, J. R. (1989). "Photosynthesis: the dependence on nitrogen partitioning" in *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants*. Eds H. Lambers, M. L. Cambridge, H. Konings and T. L. Pons. (The Netherlands: SPB Academic Publishing), 159–174.

Fichot, R., Brignolas, F., Cochard, H., and Ceulemans, R. (2015). Vulnerability to drought-induced cavitation in poplars: synthesis and future opportunities. *Plant, Cell and Environment* 38, 1233–1251. doi: 10.1111/pce.12491

Fort, C., Fauveau, M. L., Muller, F., Label, P., Granier, A., and Dreyer, E. (1997). Stomatal conductance, growth and root signaling in young oak saplings subjected to partial soil drying. *Tree Physiology* 17, 281–289. doi: 10.1093/treephys/17.5.281

Frak, E., Millard, P., Le Roux, Z., Guillaumie, S., and Wendler, R. (2002). Coupling sap flow velocity and amino acid concentrations as an alternative method to 15N labeling for quantifying nitrogen remobilization by walnut trees. *Plant Physiology* 130, 1043–1053. doi: 10.1104/pp.002139

Fraser, E. C., Lieffers, V. J., and Landhäusser, S. M. (2006). Carbohydrate transfer through root grafts to support shaded trees. *Tree Physiology* 26, 1019–1023. doi: 10.1093/treephys/26.8.1019

Frey, B. R., Lieffers, V. J., Hogg, E. H., and Landhäusser, S. M. (2004). Predicting landscape patterns of aspen dieback: mechanisms and knowledge gaps. *Canadian Journal of Forest Research* 34, 1379–1390. doi: 10.1139/X04-062

Fridley, J. D., Grime, J. P., Askew, A. P., Moser, B., and Stevens, C. J. (2011). Soil heterogeneity buffers community response to climate change in species-rich grassland. *Global Change Biology* 17, 2002–2011. doi: 10.1111/j.1365-2486.2010.02347.x

Galiano, L., Martinez-Vilalta, J., and Lloret, F. (2011). Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytologist* 190, 750–759. doi: 10.1111/j.1469-8137.2010.03628.x

Galvez, D. A., Landhäusser, S. M., and Tyree, M. T. (2011). Root carbon reserve dynamics in aspen saplings: does simulated drought induce reserve limitation? *Tree Physiology* 31, 250–257. doi: 10.1093/treephys/tpr012

Galvez, D. A., Landhäusser, S. M., and Tyree, M. T. (2013). Low root reserve accumulation during drought may lead to winter mortality in poplar seedlings. *New Phytologist* 198, 139–148. doi: 10.1111/nph.12129

Galvez, D. A., and Tyree, M.T. (2009). Impact of simulated herbivory on water relations of aspen (Populus tremuloides) seedlings: the role of new tissue in the hydraulic conductivity recovery cycle. *Oecologia* 161, 665–671. doi: 10.1007/s00442-009-1416-8

Gaudinski, J. B., Torn, M. S., Riley, W. J., Swanston, C., Trumbore, S. E., Joslin, J. D., et al. (2009). Use of stored carbon reserves in growth of temperate tree roots and leaf buds: analyses using radiocarbon measurements and modeling. *Global Change Biology* 15, 992–1014. doi: 10.1111/j.1365-2486.2008.01736.x

Geßler, A., Keitel, C., Nahm, M., and Rennenberg, H. (2004). Water shortage affects the water and nitrogen balance in central European beech forests. *Plant Biology* 6, 289–298. doi: 10.1055/s-2004-820878

Gebre, G. M., Tschaplinski, T. J., Tuskan, G. A., and Todd, D. E. (1998). Clonal and seasonal differences in leaf osmotic potential and organic solutes of five hybrid poplar clones grown under field conditions. *Tree Physiology* 18, 645–652. doi: 10.1093/treephys/18.10.645

Gersani, M., and Sachs, T. (1992). Developmental correlations between roots in heterogeneous environments. *Plant, Cell and Environment* 15, 463–469. doi: 10.1111/j.1365-3040.1992.tb00997.x

Gowing, D. J. D., Davies, W. J., and Jones, H. G. (1990). A positive root-sourced signal as an indicator of soil drying in apple, *Malus* x *domestica* Borkh. *Journal of Experimental Botany* 41, 1535–1540. doi: 10.1093/jxb/41.12.1535

Grassi, G., Millard, P., Gioacchini, P., and Tagliavini, M. (2003). Recycling of nitrogen in the xylem of *Prunus avium* trees starts when spring remobilization of internal reserves declines. *Tree Physiology* 23, 1061–1068. doi: 10.1093/treephys/23.15.1061

Guswa, A. J. (2012). Canopy vs roots: production and destruction of variability in soil moisture and hydrologic fluxes. *Vadose Zone Journal* 11. doi:10.2136/vzj2011.0159

Harrington, J. T., Mexal, J. G., and Fisher, J. T. (1994). Volume displacement provides a quick and accurate way to quantify new root production. *Seedling Planters' Notes* 45, 121–24.

Hart, A. T., Merlin, M., Wiley, E., and Landhäusser, S. M. (2021). Splitting the difference: heterogeneous soil moisture availability affects aboveground and belowground reserve and mass allocation in trembling aspen. *Frontiers in Plant Science* 12, 654159. doi: 10.3389/fpls.2021.654159

Harte, J., and Shaw, R. (1995). Shifting dominance within a montane vegetation community: results of a climate-warming experiment. *Science* 267, 876–880. doi: 10.1126/science.267.5199.876

Hartmann, H., Adams, H. D., Hammond, W. M., Hoch, G., Landhäusser, S. M., Wiley, E. et al. (2018). Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. *Environmental and Experimental Botany* 152, 7–18. doi: 10.1016/j.envexpbot.2018.03.011

Hartmann, H., and Trumbore, S. (2016). Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist* 211, 386–403. doi: 10.1111/nph.13955

Hesse, B. D., Hartmann, H., Rötzer, T., Landhäusser, S. M., Goisser, M., Weikl, F., et al. (2021). Mature beech and spruce trees under drought – higher C investment in reproduction at the expense of whole-tree NSC stores. *Environmental and Experimental Botany* 191, 104615. doi: 10.1016/j.envexpbot.2021.104615

Hillabrand, R. M. Lieffers, V. J., Martínez-Sancho, E., Menzel, A., and Hacke, U. G. (2018). Functional xylem anatomy of aspen exhibits greater change due to insect defoliation than to drought. *Tree Physiology* 39, 45–54. doi: 10.1093/treephys/tpy075

Hirota, I., Sakuratani, T., Sato, T., Higuchi, H., and Nawata, E. (2004). A split-root apparatus for examining the effects of hydraulic lift by saplings on the water status of neighboring crops. *Agroforestry Systems* 60, 181–187. doi: 10.1023/B:AGFO.0000013293.77907.64

Hoch, G., Ritcher, A., and Körner, C. (2003). Non-structural carbon compounds in temperate forest trees. *Plant, Cell and Environment* 26, 1067–1081. doi: 10.1046/j.0016-8025.2003.01032.x

Hoch, G. (2005). Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. *Plant, Cell and Environment* 28**,** 651–659. doi: 10.1111/j.1365-3040.2004.01311.x

Hoffman, W. A., Orthen, B., and Franco, A. C. (2004). Constraints to seedling success of savanna and forest trees across the savanna-forest boundary. *Oecologia* 140, 252–260. doi: 10.1007/s00442-004-1595-2

Hogg, E. H., Brandt, J. P., and Kochtubajda, B. (2002). Growth and dieback of aspen forests in northwestern Alberta, Canada, in relation to climate and insects. *Canadian Journal of Forest Research* 32, 823–832. doi: 10.1139/X01-152

Hogg, E. H., Brandt, J. P., and Michaellian, M. (2008). Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. *Canadian Journal of Forest Research* 38, 1373–1384. doi: 10.1139/X08-001

Hutchings, M. J., and de Kroon, H. (1994). Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research* 25, 159–238. doi: 10.1016/S0065-2504(08)60215-9

Hutchings, M. J., and John, E. A. (2004). The effects of environmental heterogeneity on root growth and root/shoot partitioning. *Annals of Botany* 94, 1-8. doi:10.1093/aob/mch111

Hutchings, M. J., John, E. A., and Wijesinghe, D. K. (2003). Toward understanding the consequences of soil heterogeneity for plant populations and communities. *Ecology* 84, 2322–2334. doi: 10.1890/02-0290

Hutchings, M. and Wikesinghe, D. (1997). Patchy habitats, division of labour and growth dividends in clonal plants. *Trends in Ecological Evolution* 12, 390–394. doi: 10.1016/S0169-5347(97)87382-X

IPCC (2013). "Climate Change 2013: The Physical Science Basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change". Eds T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung et al. (Cambridge, United Kingdom and New York, NY, USA: Cambridge Univserity Press), 1535

Iwasa, Y., and Roughgarden, J. (1984). Shoot/root balance of plants: optimal growth of a system with many vegetative organs. *Theoretical Population Biology* 25, 78–105. doi: 10.1016/0040-5809(84)90007-8

Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H.A., Sala, O.E., and Schulze, E.D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia* 108, 389–411. doi:10.1007/BF00333714

Joslin, J. D., Wolfe, M. H., and Hanson, P. J. (2001). Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant and Soil* 228, 201–212. doi: 10.1023/A:1004866705021

Kagawa, A., Sugimoto, A., and Maximov, T. C. (2006). $^{13}CO_2$ pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant, Cell and Environment* 29, 1571–1584. doi: 10.1111/j.1365-3040.2006.01533.x

Kaitaniemi, P., Ruohomaki, K., Ossipov, V., Haukioja, E., and Pihlaja, K. (1998). Delayed induced changes in the biochemical composition of host plant leaves during an insect outbreak. *Oecologia* 116, 182–190.

Karst, J., Gaster, J., Wiley, E., and Landhäusser, S.M. (2016). Stress differentially causes roots of tree seedlings to exude carbon. *Tree Physiology* 37, 154–164. doi: 10.1093/treephys/tpw090

Kays, J. S., and Canham, C. D. (1991). Effects of time and frequency of cutting on hardwood root reserve and sprout growth. *Forest Science* 37, 524–539.

Keel, S. G. and Schädel, C. (2010). Expanding leaves of mature deciduous forest trees rapidly become autotrophic. *Tree Physiology* 30, 1253–1259. doi:10.1093/treephys/tpq071

Keel, S. G., Siegwolf, R. T. W., Jäggi, M., and Körner, C. (2007). Rapid mixing between old and new C pools in the canopy of mature forest trees. *Plant, Cell and Environment* 30, 963–972. doi: 10.1111/j.1365-3040.2007.01688.x

Keel, S. G., Siegwolf, R. T. W., and Körner, C. (2006). Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. *New Phytologist* 172, 319–329. doi: 10.1111/j.1469-8137.2006.01831.x

Körner, C. (2003). Carbon limitation in trees. *Journal of Ecology* 91, 4–17. doi: 10.1046/j.1365-2745.2003.00742.x

Kosola, K. R., Dickmann, D. I., Paul, E. A., and Parry, D. (2001). Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates and root demography of poplars. *Oecologia* 129, 65–74. doi: 10.1007/s004420100694

Kosola, K. R., and Eissenstat, D. M. (1994). The fate of surface roots of citrus seedlings in dry soil. *Journal of Experimental Botany* 45, 1639–1654.

Kozlowski, T. T. (1973). “Extent and significance of shedding of plant parts,” in *Shedding of Plant Parts*. Eds T. T. Kozlowski (New York, USA: Academic Press Inc), 1–44.

Kozlowski, T. T. (1992). Carbohydrate sources and sinks in woody plants. *Botanical Review* 58, 107–222. doi: 10.1007/BF02858600

Kozlowski, T. T., and Pallard, S. G. (2002). Acclimation and adaptive responses of woody plants to environmental stresses. *The Botanical Review* 68, 270–334. doi: 10.1663/0006-8101(2002)068[0270:AAAROW]2.0.CO;2

Lacointe, A. (2000). Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. *Annals of Forest Science* 57, 521–533.

Lacointe, A., Deleens, E., Ameglio, T., Saint-Joanis, B., Lelarge, C., Vandame, M., et al. (2004). Testing the branch autonomy theory: a 13C/14C double-labelling experiment on differentially shaded branches. *Plant, Cell & Environment* 27**,** 1159–1168. doi: 10.1111/j.1365-3040.2004.01221.x

Lacointe, A., and Minchin, P. E. H. (2008). Modeling phloem and xylem transport within a complex architecture. *Functional Plant Biology* 35, 772–780.

Landhäusser, S. M. (2011). Aspen shoots are carbon autonomous during bud break. *Trees* 25, 531–536. doi: 10.1007/s00468-010-0532-8

Landhäusser, S. M., Chow, P. S., Dickman, L. T., Furze, M. E., Kulhman, I., Schmid, S., et al. (2018). Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. *Tree Physiology* 38, 1764–1778. doi: 10.1093/treephys/tpy118

Landhäusser, S. M., and Lieffers, V. J. (2002). Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following above-ground disturbance. *Journal of Ecology* 90, 658–665. doi: 10.1046/j.1365-2745.2002.00699.x

Landhäusser, S. M., and Lieffers, V. J. (2003). Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. *Trees* 17, 471–476. doi: 10.1007/s00468-003-0263-1

Landhäusser, S. M. and Lieffers, V. J. (2012). Defoliation increases risk of carbon starvation in root systems of mature aspen. *Trees* 26, 653–661. doi: 10.1007/s00468-011-0633-z

Landhäusser, S. M., Pinno, B. D., Lieffers, V. J., and Chow, P. S. (2012). Paritioning of carbon allocation to reserves or growth determines furture performance of aspen seedlings. *Forest Ecology and Management* 275, 43–51. doi: 10.1016/j.foreco.2012.03.010

Legates, D. R., Mahmood, R., Levia, D. F., DeLiberty, T. L., Quiring, S. M., Houser, C., et al. (2010). Soil moisture: a central and unifying theme in physical geography. *Progress in Physical Geography* 35, 65–86. doi: 10.1177/0309133310386514

Lehtila, K., Haukioja, E., Kaitaniemi, P., and Laine, K. A. (2000). Allocation of resources within mountain birch canopy after simulated winter browsing. *Oikos* 90, 160–170. doi: 10.1034/j.1600-0706.2000.900116.x

Lenth, R. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.5.1. https://CRAN.R-project.org/package=emmeans

Li, M. H., Hoch, G. and Körner, C. (2002). Source/sink removal affects mobile carbohydrates in

Pinus cembra at the Swiss treeline. *Trees – Structure and Function* 16, 331–337. doi: 10.1007/s00468-002-0172-8

Lockwood, D. W., and Sparks, D. (1978). Translocation of 14C from tops and roots of pecan in the spring following assimilation of $14CO_2$ during the previous growing season. *Journal of the American Society for Horticultural Science* 103, 45–49.

Loescher, W. H., McCamant, T., and Keller, J. D. (1990). Carbohydrate reserves, translocation, and storage in woody plant roots. *Horticultural Science* 25, 274–281. doi: 10.21273/HORTSCI.25.3.274

Loranty, M. M, Mackay, D. S., Ewers, B. E., Adelman, J. D., and Kruger, E. L. (2008). Environmental drivers of spatial variation in whole-tree transpiration in an aspen-dominated upland-to-wetland forest gradient. *Water Resources Research* 44, 1–15. doi: 10.1029/2007WR006272

Magel, E., Einig, W., and Hampp, R. (2000). "Carbohydrates in trees," in *Carbohydrate Reserves in Plants*. Eds A.K. Gupta and N. Kaur (Amerstedam: Elsevier Science), 317–336. doi: 10.1016/S0378-519X(00)80016-1

Malaguti, D., Millard, P., Wendler, R., Hepburn, A., and Tagliavini, M. (2001). Translocation of amino acids in the xylem of apple (*Malus domestica* Borkh.) trees in spring as consequence of both N remobilization and root uptake. *Journal of Experimental Botany* 52, 1665–1671. doi: 10.1093/jexbot/52.361.1665

Marchi, S., Sebastiani, L., Gucci, R., and Tognetti, R. (2005). Sink-source transition in peach leaves during shoot development. *Journal of the American Society for Horticultural Science* 130, 928–935. doi: 10.21273/JASHS.130.6.928

Mariotti, A. (1983). Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature* 303, 685–687. doi: 10.1038/303685a0

Markesteijn, L., and Poorter, L. (2009). Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. *Journal of Ecology* 97, 311–325. doi: 10.1111/j.1365-2745.2008.01466.x

Martinez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., et al. (2016). Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecological Monographs* 86, 495–516. doi: 10.1002/ecm.1231

McDowell, N., Rockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., et al. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178, 719–739. doi: 10.1111/j.1469-8137.2008.02436.x

Meier, I. C., and Leuschner, C. (2008). Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. *Global Change Biology* 14, 2081–2095. doi: 10.1111/j.1365-2486.2008.01634.x

Metz, J., and Tielbörger, K. (2016). Spatial and temporal aridity gradients provide poor proxies for plant-plant interactions under climate change: a large-scale experiment. *Functional*

Ecology 30, 20–29. doi: 10.1111/1365-2435.12599

Millard, P. (1996). Ecophysiology of the internal cycling of nitrogen for tree growth. *Journal of Plant Nutrition and Soil Science* 159, 1–10.

Millard, P., and Grelet, G-A. (2010). Nitrogen storage and remobilization in trees: ecophysiological relevance in a changing world. *Tree Physiology* 30, 1083–1095. doi: 10.1093/treephys/tpq042

Millard, P., Hester, A., Wendler, R., and Baillie, G. (2001). Interspecific defoliation responses of trees depend on sites of winter nitrogen storage. *Functional Ecology* 15, 535–543. doi: 10.1046/j.0269-8463.2001.00541.x

Millard, P., and Proe, M. F. (1991). Leaf demography and the seasonal internal cycling of nitrogen in sycamore (*Acer pseudoplatanus* L.) seedlings in relations to nitrogen supply. *New Phytologist* 117, 587–596. doi: 10.1111/j.1469-8137.1991.tb00963.x

Millard, P., Wendler, R., Grassi, G., Grelet, G. A., and Tagliavini, M. (2006). Translocation of nitrogen in the xylem of field-grown cherry and poplar trees during remobilization. *Tree Physiology* 26, 527–536. doi: 10.1093/treephys/26.4.527

Miller, H. G. (1986). Carbon x nutrient interactions – the limitations to productivity. *Tree Physiology* 2, 373–385. doi: 10.1093/treephys/2.1-2-3.373

Millett, J., Millard, P., Hester, A. J., and McDonald, A. J. S. (2005). Do competition and herbivory alter the internal nitrogen dynamics of birch saplings? *New Phytologist* 168, 413–422. doi: 10.1111/j.1469-8137.2005.01510.x

Minchin, P. E. H., and Lacointe, A. (2005). New understanding on phloem physiology and possible consequences for modelling long-distance carbon transport. *New Phytologist* 166, 771–779. doi: 10.1111/j.1469-8137.2005.01323.x

Minchin, P. E. H., Thorpe, M.R., and Farrar, J. F. (1993). A simple mechanistic model of phloem transport which explains sink priority. *Journal of Experimental Botany* 44, 947–955. doi: 10.1093/jxb/44.5.947

Münch, E. (1930). Die Stoffbewegungen in der Pflanze. Verlag von Gustav Fischer, Jena, 234.

Nakajima, H. (2018). Refoliation of deciduous canopy trees following severe insect defoliation: comparison of *Fagus crenata* and *Quercus crispula. Plant Ecology* 219, 665–675. doi: 10.1007/s11258-018-0825-3

Näsholm, T., Ekbald, A., Nordin, A., Giesler, R., Högberg, M., and Högberg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature* 392, 914–916.

Norris, C. E., Quideau, S. A., Landhäusser, S. M., Bernard, G. M. and Wasylishen, R. E. (2012). Tracking stable isotope enrichment in tree seedlings with solid-state NMR spectroscopy. *Scientific Reports* 2, 1–5. doi: 10.1038/srep00719

Ovaska, J., Ruuska, S., Rintamaki, E., and Vapaavuori, E. (1993). Combined effects of partial defoliation and nutrient availability on cloned Betula pendula saplings II: changes in net photosynthesis and biochemical properties. *Journal of Experimental Botany* 44, 1395–1402.

Palacio, S., Hester, A.J., Maestro, M., and Millard, P. (2008). Browsed *Betula pubescens* trees are not carbon-limited. *Functional Ecology* 22, 808–815. doi: 10.1111/j.1365-2435.2008.01433.x

Peltier, D. M. P., Guo, J., Nguyen, P., Bangs, M., Gear, L., Wilson, M., et al. (2020). Temporal controls on crown nonstructural carbohydrates in southerwestern US tree species. *Tree Physiology* 41, 388–402. doi: 10.1093/treephys/tpaa149

Peterson, E. B., and Peterson, N. M. (1992). "Ecology, management, and use of aspen and balsam poplar in the prairie provinces, Canada," in *Canadian Forest Service Publications Special Report*, vol 1. (Foresty Canada, Northern Forestry Centre, Edmonton, Alberta), 1–252.

Pinheiro, C., and Chaves, M. M. (2011). Photosynthesis and drought: can we make metabolic connections from available data? *Journal of Experimental Botany* 62, 869–882. doi: 10.1093/jxb/erq340

Pinheiro, J., Bates, D., DeRoy, S., Sarkar, D., and R Core Team (2016). nlme: Linear and Nonlinear Mixed Effects Models. R Package version 3.1-128. http://CRAN.R-project.org/package=nlme

Pinkard, E. A., and Beadle, C. L. (1998). Regulation of photosynthesis in *Eucalyptus nitens* (Deane and Maiden) Maiden following green pruning. *Trees* 12, 366–376. doi: 10.1007/s004680050163

Piper, F. I., and Fajardo, A. (2014). Foliar habit, tolerance to defoliation and their link to carbon and nitrogen storage. *Journal of Ecology* 102, 1101–1111. doi: 10.1111/1365-2745.12284

Piper, F. I., Gundale, M. J., and Fajardo, A. (2015). Extreme defoliation reduces tree growth but not C and N storage in a winter-deciduous species. *Annals of Botany* 115, 1093–1103. doi: 10.1093/aob/mcv038

Plavcova, L., and Hacke, U. G. (2012). Phenotypic and developmental plasticity of xylem in hybrid poplar saplings subjected to experimental drought, nitrogen fertilization and shading. *Journal of Experimental Botany* 63, 6481–6491. doi:10.1093/jxb/errs3103

Pomiès, L., Decourteix, M., Franchel, J., Moulia, B., and Leblanc-Fournier, N. (2017). Poplar stem transcriptome is massively remodelled in response to single or repeated mechanical stimuli. BMC Genomics 18, 300. doi:10.1186/s12864-017-3670-1.

Poorter, H., and Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology* 27, 595–607. doi: 10.1071/PP99173

Poorter, H., Niklas, K. J, Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist* 193, 30–50. doi: 10.1111/j.1469-8137.2011.03952.x

Prieto, I., Armas, C., and Pugnaire, F. I. (2012). Water release through plant roots: new insights into its consequences at the plant and ecosystem level. *New Phytologist* 193, 830–841. doi: 10.1111/j.1469-8137.2011.04039.x

R Development Core Team (2018). R: A Language and Environment for Statistical Computing.

Rachmilevitch, S., Lambers, H., and Huang, B. (2008). Short-term and long-term root respiratory

acclimation to elevated temperatures associated with root thermotolerance for two Agrostis grass species. *J. Exp. Bot.* 59, 3803–3809. doi:10.1093/jxb/ern233.

Reich, P. B., Teskey, R. O., Johnson, P. S., and Hinckley, T. M. (1980). Periodic root and shoot growth in oak. *Forest Science* 26, 590–598.

Reichenbacker, R. R., Schultz, R. C., Hart, E. R. (1996). Artificial defoliation effect on *Populus* growth, biomass production, and total non-structural carbohydrate concentration. *Environmental Entomology* 25, 632–642. doi: 10.1093/ee/25.3.632

Reynolds, W. D., and Clarke Topp, G. (2008). "Soil Water Desorption and Imbibition: Tension and Pressure Techniques," in *Soil Sampling and Methods of Analysis Second Edition*. Eds M. R. Carter and E. G. Gregorich (Boca Raton, FL: CRC Press), 981–997.

Rood, S. B., Patino, S., Coombs, K., and Tyree, M. T. (2000). Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees – Structure and Function* 14, 248–257. doi: 10.1007/s004680050010

Ryser, P. (1996). The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. *Functional Ecology* 10, 717–723. doi: 10.2307/2390506

Sakuratani, T. A., and Higuchi, H. (1999). Reverse flow in roots of Sesbania rostrata measured using the constant power heat balance method. *Plant, Cell and Environment* 22, 1153–1160. doi: 10.1046/j.1365-3040.1999.00464.x

Sala, A., Piper, F., and Hoch, G. (2010). Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist* 186, 274–281. doi: 10.1111/j.1469-8137.2009.03167.x

Sala, A., Woodruff, D. R., and Meinzer, F. C. (2012). Carbon dynamics in trees: feast or famine? *Tree Physiology* 32, 764–775. doi: 10.1093/treephys/tpr143

Sauter, J. J., van Cleve, B., and Wellenkamp, S. (1989). Ultrastructural and biochemical results on the localization and distribution of storage proteins in a poplar tree and in twigs of other tree species. *Holzforschung* 43, 1–6.

Schädel, C., Blöchl, A., Richter, A., and Hoch, G. (2009). Short-term dynamics of non-structural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. *Tree Physiology* 29, 901–911. doi: 10.1093/treephys/tpp034

Schäfer, K. V. R., Clark, K. L., Skowronski, N., and Hamerlynck, E. P. (2010). Impact of insect defoliation on forest carbon balance as assessed with a canopy assimilation model. *Global Change Biology* 16, 546–560. doi: 10.1111/j.1365-2486.2009.02037.x

Schmid, S., Palacio, S., and Hoch, G. (2017). Growth reduction after defoliation is independent of CO_2 supply in deciduous and evergreen young oaks. *New Phytologist* 214, 1479–1490. doi: 10.1111/nph.14484

Schott, K. M., Pinno, B. D., and Landhäusser, S. M. (2013). Premature shoot growth termination allows nutrient loading of seedlings with an indeterminate growth strategy. *New Forests* 44, 635–647. doi: 10.1007/s11056-013-9373-9

Schreiber, S. G., Hacke U. G., Chamberland, S. et al. (2016). Leaf size serves as a proxy for xylem vulnerability to cavitation in plantation saplings. *Plant, Cell and Environment* 39, 272–281. doi: 10.1111/pce.12611

Seneviratne, S. I., Corti, T., Davin, E. L., Hirschi, M., Jaeger, E. B., Lehner, I., et al. (2010). Investigating soil moisture-climate interactions in a changing climate: a review. *Earth-Sciences Reviews* 99, 125–161. doi: 10.1016/j.earscirev.2010.02.004

Sevanto, S., McDowell, N. G., Dickman, T., Pangle, R., and Pockman, W. T. (2014). How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell and Environment* 37, 153–161. doi: 10.1111/pce.12141

Silla, F., and Escudero, A. (2006). Coupling N cycling and N productivity in relation to seasonal stress in *Quercus pyrenaica* Willd. saplings. *Plant and Soil* 282, 301–311. doi: 10.1007/s11104-006-6249-1.

Snedden, J. (2013). The root distribution, artchitecture, transpiration and sapflow dynamics of mature trembling aspen (Populus tremuloides) growing along a hillslope. [Master's thesis]. [Edmonton (AB)]: University of Alberta

Sperry, J. S., and Ikeda, T. (1997). Xylem cavitation in roots and stems of Douglas-fir and white fir. *Tree Physiology* 17, 275–280.

Spicer, R. (2014). Symplasmic networks in secondary vascular tissues: parenchyma distribution and activity supporting long-distance transport. *Journal of Experimental Botany* 65, 18291848. doi: 10.1093/jxb/ert459

Staswick, P. E. (1994). Storage proteins of vegetative plant tissues. *Annual Review of Plant Physiology and Plant Molecular Biology* 45, 303–322.

Stepien, V., Sauter, J. J., and Martin, F. (1994). Vegetative storage proteins in woody plants. *Plant Physiology and Biochemistry* 32, 185–192.

Sword, M. A., Gravatt, D. A., Faulkner, P. L., and Chambers, J. L. (1996). Seasonal branch and fine root growth of juvenile loblolly pine five growing seasons after fertilization. *Tree Physiology* 16, 899–904.

Tagliavini, M., Millard, P., and Quartieri, M. (1998). Storage of foliar-absorbed nitrogen and remobilization for spring growth in young nectarine (*Prunus persica* var. *nectarina*) trees. *Tree Physiology* 18, 203–207.

Thornley, J. H. M. (1972). A balanced quantitative model for root: shoot ratios in vegetative plants. *Annals of Botany* 36, 431–441. doi: 10.1093/oxfordjournals.aob.a084602

Tixier, A., Sperling, O., Orozco, J., Lampinen, B., Roxas, A. A., Saa, S., et al. (2017). Spring bud growth depends on sugar delivery by xylem and water recirculation by phloem Münch flow in *Juglans regia*. *Planta* 246, 495–508. doi: 10.1007/s00425-017-2707-7

Tomlinson, K. W., Sterck, F. J., Bongers, F., da Silva, D. A., Barbosa, E. R. M., Ward, D., et al. (2012). Biomass partitioning and root morphology of savanna trees across a water gradient. *Journal of Ecology* 100, 1113–1121. doi: 10.1111/j.1365-2745.2012.01975.x

Trifilò, P., Casolo, V., Raimondo, F., Petrussa, E., Boscutti, F., Lo Gullo, M. A., et al.. (2017). Effects of prolonged drought on stem non-structural carbohydrates content and post-drought

hydraulic recovery in *Laurus nobilis* L.: The possible link between carbon starvation and hydraulic failure. *Plant Physiology and Biochemistry* 120, 232–241. doi: 10.1016/j.plaphy.2017.10.003

Tromp-van Meerveld, H. J., and McDonnell, J. J. (2006). On the interrelations between topography, soil depth, soil moisture, transpiration rates and species distribution at the hillslope scale. *Advances in Water Resources* 29, 293–310. doi: 10.1016/j.advwatres.2005.02.016

Trugman, A. T., Detto, M., Bartlett, M. K., Medvigy, D., Anderegg, W. R. L., Schwalm, C., et al. (2018). Tree carbon allocation explain forest drought-kill and recovery patterns. *Ecology Letters* 21, 1552–1560.

Trumbore, S., Czimczik, C. I., Sierra, C. A., Muhr, J., and Xu, X. (2015). Non-structural carbon dynamics and allocation relate to growth rate and leaf habit in California oaks. *Tree Physiology* 35, 1206–1222. doi: 10.1093/treephys/tpv097

Tuomi, J., Niemelä, P., and Sirén, S. (1990). The Panglossian paradigm and delayed inducible accumulation of foliar phenolics in mountain birch. *Oikos* 59, 399–410. doi: 10.2307/3545152

Turgeon, R. (1989) The sink-source transition in leaves. *Annual Review of Plant Physiology and Plant Molecular Biology* 40, 119–138. doi: 10.1146/annurev.pp.40.060189.001003

Tyree, M. T., Cochard, H., Cruiziat, P., Sinclair, B., and Ameglio, T. (1993). Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. *Plant, Cell and Environment* 16, 879–882. doi: 10.1111/j.1365-3040.1993.tb00511.x

Vereecken, H., Huisman, J. A., Pachepsky, Y., Montzka, C., van der Kruk, J., Bogena, H., et al. (2014). On the spatio-temporal dynamics of soil moisture at the field scale. *Journal of Hydrology* 516, 76–96. doi: 10.1016/j.jhydrol.2013.11.061

Vizoso, S., Gernat, D., Guehl, J. M., Joffre, R., Chalot, M., Gross, P., et al. (2008). Do elevation of CO_2 and nitrogen fertilization alter storage and remobilization of carbon and nitrogen in pedunculate oak saplings? *Tree Physiology* 28, 1729–1739. doi: 10.1093/treephys/28.11.1729

Volin, J., Kruger, E., and Lindroth, R. (2002). Responses of deciduous broadleaf trees to defoliation in a CO_2 enriched atmosphere. *Tree Physiology* 22, 435–448.

Wardlaw, I. F. (1990). The control of carbon partitioning in plants. *The New Phytologist* 116, 341381. doi: 10.1111/j.1469-8137.1990.tb00524.x

Warren, C. R., Dreyer, E., and Adams, A. (2003). Photosynthesis-Rubisco relationships in foliage of *Pinus sylvestris* in response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores. *Trees* 17, 359–366. doi: 10.1007/s00468-003-0246-2

Watson, M. A., and Casper, B. B. (1984). Morphogenetic constraints on patterns of carbon distribution in plants. *Annual Review of Ecology and Systematics* 15**,** 233–258.

Wiley, E. (2020). Do carbon reserves increase tree survival during stress and following

disturbance? *Current Forestry Reports* 6, 1425. doi: 10.1007/s40725-019-00106-2

Wiley, E., Hoch, G., and Landhäusser, S. M. (2017). Dying piece by piece: carbohydrate dynamics in aspen (*Populus tremuloides*) seedlings under severe carbon stress. *Journal of Experimental Botany* 68, 5221–5232. doi: 10.1093/jxb/erx342

Wiley, E., Huepenbecker, S., Casper, B. B., and Helliker, B. R. (2013). The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favour of storage? *Tree Physiology* 33, 1216–1228. doi: 10.1093/treephys/tpt093

Wiley, E., King, C. M., and Landhäusser, S. M. (2019). Identifying the relevant carbohydrate storage pools available for remobilization in aspen roots. *Tree Physiology* 39, 1109–1120. doi: 10.1093/treephys/tpz051

Wiley, E., Rogers, B. J., Hodgkinson, R., and Landhäusser, S. M. (2016). Nonstructural carbohydrate dynamics of lodgepole pine from mountain pine beetle attack. *New Phytologist* 209, 550–562. doi: 10.1111/nph.13603

Zimmermann, M. H. (1971). "Storage, mobilization and circulation of assimilates" in *Trees: structure and function*. Eds M.H Zimmermann and C.L. Brown (New York: Springer), 307–331.

Zimmerman, M. H. (1983). "The significance of plant segmentation" in *Xylem Structure and the Ascent of Sap*. Eds M. H. Zimmerman (Berlin: Springer), 171–174.

Appendix

Chapter 2

Table A-1. Adjusted p-values for detection of excess ^{13}C and ^{15}N (APE) in leaf and root samples for stem-origin and leaf and stem samples of root-origin saplings at both harvest timepoints (18 days after early leaf expansion and 3 weeks after defoliation) using the Benjamini-Hochberg correction. NA: no label detected. ($\alpha < 0.05$).

Label Origin	Tissue	^{13}C Atom Percent Excess		^{15}N Atom Percent Excess	
		Raw p-value	Adjusted p-value	Raw p-value	Adjusted p-value
Stem	Roots (18 days)	0.047	0.047	0.011	0.013
	Lower early leaves	4.1e-04	8.2e-04	9.1e-05	2.1e-04
	Upper early leaves	4.5e-05	1.4e-04	5.0e-05	2.1e-04
	Upper late leaves	1.1e-05	6.5e-05	8.8e-05	2.1e-04
	Roots (3 weeks)	NA	NA	0.0076	0.011
	Lower re-flush leaves	0.0025	0.0037	7.7e-04	0.0013
	Upper re-flush leaves	0.034	0.041	0.082	0.082
Root	Lower stem (18 days)	0.046	0.059	0.0017	0.0025
	Lower early leaves	4.4e-05	1.9e-04	2.9e-09	1.3e-08
	Upper stem (18 days)	0.039	0.058	0.011	0.012
	Upper early leaves	2.7e-05	1.905e-04	1.4e-08	4.2e-08

Upper late leaves	0.017	0.049	5.3e-10	4.7e-09
Lower stem (3 weeks)	0.030	0.055	0.0098	0.012
Lower re-flush leaves	0.022	0.049	0.0014	0.0025
Upper Stem (3 weeks)	0.082	0.092	0.096	0.096
Upper re-flush leaves	0.19	0.19	0.0014	0.0025

Table A-2. Estimated marginal means (±1 standard error) for height (cm), root collar diameter (RCD) (mm), aboveground mass (g), stem mass (g), leaf mass (g), root mass (g) and root volume (g/cm^3) for un-grafted saplings compared to grafted saplings 18 days after early leaf expansion (n = 32, $\alpha < 0.05$).

	Un-grafted saplings	Grafted saplings
Height (cm)	78.8 (3.5) **a**	71.7 (2.9) **a**
RCD (mm)	7.6 (0.4) **a**	8.3 (0.3) **a**
Aboveground Mass (g)	11.3 (1.1) **a**	9.9 (0.9) **a**
Stem Mass (g)	6.8 (0.8) **a**	6.0 (0.6) **a**
Leaf Mass (g)	4.5 (0.4) **a**	3.8 (0.3) **a**
Root Mass (g)	9.4 (1.1) **a**	8.8 (0.9) **a**
Root Volume (g/cm^3)	43.2 (5.0) **a**	40.7 (4.1) **a**

Table A-3. Estimated marginal means (±1 standard error) for height (cm), root collar diameter (RCD) (mm), aboveground mass (g), stem mass (g), re-flush leaf mass (g), leaf mass recovery (%), root mass (g) and root volume (g/cm^3) for un-grafted saplings compared to grafted saplings 3 weeks after defoliation (n = 34, $\alpha < 0.05$).

	Un-grafted saplings	**Grafted saplings**
Height (cm)	69.8 (3.7) **a**	73.4 (3.1) **a**
RCD (mm)	7.5 (0.4) **a**	8.2 (0.3) **a**
Aboveground Mass (g)	7.7 (0.8) **a**	8.3 (0.7) **a**
Stem Mass (g)	6.4 (0.8) **a**	7.0 (0.7) **a**
Re-Flush Leaf Mass (g)	1.3 (0.2) **a**	1.3 (0.1) **a**
Leaf Mass Recovery (%)	35.9 (5.0) **a**	31.3 (4.2) **a**
Root Mass (g)	8.3 (1.0) **a**	9.0 (0.8) **a**
Root Volume (g/cm^3)	41.7 (4.8) **a**	47.0 (4.0) **a**

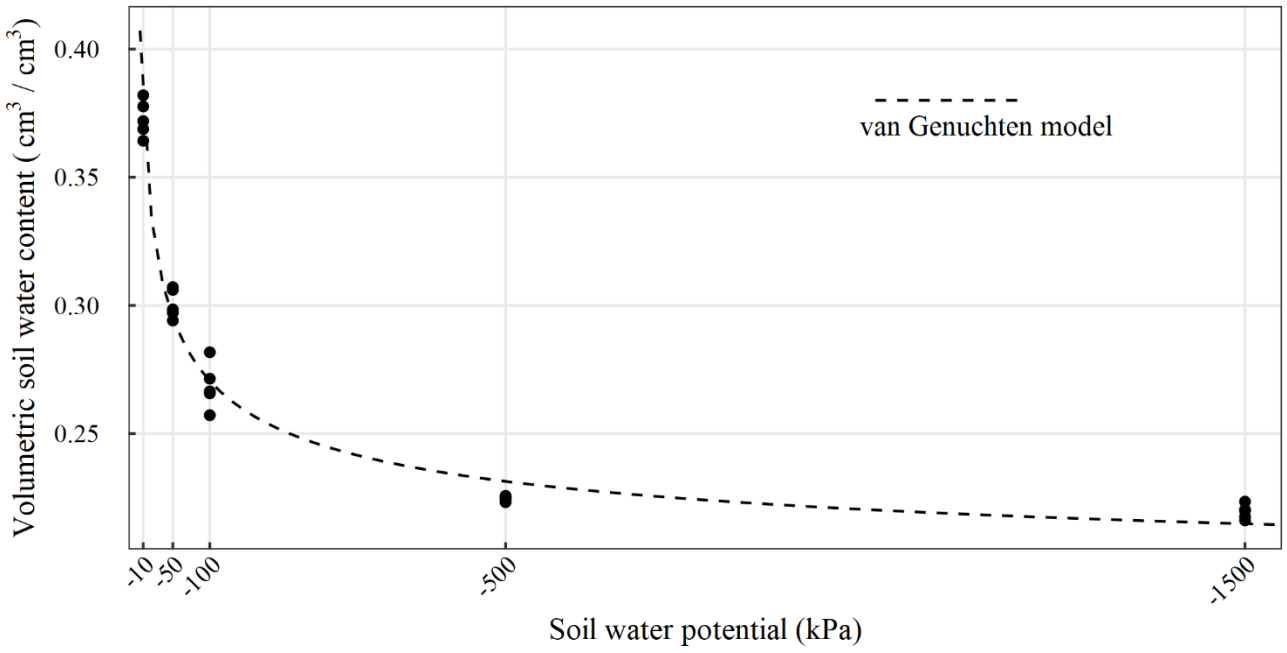

Figure A-1. Soil water retention curve determined using the pressure extractor method (Reynolds and Clarke Topp, 2008) for the sandy-loam soil used to fill split-pots. Soil hydraulic properties were assessed to determine use of both MPS2 sensors and daily weighing to produce moderate, progressive drought conditions. The van Genuchten model was used to represent the relationship between volumetric soil water content (SWC) and soil water potential (SWP); $SWC = \theta_r + \frac{\theta_s - \theta_r}{(1+(\alpha SWP)^n)^{1-1/n}}$**. The estimation of the four parameters are:** $\theta_r \sim 0.18$; $\theta_s \sim 0.49$; $\alpha \sim 0.32$; $n \sim 1.35$

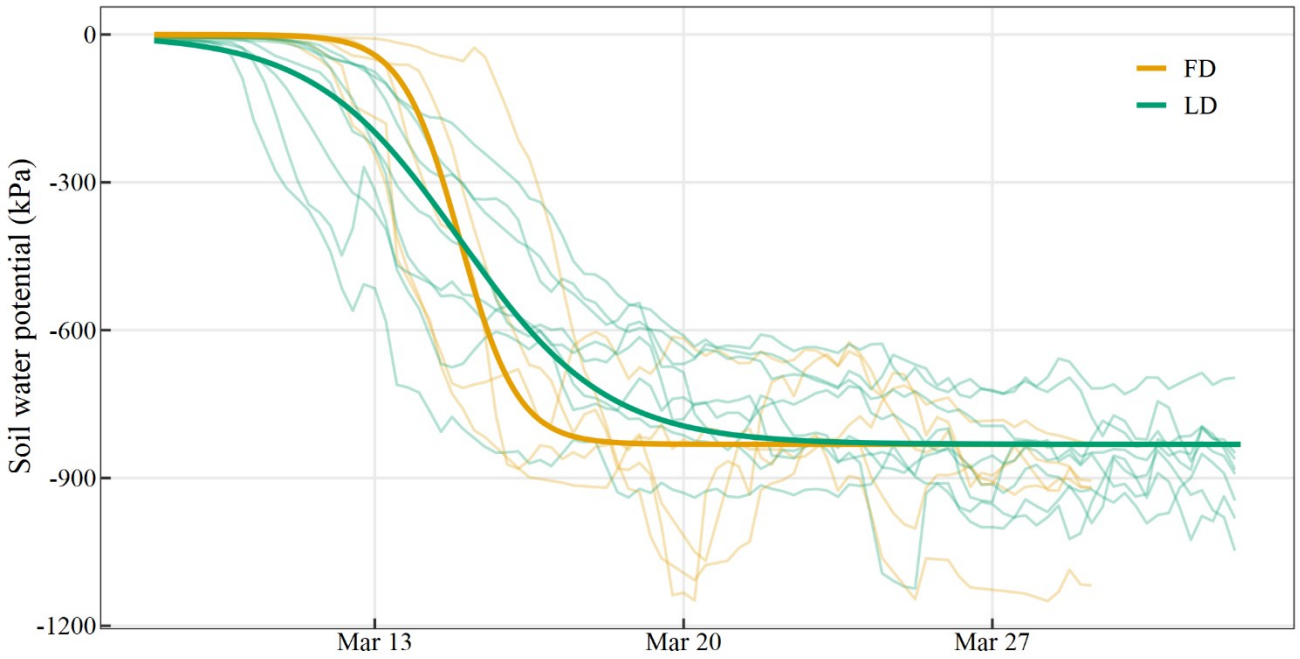

Figure A-2. Change in soil water potential (kPa) measured using MPS2 sensors for full drought (FD, yellow) and localized drought (LD, green) treatments over the four-week experimental period. A logistic function was used to represent the progressive decrease in soil water potential over time for the two soil moisture treatments: $\boldsymbol{SWP = \frac{Asym}{1+ e^{(x_{mid} - Time)/scal}}}$**.**

The estimated parameters are: $\boldsymbol{Asym \sim -831.8}$; $\boldsymbol{x_{mid} \sim 167.3}$; $\boldsymbol{scal_{FD} \sim 15.8}$; $\boldsymbol{scal_{LD} \sim 39.8}$**.**

Table A-4. Schedule of the growth chamber settings (daylight length, air temperature (°C), light level (µmol) and humidity (%) between November 21st, 2016 and March 5th, 2017 when the saplings were cycled through winter and spring conditions and during the experimental period starting March 6th, 2017.

Date	Day length	Hour interval	Temperature	Light Level	Humidity
Nov 21st 2016 – Jan 3rd 2017	0h	0:00 – 0:00	-1°C	0 µmol	Turned off
Jan 4th – Jan 19th 2017	12 h	7:00 – 10:00	5°C	500µmol	60%
		10:00 – 14:00	5°C	500µmol	60%
		14:00 – 19:00	5°C	500µmol	60%
		19:00 – 7:00	2°C	0µmol	60%
Jan 19th – Feb 3rd 2017	14 h	6:00 – 10:00	9°C	500µmol	60%
		10:00 – 14:00	12°C	500µmol	60%
		14:00 – 20:00	9°C	500µmol	60%
		20:00 – 6:00	6°C	0µmol	60%
Feb 3rd – Feb 6th 2017	16 h	5:00 – 10:00	13°C	500µmol	60%
		10:00 – 14:00	18°C	1000µmol	60%
		14:00 – 21:00	16°C	1000µmol	60%
		21:00 – 5:00	10°C	0µmol	60%
Feb 6th – Feb 22nd 2017	12 h	8:00 – 20:00	18°C	500µmol	60%
		20:00 – 8:00	16°C	0µmol	60%
Feb 22nd – April 1st 2017	24 h	0:00 – 0:00	20°C	500µmol	60%