University of Alberta

The root distribution, architecture, transpiration and root sapflow dynamics of mature trembling aspen (*Populus tremuloides*) growing along a hillslope

by

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

Master of Science
in
Water and Land Resources

Department of Renewable Resources

Fall 2013
Edmonton, Alberta

Abstract

The objectives of this study were to explore comparative controls by atmospheric and belowground variables governing transpiration of trembling aspen (*Populus tremuloides*) growing along a water limited hillslope. Vertical and horizontal root distribution, intra- and inter-clonal root connections, soil moisture and transpiration and root water uptake dynamics of several aspen clones growing along a gradient of soil moisture availability were investigated. Fine root surface area was greatest at the lower portion of the hillslope and in the surface soil layers where soil moisture was greatest. Root water uptake capability was positively and strongly correlated with transpiration where trees at lower slope positions transpired twice the water per unit leaf area than trees in upper slope positions. The description of comparative atmospheric and belowground variables on water economy of trees is novel and provides significant insight into growth and water use strategies of trees growing in water limited environments.

Acknowledgements

I would like to take this opportunity to thank my supervisors, Dr. Simon Landhäusser and Dr. Uldis Silins for their wonderful guidance and incredible support throughout this project. Without your patience, trust, enthusiasm and friendship this project would not have been a success. Thank you for keeping me focused and all your editorial comments that have helped me produce a thesis that I am proud of. I would also like to thank Dr. Karen Mock, Dr. Miles Dyck and Dr. Kevin Devito for providing guidance during the development of this project.

I would also like to thank my friends and colleagues Jocelyn Howery, Pablo Pina, Michael Wagner and Chris Williams for their spirited conversations, encouragement and support, which made my graduate experience a blast! I would also like to thank my field assistances who partook in some extreme manual labour to excavate root systems and pick leaves off trees on a south facing hillslope: Tory Culen, Jeremy Fitzpatrick, Geoff Kershaw, Kathleen Little, Jolene Lust, Jem Morrison, Pete Presant, Ryan Sherrit and Ian Tichkowsky.

I would also like to thank all those responsible for providing financial support. Thank you to Alberta-Pacific Forest Industries Inc., Albian Sands Energy, Canadian Natural Resources Ltd., Ducks Unlimited Canada, Forest Products Associated of Canada, Imperial Oil Ltd., Petro-Canada, Slave Lake AESRD, Syncrude Canada Ltd., Tolko Industries Ltd. and Total E&P Canada Ltd. Thank you to NSERC for providing the funding for this project (Drs. Uldis Silins/Simon Landhäusser), HEAD2 NCERC-CRD (Dr. Kevin Devito) and the Department of Renewable Resources for recognizing my achievements and enabling me to pursue this project. I would also like to thank West Fraser Timber for awarding me a scholarship and the Circumpolar/Boreal Alberta Research (C/BAR) Grant for providing additional funding for this project.

Finally, I would like to thank my friends for their never-ending encouragement and support. I would also like to thank my family for providing me a great foundation to start from. Thank you all for always believing in me.

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List of Symbols and Abbreviations

ga	Aerodynamic conductance (m s^{-1})
Ta	Ambient temperature (°C)
ea	Ambient vapour pressure (Pa)
ANOVA	Analysis of variance
gc	Canopy conductance (m s^{-1})
pa	Density of air (g m^{-3})
DBH	Diameter at breast height
SGA	Dynagage sapflow sensor
ca	Heat capacity of air (J g^{-1} K^{-1})
λ	Latent heat of vapourization (J g^{-1})
LAI	Leaf area index
MPS	Matric potential sensor
$Q*$	Net radiation (W m^{-2})
ET_p	Potential evapotranspiration (mm h^{-1})
γ	Psychrometric constant (Pa K^{-1})
RH	Relative humidity (%)
Root Q_v	Root volumetric sapflow rate (m^3 m^{-2} h^{-1})
es	Saturation vapour pressure (Pa)
Δ	Slope of the saturation vapour pressure versus temperature curve at a given ambient temperature (Pa $°C^{-1}$)
ψs	Soil water potential (kPa)
TDP	Thermal dissipation probe
A_L	Total leaf area of the tree
Q_L	Transpiration per unit leaf area (m^3 H2O m^{-2} AL h^{-1})
Q_v	Tree volumetric sapflow rate (cm^3 h^{-1})
URSA	Utikuma Research Study Area
D	Vapour pressure deficit (kPa)
u	Wind speed (m s^{-1})

Chapter 1: Introduction

Water is the primary limiting resource for terrestrial plants because it is needed for primary physiological processes (Rodriguez-Iturbe 2000). Transpiration is the process of water loss through plant stomata to the atmosphere during photosynthesis. Most of the water required by plants from the soil (pedosphere) is lost passively along a water potential gradient from the soil to the atmosphere through transpiration and at larger scales, this process regulates the transfer of energy between the pedosphere and the atmosphere (Jackson et al. 1999, Amenu and Kumar 2008). The transfer of energy between the pedosphere, atmosphere and plants are commonly referred to as the soil-plant-atmosphere continuum (SPAC) (Berry et al. 2006).

Plants can be viewed as consisting of three main organs: leaves, stems and branches and roots. Leaves are responsible for photosynthesis, the conversion of incoming radiation (light), water and carbon dioxide to oxygen and sugar. The stem and branches position the leaves such that they can intercept the maximum amount of light and act as conduits for the transfer of water and nutrients to the leaves (xylem) and sugars to the roots (phloem) (Jackson et al. 1999). The roots stabilize and anchor the plant and uptake water and nutrients from the soil, which are necessary for photosynthesis (Berry et al. 2006). In northern temperate regions, soil moisture availability is typically greater early in the growing season and declines later in the growing season. Early in the growing season plant growth is considerable, and as a result water loss due to transpiration at this time is high. Plants adapt to the local climate by maximizing growth and leaf area until the decline in water availability prevents further growth, and maximum leaf area is reached for that season (Kergoat 1998). As a result of the decline in soil moisture later in the growing season, plants must achieve a balance between maximizing transpiration (i.e. growth and productivity) and reducing water loss to the atmosphere (Grier and Running 1977). The balance between productivity and maintenance of internal water status is commonly referred to as hydrological equilibrium (Kergoat 1998).

1.1 Plant Adaptations to Water Stress

Plants have evolved various strategies and adaptations to balance productivity and internal water status. These adaptations can be grouped into short-term and long-term adaptations. Localized and short-term adaptations to water stress include, but are not limited to changes to stomatal closure, or short-term changes in leaf morphology such as rolling or curling of leaves, and early senescence (Hale & Orcutt 1987). At seasonal time scales, plants under water stress can reduce leaf growth and expansion of leaf area to constrain water loss (Kergoat 1998). Long-term adaptations to water stress are generally more drastic, where whole branches of crowns or in the case of aspen clones ramets can be abscised (Grier and Running 1977). While this may result in severe limitations to photosynthesis and growth, this strategy may conserve water, enabling survival under extended drought conditions.

Another long-term adaptation to water stress is the plasticity of root distribution and root architecture throughout the soil. The distribution of roots has been shown to be influenced by localized soil moisture conditions, where root growth and mortality is greatly influenced by availability of soil moisture (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Coleman 2007, Brassard et al.2009). Root hydrotropism refers to observations of greater fine root development in areas of greater soil moisture availability (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Coleman 2007, Brassard et al. 2009). However, fine roots have high seasonal turnover and impose great metabolic cost to a plant (Brassard et al. 2009). Therefore, plants must balance the metabolic costs of greater root growth against the benefits of greater water uptake from the soil.

Some plants use more dynamic adaptations to achieve hydrologic equilibrium such as sharing of resources through root connections (grafting or clonal root connections) or through processes called hydraulic lift or hydraulic redistribution of soil water. Hydraulic lift is the upward movement of water along a water potential gradient from moist deep soil layers to dry shallow soil layers via plant roots (Richards and Caldwell 1987, Caldwell and Richards 1989). In contrast, hydraulic redistribution involves the passive movement of water laterally through plant roots from areas of greater soil moisture to drier areas within the soil profile (Guswa 2012). These adaptations enable plants to access available soil moisture, which is heterogeneously distributed vertically throughout the soil profile and horizontally in the immediate area. These adaptations produce hydrological linkages that connect the atmosphere to the pedosphere through plants (Devito et al. 2005). However, it remains unclear where the dominant influences are; antecedent moisture conditions influence plant productivity, but plant productivity also influence soil moisture.

1.2 Influence of Atmospheric and Soil Moisture Conditions on Transpiration

The soil-plant-atmosphere continuum encompasses the interaction of plants with both atmospheric variables and soil moisture availability. Atmospheric moisture demand and net radiation (Q^*) strongly affect stomatal opening and thus the water potential gradient between the atmosphere, plant and soil. When soil moisture is not limiting, transpiration is strongly and positively associated with greater vapour pressure deficit (D), Q^*, air temperature (Ta) and total atmospheric demand for moisture or potential evapotranspiration (ET_p) (Granier and Breda 1996, O'Brien et al. 2004, David et al. 2004, Leuzinger et al. 2005, Bovard et al. 2005, Kume et al. 2007, Adelman et al. 2008, McLaren et al. 2008, MacKay et al. 2012). However, high atmospheric moisture demand is also associated with plant water stress. It is difficult to disentangle the influences of atmospheric variables and soil moisture availability on transpiration. Examination of the influence of atmospheric variables and soil moisture availability on plants in natural environments is very complicated because of the difficulty of finding areas to test how plants respond to different levels of soil moisture conditions under the same atmospheric variables. Soil moisture gradients exist through the soil profile and along the landscape such as along a hillslope. A gradient of soil moisture availability often exists along hillslopes, where soil moisture availability generally increases from the upper to the lower portion of the hillslope. Thus a hillslope allows for testing test how plants respond to different levels of soil moisture conditions at under the same atmospheric controls variables, and changing levels of both.

The influence of atmospheric conditions and soil moisture availability on plants varies strongly among species. *Populus tremuloides* Michx. (trembling aspen) is well adapted to survival across a broad range of environmental conditions as evidenced by the wide distribution of this species (DeByle and Winokur 1985). Trembling aspen (aspen) can be found growing throughout North America, spanning the lower elevation boreal forests, to the higher elevation southern Rocky Mountains, from moist rich transitional areas along riparian areas to the drought prone transitions zones of the prairie grasslands (Little 1971). Aspen reproduces more successfully by asexual reproduction than germinating from seed (sexual reproduction). Asexual reproduction of aspen generally occurs after a disturbance removes the aboveground portion of the tree, which stimulates root suckering from lateral roots (Bartos 2001, Frey et al. 2003). This form of asexual reproduction results in the formation of a parental root system (the surviving root system) that connects genetically identical suckers (ramets) (Stone 1974, Peltzer 2002). The parental root system continues to function after the aboveground disturbance by sequestering water and nutrients from the soil for the new suckers, producing a clonal organism (DesRochers and Lieffers 2001). The importance of the surviving parental root system in providing clonal root connections for the ramets has been shown to be important for survival and growth of the clone by facilitating the sharing of resources. Studies have shown that severing these root connections results in reduced growth of individual ramets (DesRochers and Lieffers 2001). In addition to clonal root connections, aspen can also form functional root grafts (DesRochers and Lieffers 2001). Root grafts are formed when the cambium, phloem and xylem of two or more overlapping roots fuse, which facilitates water and photosynthate exchange between the connected roots (Graham and Bormann 1966, Fraser et al. 2006). Severing a clonal or grafted root connection has shown to result in reduced productivity of aspen suckers, which suggests that root connections are important for the preservation of genetic material. However, little is known about what role these root connections could play in the distribution of water and other resources among individual trees under limiting conditions.

Root systems are comprised of laterally and vertically distributed coarse roots, which transfer water and nutrients from the soil to the plant and are used for structural support. Fine roots are more important for water and nutrient uptake from the soil (Block et al. 2006, Strong and La Roi 1983a), and can have high seasonal turnover rates (Coutts 1987, Brassard et al. 2009). The distribution of coarse and fine roots of mature trees growing in natural environments is poorly understood because of difficult logistical issues in excavating large root systems. As a result, researchers have typically used small-scale studies to enable them to gain insight into the root architecture of mature trees using soil cores (Bauhus and Messier 1999) or smaller soil pits (Strong and La Roi 1983a). However, a limited number of studies have excavated areas around single stems or small sections of clones (Gifford 1966, De Boyle and Winokur 1985, Strong and La Roi 1983b, Puri et al. 1994, DesRochers and Lieffers 2001) to enable more thorough description of root system architecture of mature trees. However, these have not previously been used to describe distribution of roots in response to variation in environmental factors such as soil moisture gradients. Thus, information on root system architecture in response to environmental gradients provided by such studies remains limited, because lateral roots in aspen can spread many times further than the crown radius of an individual tree in mature forests (Gifford 1966).

Plasticity of root distribution is a key adaptation that enables plants to grow and survive in water limiting environments, such as those existing at the upper portion of a hillslope. However, little is known about the spatial variability of root growth and root occupancy along hillslopes in mature forests, and how trees allocate resources for root growth to areas where soil moisture is more available as a strategy for growth and survival. There is a large body of research that confirms atmospheric conditions greatly influence productivity in plants (Granier and Breda 1996, O'Brien et al. 2004, David et al. 2004, Leuzinger et al. 2005, Bovard et al. 2005, Kume et al. 2007, Adelman et al. 2008, McLaren et al. 2008, MacKay et al. 2012); while another body of research concludes that soil moisture availability has a greater influence on productivity (Pataki et al. 2000, Schwarz et al.2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Dalla-Salda et al. 2009, Dalla-Salda et al. 2011, MacKay et al. 2012). The combined influence of atmospheric variables and soil moisture availability on transpiration of mature trees has not been extensively studied. Thus the interaction of these variables as key regulators of water use and productivity remains unclear (Hogg et al. 2000, Pataki et al. 2000, Schwarz et al. 2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Tromp-van Meerveld and McDonnell 2006, Berry et al. 2006). Additionally, the influence of seasonal variation in soil moisture availability and atmospheric demand on transpiration dynamics in trees is not fully understood.

1.3 Research Outline/Objectives

The overall objectives of this research were to explore the influence of atmospheric variables and soil moisture availability on root architecture and transpiration of mature aspen trees growing along a hillslope.

The objectives of the study reported on in Chapter 2 were to describe the three dimensional spatial distribution of root systems of mature aspen clones growing along a hillslope. Since a hillslope represents a spatial gradient in soil water availability, this study was used to describe relationships between soil water availability and the horizontal and vertical distribution of fine and coarse roots. A related objective was also to identify the role of inter- and intra-clonal root connections for uptake and distribution of water among trembling aspen clones growing along a hillslope.

The objectives of Chapter 3 were to explore the comparative influence of atmospheric variables and soil moisture availability on transpiration of mature aspen trees growing along a hillslope representing a gradient in moisture availability. A related objective was to more closely examine whether the relationship between atmospheric variables, soil moisture and transpiration of aspen will change over the growing season (wetter to drier conditions) depending on the hillslope position.

In Chapter 4, the results from these two studies are synthesized and the implications that can be drawn from this work are discussed. Outstanding research issues and future research needs are proposed.

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Chapter 2: Vertical and Horizontal Root Distribution of Mature Aspen Clones Along a Hillslope: Potential Mechanisms for Drought Avoidance in Water Limited Environments

2.1 Introduction

Tree root systems provide structural support, anchor the aboveground stem and crown structure and secure supply of water and nutrients to support the leaf area required for growth. Distribution of roots (root architecture) varies among tree species with the spatial and temporal availability of belowground growth resources (Coleman 2007, Hutchings and John 2003, 2004). Many tree species growing in the boreal forest have large laterally spreading root systems where most roots occupy the top 5 - 15 cm of the soil profile (Gifford 1966, Strong and LaRoi 1983a,b, Puri et al. 1994), which is generally considered to reflect the vertical variation in soil nutrient and water availability (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Coleman 2007, Brassard et al. 2009). However, other variables such as climate, stand age, stem density (Pinno et al. 2010), basal area and belowground competition (Bauhus and Messier 1999) have also been shown to influence root distribution.

Lateral roots are generally considered to exploit resources such as nutrients and water that are heterogeneously distributed throughout the upper layers of the soil profile, while vertical roots permit access to resources (mostly water) from the deeper soil layers (Brassard et al. 2009, Coutts 1987, Hutchings and John 2003). Large diameter roots are essential for structural stability and transport of water and nutrients, while small diameter roots and fine roots are critical for acquisition of water and nutrients (Block et al. 2006, Strong and La Roi 1983a). Fine roots can have high seasonal turnover rates and impose significant metabolic costs (e.g. growth and maintenance respiration) to trees (Coutts 1987, Brassard et al. 2009). Additionally, fine roots are commonly associated with mycorrhizal fungi, which can increase absorption surface area by 100 to 1000 fold (Egerton-Warburton et al. 2007, Larcher 1995). Belowground competition for resources can also vary among root size classes and rooting depths. The shallow lateral roots often grow where root competition for resources with other herbaceous and woody plants can be high, while the deeper vertical roots are generally exposed to much less root competition (Mundell et al. 2007).

Many studies have demonstrated that root growth is often hydrotropic or greatest in areas where there is adequate soil moisture (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Coleman 2007, Brassard et al. 2009). Hillslopes often have moisture gradients because the lower portion of a hillslope generally experiences greater soil water recharge through overland flow or sub-surface flow from the upper portions of the hillslope (Chamran et al. 2002), or the hillslope has a moisture gradient due to varying distances to a waterbody. However, little is known about the spatial variability of root growth and root occupancy along hillslopes in mature forests, and how trees allocate resources for root growth to areas where soil moisture and nutrient availability is more favourable.

Describing the distribution of roots and root architecture of mature trees in natural stands is very difficult, and has been a primary limitation to research in this field. Many previous studies have used soil cores (Bauhus and Messier 1999) or soil pits (Strong and La Roi 1983a). Other studies have excavated areas around single stems or small sections of clones (Gifford 1966, De Boyle and Winokur 1985, Strong and La Roi 1983b, Puri et al. 1994, DesRochers and Lieffers 2001) to provide insight into spatial distribution of tree roots. While these approaches have provided valuable insights into tree root systems, the information on root system architecture in response to environmental gradients provided by such studies remains limited, because lateral roots in trembling aspen (*Populus tremuloides* Michx.) can spread many times further than the crown radius of an individual tree in mature forests (Gifford 1966).

Trembling aspen (aspen) is a widely distributed tree species across North America, spanning the lower elevation boreal forests, the higher elevation sites in the southern Rocky Mountains and the drought prone transitions zones of the prairie grass lands (Little 1971). Accordingly, aspen is well adapted to grow on a wide range of site and climatic conditions (DeByle and Winokur 1985). Aspen predominately regenerates asexually through root suckering from its lateral roots after a disturbance has killed the aboveground portion of the tree (Bartos 2001, Frey et al. 2003). As a result, the surviving root system (parent root system) connects genetically identical ramets, producing a clonal organism (DesRochers and Lieffers 2001). Severing these root connections has been shown to reduce growth of the individual ramets (Stone 1974, Peltzer 2002), suggesting that these root connections are important for growth and performance of the clone, and allow for the sharing of resources. Aspen roots can also form functional grafts (DesRochers and Lieffers 2001), which are produced by a fusion of the cambium, phloem and xylem of two or more overlapping roots, allowing the exchange of water and photosynthates between grafted roots (Graham and Bormann 1966, Fraser et al. 2006). However, little is known about what role these root connection could play in the distribution of water and other resources among individual trees under limiting conditions.

The objectives of this study were to describe the spatial (3 dimensional) distribution of root systems of mature trembling aspen clones growing along hillslopes and explore relationships between water availability and the horizontal and vertical root mass distribution and the role of inter- and intra-clonal root connections in the acquisition and distribution of water.

2.2 Materials and Methods

2.2.1 Site Description

This study was conducted in the Utikuma Research Study Area (URSA) near Utikuma Lake (56°04’ 45.05”N, 115°28’58.74”W) in north-central Alberta, Canada (Devito et al. 2005). The study area is located within the Boreal Plains ecozone. The climate in the Boreal Plains ecozone is typically sub-humid with short, warm and moist summers (80 - 180 frost-free days; average temperature 9.6 °C, average summer precipitation 336 mm) and long, cold and dry winters (average temperature -14.9 °C, average winter precipitation 142 mm) (Ecoregions Working Group 1989, Natural Regions Committee 2006).

To study root distribution of mature aspen stands along a moisture gradient, a south-facing hillslope (slope > 10°, > 30 m in slope length and > 800 m wide) dominated by mature aspen (approximately 60 yrs. old, 14 m height, stem density of 2800 stems ha^{-1} and diameter at breast height of 13 cm) was selected. Five area-based transects (A to E) were established along the hillslope in the aspen dominated section of the hillslope. The soil type on the entire hillslope was very uniform and was classified as an eutric Brunisol with a silty-sand texture (Soil Classification Working Group 1998). The moisture regime was considered subxeric, rapidly draining and the understory vegetation indicated a medium to poor nutrient regime. The hillslope was situated in close proximity (< 50 m) to a lake which was needed as a water supply for the hydraulic excavation of the root systems.

2.2.2 Clone and Transect Selection

Initially, potential single clones were identified along the hillslope using phenological and morphological characteristics (i.e. stage of leaf flush, bark colour and roughness). To verify the actual clonal composition genetic analysis was used (see Section 2.2.4). The five transects (A to E) were selected along the hillslope by selecting an area where one group of trees (one potential clone) was identified on the lower portion of the slope and a second group of trees (potentially another clone) was clustered directly above on the upper portion of the same slope. Transects were between 10 and 14 m long, 4.5 and 6 m wide and between 25 and 120 m apart. Transects varied somewhat in size in an attempt to encompass the two groups of aspen in each transect. The base of the transects were established above the riparian vegetation, approximately 1 to 1.5 m above the water table, where the hillslope vegetation was dominated by aspen.

The genetic analysis showed that there were actually twelve different genotypes present in the five transects (Table 2-1). Interestingly, some of the groups identified using morphological characteristics were later found to contain ramets belonging to up to four different genotypes (Table 2-1). In one transect (E) ramets turned out to be only from one genotype, even though there appeared to be clear distinguishing characteristics in the bark and the timing of spring bud flush between the trees located at the upper and lower slope position (Table 2-1). Furthermore, ramets of some of the detected genotypes were also present in adjacent transects (Figure 2-8).

2.2.3 Aboveground Measurements

To describe the aboveground parameters of the tree groups and individual trees in each transect, the height and diameter at breast height (1.3 m) were measured on every tree prior to the excavation of the root systems. Prior to excavation, each tree was felled with a chainsaw, and a cross-section of the stem was collected at breast height. For the genetic identification of each ramet (see Section 2.2.4), leaves from the tip of four different branches were clipped from each tree, wrapped in tin foil, placed in a cooler filled with dry ice and transferred to a freezer (-20 °C) within 8 hrs. In transect D only, the total leaf area of each tree was individually measured by hand-picking all leaves off each felled tree and weighing them using a Berkley® 50 lb digital scale (Pure Fishing Inc., Portage la Prairie, MB) to determine leaf fresh mass. A subsample of the fresh leaves from each tree was then used to determine projected leaf area by scanning the leaves using WinDENDRO (REGENT Instruments, Quebec City, Quebec). The relationship between leaf fresh mass and leaf area was developed, and used to estimate total leaf area for each individual tree for all of the remaining the transects in this study.

To determine the age and the sapwood area, a stem disc was collected of each tree at 1.3 m were dried and sanded with 220-grit sanding paper. Stem cross-sections were aged based on the number of annual growth rings using WinDENDRO (REGENT Instruments, Quebec City, Quebec). To enhance the visual discrimination of sapwood from heartwood, a 4.13 % bromoscreol green ethanol solution was applied liberally with a sponge to the sanded stem discs (Kutsch and Sachs 1962). The discs were then scanned immediately after the dye application from which the sapwood area was delineated and measured digitally using Sigma Scan (Systat Software Inc., Chicago, IL).

2.2.4 Genetic Analysis

Genetic analysis was used to verify the genotypic compositions of the transect trees, which were initially identified using morphology and phenology. One leaf from each of the frozen leaf samples (above) was randomly selected and dried in a small paper envelope on silica gel for two days prior to genetic analysis. Genomic DNA was extracted from sampled tissues using a QIAGEN DNEasy 96 Plant Kit. Nine nuclear microsatellite loci were amplified using the methods described in Mock et al. (2008). The loci included WPMS14, WPMS20, WPMS15, WPMS17 (Smulders et al. 2001), PMGC2571, PMGC576, PMGC510, PMGC2658 and PMGC485 (http://www.ornl.gov/sci/ipgc/ssr_resources.htm). Samples were pooled into twelve unique multilocus genotypes (genets) which differed from each other by a minimum of five out of the total of nine loci. There were no mutational differences among samples within genets. The probability of identity for siblings (a conservative metric) was 2.1×10^{-10} (Waits et al. 2001), indicating that the panel of loci used was sufficiently powerful to identify unique genets. Genotypic matches and probability of identity were calculated using GenAlEx software (Peakall and Smouse 2006).

2.2.5 Transect Layout and Root Excavation

After the aboveground portion of the trees had been removed, each transect was overlain by a grid (that was either 1.5 × 1.5 m, 1.5 × 2 m, 1.6 × 2 m or 2 × 2 m), and each grid corner was marked with a 1.5 m long galvanized metal conduit pipe. The bottom 60 cm of each conduit pipe was marked at 10 cm intervals to later guide the depth of soil removal during root excavation. The conduit pipes were hammered into the ground until the top 10 cm marker was flush with the top of the mineral soil layer.

To describe the fine-scale topography of the hillslope, each transect was surveyed for change of elevation along a 50 cm grid of survey points. The elevation at each grid corner was determined using a level and sight mounted on a tripod and a survey rod. Within transects, each elevation value was subtracted from the maximum elevation value for a particular transect, which scaled the slope elevation relative to the lowest elevation point on that transect (i.e. the datum for each transect).

Prior to root excavation, understory vegetation was cut and rakes were used to remove most of the litter layer. Beginning at the top of a transect and working first across the slope and then down the slope, the mineral soil in each grid square was hydraulically removed to a depth of 10 cm (layer 1) using a Wajax® Mark3 forest fire pump (Wajax Manufacturing Ltd., Montreal, Canada) with a spray/straight stream combination nozzle. Only six grid squares were excavated at one time to reduce the drying of exposed roots, and after the soil had been removed from each group of six squares, exposed roots were covered with tarps to reduce desiccation during root measurements and sampling.

2.2.6 Root Measurements

Aspen roots were categorized into the following diameter classes [0.05 - 1 cm (fine roots), 1 - 2.5 cm (coarse roots) and > 2.5 cm (structural roots)]. Root diameter was measured using manual calipers. All roots in a given diameter class were clipped and placed on a tarp. The fresh mass of roots in all diameter classes was determined using a 50 lb digital scale. After all roots had been clipped and weighed in the first grid square, the next grid square was uncovered and the same procedure was repeated until all six exposed grid squares were processed.

To capture information on the location, direction and length of the coarse lateral root system, the coarse and structural roots were not clipped immediately in the grid locations, but were gently excavated to enable mapping of the direction and destination of these roots. A sketch of each transect was drawn showing each tree and the location of coarse and structural roots in relation to the transect grid.

The occurrence of root grafts and parental root connections were identified in each grid square, counted and mapped for each transect to assess the connectivity between and within clones. Two categories of root connections were recorded. Connections were identified as parental root connection where two or more trees had originated from a common (parent) root after the last disturbance (e.g. DesRochers and Lieffers 2001) or as functional graft where two or more roots overlapped and were fused together. After all grid squares in each transect had been excavated to 10 cm depth, the grid squares were further excavated to 20 cm depth (layer 2) and again to 30 cm depth (layer 3). After the hillslope was excavated to 30 cm, trees with taproots were excavated further.

Taproots were defined as a root growing vertically directly beneath the stem to a depth greater than 30 cm. To determine taproot length, taproots were excavated until they ended, turned and grew horizontally, or extended deeper than 3 m (for safety reasons). The diameter of the taproot was measured at the proximal and distal end.

Root segments (subsamples) from a range of root diameters within each diameter class were collected in the field in order to estimate the relationships between root surface area and root fresh mass, and the relationship between the root fresh mass and root dry mass. Root subsamples were placed in large plastic bags, sealed and placed in a cooler to transport to the lab, where they were stored frozen. Since fine roots serve primarily for resource uptake, fine root mass was expressed on a surface area basis. To estimate the surface area and root volume of fine roots, the frozen roots of a range of diameters were cut into 10 cm segments that were straight and without taper. Diameter, length and fresh mass of the root subsample were measured. Surface area was calculated from diameter and length measurements, assuming roots were cylinders. To determine the relationship between the root fresh and dry mass, all root segments of all three size classes were weighed and dried at 75 °C until reaching a constant mass.

2.2.7 Soil Matric Potential

Soil water potential (ψ_s; kPa) was measured using soil matric potential sensors (MPS-1, Decagon Devices, Pullman, WA, USA) for each cluster of aspen trees (upper and lower slope positions) at two soil depths (15 and 30 cm) within transect D. A total of six matric potential sensors were installed at each of two depths (15 and 30 cm) at both upper and lower slope positions. Soil matric potential was sampled every 5 seconds, and averaged after 20 minutes using a CR1000 datalogger and Multiplexer (DNX 1000 Datalogger and AM 16/32 Multiplexer, Dynamax, Houston, TX, USA) at hourly time-steps from (May 29 to August 25, 2009).

2.2.8 Data Analysis

Differences in tree height, DBH, sapwood area, leaf area and age between trees growing in the upper and lower portions of the hillslope were tested using two-sample t-test. The relationship between leaf area and sapwood area were tested using linear regression (PROC REG in SAS). Differences in root dry mass and root surface area distribution due to root diameter and soil depth were tested using a two-way ANOVA using PROC GLM in SAS (Ver. 9.2 SAS Cary, NC). The linear model was $Y_{ijk} = \mu + D_i + S_j + D_iS_j + \varepsilon_{ijk}$, where Y_{ijk} is the response variable (e.g. root surface area or root dry mass), μ is the overall average, D_i is the effect of the i^{th} level of soil depth, S_j is the effect of the j^{th} level of root size class, D_iS_j is the interaction term between soil depth and root size class and ε_{ijk} is the random error in the experimental design. There was no interaction between soil depth and root diameter, so differences in root dry mass and root surface area distribution soil depth were tested using one-way ANOVA. The relationship between root dry mass, root surface area and taproot length with changes in relative elevation along the hillslope were tested using linear regression (PROC REG in SAS, Ver. 9.2 Cary, NC). Root surface area and dry mass were not summarized by upper and lower slope categories because of the presence of the asymmetric root system of trees located at the upper portion of the hillslope (see below). Due to the small sample size in this study an alpha of 0.10 was accepted as the level of significance for all tests, unless otherwise indicated. All data met the statistical assumptions of normality and homogeneity of variance.

2.3 Results

While generally similar stand characteristics were present on the hillslope, trees on transects A to C were slightly smaller and younger than those on transects D and E (Table 2-1). Mean tree age, stem density, height and DBH ranged from 52-74 years. old, 1550-3400 stems ha^{-1}, 13-14 m high and 12-13 cm DBH across the five transects. Average tree height, diameter at breast height (DBH) and sapwood area did not differ among upper and lower portions of the hillslope (Table 2-2). However, mean leaf area per tree was significantly less on the upper portion of the hillslope: 11 m^2 compared to 17 m^2 observed on trees on the lower portion of the hillslope (Table 2-2). Although soil moisture was uniform along the hillslope in the early summer period, soil matric potential at 15 cm depth was 109.5 kPa at the lower portion of the hillslope and significantly drier (-220.9 kPa, $P < 0.001$) at the upper portion of the hillslope (Table 2-3). Although the soil was much wetter at 30 cm depth, the same trend was observed where the soil matric potential was -77.5 kPa at the lower portion of the hillslope and significantly drier (-122.6 kPa, $P < 0.001$) at the upper portion of the hillslope (Table 2-3). The relationship between leaf area and sapwood area of dominant trees differed greatly between trees located at the upper and lower portion of the hillslope (Figure 2-1). The average leaf area to sapwood area ratio of dominant trees growing at the upper portion of the hillslope was almost half that of those growing at the lower portion of the hillslope ($P = 0.002$) (Figure 2-2).

Root distribution of fine (0.05 - 1 cm), coarse (1 - 2.5 cm) and structural (> 2.5 cm) roots was different between the three soil layers of the soil profile and also showed differences between the upper and lower portions of the hillslope. Within the first 30 cm of the soil profile, fine roots comprised 60 % of the total root surface area ($P < 0.001$). Fine root surface area decreased from 3 m^2 root m^{-3} soil in the first 10 cm (layer 1) to 1 m^2 root m^{-3} soil between 20 and 30 cm soil depth (layer 3) ($P = 0.069$, Figure 2-3). The pattern of coarse root occupancy was similar to that of fine roots (Plate 2-1 a-c). Coarse root dry mass decreased from 3.8 kg m^{-3} in the first 10 cm layer to 1.5 kg m^{-3} in the 30 cm layer ($P = 0.004$; Figure 2-4). Both fine and coarse root distribution was highly variable in the top 20 cm of the soil profile (Figures 2-3 and 2-4), but dry mass of structural roots (>2.5 cm) throughout the soil profile did not change with depth ($P = 0.503$; data not shown).

The total surface area of fine roots was greater in the lower hillslope position than in upper slope as reflected in the significant decrease in fine root surface area with an increase in elevation (Figure 2-5). Average fine root surface area decreased by 1 m^2 root m^{-3} soil (30 %) over an elevation gain of less than 3 m (Figure 2-5, $P = 0.041$, $R^2 = 0.80$). Correspondingly, coarse root mass decreased by 1 kg m^{-3} with the same elevation gain (Figure 2-6, $P = 0.085$, $R^2 = 0.68$). Closer examination of the distribution of fine and coarse roots indicates an increase in fine root surface area or coarse root dry mass in the middle of the hillslope, (Figure 2-5 and 2-6). In contrast to fine and coarse root distribution, hillslope position had no effect on the structural roots (data not shown).

The direction and length of coarse lateral roots growing from trees located on the upper slope was different from those growing on the lower slope. The spatial arrangement of coarse roots of trees growing in the upper portion of the hillslope was asymmetric, with a higher proportion of roots growing down slope compared to roots growing up or across the slope (Plate 2-2). Generally, these down slope (lateral) roots were 6 to 10 m long, but some reached lengths of over 14 m. Additionally, most of these lateral roots remained within the upper 20 cm of the soil profile, root diameter tapered very little, were highly branched along the length of the root and eventually produced a mesh of fine roots at the terminal end. There were also instances of lateral roots growing upwards towards the top of the slope, though these roots were much shorter than roots growing down slope. Roots oriented down slope, growing from trees located at the lower slope were also observed, but they tapered much more rapidly and were shorter compared to roots from the trees growing at the upper slope position, resulting in a more symmetric root system around the stems. Interestingly, in one transect, a tree located at the bottom of the slope produced a lateral root that grew initially upslope, only to turn down slope after a few meters.

Sixty percent of all excavated trees had a taproot, with lengths ranging between 0.4 m to 2.7 m. The length of taproots was significantly affected by slope positions where trees in the upper slope positions had longer taproots than trees growing in the lower slope positions (Figure 2-7, $P = 0.046$; $R^2 = 0.15$). Generally, taproots were greater than 2.5 cm diameter at the base of the stem, but tapered rapidly to less than 1 cm within the first 15 cm.

Root grafts were relatively common among the 92 trees excavated across the five transects, with root grafts present in 46 % of all ramets. Generally, most grafted root connections were observed within 1 m of the stump, and parental root connections usually existed only where trees were in close proximity (less than 2 m apart). The occurrence of each type of root connection also differed greatly in each transect (Figure 2-8). The greatest number of parental root connections (6) was observed in transect C, and the greatest number of grafts (8) occurred in transect E. No root grafts, and only two parental root connections, were observed in transect A, which had the highest tree density of the five transects. Overlapping roots were often observed; however, many of those did not result in grafting, particularly when they were located more than 1 m away from the stump. Genetic analyses revealed that in four instances, grafting occurred between roots from different clones (genotypes) (two in each of transects C and D). Additionally, it was observed that of the trees without taproots, 15 % were grafted to another tree and 23 % had a parental root connection to another tree with a taproot. The remaining 62 % of trees without taproots had long lateral roots that usually extended down slope, which were observed to be more prominent than those with taproots or root connections.

2.4 Discussion

Hillslope position and distance to water table strongly influenced root distribution of fine and coarse roots in aspen and the clonal nature of aspen likely played a smaller role in the root distribution along coarse textured hillslopes than soil moisture availability. Fine root surface area was greatest at the lower portion of the hillslope and in the upper surface layers of the soil where soil moisture was more available. This is consistent with a study using seedlings of *Populus deltoides* (Bartr.), *Platanus occidentalis* (L.), *Liquidambar styraciflua* (L.), *Pinus taeda* (L.) and *Pinus massoniana* (Lamb.) showing increased fine root occupancy in response to higher soil moisture availability (Tsutsumi et al. 2004, Coleman 2007). The production of coarse roots, where the majority of clonal connections among ramets were found, appears to be driven by moisture availability and also followed a similar trend as observed in fine roots, where more coarse roots occurred at the lower slope position.

Observation of the root system distribution along the hillslope correlates well with the observation of greater leaf area carried by the trees growing at the lower portion of the hillslope, suggesting greater productivity with the increased availability of soil moisture in this position. Although trees at the bottom of the slope had significantly more leaf area than the trees at the top of the slope, the size of the trees (including sapwood cross-sectional area) was not strongly affected by slope position. As a result, the average leaf area to sapwood area ratio was about 50 % lower in trees growing at the upper portion of the hillslope compared to the lower hillslope. This indicates that trees located at the bottom of a slope required less sapwood area to support a unit of leaf area, and this leaf area development was closely related to soil moisture availability. In *Quercus pubescens* (Willd.; downy oak) hillslope position was also found to affect leaf area, and was tightly coupled with soil moisture, while stem DBH and sapwood area was not significantly influenced by slope position (Barij et al. 2007). Sapwood hydraulic conductivity has been shown to be influenced by slope position, where stem wood density was greater as a result of smaller vessels, in positions that had moisture limitations (Meinzer 2003, Bucci et al. 2004, Barij et al. 2007), reducing the potential risk of cavitation (Martinez-Meier et al. 2008, Dalla-Salda et al. 2009, 2011). Interestingly, my study does not support the notion that root mass increases relative to shoot mass under soil moisture limited conditions (Coutts 1987, 1989, Mahoney and Rood 1991, Coleman 2007, Brassard et al. 2009). There could be two reasons for that: firstly my study did not distinguish root mass of individual ramets or clones as it was soil volume based, and secondly the lateral roots originating from trees growing at the upper slope position and extending to the lower slope position acted to increase the root mass in the lower portion of the hillslope.

The root growth strategy of trees growing at the upper slope position producing long lateral roots towards the lower slope positions was a particularly interesting observation. This growth pattern resulted in a distinct asymmetrical root system in the trees growing at the upper slope compared with the trees growing at the lower slope (Plate 2-2). This has not been previously described for aspen in the literature, and could be interpreted as an adaptive strategy of aspen to avoid drought stress on water limited regions of hillslopes. Previous studies investigating root distribution in aspen have found that lateral roots produced from the stem of the tree expand symmetrically (Strong and La Roi 1983b). However, trees are understood to allocate more resources to root systems for increased structural support and/or resource uptake to maintain and increase support of aboveground biomass (Brassard et al. 2009). Asymmetries in mature tree root systems have been previously observed, but mostly interpreted as the result of increased structural support in exposed areas or of mechanical impedance for root growth in *Quercus pubescens* (Willd.) (Di Iorio et al. 2005), *Picea sitchensis* (Bong. Carr.) (Coutts et al. 1999), *Pinus halepensis* (Mill.) and *Pinus brutia* (Ten.) (Ganatsas and Spanos 2005).

Excavation of the hillslope in three 10 cm layers revealed a noticeable decrease in fine and coarse root mass with soil depth. Independent of slope position, fine (0.05 - 1 cm diameter) and coarse roots (1 - 2.5 cm diameter) dominated the first 20 cm of the soil profile (Figure 2-3 and 2-4), with the exception of taproots, which were often observed at depths up to 2.7 m. This result is consistent with what has been found in other studies on aspen (Strong and La Roi 1983b), *Populus deltoides* (Puri et al. 1994), along with general observations from root studies on other boreal tree species (Jackson et al. 1996). The pattern of decreasing vertical distribution of fine root surface area may indicate that nutrient and water uptake predominately occur in the upper 20 cm of the soil (Coleman 2007). Hillslope position also greatly influenced the length of the aspen taproots. Taproot length was found to increase with slope elevation, which is consistent with other studies that have observed taproot to be greater in trees experiencing drought stress (Mahoney and Rood 1991, Hendrick and Pregitzer 1996, Hutchings and John 2003). Interestingly, on my site, the taproot length of trees at the upper portion of the hillslope was much greater than what has been previously published in the literature for aspen: 1 m (Strong and La Roi 1983b) and 2.4 m (Gifford 1966). This is likely unrelated to soil texture as all three studies occurred on well drained sandy soils; however, the distance to the water table at the upper portion of the hillslope in this study was likely much greater than that of either Strong and La Roi (1983b) or Gifford (1966). It appears that trees growing along the hillslope in my study utilized a combination of both strategies (taproots and asymmetrically distributed roots) to increase water uptake where soil moisture is limited.

The clonal root system of aspen (parent roots and functional grafts) also allows for the sharing of resources between individuals and ramets (DeByle 1964, DesRochers and Lieffers 2001, Peltzer 2002, Jelinkova et al. 2009). Interestingly, although there were multiple clones within each transect, and a number of root grafts and parental root connections were found, root connections between trees of the upper and lower hillslope positions that could have facilitated significant direct resource sharing between trees were not found, as originally expected. However, 85 % of the trees located at the upper portion of the hillslope did not have a taproot, and of these, 23 % were connected to a tree with a taproot while the other 62 % had long lateral roots extending down slope. This evidence suggests that clonal connection might play a role in the resource sharing under stressed conditions, but likely over short distances. As a result, both lateral root expansion and taproots appear to be the most important mechanisms for aspen to access water on upper hillslopes where water limiting conditions exist.

2.5 Conclusion

This study has provided fundamental new insights into root system architecture of mature aspen and aspen clones growing along hillslopes. Exploring these relationships would not have been possible without large scale excavations. The vertical and horizontal root system structure, and clonal root connections that I observed support the notion that a combination of 1) developing deep taproots, 2) growing asymmetric lateral roots down slope and 3) intra- and inter-clonal root system connections are three possible mechanisms for trembling aspen to access water and other limiting resources when growing in an upper slope position. In my study the development of an asymmetric root system appeared to play the greatest role in maintaining productivity of trees on the upper portion of a hillslope rather than the development of a taproot or clonal connections; however, the importance and significance of water supply through these alternative pathways is still poorly understood.

2.6 Tables and Figures

Table 2-1 Summary of aboveground variables for each transect. Leaf area per tree was estimated for transects A, B, C and E based on actual measurements taken on the 13 trees in transect D.

Transect Totals:	Transect A	Transect B	Transect C	Transect D	Transect E
Clones (1-12) present:	2, 4, 10, 11	2, 4, 8, 9	2, 3, 9, 12	1, 5, 6, 7	5
Number of trees	19	17	27	13	16
Transect size (m^2)	60	50	96	84	54
Average age (yrs)	54	52	52	74	72
Average tree height (m)	10	11	12	14	17
Average DBH (cm)	11	10	11	17	17
Average sapwood area (cm^2)	67	40	54	146	112
Average leaf area (m^2)	13	4	6	43	18

Table 2-2 Average (standard error of the mean) of aboveground variables for aspen trees growing at the upper and lower portions of the hillslope (n=5).

Slope Position	Age (years)	Tree height (m)	DBH (cm)	Sapwood area (cm^2)	Leaf area[1] (m^2) *
Upper	58 (± 1.4)	13 (± 0.5)	12 (± 0.6)	72 (± 8.3)	11 (± 2.3)
Lower	61 (± 1.8)	14 (± 0.6)	13 (± 0.8)	85 (± 11.1)	17 (± 3.7)

1Leaf area per tree was estimated using the two equations for upper and lower slope shown in Figure 2-1, and are based on data collected in transect D

*Indicates significant difference at $\alpha = 0.1$

Table 2-3 Average soil matric potentials (kPa) at 15 and 30 cm depths at the upper and lower portion of the hillslope.

Slope Position	Root Depth	Average Soil Matric Potential (kPa)
Upper Slope	Shallow (15 cm)*	-220.9 (± 4.6)
	Deep (30 cm)^	-122.6 (± 2.8)
Lower Slope	Shallow (15 cm)	-109.5 (± 2.5)
	Deep (30 cm)	-77.5 (± 2.0)

* Indicates significant difference at α = 0.05
^Indicates significant difference at α = 0.05

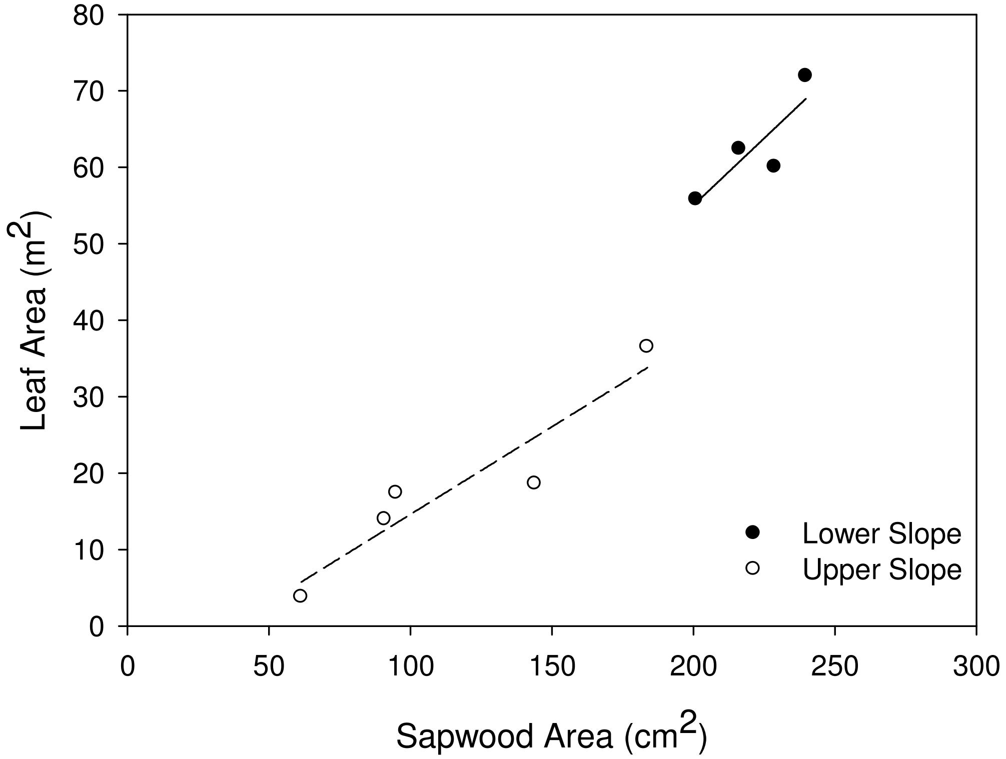

Figure 2-1 Relationship between leaf area and sapwood area for dominant trees located at the upper portion of the hillslope (y = 0.3508x - 15.11, R^2 = 0.74, P = 0.142) and the lower portion of the hillslope (y = 0.2292x - 8.2899, R^2 = 0.88, P = 0.018) in transect D.

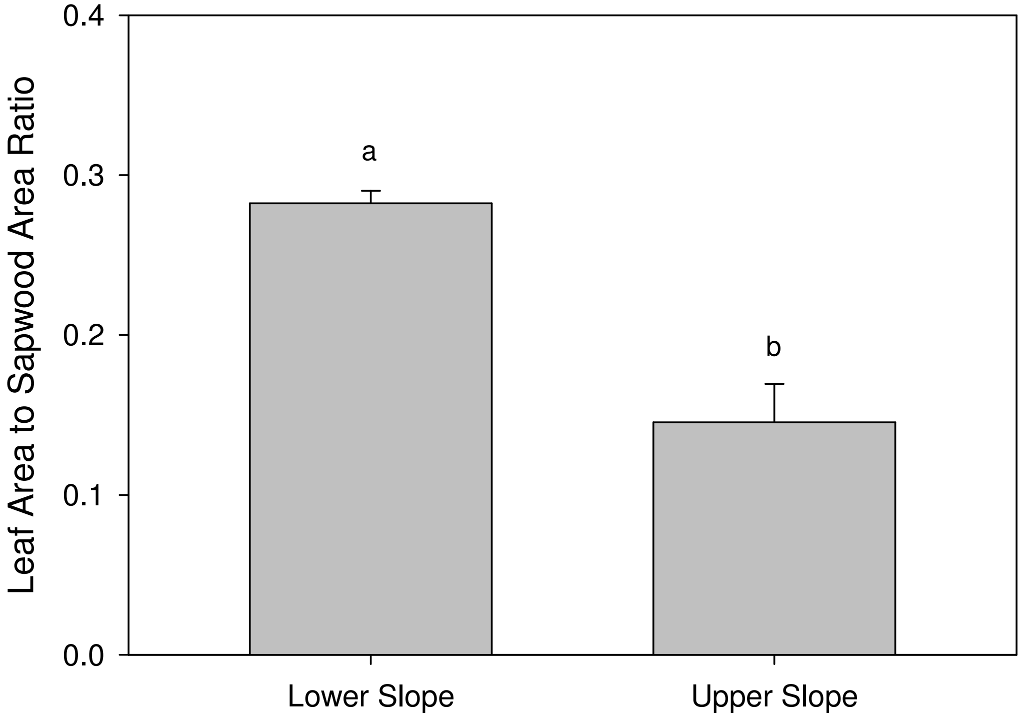

Figure 2-2 Average leaf area to sapwood area ratio for dominant trees located at the upper and lower portion of the hillslope. Different letters indicate significant difference between means (α = 0.1) and bars represent one standard error of the mean (n = 5).

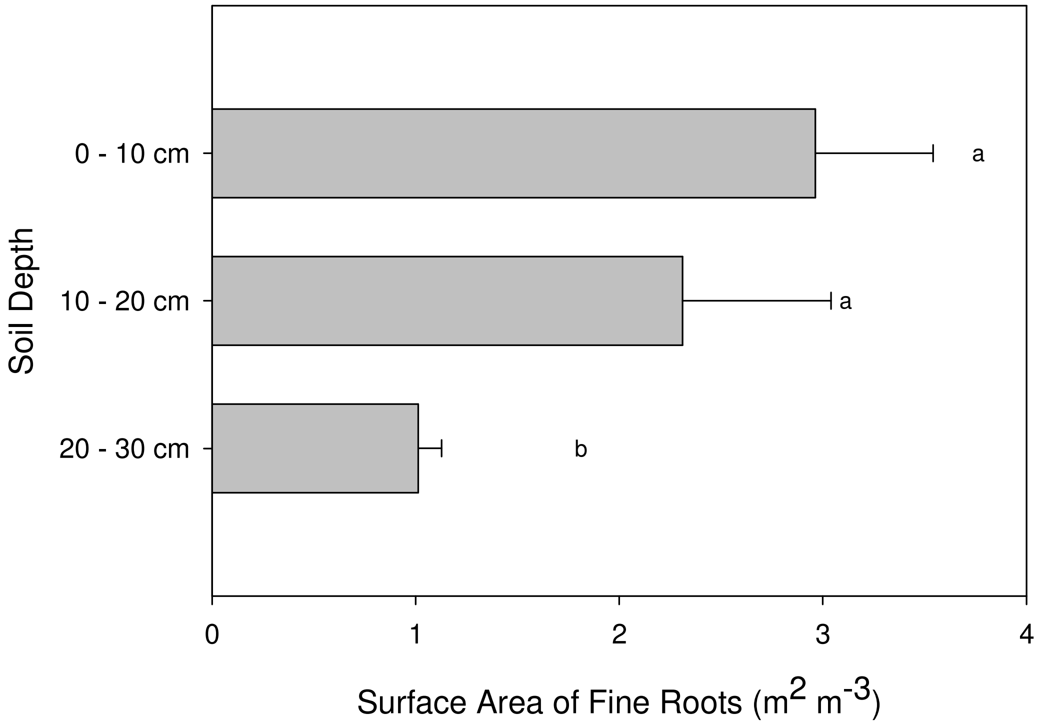

Figure 2-3 Average root surface area of fine (0.05 - 1 cm diameter) roots at three 10 cm mineral soil depth increments. Different letters indicate a significant difference between treatment levels means (α = 0.1). Bars represent standard error of the mean (n = 5).

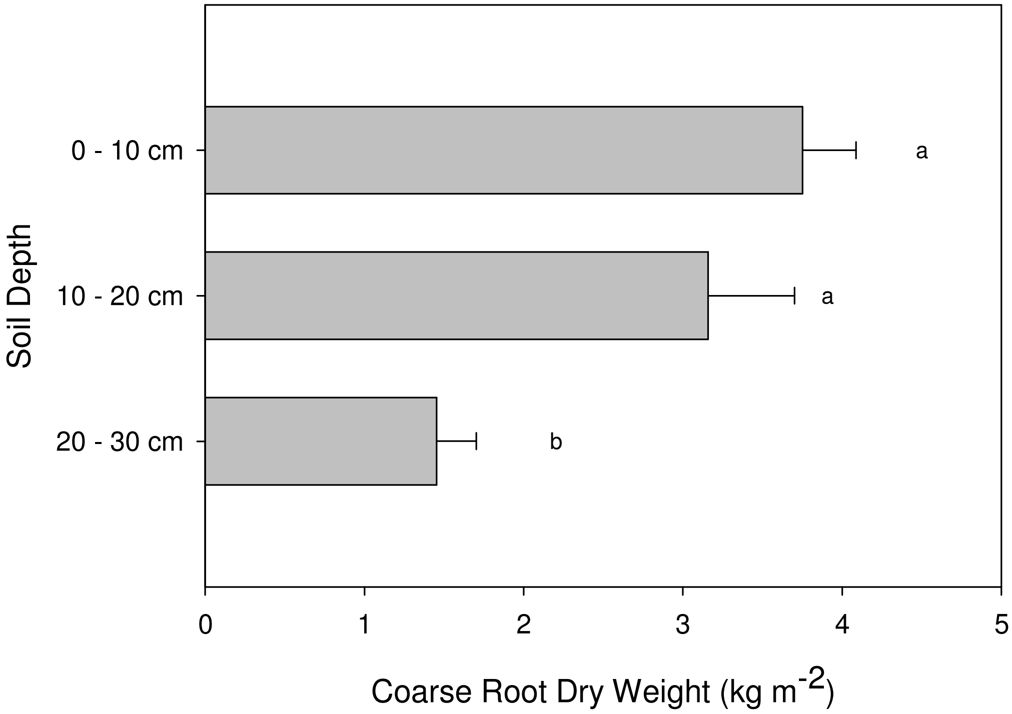

Figure 2-4 **Average coarse root (1 – 2.5 cm) dry mass at three 10 cm mineral soil depths. Different letters indicate significant differences between treatment means ($\alpha = 0.1$). Bars represent standard error of the mean ($n = 5$).**

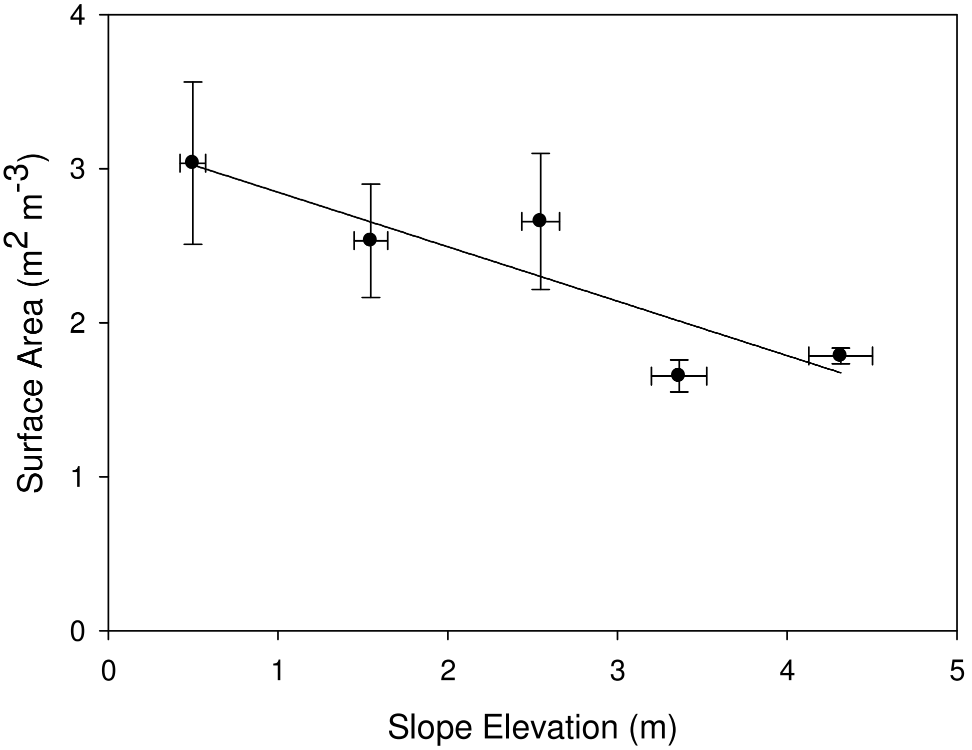

Figure 2-5 **The relationship between average root surface area of fine roots (0.05 - 1 cm diameter) in the top 20 cm of the mineral soil and slope elevation. Elevation of 0 m corresponds to the bottom of the slope ($y = -0.3537x + 3.2004$; $P = 0.041$; $R^2 = 0.80$). Error bars represent standard error of the mean ($n = 5$).**

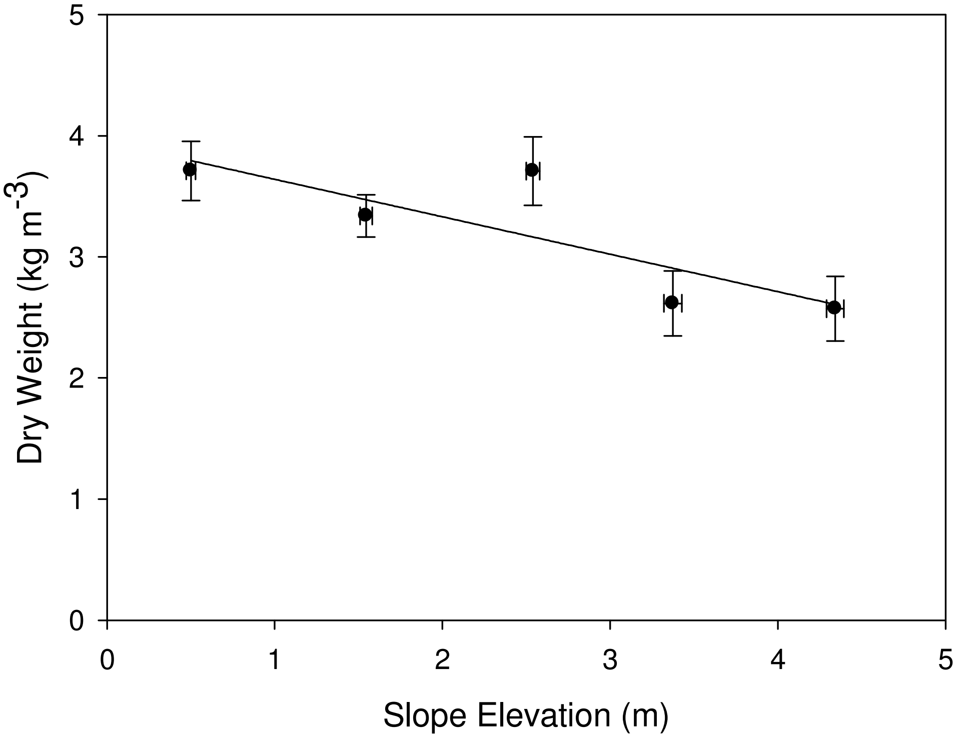

Figure 2-6 The relationship between average root dry mass of coarse roots (1 – 2.5 cm) present in the top 20 cm of the mineral soil and slope elevation. Elevation of 0 m correspond to the bottom of the aspen dominated hillslope ($y = -0.3092x + 3.9497$; $P = 0.085$; $R^2 = 0.68$). Error bars represent standard error of the mean ($n = 5$).

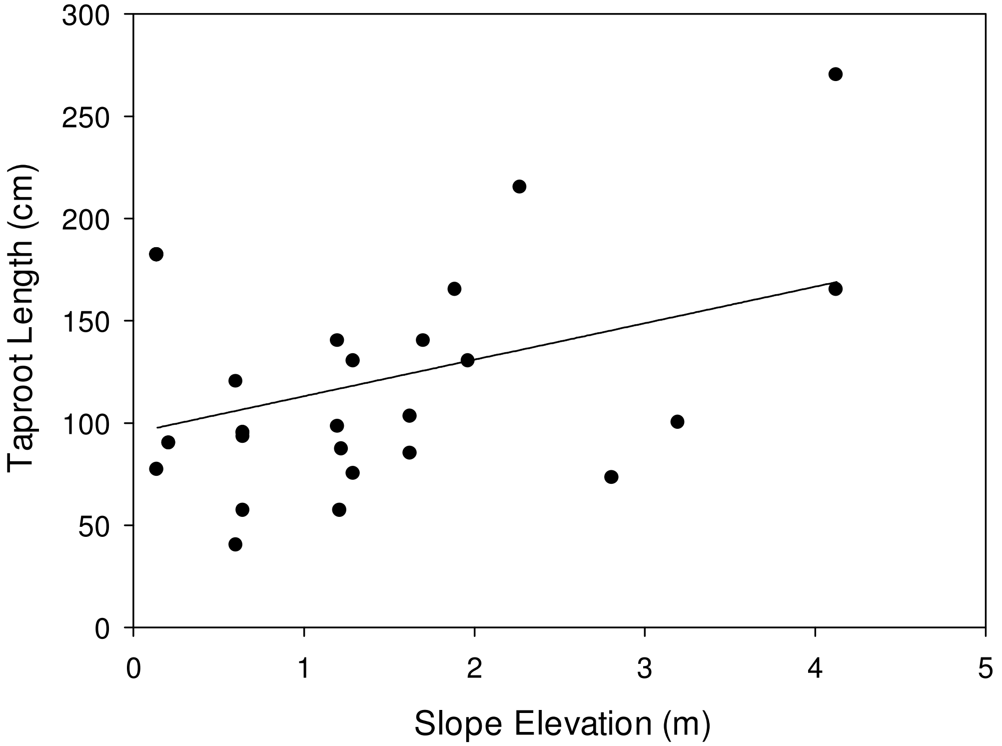

Figure 2-7 Relationship between taproot length and slope elevation. Elevation of 0 m corresponds to the bottom of the aspen dominated hillslope (y = 17.878x + 95.176; P = 0.046; R^2 = 0.15).

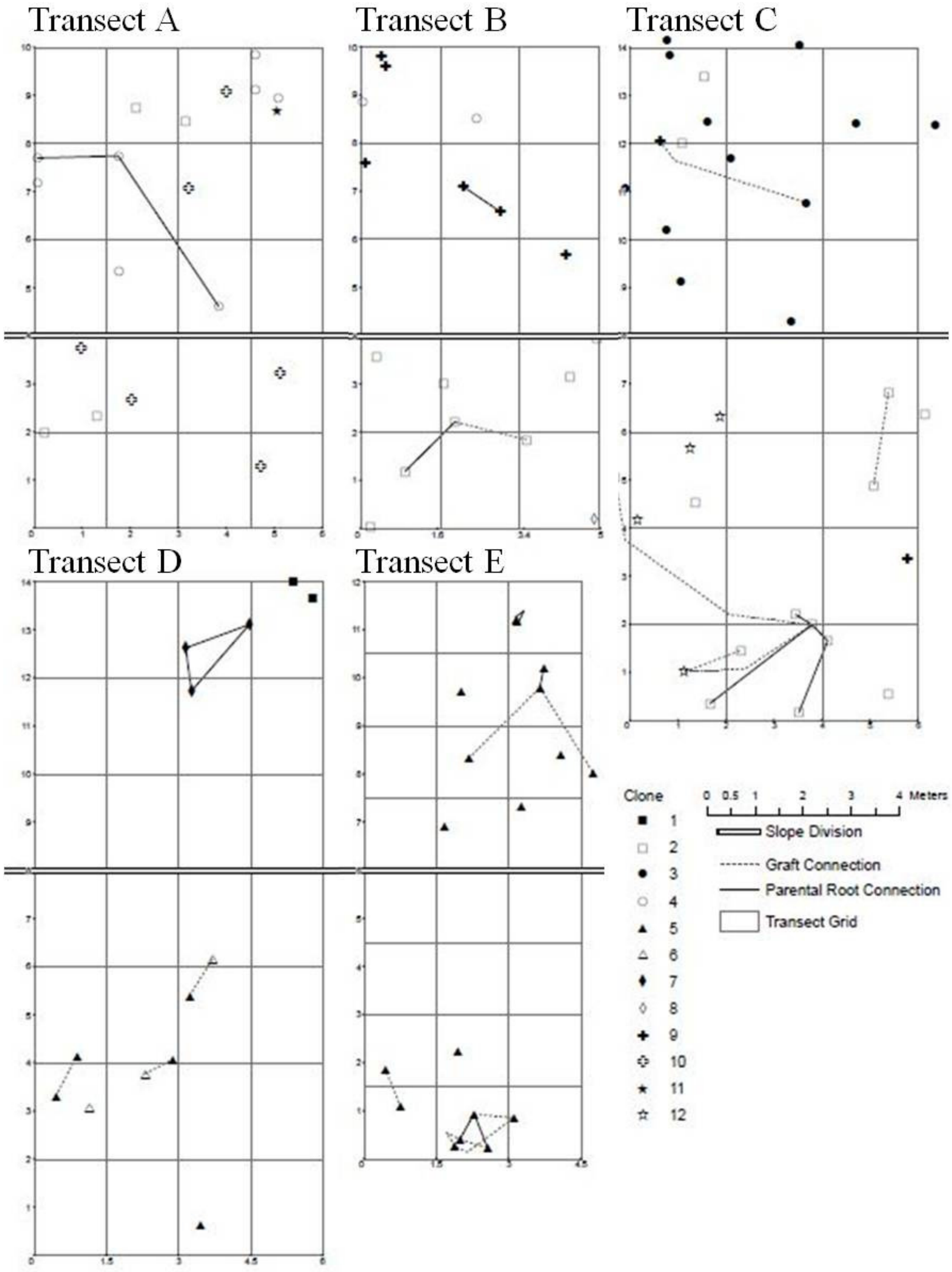

Figure 2-8 Intra- and inter-clonal connections between trees in each of the five transects. Like symbols indicate trees are the same genotype. Solid lines indicate parental root connections, hatched lines indicate root grafts.

Plate 2-1 Root distribution at each 10 cm mineral soil layer (a) 0 – 10 cm, (b) 10 – 20 cm and (c) 20-30 cm.

Plate 2-2 Example of asymmetric root system.

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Chapter 3: Effect of Atmospheric Variables and Soil Moisture Availability on Transpiration and Water Uptake of Aspen along a Hillslope

3.1 Introduction

Strong links exist between water cycling and the state of forest vegetation. Forest stand leaf area usually increases rapidly after disturbance (Oliver and Larson 1996), and a large body of watershed scale research has demonstrated that initial increases in water yield after forest disturbance are followed by declining water yields which are associated with the recovery of evapotranspiration (*ET*) as forest vegetation re-develops (Bosch and Hewlett 1982, Stednick 1996). A similarly strong link also exists between root water uptake and leaf area development for photosynthesis and growth which is thought to be limited by both soil moisture availability and meteorological conditions that regulate atmospheric moisture demand (Grier and Running 1977, Gholz 1982, Kergoat 1998).

Soil moisture availability is highly variable, both spatially and temporally. Strong spatial variation in soil moisture is usually evident both vertically in soil profiles and horizontally across the landscape (Chamran et al. 2002, Berry et al. 2006, Tromp-van Meerveld and McDonnell 2006, Loranty et al. 2008, Guswa 2012, Angstmann et al. 2012). A hillslope is an example of strong moisture gradients which typically occur both horizontally along the hillslope and vertically down the soil profile (Chamran et al. 2002). This variation in soil moisture is strongly coupled to variation in plant productivity. Greater forest productivity in regions of greater soil moisture availability (both large and small scale) has been shown for many tree species and geographic regions (Pataki et al. 2000, Schwarz et al.2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Dalla-Salda et al. 2009, Dalla-Salda et al. 2011, MacKay et al. 2012).

Similarly, strong seasonal variation in soil moisture availability is evident in many environments. In northern temperate and boreal regions, soil moisture availability is typically greater early in the growing season and declines later in the growing season. The seasonal decrease in availability of soil moisture is well coupled to the timing of maximum leaf expansion reached later in the growing season (MacKay et al. 2012). Plants have the ability to respond to limitations in soil moisture availability using one or more strategies related to adaptations at the leaf, stem and root level (Guswa 2012). To optimize root water uptake, plants will allocate more resources to areas where more soil water is available (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Coleman 2007, Brassard et al. 2009). Some species maintain growth in dry environments by accessing deeper soil water through taproots (Mahoney and Rood 1991, Hendrick and Pregitzer 1996, Hutchings and John 2003). In some species, soil water can also be redistributed from wetter areas (high soil matric potential) to areas of the soil that are dryer (low soil matric potential) through a root mediated hydraulic redistribution mechanism (Richards and Caldwell 1987, Caldwell et al. 1998, Brooks et al. 2006). Thus both plasticity in rooting depth and hydraulic redistribution of soil moisture are considered key mechanisms allowing plants and trees to survive in moisture limited environments.

Transpiration is governed by water potential gradients between the leaf and the atmosphere, and stomatal regulation of water loss is another key mechanism utilized by plants and trees to survive in moisture limited environments. When the leaf experiences water stress the hydrostatic pressure decreases within the guard cells, and relaxes guard cell walls, which close over the stomatal opening. This process stops the loss of water vapour from the leaf to the atmosphere, but also blocks the uptake of CO_2 for use in photosynthesis (Tyree and Ewers 1991, Berry et al.2006, MacKay et al. 2012). The rapidity of the response of the stomata to water stress is dependent on many molecular components that are specific to each species and phenotype (Luan 2002).

Atmospheric demand for moisture drives the water potential gradient between the atmosphere, plants and soil. Atmospheric moisture demand is comprised of several atmospheric variables such as vapour pressure deficit (*D*), ambient air temperature (*Ta*) and potential evapotranspiration (ET_p) or total atmospheric moisture demand. Additionally, net radiation (*Q**) is known to stimulate stomatal opening, and thus strongly influences the interaction of plants and the atmosphere (Figure 3-1). When water is not limiting, transpiration is strongly and positively associated with greater *D, Q*, Ta and* ET_p (Granier and Breda 1996, O'Brien et al. 2004, David et al. 2004, Leuzinger et al. 2005, Bovard et al. 2005, Kume et al. 2007, Adelman et al. 2008, McLaren et al. 2008, MacKay et al. 2012). However, high atmospheric moisture demand is also associated with plant water stress. Stomatal regulation of transpiration to prevent xylem cavitation and severe plant stress is also a commonly observed strategy to balance photosynthesis for growth and survival with water availability in many tree species (Kozlowski and Pallardy 2002).

While many studies have explored the relationship between tree productivity and atmospheric variables regulating atmospheric moisture demand (*Q**, *D, Ta* and ET_p) (Granier and Breda 1996, O'Brien et al. 2004, David et al. 2004, Leuzinger et al. 2005, Bovard et al. 2005, Kume et al. 2007, Adelman et al. 2008, McLaren et al. 2008, MacKay et al. 2012), another large body of research has explored the relationships between productivity and soil moisture availability (Pataki et al. 2000, Schwarz et al.2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Dalla-Salda et al. 2009, Dalla-Salda et al. 2011, MacKay et al. 2012). However, few studies have evaluated transpiration response to both atmospheric variables and soil moisture availability in mature trees. Thus the interaction of these variables as key regulators of water use and productivity remains unclear (Hogg et al. 2000, Pataki et al. 2000, Schwarz et al. 2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Tromp-van Meerveld and McDonnell 2006, Berry et al. 2006). This includes a basic understanding of how seasonal variation in soil moisture availability and atmospheric conditions affect transpiration dynamics in trees.

Trembling aspen (*Populus tremuloides* Michx.) is a widely distributed tree species across North America (Little 1971). The distribution of trembling aspen

(aspen) spans the lower latitudes of the boreal forests, the mid elevations in the Rocky Mountains and the drought prone transition zones between the prairie grasslands and boreal forest. Accordingly, aspen is an ideal species for studying adaptive strategies of trees to environmental stresses (e.g. growing along a hillslope) because this species is well adapted to grow on a wide range of site and climatic conditions, and likely utilizes many adaptive strategies (DeByle and Winokur 1985).

The broad objective of this research was to explore the comparative role of both aboveground (atmospheric) and belowground (soil moisture availability) controls on regulation of water use of aspen growing along a hillslope. Specific objectives were to: 1) describe the change in tree water relation variables (leaf area, sapwood area, root surface area, transpiration, water use and root sapflow of aspen) in relation to available moisture along the hillslope, 2) explore the interaction of atmospheric variables (net radiation, vapour pressure deficit, ambient temperature, potential evapotranspiration and wind speed) and soil moisture availability on sapflow of aspen growing in different hillslope positions and 3) test whether these relationships change between wetter to drier growing season conditions.

3.2 Materials and Methods

3.2.1 Site description

This study was conducted in the Utikuma Research Study Area (URSA) near Utikuma Lake (56°04’ 45.05”N, 115°28’58.74”W) in north-central Alberta, Canada (Devito et al. 2005). The study area is located within the Boreal Plains ecozone (Ecoregions Working Group 1989). The climate is typically sub-humid with short, warm and moist summers (80 - 180 frost-free days; average temperature 9.6 °C, summer precipitation 336 mm) and long, cold and dry winters (average temperature -14.9 °C, winter precipitation 142 mm) (Natural Regions Committee 2006).

To study transpiration dynamics of aspen along a hillslope, a south facing hillslope (slope > 10°, >30 m in slope length and >500 m wide) dominated by mature aspen was selected. The soil along the hillslope was very uniform and was classified as a eutric Brunisol with a silty-sand texture (Soil Classification Working Group 1998). The moisture regime was considered subxeric, rapidly draining and the understory vegetation indicated a medium to poor nutrient regime.

3.2.2 Plot Layout and Tree Characteristics

To study the influence of hillslope position on the transpiration and root sapflow dynamics on mature aspen, a 14 m long by 6 m wide transect (plot) was established along the hillslope extending from base of the slope upwards to near the top of the slope in early May 2009. This plot encompassed two discrete groups or clusters of aspen trees with one clump situated at the lower hillslope position (eight trees), below another cluster of aspen (five trees) growing at the upper hillslope position (Figure 3-2). To describe the aboveground characteristics of trees within these two groups, the total height, height to live crown, diameter at breast height (1.3 m) were measured on every tree. To calculate the whole-tree transpiration from sap velocity measurements (see below) all thirteen trees were destructively sampled in late August 2009 after transpiration and root sapflow measurements were completed and the sensors were removed. After harvesting, stem cross-sections were collected at 1.3 m height to determine the age and sapwood area of each tree. Stem cross-sections were air dried in the laboratory and sanded with coarse to fine-grit sand paper, finishing with 220-grit sanding paper. Stem cross-sections were aged based on the number of annual growth rings using WinDENDRO (REGENT Instruments, Quebec City, Quebec). To enhance the visual discrimination of sapwood from heartwood, a 4.13 % bromoscreol green ethanol solution (Kutsch and Sachs 1962) was applied liberally with a sponge to the sanded stem discs. The discs were then scanned immediately after the dye application from which the sapwood area was delineated and measured digitally using Sigma Scan (Systat Software Inc., Chicago, IL).

Total leaf area of each tree was measured by removing all leaves by hand from the crown after felling the trees. Leaf fresh weight was determined using a

Berkley® 50 lb digital scale (Pure Fishing Inc., Portage la Prairie, MB). An average subsample of fresh leaves from different parts of the crown in each tree was then collected, weighed and leaf area was measured by scanning the leaves using WinDENDRO (REGENT Instruments, Quebec City, Quebec). Leaf area to fresh weight ratios were calculated for each tree and used to estimate total crown leaf area. Leaf area index (LAI) was calculated by summing the total leaf area of each aspen cluster and dividing this value by the area of ground each aspen cluster's canopy shadowed.

3.2.3 Atmospheric and Soil Variables

A meteorological station was established at the base of the hillslope on May 25, 2009 to measure atmospheric variables regulating transpiration of aspen trees on the hillslope. All meteorological instruments except a precipitation gauge were mounted on a radio tower (4.6 m height). Net radiation (Q^*; W m^{-2}) data was collected using a NR-Lite Net Radiometer (Campbell Scientific, Logan, UT, USA), wind speed (u; m s^{-1}) was measured using a RM Young anemometer (Wind Monitor, 05103-10, Campbell Scientific, Logan, UT, USA) and precipitation (mm) was measured using a tipping bucket rain gauge (TW 525M, Campbell Scientific, Logan UT, USA). Ambient temperature (Ta; °C) and relative humidity (RH; %) data were measured at 1 m height using a HOBO H8 Pro Series Temp/RH Intrinsically Safe Logger (H08-032-IS, Onset Hobo Data Loggers, Pocasset, MA, USA) and were used to calculate ambient vapour pressure (e_a; Pa), saturation vapour pressure (e_s; Pa) and vapour pressure deficit (D; kPa). Soil water potential (ψ_s; kPa) was measured using soil matric potential sensors (MPS-1, Decagon Devices, Pullman, WA, USA) for each cluster of aspen trees (upper and lower slope positions) at two soil depths (15 and 30 cm). The soil matric potential sensors were installed in specific locations that were paired with a Dynagage sapflow sensor (Dynamax, Houston, TX, USA) (see below) to reduce ground disturbance. A total of 6 matric potential sensors were installed at each of two depths (15 and 30 cm) in both upper and lower slope positions. Atmospheric variables and soil moisture availability were sampled every 5 seconds, and averaged after 20 minutes using a CR1000 datalogger and Multiplexer (DNX

1000 Datalogger and AM 16/32 Multiplexer, Dynamax, Houston, TX, USA) at hourly time-steps from May 29 to August 25, 2009.

Potential evapotranspiration (ET_p; mm h^{-1}) was calculated from atmospheric demand for moisture using atmospheric variables and the Penman-Monteith equation:

Equation 3-1:
$$ET_p = \frac{\Delta Q^* + p_a c_a D g_a}{\lambda\left[\Delta + \gamma\left(1 + \frac{g_a}{g_c}\right)\right]}$$

where Δ is the slope of the saturation vapour pressure versus temperature curve at a given ambient temperature (Pa °C-1), Q* is net radiation (W m-2), pa is the density of air (g m-3), ca is the heat capacity of air (J g-1 K-1), D is the vapour pressure deficit (kPa), ga is the aerodynamic conductance of the aspen tree along the hillslope (m s-1), λ is latent heat of vapourization (J g-1); γ is the psychrometric constant (Pa K-1) and gc is canopy conductance (m s-1), which was calculated using the Monteith and Unsworth (1990) equation[1].

3.2.4 Transpiration and Root Sapflow Measurements

Thermal dissipation probe (TDP) sapflow sensors (Dynamax Inc., Houston, TX) were used to measure sapflow of each of the thirteen trees along the hillslope. The TDP sensors consist of two thermocouple needles inserted into the tree to measure sapwood temperature where the upper needle also contains an electric heater. Difference in sapwood temperature between the heated and unheated needles is proportional to the sap velocity. Two sizes of TDP probe were used in this study to accommodate both small and large diameter trees (TDP 50 or TDP 30; 50 and 30 mm length, respectively). The size of the sensor was determined based on the diameter of the tree at 1.3 m. One TDP probe pair was installed on the north side of each tree just before leaf flush on May 28, 2009. The sensors and tree stems were wrapped in reflective bubble wrap, and covered with aluminum foil to protect the sensors from solar heat gain. The bubble wrap was sealed with duct tape to protect the sensors from rain. Data from each sensor was

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[1] g_c was multiplied by leaf area index before it was used in the Penman-Monteith equation

recorded using a CR1000 datalogger (DNX 1000 Datalogger) recording at 10 second intervals, which were averaged every 20 minutes. This system enables measurement of sap velocity throughout the day, which can be converted to a volumetric sapflow rate (Q_v; cm^3 h^{-1}) by multiplying the sap velocity by cross-sectional area of tree sapwood to permit estimation of total transpiration (Granier 1985). Transpiration was expressed in two ways: 1) transpiration per unit leaf area (transpiration; Q_L; m^3 H_2O m^{-2} A_L h^{-1}) and 2) total water use per unit ground area (mm season^{-1}). Transpiration per unit leaf area allows for direct comparison of transpiration rates between trees was calculated using Ewers and Oren (2000):

Equation 3-2:

$$Q_L = \frac{Q_v}{A_L}$$

where Q_v is the sapflow velocity (sapflow) and A_L is the total leaf area of the tree. Total water use was also calculated to enable comparison of transpiration with precipitation inputs. Water use for each tree was calculated by multiplying transpiration (Q_L) by the corresponding leaf area index (LAI) for either the upper slope or lower slope group of aspen trees to normalize transpiration to a unit ground area.

Sapflow of roots was concurrently measured using heat balance techniques (Dynagage, Dynamax Inc., Houston, TX). The basis of this technique differs from the TDP sensors above in that the measurement of root sap velocity is based on externally mounted paired thermocouples, and a heater to measure radial and axial heat fluxes to estimate the axial transport of heat flowing in sap during transpiration. Since the specific heat of water (sap) is known (4.186 J g^{-1} °C^{-1}), this axial heat flux can be expressed in terms of mass of axial water flow or sapflow (g s^{-1}). This technique was used to measure sapflow of shallow (0-15 cm) and deep (15-30 cm) roots beneath tree groups located in upper and lower hillslope positions. Small soil pits were carefully excavated by hand beneath upper and lower tree groups to temporarily expose roots. Dynagage sapflow sensors (SGA 13) were installed in pairs where two roots were found such that one root was located at approximately 15 cm depth (shallow) and the other at approximately 30 cm depth (deep). Six pairs of shallow and deep roots were instrumented with Dynagage sensors in both upper and lower slope position tree groups. The mean diameters of both deep and shallow roots instrumented with SGA root sapflow sensors were 14-15 mm. All SGA sensors were wrapped in plastic and sealed with tape to prevent water from reaching the sensors. The soil was replaced around the root and sensors. Heat flux from both models of Dynagage sapflow sensors was recorded every10 seconds using CR1000 dataloggers (DNX 1000 Datalogger) and averaged every hour. As mentioned above, the specific heat of water is known (4.186 J g^{-1} $°C^{-1}$), and it is assumed that the specific heat capacity of sap is equal to that of water. Because the specific heat flux of water is known, the axial heat flux can be expressed in terms of mass of axial water flow or sapflow (g s^{-1}) by using a series of energy balance equations from section 3 of the Flow32 Dynagage Manual (Dynamax 2007), which eventually require the multiplication of heat flux by sapwood area to calculate volumetric sapflow through roots.

The entire system measuring atmospheric variables and soil moisture availability, transpiration and root sapflow measurements described above was powered with four solar panels (MSX60R, 65 Watt, Dynamax Inc., Houston, TX) mounted to the radio tower, with power storage in six 12 volt deep cycle batteries. All instrumentation was tested and calibrated in the laboratory prior to field installation. All measurements started on May 29th, 2009 and continued through August 25th, 2009.

3.2.5 Root Excavation and Measurement

To describe the distribution of aspen roots along the hillslope the 14 m long by 6 m wide transect was overlain by a grid (2 × 1.5 m), and each grid corner was marked with a 1.5 m long galvanized metal conduit pipe (see also Chapter 2). The base of the transect was approximately 1 m above the water table. The bottom 60 cm of each conduit pipe was marked at 10 cm intervals to later guide the depth of soil removal during root excavation. The conduit pipes were hammered into the ground until the top 10 cm marker was flush with the top of the mineral soil layer.

Prior to root excavation, understory vegetation was cut and rakes were used to remove most of the litter layer. Beginning at the top of a transect and working first across the slope and then down the slope, the soil in each grid square was hydraulically removed to a depth of 10 cm (layer 1) using a Wajax® Mark3 forest fire pump (Wajax Manufacturing Ltd., Montreal, Canada) with a spray/straight stream combination nozzle. Only six grid squares were excavated at one time to reduce the drying of exposed roots, and after the soil had been removed from each group of six squares, exposed roots were covered with tarps to reduce desiccation during root measurements and sampling.

Aspen roots were categorized into the following diameter classes [0.05 - 1 cm (fine roots), 1 - 2.5 cm (coarse roots) and > 2.5 cm (structural roots)]. Root diameter was measured using manual calipers. All roots in a given diameter class were clipped and placed on a tarp. The fresh mass of roots in all diameter classes was determined using a 50 lb digital scale. After all roots had been clipped and weighed in the first grid square, the next grid square was uncovered and the same procedure was repeated until all six exposed grid squares were processed.

Root segments (subsamples) from a range of root diameters within each diameter class were collected in the field in order to estimate the relationships between root surface area and root fresh mass, and the relationship between the root fresh mass and root dry mass. Root subsamples were placed in large plastic bags, sealed and placed in a cooler to transport to the lab, where they were stored frozen. To calculate the surface area of roots, the frozen roots of a range of diameters were cut into 10 cm segments that were straight and without taper. Diameter, length and fresh mass of the root subsample were measured. Surface area was calculated from diameter and length measurements, assuming roots were cylinders. To determine the relationship between root fresh and dry mass, all root segments of all three size classes were weighed and dried at 75 °C until reaching a constant mass.

3.2.6 Data Analysis

Two-tailed t-tests were used to test for differences in tree age, diameter at breast height, tree height, sapwood area and leaf area, root surface area, soil matric potential, transpiration per unit leaf area, tree sapflow and total water use between clusters of aspen growing at the upper and lower slope position (PROC TTEST, Ver. 9.2 SAS Cary, NC). Two-tailed t-tests were also used to test the difference in net radiation, vapour pressure deficit, ambient temperature and potential evapotranspiration early (June 13 – July 22, 2009) and late (July 23 – August 25, 2009) in the growing season. Additionally, two-tailed t-tests were used to test the difference in transpiration per unit leaf area and root sapflow at the upper and slope positions early and late in the growing season. Finally, two tailed t-tests were used to test the difference in deep and shallow root sapflow and soil matric potential at upper and lower slope positions both early and late in the growing season.

Regression analysis was used to test for differences in the relationships between transpiration per unit leaf area, ambient temperature, vapour pressure deficit, potential evapotranspiration and wind speed as well as root sapflow of deep and shallow roots early in the season in comparison to late in the growing season (PROC REG, Ver. 9.2 SAS Cary, NC). Nonlinear regression was used to test for differences in the relationship between transpiration per unit leaf area and net radiation (PROC NONLIN, Ver. 9.2 SAS Cary, NC) early in the season in comparison to later in the season. Differences in slopes among regression relationships between upper and lower slope position transpiration per unit leaf area and root sapflow, net radiation, ambient temperature, vapour pressure deficit, potential evapotranspiration, wind speed and soil moisture availability were evaluated using tests for overall coincidental regression after Zar (2010). An alpha of 0.05 was used as the level of significance for all tests. All data was tested for and met the statistical assumptions of normality.

3.3 Results

3.3.1 Tree Characteristics

Average tree age, diameter at breast height (DBH) and height were not different between trees on the upper and lower hillslope position. However, there were large differences in sapwood and leaf area per tree among clusters of aspen trees growing at the upper and lower hillslope position (Table 3-1). Mean tree age, DBH and height of trees growing at the lower hillslope position were 74 yr, 18 cm DBH and 16 m tall and 76 yr, 17 cm DBH and 15 m tall at the upper hillslope position. Although not significant, sapwood area tended to be greater in trees at the lower slope compared to those at the upper slope (Table 3-1). Average sapwood was 166 cm^2 at the lower hillslope position and 155 cm^2 for trees at the upper hillslope position. Average leaf area per tree for aspen at the lower slope position was 37 m^2, which was significantly greater than the average leaf area per tree measured at the upper hillslope position (18 m^2, $P = 0.05$). Leaf area index (LAI) of the cluster of aspen trees growing at the upper hillslope was 3.8, where the LAI of the aspen cluster at the lower hillslope position was 6.2, which was consistent with leaf area per tree observations. The root surface area of roots less than 1 cm in diameter at the lower slope was 66 m^2 m^{-3}, compared to 55 m^2 m^{-3} at the upper slope position.

3.3.2 Atmospheric and Soil Conditions

Strong daily variation in both atmospheric variables (net radiation, ambient air temperature, vapour pressure deficit, precipitation) and soil matric potential were observed throughout the 2009 growing season (May 29 to August 25, Figure 3-3 a-f). In particular, these environmental variables were notably different between two key time periods; early (May 29 - July 22) and later (July 23 – August 25) in the growing season. Mean daily daytime (05:00-22:00) net radiation (Q^*) was 226 W m^{-2} early in the growing season, which was significantly greater than mean net radiation later in the growing season (337 W m^{-2}; $P < 0.001$; Figure 3-3a). Mean daytime ambient air temperature was generally between 10-23 $^{\circ}C$ throughout the growing season reaching a peak (23

°C) in mid-July, and then declined towards the end of August (Figure 3-3b). Vapour pressure deficit (*D*) did not vary strongly between early and late season and averaged 1 kPa (Figure 3-3c) over the growing season, though *D* was slightly lower near the latter part of the season. Potential evapotranspiration (ET_p) was also variable throughout the season, but was generally greater earlier in the season (average of 2.6 mm day^{-1}) compared to mean ET_p of 1.8 mm day^{-1} in the latter part of the season (Figure 3-3e). Total seasonal precipitation from May 29th to August 25th was 95 mm, which was distributed among 23 precipitation events. The largest event occurred on August 2nd where 16.5 mm of rain fell, but in nine of the 23 events, less than 2 mm of rain fell (Figure 3-3d).

Soil matric potential ($\psi_{s)}$ also varied strongly during the season, and among slope positions ($P < 0.001$). The soil was evenly wet at the beginning of the growing season across the hillslope, likely due to snow melt and early season precipitation. These moisture soil conditions remained relatively constant until the middle of June, when the soil matric potential began to decrease. On June 20th there was a large 13.5 mm rain event which recharged soil moisture and temporarily increased soil water potentials at both upper and lower slope positions (Figure 3-3d). As the season progressed and soil moisture was depleted, differential soil matric potential began to develop between the upper and lower slope positions. The soil matric potential at the upper slope positions decreased faster than at the lower hillslope position (Figure 3-3d). There was a 16.5 mm rain event on August 2nd, and there was evidence of some soil water recharge at both 15 and 30 cm depths; however, this was very short in duration as soil matric potential decreased rapidly soon after the event. Again, soil matric potential decreased faster and became more negative in the upper hillslope position compared to the lower hillslope position (Figure 3-3f).

3.3.3 Relationship between Transpiration and Root Water Uptake

Mean seasonal transpiration and total sapflow was greater in trees growing at the lower slope than on trees growing at the upper slope position (Table 3-2). Average total seasonal sapflow velocity was three times greater in trees growing on the lower hillslope position (284 cm h^{-1}) compared to the upper hillslope position (94 cm h^{-1}; $P < 0.001$). Since trees located at the lower hillslope position had approximately twice the leaf area than trees in the upper slope (Table 3-1), mean seasonal transpiration on a per unit leaf area basis (Q_L) of trees on the lower position was approximately 45 % higher at the lower slope ($P < 0.001$, Table 3-2). However, as a result total seasonal water use (May 29 - August 25, 2009) was 2.4 times greater in trees growing on the lower slope position (272 mm) compared to trees on the upper position (113 mm).

Differences in mean transpiration among upper and lower slope positions was also reflected in strongly differential patterns of seasonal water use among trees situated in these two slope positions. Water use was generally low early in the growing season for trees on the upper and lower slope (Figures 3-4a and 3-4b). In early June, water use in trees in the upper slope positions increased to 2 mm day^{-1} and remained relatively stable until July 22nd, after which daily water use began to decrease (approximately linearly) to about 0.6 mm day^{-1} by August 17th (Figure 3-4a). While the same general seasonal trend of more water use early in the growing season, decreasing later in the growing season was observed in trees growing at both upper and lower slope positions, at the lower slope position, total water use was significantly greater ($P < 0.001$; Figure 3-4b). In early June, water use of lower slope trees had increased to about 5 mm day^{-1}, and remained relatively constant until July 22nd, when water use decreased considerably to 1 mm day^{-1} at the end of the growing season. Average daily water use over the growing season was 1.3 mm day^{-1} at the upper slope and 3.1 mm day^{-1} at the lower slope.

Differences in transpiration of trees in upper and lower slope positions during the growing season were reflected in the root sapflow. Mean root sapflow (Root Q_v) was 0.08 $m^3\ m^{-2}\ h^{-1}$ and mean transpiration per unit leaf area (Q_L) was 4.8 $x10^{-6}\ m^3\ m^{-2}\ h^{-1}$ (Table 3-2 and 3-3) for upper slope trees, which was almost half that measured at the lower slope (0.14 $m^3\ m^{-2}\ h^{-1}$ and 7.0 $x10^{-7}\ m^3\ m^{-2}\ h^{-1}$ respectively) (Table 3-2 and 3-3). Similarly, the high transpiration rates in the early part of the growing season for both upper and lower slope positions was also coupled with high root sapflow rates. Q_L was 7.3 x10^{-6} m^3 m^{-2} h^{-1} early in the growing season, but decreased to 3.5 x10^{-6} m^3 m^{-2} h^{-1} later in the growing season. The same trend was observed in root Q_v, which decreased from 0.13 m^3 m^{-2} h^{-1} early in the season to 0.09 m^3 m^{-2} h^{-1} later in the growing season (Table 3-3). Overall, trees in the upper and lower slope positions showed the same pattern of strongly declining root sapflow and transpiration rates from early to late in the growing season.

Transpiration of trees at upper and lower slope positions were also linked to root sapflow in upper and lower slope positions observed at the two depths; shallow (15 cm) and deep (30 cm). Shallow roots at both the upper and lower slope position had generally similar sapflow rates (Figure 3-4a & 3-4b) ranging between 0.04 and 0.15 m^3 m^2 h^{-1} in the upper slope, and 0.04 and 0.2 m^3 m^2 h^{-1} in the lower slope ($P < 0.001$). However, sapflow of deep roots (30 cm) located at upper and lower slope positions differed significantly ($P < 0.001$). Sapflow of deep roots at the lower slope position had significantly greater sapflow (0.18 m^3 m^{-2} h^{-1}) compared to deep roots at the upper hillslope position (0.11 m^3 m^{-2} h^{-1}). While root sapflow generally paralleled seasonal patterns of transpiration at both slope positions, the temporal correspondence between sapflow of roots with water use varied among clusters of aspen trees growing at upper and lower hillslope positions. Sapflow of both shallow and deep roots was strongly coupled with water use in the trees in the upper slope position from the start of the growing season until approximately July 22nd, when sapflow of shallow roots began to decline while those of deep roots did not (Figure 3-4a). This was reflected in relationships between root sapflow and transpiration that indicate both shallow and deep roots in upper slope positions contributed similar root sapflow supporting transpiration during the early part of the season (0.11 m^3 m^{-2} h^{-1}; Figure 3-5a; Table 3-4). However, later in the growing season deeper roots contributed more water (0.11 m^3 m^{-2} h^{-1}) compared to shallow roots (0.09 m^3 m^{-2} h^{-1}; $P < 0.001$, Figure 3-5b, Table 3-4). In contrast, root sapflow of shallow and deep roots did not appear well synchronized in trees on the lower slope position until mid-summer (Figure 3-4b). During this period, sapflow of both deep and shallow roots in the lower slope was strongly coupled with transpiration ($P < 0.001$), deep roots contributed more water to support transpiration than did shallow roots ($P < 0.001$; Figure 3-5c, Table 3-4). However, later in the season both deep and shallow roots appeared to contribute more similar water flow supporting transpiration, while deep roots still contributed significantly more to transpiration ($P < 0.001$; Figure 3-5d, Table 3-4).

3.3.4 Atmospheric demands on Transpiration

Transpiration of trees from upper and lower slope positions was influenced by atmospheric variables such as net radiation, air temperature, vapour pressure deficit and potential evapotranspiration (evaporative demand) (Figure 3-6a-e; Appendix Table A-1). At the same magnitude of atmospheric demand, transpiration of trees at the lower slope position was significantly greater compared to that at the upper slope position ($P < 0.001$). While the relationships between transpiration and these atmospheric variables differed between trees from upper and lower slope positions, transpiration was much more sensitive to these atmospheric variables earlier in the season compared to later in the growing season. The relationship between transpiration and net radiation was nonlinear, and transpiration was significantly more sensitive to net radiation early in the season while later in the season this relationship was weaker ($P < 0.001$, Figure 3-6a). Significant linear relationships between transpiration and air temperature were observed both early and later in the season (Figure 3-6b) though transpiration was much more sensitive to variation in air temperature earlier in the season ($P < 0.001$). Similarly, linear relationships between transpiration and vapour pressure deficit and potential evapotranspiration were significant both earlier and later in the growing season (Figure 3-6c and 3-6d), with transpiration most sensitive to variation in these variables earlier in the season ($P < 0.001$ and $P < 0.001$) for D and ET_p, respectively). The weak linear relationship between transpiration and wind speed was significant early in the growing season ($P < 0.001$), but transpiration was not influenced by wind speed later in the growing season ($P = 0.323$).

3.3.5 Soil Moisture Availability and Root Sapflow

Seasonal variation in tree water use and root water uptake generally paralleled the variation in soil matric potential (ψ_s) during the growing season. Soil Ψ declined from peak values (near saturation) after snowmelt to late summer, with the exception of a temporary recharge of the soil moisture that resulted from a large precipitation event on August 2, 2009, where 16.5 mm of precipitation was measured (Figure 3-3f). Shallow root water uptake in the upper hillslope position decreased from 0.11 $m^3 m^{-2} h^{-1}$ early in the season to 0.07 $m^3 m^{-2} h^{-1}$ late in the growing season, but deep root sapflow in the upper hillslope remained at 11 $m^3 m^{-2} h^{-1}$ throughout the growing season (Table 3-4). This corresponded with a decline in mean soil Ψ of shallow soils in the upper hillslope position from -62.5 kPa early in the season to -477.1 kPa late in the growing season, whereas soil Ψ deeper in the profile declined from -37.7 kPa to -260.2 kPa during this same period (Table 3-4). Similarly, shallow root water uptake in the lower hillslope position generally co-varied with seasonal variation in soil Ψ decreasing from 0.13 $m^3 m^{-2} hr^{-1}$ (early) to 0.09 $m^3 m^{-2} hr^{-1}$ (late), whereas deep root water uptake declined 45 % from early to later in the growing season (Table 3-4). In both upper and lower hillslope position, while sapflow of deeper roots was at least equal (or greater) than that of shallow roots early in the season, sapflow of deeper roots was consistently greater than shallow roots later in the season when soil moisture was limiting and soil water potentials were lowest.

3.4 Discussion

Water availability exerted the strongest influence on transpiration of aspen trees along the hillslope. Across the entire growing season, mean soil matric potential at the lower hillslope position was 29 % greater than what was observed at the upper hillslope position (Figure 3-3f, Table 3-4). Greater soil water potential at the lower hillslope position was strongly associated with greater root sapflow (Root Q_v; 0.14 $m^3 m^{-2} h^{-1}$), tree sapflow (Q_v; 284 $cm^3 h^{-1}$), transpiration per unit leaf area (Q_L; 7.0 $m^3 H_2O m^{-2} A_L h^{-1}$) and water use (272 mm $season^{-1}$) (Tables 3-2 and 3-3). Furthermore, greater soil water availability was also strongly associated with greater tree leaf area, sapwood area, root surface area, and with that indirectly to productivity (Table 3-1).

Although the effect of soil moisture availability on transpiration and water relations of trees has been studied in many species and regions in the context of drought and spatial heterogeneity of soil moisture, none of these studies provided a comprehensive description of differential atmospheric or soil moisture availability on tree water use and productivity including effects of spatial and temporal (seasonal) heterogeneity of soil moisture availability on the landscape. The results of my study are consistent with previous research, reporting strong positive covariance between soil moisture availability and sapwood area, leaf area, and with that the productivity of trees. All of these studies have reported greater tree productivity reflected in greater transpiration where soil moisture is not limiting (Pataki et al. 2000, Schwarz et al. 2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Dalla-Salda et al. 2009, Dalla-Salda et al. 2011, MacKay et al. 2012). Similar to my study, these studies were also conducted within one growing season and were subject to that year's environmental condition, such as amount and timing of precipitation. As a result, the studies listed above were only able to examine the influence of atmospheric variables on transpiration and plant productivity when soil moisture was not limiting. This study was able to examine the influence of atmospheric variables on transpiration and plant productivity when soil moisture was and was not limiting.

Seasonal availability of soil moisture differs regionally in length of time and magnitude, but similar trends of greater soil moisture availability early in the growing season and declining towards the end of the growing season are consistent for most regions (MacKay et al. 2012). Likely due to snow melt, soil moisture was greatest at the start of the growing season resulting in little differences in soil water availability between hillslope positions. Seasonally mean soil matric potential was 84 % greater early in the season compared to later in the season, which corresponded closely with the seasonal variation in transpiration and root water uptake. Increased transpiration and root water uptake early in the growing season is likely the result of the physiological response (i.e. leaf flush, reduced control of stomata – data not shown) of the aspen trees to the start of the growing season (i.e. increase in net radiation, and soil and air temperature) (Landhäusser et al. 2003).

Although transpiration and root water uptake were greater earlier in the growing season, there was a steady decline in transpiration and root water uptake, which corresponded to a steady decline in soil matric potential leading up to midsummer (Figure 3-3f). However, after a late summer rain, soil moisture was temporarily recharged (Figure 3-3f), and an increase in both transpiration and root water uptake were observed during this short period (Figure 3-4a and 3-b). This increase in transpiration and root water uptake was more pronounced in trees growing at the lower hillslope position. This result is in contrast with observations by Tromp-van Meerveld and McDonnell (2006) who observed trees growing at the upper hillslope position showed a greater increase in transpiration after a late season rain fall compared to trees at the lower hillslope position in an oak-(*Quercus* sp.) hickory (*Carya* sp.) forest in Georgia, USA. However, soils in mid- and lower hillslope positions in their study were much shallower than those in my study and were not moisture limited in the late season as was the case in this research.

While limitations in soil moisture had a large effect on transpiration in aspen trees along the hillslope, atmospheric variables still had a large influence on transpiration. While transpiration was strongly associated with all atmospheric variables both early and late season, this association was much stronger early in the season when soil water was less limiting (Figure 3-3a-e). Early in the growing season transpiration was more sensitive (steeper regression slope) to net radiation ($Q*$), followed by air temperature (Ta), vapour pressure deficit (D), potential evapotranspiration (ET_p) then wind speed (μ) (Figure 3-4a-e). Interestingly, later in the growing season when soil moisture was limiting, the strength of the relationship between transpiration and atmospheric variables was weaker (the regression slope was less steep) compared to earlier in the growing season. Likely the reduction in soil moisture availability caused the aspen trees to increase water conservation. Although stomatal conductance was not measured in this study, the reduction in transpiration observed later in the growing season when the tree was exposed to the same magnitude of atmospheric variables suggests that stomatal regulation may have been used to reduce water loss to the atmosphere (Tyree and Ewers 1991, Berry et al. 2006, MacKay et al. 2012). Furthermore, after the late summer rain event, the root water uptake (root sapflow) of deep roots appeared decoupled from transpiration (Figure 3-4a) as the response of increased root water uptake after this event was not reflected in any clear transpiration response. This result is in contrast to the theory of the passive water flow through plants following a water potential gradient (Tyree and Ewers 1991, Ewers et al. 2000). Additionally, when soil moisture becomes limiting later in the growing season this likely caused the onset of senescence in the aspen trees, and may also account for the decoupling of root water uptake and transpiration.

The hydraulic architecture of trees is complex, but essentially plants connect the soil and atmosphere through the soil-plant-atmosphere continuum (Ewers et al. 2000, Berry et al. 2006, Amenu and Kumar 2008). When stomates are open the difference in water potential between leaves and roots is great and water passively moves water from the roots to the leaves through the xylem. When stomatal closure occurs (i.e. to reduce water loss from the leaves to the atmosphere) the difference in water potential between the leaves and the roots is small and water uptake from the soil decreases (Ewers et al. 2000, Berry et al. 2006, Amenu and Kumar 2008). Late in the growing season, soil moisture and transpiration decreased significantly. Likely stomatal opening was tightly regulated to reduce water loss to the atmosphere from the leaves at this time, and although atmospheric demand was high, transpiration was significantly lower. However, after the late summer rain fall event, during which soil moisture was temporarily recharged and atmospheric demand was high, it was expected that transpiration of the aspen trees would have increased significantly and would be similar to the level earlier in the growing season. However, after the late summer rain fall event transpiration did not increase to the level measured earlier in the growing season. To my knowledge, change in response to atmospheric variables and the impact of soil moisture on transpiration has not been described previously in the literature. The difference in early and later regression slopes between transpiration and atmospheric variables suggests that atmospheric variables are very important; however, my results suggest atmospheric variables have a strong influence on transpiration only when soil moisture is less limiting.

Distinguishing between which variable, or combination of variables, influence transpiration at any given time is very difficult. Few studies can concretely determine whether atmospheric variables or soil moisture availability have greater influence over transpiration of mature trees growing in a natural setting. Several studies have found that transpiration increases linearly with increased *D* until a threshold is reached (between 0.8 and 1.0 kPa) where transpiration then decouples from *D* (MacKay et al. 2012, McLaren et al. 2008, Kume et al. 2007, Leuzinger et al. 2005, Bovard et al. 2005, David et al. 2004, O'Brien et al. 2004, Granier and Breda 1996). Only Bovard et al. (2005) also found that when soil moisture was limiting later in the growing season, that the *D* threshold decreased, which is consistent with the results from this study. In the hierarchy of limiting factors, water is consistently considered the most important limiting resource for tree growth (Berry et al. 2006, Tromp-van Meerveld and McDonnell 2006, Wullschleger and Hanson 2006, Bovard et al. 2005, Schwarz et al. 2004, Hogg et al. 2000, Pataki et al. 2000). Early in the season when moisture was not limiting transpiration showed a much higher degree of sensitivity to atmospheric variables. A common adaptive strategy for plants is to grow when soil moisture is available and limit growth when soil moisture becomes limiting (Mackay et al. 2012). This was observed in this study as a reduction in transpiration later in the growing season, and is consistent with the correspondence of maximum leaf area occurring when soil moisture becomes limiting (Berry et al. 2006). Results of this study strongly suggest that soil water availability appear as the dominant limiting factor for trees growing on this hillslope. Atmospheric variables (*Q**, *D*, etc.) showed only moderate control over transpiration when soil moisture conditions were favorable. The influence of atmospheric variables was much weaker when soil moisture availability became limiting.

3.5 Conclusion

Both atmospheric variables and soil moisture availability strongly influenced transpiration and root sapflow of aspen growing on a well-drained hillslope; however, atmospheric variables seem to only exert greater control over transpiration when soil water was not limiting. Of the atmospheric variables net radiation showed to have the greatest influence on transpiration; however, the temporal variation of the relationship between transpiration and atmospheric variables was explained by the variation in soil moisture availability that occurs throughout the growing season. This conclusion furthers our understanding of soil, plant and atmospheric relationships and provides important insights into the hierarchy of differential controls on transpiration and plant productivity.

3.6 Tables and Figures

Table 3-1 Summary of tree characteristics for aspen trees growing along the upper and lower hillslope positions.

Slope Position	Mean Age (yrs)	Mean DBH (cm)	Mean Tree Height (m)	Mean Sapwood Area per Tree (cm^2)	Mean Leaf Area per tree (m^2)*	Total Root Surface Area (m^2 m^{-3})^
Lower	74 (± 2)	18 (± 2)	16 (± 1)	166 (± 37)	37 (± 10)	66
Upper	76 (± 1)	17 (± 1)	15 (± 1)	115 (± 22)	18 (± 5)	55

NOTE: Numbers in brackets represent standard error of the mean. $n = 13$

*Indicates significant difference at $\alpha = 0.05$

^Sum of root surface area of roots 0.05 - 1 cm

Table 3-2 Average daytime transpiration per unit leaf area, sapflow and total water use of trees located at the upper and lower hillslope position.

Slope Position	Mean Transpiration per Unit Leaf Area (Q_L, m^3 m^{-2} h^{-1})*	Mean Sapflow (Q_v, cm^3 h^{-1})*	Total Water Use (mm season^{-1})*
Upper	4.8×10^{-6} ($\pm 7.1 \times 10^{-8}$)	94 (± 2)	113
Lower	7.0×10^{-6} ($\pm 1.1 \times 10^{-7}$)	284 (± 4)	272

NOTE: Numbers in brackets represent standard error of the mean. n = 176

*Indicates significant difference at $\alpha = 0.05$

Table 3-3 Average daytime transpiration per unit leaf area ($m^3\ m^{-2}\ h^{-1}$) and average root sapflow ($m^3\ m^{-2}\ h^{-1}$) early and late in the growing season for all aspen growing at the upper and lower hillslope positions.

Slope Position	**Average Transpiration per Unit Leaf Area (Q_L; $m^3\ m^{-2}\ h^{-1}$) ***		**Average Root Sapflow (Root Q_v; $m^3\ m^{-2}\ h^{-1}$) ^**		
	Early	**Late**	**Early**	**Late**	**Mean**
Upper Slope *^	5.8×10^{-6} ($\pm 1.8 \times 10^{-7}$)	3.1×10^{-6} ($\pm 1.1 \times 10^{-7}$)	0.09 (± 0.002)	0.07 (± 0.002)	0.08 (± 0.002)
Lower Slope *^	8.9×10^{-6} ($\pm 2.8 \times 10^{-7}$)	4.0×10^{-6} ($\pm 1.6 \times 10^{-7}$)	0.17 (± 0.005)	0.10 (± 0.003)	0.14 (± 0.003)
Mean	7.3×10^{-6} ($\pm 2.8 \times 10^{-7}$)	3.5×10^{-6} ($\pm 1.6 \times 10^{-7}$)	0.13 (± 0.003)	0.09 (± 0.002)	

NOTE: Numbers in brackets represent standard error of the mean. n = 176

*Indicates significant different at $\alpha = 0.05$ for difference among mean transpiration per unit leaf area early and late season (n = 168)

^Indicates significant different at $\alpha = 0.05$ for difference among mean root sap flow early and late season (n = 4142)

Table 3-4 Mean root sapflow (m^3 m^{-2} h^{-1}) and soil matric potentials (kPa) for roots at 15 and 30 cm depths at the upper and lower hillslope positions and root sapflow of long lateral roots from trees at the upper slope.

Slope Position	Root Depth	Mean Root Sapflow (Q_v; m^3 m^{-2} h^{-1}) *			Mean Soil Matric Potential (ψ_s; kPa) ^		
		Early	**Late**	**Mean**	**Early**	**Late**	**Mean**
Upper Slope	**Shallow * ^**	0.11 (± 0.004)	0.07 (± 0.003)	0.09 (± 0.004)	-62.5 (± 1.9)	-477.1 (± 2.2)	-220.9 (± 4.6)
	Deep ^	0.11 (± 0.004)	0.11 (± 0.004)	0.11 (± 0.003)	-37.7 (± 1.0)	-260.2 (± 3.6)	-122.6 (± 2.8)
	Ratio Shallow:Deep	*1.0*	*0.6*				
Lower Slope	**Shallow * ^**	0.13 (± 0.60)	0.09 (± 0.50)	0.12 (± 0.60)	-28.1 (± 0.4)	-241.1 (± 2.7)	-109.5 (± 2.5)
	Deep * ^	0.22 (± 0.03)	0.12 (± 0.02)	0.18 (± 0.02)	-22.4 (± 0.3)	-166.8 (± 3.2)	-77.5 (± 2.0)
	Ratio Shallow:Deep	*0.6*	*0.8*				

NOTE: Numbers in brackets represent standard error of the mean.

*Indicates significant different at α = 0.05 for difference among mean root sapflow early and late season (n = 2071)

^Indicates significant different at α = 0.05 for difference among mean soil matric potential early and late season (n = 2118)

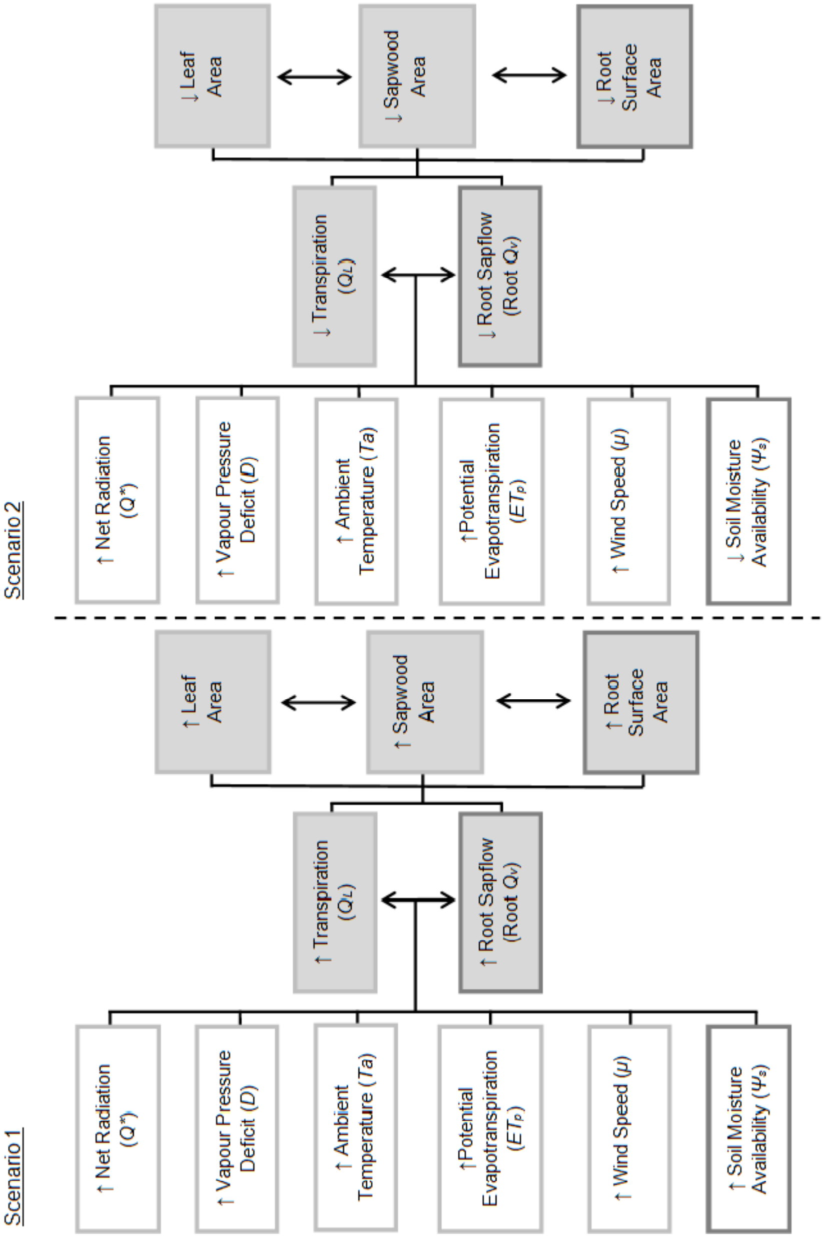

Figure 3-1 **Hypothesized influence of atmospheric variables and soil moisture availability on transpiration and root sapflow. Scenario 1 represents early growing season or lower hillslope position, and Scenario 2 represents late growing season or upper hillslope position. Atmospheric and belowground variables are represented by light and dark border, respectively.**

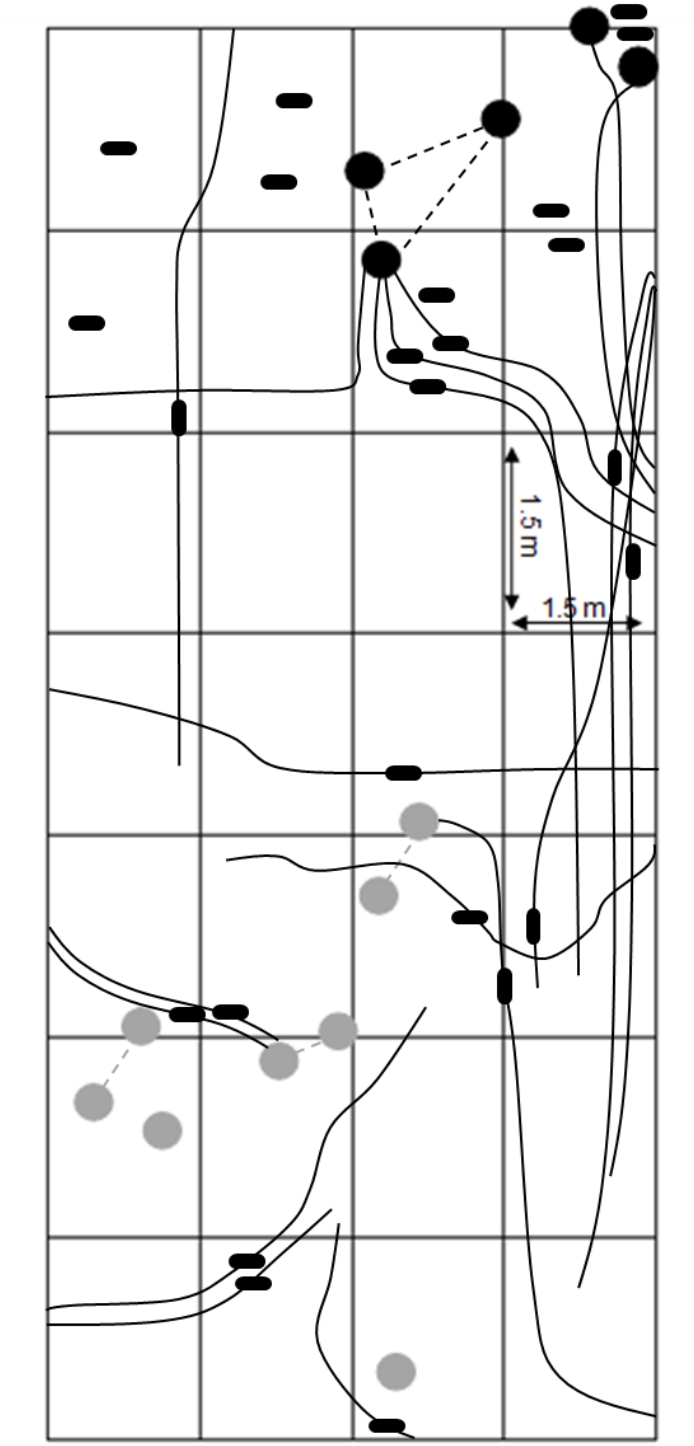

Figure 3-2 Transect grid layout and location of aspen clusters at the upper (black circles) and lower (grey circles) hillslope positions, the location of root sapflow sensors (black ovals), clonal root connections (black hatched lines), root grafts (grey hatched lines) and long lateral roots (solid black lines).

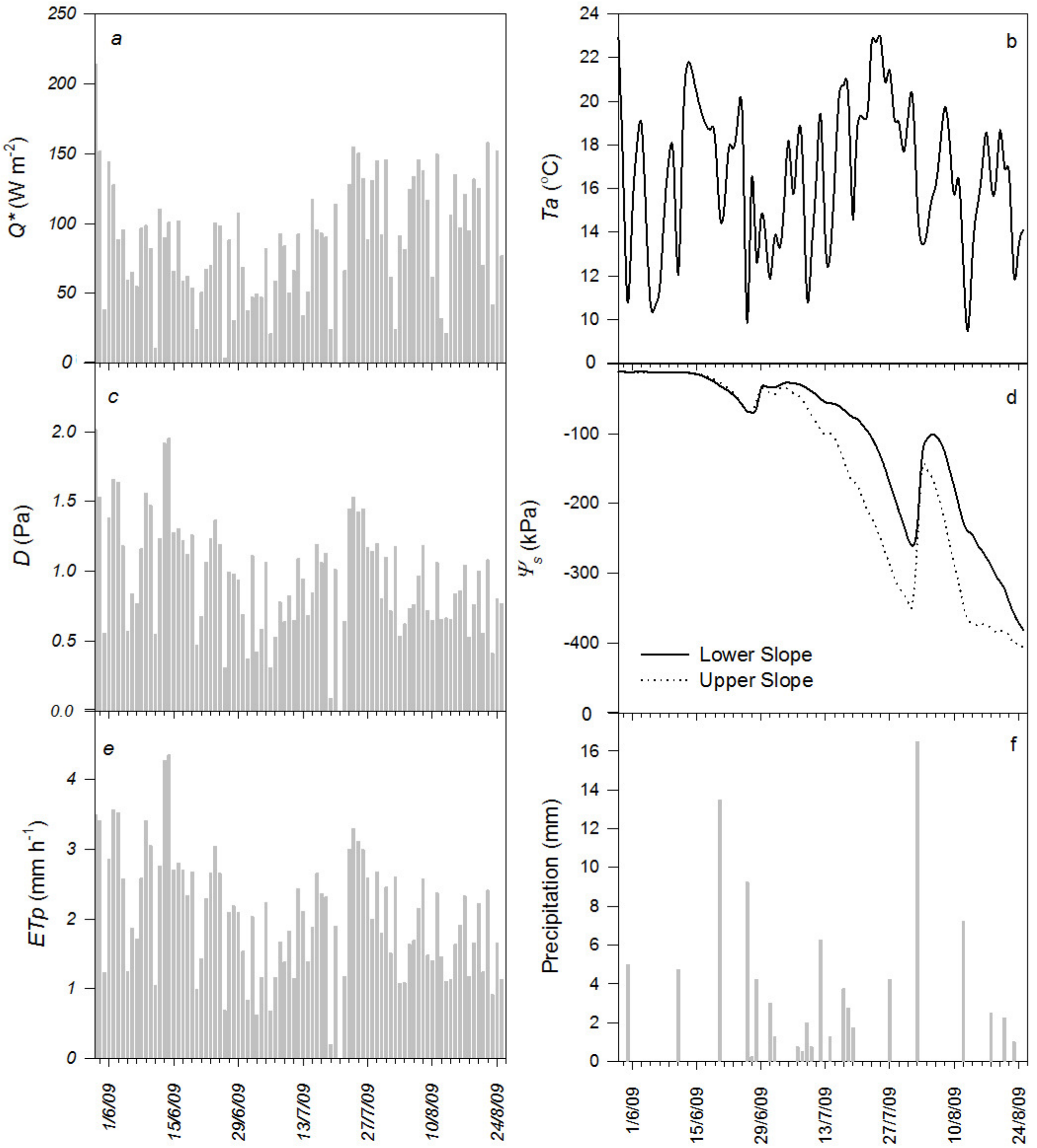

Figure 3-3a-f Mean daytime (05:00-22:00) (a) net radiation, (b) ambient air temperature, (c) vapour pressure deficit, (d) soil matric potential, (e) total daytime potential evapotranspiration and (f) total daily precipitation (MDT; May 29 – August 25, 2009).

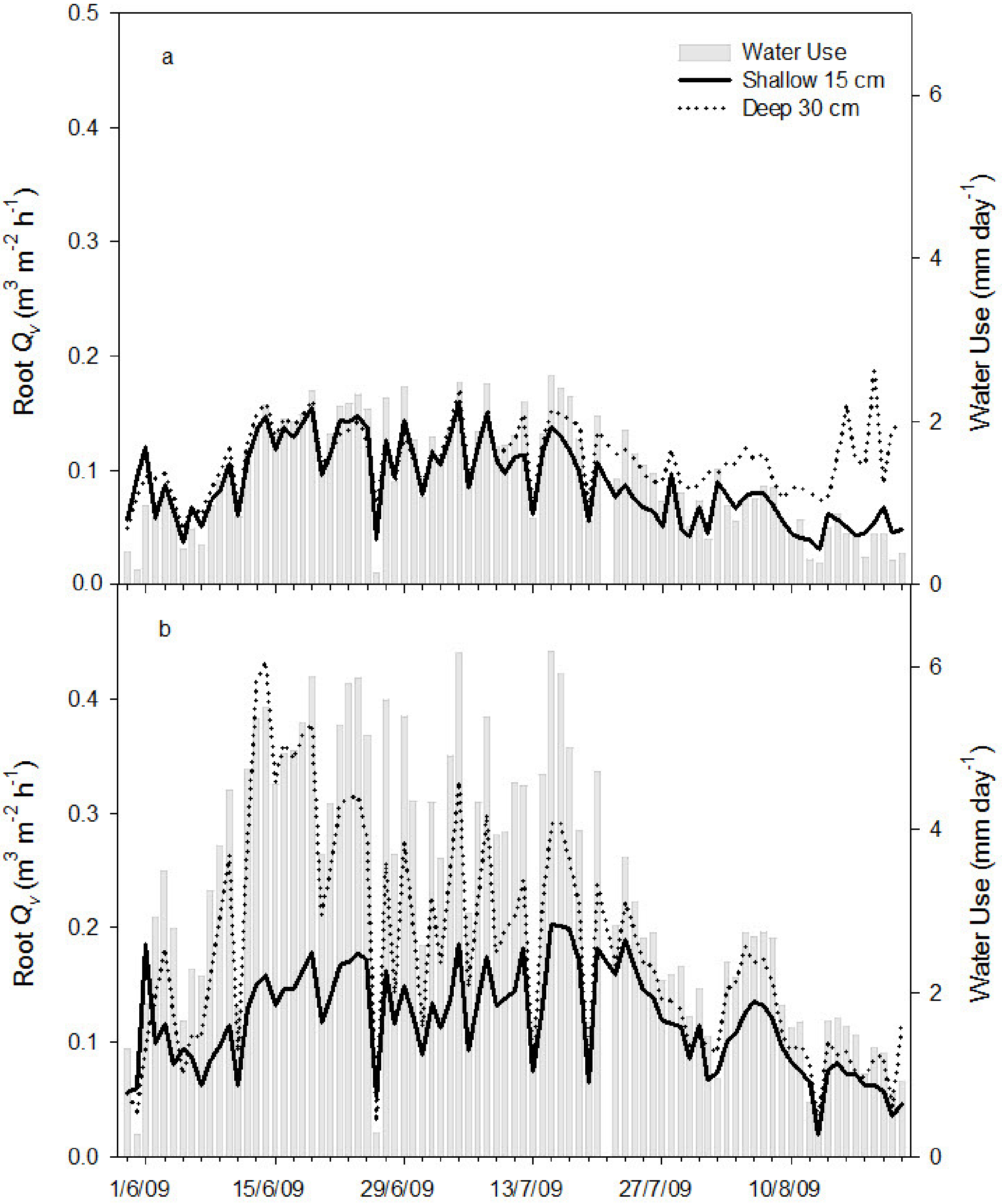

Figure 3-4a-b Daily water use and average root sapflow of shallow and deep roots at the (a) upper and (b) lower hillslope positions (May 30 – August 22, 2009).

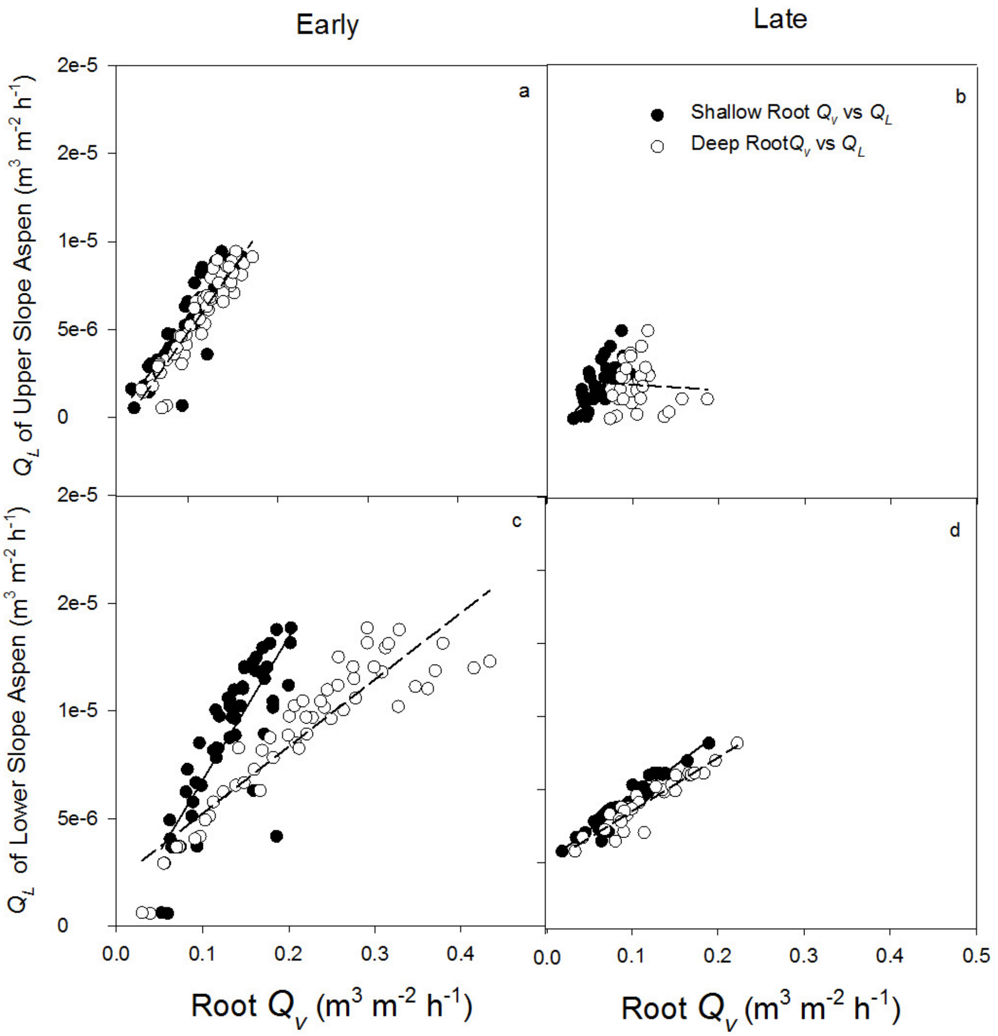

Figure 3-5a-d Regression of daily transpiration per unit leaf area (Q_L) and root sapflow (Q_V) of trees growing at the upper (a & b) and lower (c & d) hillslope positions, early (a & c) and later (b & d) in the growing season. See Appendix A, Table A-1 for equations and regression statistics.

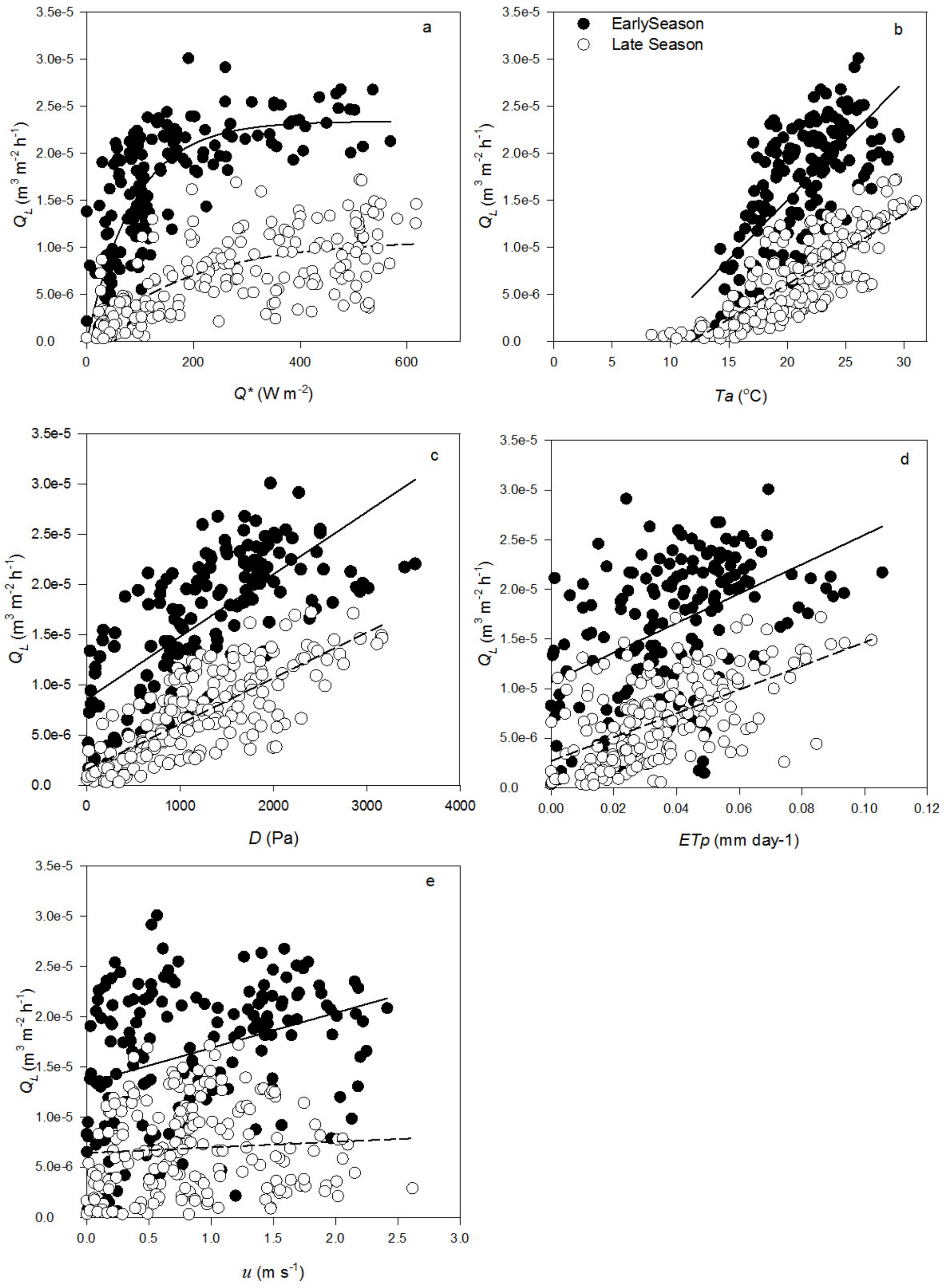
a
b
c
d
e
● EarlySeason
○ Late Season
Q_L (m^3 m^{-2} h^{-1})
3.5e-5
3.0e-5
2.5e-5
2.0e-5
1.5e-5
1.0e-5
5.0e-6
0.0
0
200
400
600
Q^* (W m^{-2})
0
5
10
15
20
25
30
Ta (°C)
0
1000
2000
3000
4000
D (Pa)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
ETp (mm day-1)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
u (m s^{-1})

Figure 3-6a-e

Figure 3-6a-e Response of early (June 13 – July 22, 2009) and late season (July 23 – August 25, 2009) transpiration per unit leaf area (Q_L) to (a) average net radiation (Q^*) (900-1500), (b) air temperature (Ta) (0500-2200), (c) vapour pressure deficit (D) (0500-2200), (d) potential evapotranspiration (ET_p) (0500-2200) and (e) wind speed (u) (0500-2200) and MDT. See Appendix A, Table A-2 for equations and regression statistics.

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Chapter 4: Synthesis

The overall objectives of this research were to explore the influence of atmospheric variables and soil water availability on root architecture and transpiration of mature aspen trees growing along a hillslope. The first study chapter (Chapter 2) described the three dimensional spatial distribution of root systems, and identified the role of inter- and intra-clonal root connections for uptake and distribution of water among trembling aspen clones growing along a hillslope. The results from Chapter 2 showed that the distribution of roots and root architecture of aspen trees was influenced by the moisture gradient along the hillslope. The hillslope represented a soil moisture gradient, where soil water potential was 29 % greater at the lower hillslope position than that observed upper hillslope position. Greater root surface area (root occupancy) of fine and coarse roots was observed at the lower hillslope position where there was greater soil moisture availability. The hydrotropism of root systems is not a new phenomenon and has been observed in many species across many regions (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Tsutsumi et al. 2004, Coleman 2007, Brassard et al. 2009). The observation of increased abundance of coarse and fine roots correlated well with the observation of greater leaf area of aspen trees growing at the lower hillslope position, which suggests that greater soil moisture availability likely results in greater tree productivity. However, although aspen trees at the lower hillslope position had significantly greater leaf area, the average leaf area to sapwood area ratio was about 50 % lower in trees growing at the upper hillslope position. This indicates that aspen trees growing where there is more readily available soil moisture likely require less sapwood area to support a similar amount of leaf area. This result is consistent with a study using *Quercus pubescens* growing along a hillslope (Barij et al. 2007), but is contrary to root mass and shoot mass increase relative to each other, which other studies have found (Coutts 1987, 1989, Mahoney and Rood 1991, Coleman 2007, Brassard et al. 2009). This may be the result of the root growth strategy of aspen trees growing at the upper hillslope position as an adaptation to water stress.

The results of Chapter 2 also showed that the clonal connections of aspen trees along this hillslope were probably not as important an adaptation as root distribution along the hillslope. Clonal root connections have been shown to be an important adaptation to overcome water stress because they facilitate sharing of water and nutrients between ramets (DeByle 1964, DesRochers and Lieffers 2001, Peltzer 2002, Jelinkova et al. 2009). However, although there were multiple clones within each transect, with a number of root grafts and parental root connections, there were no root connections observed between trees at the upper and lower hillslope positions, which if they did exist, may have allowed water to move up the hillslope through preferential root pathways.

In addition to trees allocating more energy to produce roots where soil moisture is greater, the trees located at the upper hillslope position were observed to have produced long lateral roots growing towards the lower portion of the hillslope (root asymmetry). This result, to my knowledge, has not been previously described in the literature, and could be interpreted as an adaptation to overcome water stress. Root asymmetry is not uncommon in mature root systems, but is usually interpreted as an adaptation to increase structural support in steep and exposed areas or because of mechanical impedance of root growth (Coutts et al. 1999, Di Iorio et al. 2005, Ganatsas and Spanos 2005).

In Chapter 3, I investigated the influence of soil moisture availability on stand characteristics and the influence of atmospheric variables and soil moisture availability on transpiration of aspen trees along a hillslope. The gradient in soil moisture corresponded well with the distribution of roots observed within this study as described above. Root sapflow sensors were installed on roots along the hillslope at 15 and 30 cm depth on roots 14 - 15 mm in diameter. It was not possible to install these sensors on roots smaller than this, but these sensors enabled me to monitor the root water uptake of the root matrix connected to the sensored roots. Root water uptake (root sapflow) from deep roots was significantly more than that of shallow roots. Furthermore, root water uptake was significantly greater from roots located at the lower hillslope position compared to the upper hillslope position. Greater root water uptake from areas where there is greater soil moisture is consistent with the theory of root hydrotropism (Coutts 1987, Hendrick and Pregitzer 1996, Casper and Jackson 1997, Hutchings and John 2003, Coleman 2007, Brassard et al. 2009). However, increased root water uptake from deeper roots contradicts the conclusion that greater root water uptake occurs where there is greater root surface area (Eissenstat 1992, Casper and Jackson 1997).

Many studies have examined the effect of soil moisture availability on the productivity of trees in the context of drought (Pataki et al. 2000, Schwarz et al. 2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Dalla-Salda et al. 2009, Dalla-Salda et al. 2011, MacKay et al. 2012) and spatial heterogeneity of soil moisture (Berry et al. 2006, Tromp-van Meerveld and McDonnell 2006, Loranty et al. 2008, Guswa 2012, Angstmann et al. 2012). The results of Chapter 3 were consistent with previous studies showing strong positive covariance between soil moisture availability and sapwood area, leaf area, and with that, the productivity of trees (Hogg and Hurdle 1997, Ewers et al. 2000, Hogg et at. 2000, Bond-Lamberty et al. 2002, Schwarz et al. 2004, Ewers et al. 2005, Loranty et al. 2008).

Soil matric potential, which is an indicator of soil moisture availability, was 84 % greater early in the growing season compared to later in the growing season. The greater soil moisture availability early in the growing season was associated with greater transpiration and root water uptake by aspen trees growing along the hillslope. Later in the growing season both transpiration and root water uptake were significantly less, which is consistent with the concept of hydrological equilibrium in plants. Along this hillslope transpiration and water loss to the atmosphere was high, but as soil moisture became limiting, these aspen trees may not have been able to continue to support that level of transpiration and likely further growth was prevented and maximum leaf area was reached (Grier and Running 1977, Kergoat 1998).

In the study reported on in Chapter 3 I also investigated the effect of atmospheric variables on transpiration. Transpiration of aspen trees was positively related to Q^*, T_a, D and ET_p consistent with what has been found in other studies (Pataki et al. 2000, Schwarz et al. 2004, Bovard et al. 2005, Wullschleger and Hanson 2006, Dalla-Salda et al. 2009, Dalla-Salda et al. 2011, MacKay et al. 2012). However, I found that when soil moisture became limiting the relationship between transpiration and atmospheric variables changed. Early in the growing season transpiration was highly sensitive (steeper regression slope) to atmospheric variables; however, later in the growing season when soil moisture decreased, the relationship between atmospheric variables and transpiration was weaker (regression slope was moderate). The difference in early and later regression slopes between transpiration and atmospheric variables suggests that atmospheric controls are very important; however, my results suggests atmospheric controls only have a strong influence on transpiration when soil moisture is less limiting. To my knowledge, this result has not been described previously in the literature.

4.1 Management Implications:

Aspen is an early successional species that is widely distributed across North America. Aspen is known to naturally grow on a variety of soil and climatic conditions throughout its range extent from northern Mexico through to Alaska (Little 1971), and from the results of this and other studies, has been found to be relatively drought tolerant. The adaptive strategies employed by this species that were discovered during this study, such as asymmetrical root distribution, clonal root connections and root grafts and increased root water uptake from areas where there is more soil moisture availability, provide fundamentally new insights into what enables this species to exploit soil resources in water limited environments.

Open pit mining is a common practice for resource extraction in Alberta. It requires the removal of all vegetation, soil, overburden and eventually the mined material (Rowland et al. 2009). This process leaves large areas that require reclamation. Currently, provincial guidelines require companies to reclaim these areas to forests within the natural range of ecotypes (Alberta Environment 2010); however, the long-term success of these reclamation efforts cannot yet be measured because the reclaimed areas have not yet reached maturity. Mine reclamation of upland sites is often restricted to contoured hillslopes. These sites are vulnerable to soil erosion; therefore, it is common practice to establish grass and legume species for erosion control (Emerson et al. 2009, Franklin et al. 2012, Evans et al. 2013). Although, establishment of grass and legume species facilitate development of soil structure through root development, they have also been found to reduce the success of tree establishment and growth because these pioneer species outcompete other native forbs and grasses (Evans et al. 2013). These species can also inhibit natural revegetation through seeding-in, but also damage tree and shrub species due to snow press (Eis 1981, Lieffers et al. 1993). This was evidenced in a recent study by Rowland et al. (2009) that compared reclaimed oil sand sites to natural areas and found that reclaimed sites had more bare ground, grass and forb cover than shrub and tree cover. Therefore, some reclamation practices are not ideal for the establishment of native species, and will likely not result in these areas being successfully reclaimed to native forest.

Furthermore, poor establishment and survival of target reclamation species is the result of several factors including reduced organic layer thickness, nutrient levels being outside the natural range of variation and large weed infestations (Rowland et al. 2009). Additionally, due to the reduced organic layer thickness reclaimed sites generally have large fluctuations in soil temperature and reduced carbon cycling (Yu et al. 2002). The use of aspen as a reclamation species has great potential because it is a fast growing deciduous species, and quickly produces an extensive root systems (Martens et al. 2007). The establishment of aspen along a reclaimed hillslope will likely reduce erosion and increase soil development through root growth and production of leaf litter. The deciduous nature of this species will also shade other pioneer weedy or non-desirable species that are not shade tolerant (Lieffers and Stadt 1994, Landhäusser et al. 2003). Additionally, during the spring and fall when the aspen trees are bare, target reclamation species such as spruce (*Picea glauca* (Moench) Voss) will be exposed to increased light levels, and will enable these species to maximize photosynthesis during this time (Man and Lieffers 1997).

This study showed that the adaptive strategies of aspen enable this species to effectively access soil resources, which increase productivity and tolerance to water stress. Establishment of aspen can aid in weed and non-desirable species suppression, the leaf litter increases nutrient cycling and will provide shade and microclimate conditions ideal for the establishment and growth of other desirable species such as white spruce (Landhäusser et al. 2003, Macdonald et al. 2013).

4.2 Future Research:

Although this research increased the understanding of atmospheric and soil moisture availability on the distribution of roots, root water uptake and transpiration of mature aspen trees, it also generated several unanswered questions that need to be studied further to fully understand these processes.

1. **Evaluate the role of the asymmetry in aspen root systems.** This study found that aspen at the upper hillslope position grew long lateral roots toward the lower hillslope position. This asymmetry could be used to increase water uptake from the soil. Future research should include root sapflow measurement of these roots in relation to stem sapflow along a hillslope. Measurement of lateral root sapflow will give a more complete understanding of the water uptake and use by mature aspen trees.

2. **Test whether aspen use hydraulic redistribution as an adaptation to overcome water stress.** Hydraulic redistribution is used by several species, but has not been investigated in aspen. Two root sapflow sensors should be installed in opposite flow directions on each monitored root to test whether root sapflow is bidirectional in aspen. Additionally, analysis of soil matric potential at different soil layers and locations along a hillslope for a growing season may also show evidence of hydraulic redistribution.

3. **Examine the adaptive strategies employed by aspen during establishment and growth on reclamation sites in the oil sands region.**

What adaptive strategies to juvenile aspen use, and how do these adaptations influence the growth and development of surrounding species?

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Appendix A

Table A-1 Equations and regression statistics for daily transpiration per unit leaf area and root sapflow of trees growing at the upper and lower hillslope positions, early and later in the growing season.

Season	Hillslope Position	Soil Depth	Regression Equation	R^2	P-value
Early	**Lower**	**Shallow**	$y = 1.41\text{x}10^{-7} + 6.64\ \text{x}10^{-5} * x$	0.68	<0.001
		Deep	$y = 2.13\text{x}10^{-6} + 3.12\text{x}10^{-5} * x$	0.84	<0.001
	Upper	**Shallow**	$y = -1.40\text{x}10^{-6} + 6.87\text{x}10^{-5} * x$	0.82	<0.001
		Deep	$y = -2.41\text{x}10^{-6} + 7.25\text{x}10^{-5} * x$	0.88	<0.001
Late	**Lower**	**Shallow**	$y = 5.50\text{x}10^{-9} + 4.41\text{x}10^{-5} * x$	0.93	<0.001
		Deep	$y = -1.70\text{x}10^{-7} + 3.68\text{x}10^{-5} * x$	0.88	<0.001
	Upper	**Shallow**	$y = -7.47\text{x}10^{-7} + 6.63\text{x}10^{-5} * x$	0.55	<0.001
		Deep	$y = 3.62\text{x}10^{-6} - 3.45\text{x}10^{-6} * x$	0.003	0.759

Table A-2 Equations and Regression statistics for response of early (June 13 – July 22, 2009) and late season (July 23 – August 25, 2009) transpiration per unit leaf area (*Q_L*) to average net radiation (*Q^**) (900-1500), air temperature (*Ta*) (0500-2200), vapour pressure deficit (*D*) (0500-2200), wind speed (*u*) (0500-2200) and potential evapotranspiration (*ET_p*) (0500-2200), MDT.

Season	Variable	Regression Equation	R^2	P-value
Early	Q^*	$y = 2.34\text{x}10^{-5} (1 - e^{-0.011*x})$	0.54	<0.0001
	Ta	$y = -1.026\text{x}10^{-5} + 1.26\text{x}10^{-6} * x$	0.50	<0.001
	D	$y = 8.568\text{x}10^{-6} + 6.225\text{x}10^{-9} * x$	0.53	<0.001
	u	$y = 1.338\text{x}10^{-5} + 3.498\text{x}10^{-6} * x$	0.12	<0.001
	ET_p	$y = 1.066\text{x}10^{-5} + 1.485\text{x}10^{-4} * x$	0.23	<0.001
Late	Q^*	$y = 1.08\text{x}10^{-5} (1 - e^{-0.005*x})$	0.48	<0.0001
	Ta	$y = -8.713\text{x}10^{-6} + 7.404\text{x}10^{-7} * x$	0.65	<0.001
	D	$y = 1.554\text{x}10^{-6} + 4.555\text{x}10^{-9} * x$	0.61	<0.001
	u	$y = 6.420\text{x}10^{-6} + 5.555\text{x}10^{-7} * x$	0.01	0.323
	ET_p	$y = 2.708\text{x}10^{-6} + 1.198\text{x}10^{-4} * x$	0.34	<0.001