Determining tree water acquisition with stable isotope analysis
in a temperate agroforestry system

by

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Abstract

The water acquisition strategies of trees in agroforestry systems may affect adjacent crops through competition for resources. This study characterizes the water uptake zones of *Juglans nigra* (walnut) and *Populus sp.* (hybrid of *Populus deltoids X Populus nigra clone DN-177*) (poplar) in a temperate agroforestry system. Isotopic analysis ($\delta^{18}O$) of soil water and tree xylem water occurred in early season and late season samples from the Agroforestry Research Station in Guelph, Ontario, Canada. Direct inference and multiple source mass-balance approaches showed that poplar exhibited a primary soil water uptake zone at 20 cm in early season, while walnut uptake was higher in the soil profile at 10 cm. Late season water uptake zones shifted to lower in the soil profile (40-70 cm) for both poplar and walnut trees. This study indicates: i) species dependent water acquisition zones, and ii) a shift to lower in the soil profile later in the growing season.
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Chapter 1
Introduction

1 Agricultural water use

1.1 Research context

In agricultural systems, water is one of the most limiting resources. A large portion of water is lost through evapotranspiration; 97% of the water absorbed by plant roots is lost through evaporation in the leaves (Poorter and Nagel, 2000; Taiz and Zeiger, 2010) and the productivity of an ecosystem is directly related to annual precipitation rates (Tian et al., 2010; Waring and Running, 2010). For this reason, the understanding of source water utilization patterns and the water uptake strategies of trees, herbs and annual crops in modern food production systems is essential to ensuring productivity and security (Tian et al., 2010).

Industrialized agriculture has significantly altered the global landscape through reduction of biodiversity, nutrient leaching, soil and water contamination and erosion (Smith and Olesen, 2010; Wilhelm et al., 2010). The current reliance on monoculture agriculture necessitates large inputs of fertilizer, pesticides and insecticides, which have been demonstrated to pollute ground water systems and harm human and environmental health (Horrigan et al., 2002; Hoekstra and Chapagain, 2006) services.

Agriculture is responsible for two-thirds of all water use around the world, making it a larger consumer than either industrial or municipal uses (Horrigan et al., 2002). Managing and limiting the water used in irrigation is of paramount importance because irrigation practices alter water flow, water quality and freshwater habitats to a greater extent than municipal and industrial uses (Echeverria et al., 2001). Irrigation is currently a very ineffective system, with
70% of all water withdrawals in the United States directed towards irrigation, but less than half of the water from these irrigation systems will ever make it to the crops because of leakage, evaporation and distribution problems (Echeverria et al., 2001). These figures are concerning as industrialized agriculture has also been demonstrated to increase desertification in temperate countries like the United States and around the world by over-cultivation and over-use of water (Horrigan et al., 2002).

Since the Rio +10 conference on climate change, the issue of available freshwater for agriculture has been a topic of discussion (Vano et al., 2010). The effects of climate change on the agricultural sector worldwide are predicted to include: lower productivity, soil erosion, reduced soil quality, lower quality produce, reduced ability to support livestock and decreased freshwater for irrigation (Lal et al., 2012). In the temperate zone of Canada and the United States these predicted negative impacts of climate change on the agricultural sector are of importance for a number of reasons. Population increases predicted in Canada will mean an increase in production is needed at a time when available agricultural land and freshwater resources are decreasing (Jamieson 2012; Smith et al., 2012; Schoeneberger et al., 2012). Agriculture and Agri-Food Canada released a 2012 report on the predicted effects of climate change on the Canadian agricultural industry in which they report Canada will experience higher than global average temperature increases which will negatively affect the agricultural and forestry sectors and have serious social and environmental ramifications (Jamieson, 2012).

Canada’s current agricultural systems rely upon large amounts of irrigation and chemical inputs that have led to nutrient leaching, soil and water contamination and erosion (Gibbs et
Water related issues associated with these practices include; pollution of surface and groundwater sources, waterlogged and salinized soils, wetland loss and ineffective irrigation systems (Marlow et al., 2009). Throughout Canadian history severe droughts have crippled Canada’s agricultural sector and have had significant impact on the economy. As the largest vegetable-growing region in the country, Southern Ontario was heavily impacted by the droughts of 2001-2002. In August 2002, Southwestern Ontario received 20% less than normal precipitation. Crop yields dropped significantly in numerous vegetables including carrots, onions, cabbages, potatoes and corn (Bonsal and Regier, 2007; Wheaton et al., 2008; Bonsal et al., 2011). In Ontario alone the economic cost of the drought was $27.5 million in 2001 and $16.5 million in 2002 (Wheaton et al., 2008). It is evident that incorporating farming solutions that utilize less water and maximize available resources can address current concerns and add resilience for the future (Tian et al., 2010; Smith et al., 2012).

Alternative forms of agriculture have received renewed interest in the last twenty years because increases in marginal land and shortages of fresh water for irrigation are threatening food production systems worldwide (Echeverria et al., 2001; Brazilian et al., 2001). Agroforestry, specifically tree-based intercropping (TBI), where annual crops are planted in alleys between rows of trees, is one practice which has been shown to reduce these effects (Thevathasan and Gordon, 2004; Reynolds et al., 2007; Evers et al., 2010). TBI systems mitigate environmental damage by two principle mechanisms that allow for complementary resource use (van Noordwijk et al., 1996; Allen et al., 2004; Bergeron et al., 2011). One process is greater nutrient recycling through the spatial distinction between agricultural crop
roots and the tree roots which results in resources being absorbed from different soil horizons minimizing interspecific competition for nutrients and water (Stratton et al., 2000; Meinzer et al., 1999; Meinzer et al., 2001). The second factor is that perennial tree species have different temporal nutrient uptake patterns from annual crops, providing continuous groundcover and soil stabilization, reduced leaching, increased water retention and providing continuous biomass input for nutrient recycling (Jose et al., 2000; Bergeron et al., 2011; Smith et al., 2012). TBI systems also provide numerous other benefits such as ecological diversity for peripheral species (e.g., beneficial insects and other animals), rejuvenation of marginal land, and ecosystem resilience for climate change mitigation and adaption (Schoeneberger et al., 2012). Research in agroforestry systems is needed to inform management practices that are responsive to the increased requirement for water efficiency and water quality.

1.2 Research background, objectives and hypotheses

Trees in agricultural landscapes have been shown to beneficially alter microclimates by decreasing fluctuations in soil moisture, soil temperature, humidity and solar radiation amongst other environmental benefits (Lin, 2007; Vandermeer et al., 1998). Despite these benefits it has also been acknowledged that resource competition can exist at the tree-crop interface in TBI systems (van Noordwijk and Lusiana, 1999; Rivest et al., 2009; Toor et al., 2012). One hypothesis for reduced yields is water competition in the rooting zone between trees and crops. Plant water uptake patterns from different soil depths vary spatially and temporally between different plant functional types and this directly influences soil water dynamics during the growing season (Asbjornsen et al., 2007; Asbjornsen et al., 2008). Traditionally, trees are thought to take water from deeper in the soil profile (below 50 cm);
although certain studies have demonstrated that some trees rely on a shallower horizon (Fernandez et al., 2008; Singer et al., 2013). Many herbaceous crops in Canada, including maize, barley, wheat and soy, have a shallow soil profile range for water acquisition (Zhuang et al., 2001; Misra et al., 2010).

Several questions remain as to the interaction of species in TBI systems with regard to use of water resources. Specifically, there is a need to broaden current understanding of water uptake, water partitioning and interspecific competition in agroforestry systems. Key questions include:

1) What is the location of source water for various crops and trees in TBI systems?

2) Do different species have alternate strategies at the same TBI site?

3) Do trees show a seasonal difference in water uptake zones in TBI systems?

Knowledge of tree root distribution is critical for understanding the effects of abiotic stress in these multispecies systems (Isaac and Anglaaere, 2013). However, the existence of roots does not necessarily denote an active water uptake zone (Green and Clothier, 1999; Comas et al., 2010). Techniques for studying tree root water acquisition in-situ have, to date, been limited. In this research, rooting zone activity will be studied using the $\delta^{18}$O signature of water in trees and soil as a non-destructive, natural tracer of the water uptake process. Previous work on my study site has shown that the majority of the fine roots for walnut $(Juglans nigra L.)$ and hybrid poplar $(Populus deltoids X Populus nigra clone DN-177)$, my two test tree species, are located within 1 m horizontally from the base of the tree and within 1 m vertically from the surface (Borden et al., 2013). This study will examine the isotopic gradient of the soil profile associated with these tree species and compare it to the isotopic
signature of the xylem tissue using multiple models to determine water acquisition zones of these common TBI tree species.

I propose the following hypotheses for the intercropped trees and herbaceous plants:

1) Measurement of oxygen isotopes in soil and plant xylem tissue water will be an effective natural tracer method to determine water acquisition zones in trees of TBI systems.

2) In TBI systems, tree species will vary in their water acquisition zones.

3) A time series sampling, spring (early season) and summer (late season), will demonstrate a seasonal shift in uptake pattern for the tree species. Root plasticity will be demonstrated showing a shift to water acquisition to lower in the soil profile in the late season.

It is expected that this study should demonstrate if trees have different water uptake zones in early and late season. Knowledge of the water uptake characteristics of trees will allow us to draw some conclusions about which of the two tree species, hybrid poplar or black walnut, will be best suited in a TBI system associated with shallow rooted intercropped plants. Answers to these questions are foundational for developing an understanding of the nature of water partitioning between trees and crops, and the level of direct competition for the same resource. Future tree-based intercropping design should incorporate research-based management techniques for maximizing water uptake and minimizing losses in order to promote system resilience. This work could contribute to this goal and provide insight on areas for future research.
2 Tree based intercropping systems

Agroforestry sites are multifunctional systems that incorporate trees within an agricultural context. There are six categories of agroforestry including: 1) riparian forest buffers, 2) windbreaks, 3) silvopasture, 4) tree-based intercropping or alley cropping, 5) forest farming and 6) special applications for remediation services (Reynolds et al., 2007; Schoeneberger et al., 2012). As multi-use environments, agroforestry systems have been shown to provide a diverse range of benefits including economic, cultural and environmental such as microclimate modification for crops (Smith et al., 2012; Varah et al., 2013). The focus of this literature review will be on tree-based intercropping, as these systems provide crop production, and economic and environmental benefits (Reynolds et al., 2007).

Tree-based intercropping (TBI), where rows of trees are spaced by an annual crop, is an important type of agroforestry in Ontario as these systems maintain production while contributing to environmentally sustainable agriculture. TBI systems are recognized in North America for environmental and economic benefits, providing income from agricultural and forestry crops for short and long term production (Reyonlds et al., 2007; Jose et al., 2010). For the last 20 years, TBI systems have increased soil fertility and maintained long term soil productivity (Udawatta, 2006; Moreno 2007; Jose, 2009). TBI systems have numerous soil quality benefits including a decrease in the disturbance to the uppermost soil areas around the trees, tree biomass expansion that increases water infiltration, a decrease in evaporative loss and the creation of a thriving habitat for beneficial soil fauna (Stavi and Lal., 2013; Jose,
The incorporation of organic matter from both above and belowground sources influences the release and recycling of nutrients and enhances soil physical, chemical and biological properties (Sauer et al., 2007).

The chemical and biological effects in these systems include nitrogen enhancement and increased carbon storage. Several studies have demonstrated that soil carbon and nitrogen concentrations are higher near the base of a tree than in the crop row (Sharrow and Ismail., 2004; Nair et al., 2009; Schoeneberger, 2009). Soil organic carbon is increased in agroforestry systems through above ground and belowground sources and TBI systems have the second highest storage potential of any land use system after forests, even surpassing tree plantations (Nair et al., 2009). TBI systems in Canada have reported a carbon-sequestration potential of 78.5 (Mg ha⁻¹) in a 13-year-old hybrid poplar and barley (Hordeum vulgare) system (Peichl et al., 2006). The physical biomass inputs from the pruning/litterfall of trees have been shown to be sufficient in meeting crop nutrient requirements, with the exception of phosphorus, in numerous studies (Palm, 1995). Soil porosity has been demonstrated to increase; this improves soil aggregate stability, which in turn improves carbon storage, soil nitrogen and soil enzyme activity (Udawatta et al., 2008). The windbreak mechanism provided by the trees reduces the wind speed that reaches the soil, thereby reducing evaporation at the soil surface and the effects of wind erosion. The windbreak effect is credited with improving the distribution and utilization of irrigation water, reducing evapotranspiration and improving crop water use efficiency (Kuemmel 2003; Thaler et al., 2012).
When species do compete for the same resources at the tree-crop interface, it will affect growth/production (Rivest 2009; Jose et al., 2000). Historically, when crop yields have been relatively lower in TBI systems (Onge 1991; Lin et al., 1998; Benjamin et al., 2000), it is thought to be as a result of a competition for resources, mainly water, light or mineral elements, between the trees and the crops (Miller and Pallardy, 2001; Jose, 2009). It is therefore important to take into consideration how the plants in an agroforestry system interact with the soil and each other in order to better understand water use by various species, the effect of plants on soil moisture and nutrients, and interspecific competition for water resources.

2.1 Water use in TBI systems

There are three main positive water benefits in TBI systems. First, the deeper roots of trees provide what is termed a “safety net”, where the roots take up water and nutrients that are below the rooting zone of the herbaceous plants which can be recycled into usable nutrients by root turnover and litterfall (van Noordwijk et al., 1996; Nair and Graetz, 2004). The second positive benefit is through improving water quality. Trees affect nutrient application runoff in two ways; the first being that in conventional monoculture crop systems less than half of applied fertilizers (phosphorous or nitrogen) are taken up by the crops. These nutrients are then washed off the field causing nutrient loading (Cassman et al., 2002). Trees have been shown to be very effective at removing and taking up such excess nutrients. For instance, in a study comparing a pecan-cotton alley cropping system to a mono-culture cotton system in Florida, Allen, et al. (2003) found that nitrates were reduced by 72% at a 0.9 m soil depth as compared to a mono-crop cotton field (Allen et al., 2003). However, it has also been
acknowledged that more studies are needed and results are typically site specific (Allen et al., 2003; Nair and Graetz, 2004). The third benefit for TBI systems for water is that trees increase the pool of water available for crop use. Through a process called hydraulic lift, deeply rooted trees access water at lower levels in the soil profile and transport it higher in the profile. This nocturnal process is driven by soil water potential gradients from the roots to drier soil layers where it resorbed by the tree or crop when transpiration demand is greater than the water available at lower layers (Dawson 1993; Wan et al., 2000; Strelcova et al., 2013). This water is transferred to surface soil where it is available for use not only for the trees, but for their neighbors, including the crops (Sekiya et al., 2011). Evidence has shown that hydraulic lift is an active process in TBI systems (Jose et al., 2004; Fernández et al., 2008). Particularly, it has been shown that the soil moisture benefits of hydraulic lift can increase the overall nutrients available for plants by increasing the supply of available water for rhizosphere microorganisms and soil fauna (Liste and White, 2008).

Water cycling in TBI systems is comprised of complex belowground interactions where roots compete for water resources (Anderson and Sinclair, 1993; Rossatto 2013). When considering root interspecific interactions, root length density, mycorrhization and flexibility in response to water and nutrient zones in the soil have to be considered (Schroth, 1999). TBI systems have demonstrated a positive impact on water uptake in plants because tree root systems have been shown to demonstrate a great deal of root plasticity (Schroth, 1999; Dawson 1996; Mulia and Dupraz, 2006). Zamora et al., 2007 demonstrated that cotton (Gossypium hirsutum L.) root shoot ratio, root biomass, total root length and root length density were positively affected in the TBI system incorporating pecan (Carya illinoensis K.
Koch) as compared to a mono-crop system (Zamora et al., 2007).

Tree roots are known to partition soil water in two ways (Meinzer et al., 2001). The first is temporally through seasonal displacement of leaves which controls transpirational water loss (Liang et al., 2010). The second is spatial partitioning of roots at different active layers and through horizontal spacing (Tang et al., 2005; Weltzin and McPherson, 1997). This soil partitioning is dependent on the seasonality and quality of precipitation and affects how trees access water in competition with other species (Dawson et al., 1991; Weltzin and McPherson 1997).

Many studies draw conclusions on water acquisition based on inferences related to soil moisture content and performance metrics, although the mechanisms are unclear (Jose et al., 2000; Miller and Pallardy, 2001; Wanvestraut et al., 2004; Senaviratne et al., 2012). Quantification of this important, yet understudied aspect of TBI systems, requires new and innovative approaches to characterize water use efficiency. Stable isotopic techniques may be able to provide this information.

2.2 Water isotopes in the environment

Isotopes are forms of elements, which differ by the number of neutrons. Both hydrogen and oxygen have different isotopic profiles (Michener and Lajtha, 2008; Werner 2012). Hydrogen has two stable isotopes $^1\text{H}$ (99.98%), protium and $^2\text{H}$ (0.0156%), deuterium (D). Oxygen exists in the environment in three stable isotopes, $^{16}\text{O}$ (99.759%), $^{17}\text{O}$ (0.037%), $^{18}\text{O}$ (0.204%) (VSMOW). Isotopic composition is expressed as a ratio of heavy to light atoms. For $^{18}\text{O}$ it is expressed in delta notation as $\delta^{18}\text{O}$. The relationship is:
\[
\delta^{18}O = \left( \frac{\frac{^{18}O_{sample}}{^{16}O_{sample}}}{\frac{^{18}O_{standard}}{^{16}O_{standard}}} - 1 \right) \times 1000 \%
\]

This equation gives the value “delta-oxygen-18” which is used for comparison of the amount of \(^{18}\text{O}\) to \(^{16}\text{O}\) in different sources, reported in parts per thousand. The symbols \(^{18}\text{O}_{\text{standard}}\) and \(^{16}\text{O}_{\text{standard}}\) are as defined by the VSMOW (Vienna Standard Mean Ocean Water) standard which is a reference standard. Possible conditions found in a sample are: \(\delta^{18}\text{O}\) is less than zero which means that the sample is depleted in \(^{18}\text{O}\) compared to VSMOW, and \(\delta^{18}\text{O}\) is greater than zero which means that the sample is enriched in \(^{18}\text{O}\) compared to VSMOW (Dawson et al., 2002; Werner et al., 2012).

The significantly heavier molecular mass of water with \(^{18}\text{O}\) over \(^{16}\text{O}\) (10\%) leads to differential phase change rates; liquid water containing \(^{16}\text{O}\) evaporates preferentially from a sample, while water in the gas phase containing \(^{18}\text{O}\) would condense first (Craig, 1961; Gat, 1995; Luz and Barkan, 2010). There are several processes that affect the ratio of heavy to light isotopes. The mass difference between isotopes of the same element is very important because it leads to the possibility of physical separation (fractionation) of isotopes of an element through naturally occurring processes such as chemical reactions, adsorption and phase changes. In these reactions the heavier isotope has greater bond strength or lower velocity; as a result the lighter isotopes are more mobile, thus causing fractionation (Michener and Lajtha, 2008; Werner et al., 2012).

Natural environments are open systems in most instances and, therefore, reactions do not
occur in equilibrium. As a result, the above processes can cause isotopes to separate naturally in the environment in a predictable and traceable fashion (Hofmann and Pack, 2012).

Precipitation causes fractionation in an air mass as a result of vaporization, sublimation, condensation and solidification of water across different temperature and elevation gradients (Luz and Barkan, 2010). This process occurs repeatedly and bi-directionally from the vapour, liquid and ice phases (Luz and Barkan, 2010). Water in the atmosphere is fractionated both through water phase equilibria and as water vapor diffuses in air (Gat 1996). Water evaporated over the ocean, for example, has higher levels of $^{16}\text{O}$, as $^{16}\text{O}$ evaporates preferentially over $^{18}\text{O}$ (Araguás-Araguás et al., 2000). As air moves across land masses, the isotopic composition changes. When water condenses $^{18}\text{O}$ is the first to condense. For this reason there is more "heavy" $^{18}\text{O}$ water in rain than there is in snow which condenses further from the ocean (Araguás-Araguás et al., 2000; Angert et al., 2008). In groundwater (unless ancient) the isotopic composition is a weighted average of the annual precipitation inputs (Ehleringer et al., 1992; Werner 2012).

2.3 Water isotopes as a tool for studying water uptake

Stable isotope analysis is a cutting edge technique for evaluating water interaction in the environment as it has two main benefits over other forms of analysis to detect water uptake in plants. First, very little water is needed for analysis, so the method is virtually non-destructive. Second, unlike the use of radioactive techniques, continuous information of uptake zones is available over time (Adams and Griesron, 2001; Dawson et al., 2002; Moreno-Gutiérrez, 2012). Water isotopic analysis, using $\delta^{18}\text{O}$ and $\delta\text{D}$ is useful as a tool in identifying the source of water uptake if two conditions are met. The first is that possible
source areas must have distinct values of isotope ratios that are consistent and significantly different from each other and the range of values must be greater than what would normally occur in a plant. The second condition is that there must not be significant fractionation of isotopes as the water molecules travel up the plant (Ehleringer and Dawson, 1992; Dawson et al., 2002). When investigating discreet soil moisture depths as possible distinct sources of water, there are often significant differences in isotopic profiles based on the depth of soil. Through differences in evaporation rates of lighter isotopes near the soil surface and differential precipitation and infiltration rates, the soil near the surface often demonstrates an enrichment in $^{18}$O as compared to soil depths further from the surface (Friedman et al., 1964; Gat, 1996). The second condition is met because once plant roots take up the water through active transport, there is no fractionation within the plant itself suggesting constant isotopic composition (Werner et al., 2012). There are several studies that highlight the importance of using $^{18}$O tracing techniques as the main tool of investigation tree water acquisition because D has been demonstrated to undergo fractionation in trees (Ellsworth and Williams, 2007; Kahmen et al., 2009; Hao et al., 2011).

2.4 Water isotope is temperate agroforestry

Water isotope tracing using $\delta^{18}$O has been a tool in several studies on water use by trees. Areas of research have included: 1) reliance of a species on shallow versus deep soil water (Dawson and Ehleringer, 1991; Brunel et al., 1995), 2) tree utilization of surface runoff, or stream water versus soil water (Dawson, 1996), 3) which members of a plant community rely on different water sources (Meinzer et al., 1999), 4) mapping the relationship between plant distribution along natural gradients of water availability and the depth at which plants obtain
their water (Fernández et al., 2008), 5) seasonal variation in water uptake between zones (Nie et al., 2012), 6) changes in plant size in relation to the use of groundwater vs. precipitation recharged soil water (Chimner and Cooper, 2004).

Despite the ability of water isotopes to provide information on how trees use water, limited work has been conducted in agroforestry systems, and a small portion of that has been done in temperate agroforestry. Three key studies have been conducted to show how plants compete for water resources in agroforestry systems. An important study was conducted by Asbjornsen et al. (2007) in which contrasting agricultural and native plant communities in the Midwestern U.S. were compared for water use profiles. This study found that landscape changes from native perennial vegetation to annual cropping had changed the ecohydrological balance in the area (Asbjornsen et al., 2007). It also demonstrated the importance of site-specific data because the results showed that the isotopic profiles in the soil gradients were not universal across the sites. Interestingly, they found that given identical soil conditions and precipitation history at sites that were in close proximity, the $\delta^{18}O$ profile of the soil varied depending on the plants that were present at a particular site. This indicates that plants mediate transport of water through the soil (Asbjornsen et al., 2007).

Schwendenmann et al. (2010) were the first to look at drought conditions in TBI systems using stable isotopes. The results showed that the crop was taking water mainly from the upper 0.3 m while the trees were using water from below 0.3 m. This demonstrates a complementary water use in a TBI system because the crop and trees were using water from different vertical horizons (Schwendenmann et al., 2010). Finally, Isaac and Anglaere (2013) recently showed that tree crops in an agroforestry system in Ghana modified their
water acquisitions with edaphic conditions, illustrating narrower uptake zones on uniform sandstones as compared to patchy phyllite-granites.
3 Site Description

3.1 Agroforestry Research Station, University of Guelph

Field studies were conducted at the Agroforestry Research Station, University of Guelph, Lot 10, Concession 1, Wellington County, Ontario (43º32’49”N, 80º12’ 44”W). The research center was originally established in 1987 as 30 ha of land dedicated to studying the implementation of agroforestry systems including the biological and ecological interactions, as well as the economic and political implementation considerations. The land consists of class 3 land in the Canada Land Inventory (Agriculture) designation (Gray, 2000). The location is on a western slope of a drumlin resulting in the presence of glacial till with an undetermined depth of bedrock. The site has an average slope of 6% with a maximum elevation of 346.2 m above sea level and a minimum elevation of 330.7 m above sea level (Oelbermann, 2002). Drainage varies from imperfect to moderately well-drained. The soil is classified as a gray brown luvisol in the Canadian System of Soil Classification, with a sandy loam texture consisting of 65% sand, 25% silt and 10% clay (Oelbermann and Voroney, 2007). Under the FAO system the soil is classified as a albic luvisol, with a pH of 7.4 within the top 30 cm and 7.7 between 30-40 cm depth (Peichl et al. 2006; Oelbermann, 2002). The site has temperate climate with a hot humid summers and cold winters. Annually, the average daily temperature is 7.2°C, the average frost-free period is approximately 136 days (May 15-September 28), and annual precipitation is 830 mm, with 432 mm falling during the growing season (May-September) (Environment Canada Climate Normals; Oelbermann, 2007).
The experimental design consists of two split plot fields, one consisting of tree intercropping (agroforestry), and one without tree-intercropping, the control monocrop field (Gray, 2000). The control field is separated by at least a 2 m buffer and there are no established rows of trees. Before the tree-based intercropped field was converted to agroforestry it was continuously planted with crops or hay (Oelbermann, 2002). Twelve species of trees were planted on the site in the spring of 1988 and between 1989-1994 seven more tree species were added (Simpson, 1999). Among the tree species planted were *Juglans nigra*, *Picea abies*, *Quercus rubra*, *Thuja occidentalis* and *Populus sp.* (hybrid of *Populus deltoids X Populus nigra* clone DN-177) (Peichl et al., 2006). All tree species were planted in rows between 12.5 m and 15 m apart and a within-row spacing of 6 m for a density of 111 trees ha\(^{-1}\). Beginning in 1991, four different crops have been planted in the alleys between the trees. These crops include: *Glycine max* L., *Zea mays* L., *Hordeum vulgare* L. and *Triticum aestivum* L. (Peichl et al., 2006; Abohassan, 2004). The crops were under conventional crop management with commercial fertilizer application (Abohassan, 2004).

3.2 Sampling Protocol

Two tree species poplar, (*Populus sp.* (hybrid of *Populus deltoids X Populus nigra* clone DN-177)) and walnut (*Juglans nigra*) were subselected for my study experimental design. From this point on they will be referred to by the common names, poplar and walnut. These two species were subselected based on two criteria: i) differentiated aboveground morphology and ii) variable root stratification (Borden, 2013). Previous geo-imaging data at the research station using Ground Penetrating Radar (GPR) in the summer of 2012 demonstrated that the dominant rooting zones of poplar and walnut tree species to be 1m from the tree base.
(Borden, 2013). During sample collection, these two species were intercropped with winter barley, *Hordeum vulgare* L. which was planted mid-May and harvested in the late August. Tree height, DBH (diameter at breast height) and canopy radius of each tree was collected.

The location of the rows was chosen to correlate with other research currently being conducted at the site and for close proximity to each other in order to minimize the effects of topographic features including slope and elevation. Three trees were chosen for each species: poplar (one, two and three) and walnut (one, two and three). The locations were: poplar one (43°32'44.32"N, 80°12' 35.28"W), poplar two (43°32'44.81"N, 80°12' 36.24"W) poplar three (43°32'45.59"N, 80°12' 37.34"W), walnut one (43°32'46.41"N, 80°12' 35.18"W), walnut two (43°32'46.65"N, 80°12' 35.78"W) and walnut three (43°32'47.12"N, 80°12' 36.49"W). All coordinates were taken at the base of the tree on the southwest side (Figure 1).

Samples were collected at two phases. The first collection period was in the spring (early season) before the planting of barley, during the first week of May 2013 (Figure 2). The second set of samples was collected during the late summer (late season) in the second week of August 2013 (Figure 3). Early season sampling was conducted within a two-day period with no rain or significant weather changes prior to or during collection to alter the isotopic gradient. On May 1<sup>st</sup> the daily average temperature was 16.6°C, with a high of 22.6°C and a low of 11.9°C. Similarly, for the late season sampling date (August 9<sup>th</sup>), no rainfall event had occurred for 2 days prior to collection, the daily average temperature was 19.6 °C, with a high of 24.1 °C and a low of 16.1°C (Guelph Turfgrass Institute).
Figure 1: Map of Guelph Research Station agroforestry plot, Guelph, ON with trees used in study highlighted.
Figure 2: Guelph Research Station agroforestry plot in early season. Leaf litter traps used in biomass estimate studies can be seen in the lower center of the photo.
Figure 3: Guelph Research Station agroforestry plot in late season. Barley planted in June 2013 can be seen in the crop row.
3.3 Soil and plant sampling

Soil samples were collected with a hand auger at 1 m in the southwest direction from the base of each tree. Soil samples were taken at depths of 5, 10, 20, 30, 40, 50, 60 and 70 cm. The soil was removed to the depth of the interval, at which time a small soil sample was immediately collected in 10 ml BD vacutainer serum tubes (BD Franklin Lakes, New Jersey USA). The closed tubes were immediately sealed with parafilm to prevent evaporative fractionation and placed in a cooler. A soil sample of approximately 100-150 g was also collected at each depth interval and sealed in a Ziploc® bag for subsequent moisture content analysis.

Non-photosynthetic tissue (tree core) was collected from each tree using a tree corer (1 cm diameter) at a tree height of 1.5 m. The trees were cored to a depth of 20 cm to ensure that the coring would reach the center of the tree. After the core was drawn from the tree, the bark was removed and the remaining core was divided into three sections 0-5 cm, 5-10 cm and 10-15 cm. These samples were immediately placed in vacutainers and sealed with parafilm and placed in a cooler. The tree core samples were divided in three in order to investigate the isotopic differences between the various stem radii. In order to further ensure that the water in the xylem tissue which is sampled is a direct representation of the soil water it is important that xylem water is not contaminated with the phloem water (Schwendenmann et al., 2010). This protocol was followed as the sapwood provides the most accurate representation of the water recently absorbed by roots (Busch, 1992; Beauchamp, 2013). In late season, isotopic samples were collected exclusively at poplar one and walnut one using the same procedure as early season sampling. A total of 29 isotopic samples (24 soil water, 5 xylem water) were collected during the early season per species and 9 samples in the late season (8 soil water, 1 xylem water) per species.
Soil moisture samples were processed by the gravimetric method. Samples were weighed, dried in a drying oven at 110°C for 48 hours and then reweighed. Soil moisture content was expressed as a percentage (m_{\text{water}}/m_{\text{dry soil}} \times 100\%).

3.4 Fine root biomass collection

Crops (barley) fine root data was collected during the second subset of sampling in mid-August. A root auger (6.35 cm diameter) was used to collect samples 1 m from the base of each tree in the southwest direction. The root zone was also measured. Soil cores collected from the root auger were hand washed on a 2-mm sieve and gently shaken to remove fine roots (< 2 mm in diameter measured with calipers). Using tweezers, the fine roots were collected, weighed and dried. Fine root biomass was expressed as dry mass per volume (100 cm³).

3.5 Isotopic analysis

Soil samples and plant xylem tissue samples in the scintillation tubes were refrigerated at -20°C until they were extracted using a vacuum distillation line (Ehleringer and Osmond 1989; West et al. 2006). The vacuum distillation line extracts all of the water of any given sample by a continuous process of heating and cooling that separates and conservatively traps all of the water out of the sample. Once the vacuum line has been completely cleared of gasses, the vacutainer is attached by inserting a needle into the top stopper but not through the stopper. The sample is frozen solidly in a slurry made of liquid nitrogen and ethanol at -70°C. Once the sample is frozen, the needle is pushed through the vacutainer and any gases present in the vacutainer are pumped out creating a vacuum within the container. All of the water is then removed from the soil sample into the vacuum distillation line by creating a strong pressure gradient. A liquid nitrogen bath is placed in the center of the trap (Figure 4) and the sample is immersed in boiling water. After around 30 minutes of heating,
Figure 4: Vacuum distillation line showing the boiling of the sample vacutainer on the left and the liquid nitrogen trap in the center (Environmental Isotope Lab, Western University, Ontario, Canada).
vacuum distillation and trapping, all of the water has left the sample and is now trapped within the vacuum. The non-condensable gases and carbon dioxide are then removed.

Subsequent analysis through mass spectrometry identifies and corrects for any possible residual volatile organics in the extracted water. All of the water is then transferred from the center line into a 5 ml receiving vacutainer by submersing the receiving containers in liquid nitrogen and heating the line with a blow torch. This process ensures that all water transfers to the receiving container. The isotopic composition (δ^{18}O and δD) of the soil and core water were analyzed with a Picarro H_{2}O Cavity Ring-Down Spectrometer model L1102i (Picarro, Santa Clara, CA) at the Laboratory for Stable Isotope Sciences, Western University, Ontario, Canada.

3.6 Data analysis

The use of direct matching of plant and soil isotopic signatures to determine the location of source water in a plant has been shown to be an effective method (Brunel et al. 1995). Soil depth versus δ^{18}O was plotted for each individual tree (Appendix 1). A vertical line was drawn on these plots at the δ^{18}O value of the xylem tissue of the tree associated with that subset of soil samples. The soil depth which matched the tree xylem was determined by the point(s) closest to this line. In the event of more than one matching value, trees of the same species were considered. The source of the soil water was the depth which was conjunct for all three trees (for a particular season) (Wang, 2010).

Several studies have demonstrated that plants may extract water from several zones simultaneously (Wang, 2010). To account for this, a multiple source mass-balance approach, where the isotopic value for the stem water is reached by proportionally mixing the sources, was used (Phillips and Gregg, 2003). To do this, I used the computer program IsoSource which was created and described by Phillips and Gregg and which is available through the
United States Environmental Protection Agency website (Phillips, Newsome, & Gregg, 2005; Phillips & Gregg, 2003). This multiple-source mass-balance approach assesses all of the possible mixing combinations of the contributing sources which can be combined to create the target mixture, in my case the tree xylem signature. In this case, eight soil depths were considered as possible sources (soil at 5, 10, 20, 30, 40, 50, 60 and 70 cm depths) in the first round of analysis and three aggregated soil depths (5-10 cm, 20-30 cm and 40-70 cm) were considered in a second round of analysis. When considering many sources, there is not a single unique solution that can achieve the resultant mixture (Phillips and Gregg, 2003).

Histographs were generated which depict the percent of the total number of feasible solutions that a particular source (soil depth) contributes successfully to create the target mixture at each incremental level. The program mixes these contributing sources in various incremental values, e.g., 1% or 2%, etc. Phillips and Gregg (2003) recommend using small increments of 1% change in contribution of each source (depth), with a tolerance ±0.1‰ for a resultant mixture. In my study, the percentage of solutions that two of the eight soil depths contributed were altered by 1%, while keeping the other six constant. The program allows the user some flexibility for sensitivity in the analysis in that increment levels can be smaller or larger. Trial analyses were also run at 2%, 5% and 20% increment levels. The number of feasible solutions dropped, but both the shape of the histographs and the estimated range for each source level varied minimally as was also observed in other studies (Phillips and Gregg, 2003; Asbjornsen et al., 2007). I therefore present data with only 1% incremental levels.

The tolerance level is the second variable that can be adjusted with the program. The significance of tolerance level is that in order to have a “possible solution”, the value obtained from the hypothetical mixing must be within a range plus or minus the tolerance
level. For example, for the core value of -9.58‰ a tolerance level of 0.1‰ would mean that any solution providing a mixture that fell between -9.48‰ to -9.68‰ was considered successful. The tolerance level was set at ±0.1‰ as this level of precision would be similar to that achieved in the replicates of the isotopic analysis.

The soil profile showed a pattern of increasingly negative δ\textsuperscript{18}O across samples taken from each tree site. This pattern was used to aggregate soil levels with similar isotopic contributions in order to simplify the analysis and to identify levels of contribution required from these soil regions. The levels chosen were 5-10 cm, 20-30 cm, and 40-70 cm depths. Individual δ\textsuperscript{18}O values in each of these intervals were averaged. In this analysis the incremental contributions remained at 1%, but the mixture tolerance was broadened to ±0.2‰. The results of both analyses are considered below. It is important to note that while the percent frequency of a particular soil level contributing a specific proportion will be reported, a definite solution to the mixing problem cannot be determined due to the large number of possible sources and therefore the number of possible solutions.
4 Results

4.1 Plant Characteristics

Mean (±S.E.) poplar and walnut tree height was 18.87 m (± 2.61) and 14.80 m (±0.83), respectively (Table 1). Tree diameter at breast height (DBH) was larger for poplar trees at 43.57 cm (±1.91) than walnut trees at 31.33 cm (±5.0) (Table 1). However, the width of the tree canopy was similar in both species. The canopy width for poplar was 4.44 m (±1.48) and walnut canopy width was 4.43 m (±0.47) (Table 1).

Crop (barley) root biomass density taken 1 m from the poplar tree was 2.86 kg m\(^{-3}\) (±2.58), slightly higher than the root biomass density near the walnut trees at 2.35 kg m\(^{-3}\) (±2.61). The rooting depth of the barley plants 1 m from the poplar trees was 7.0 cm (±1.5), while the rooting depth of the barley plant 1 m from the walnut trees was 8.0 cm (±1.0) (Table 1), 8% deeper than near the poplar trees.

4.2 Soil moisture content

Soil moisture content (SMC) was sampled in both May and August at a distance of 1 m from the base of the trees. The results for both sample periods show a decrease in soil moisture with soil depth (Figures 5 and 6). In early season, SMC ranged from 30.85% to 10.77% at 5 cm and 50 cm depth, respectively, near the poplar. Near the walnut, SMC ranged from 23.28% to 9.12% at 20 cm and 40 cm, respectively, showing the highest soil water lower in the profile as compared to soils near poplar. In late season, the maximum SMC for soil near poplar was 17.83% soil at a 5 cm and the minimum was 6.11% at a 40 cm depth (Figure 5).
Table 1: Tree characteristics [DBH (cm), height (m) and canopy width (m)] of individual poplar and walnut trees. Barley crop root depth (cm) associated with individual trees, taken at 1.5 m from base of tree (August).

<table>
<thead>
<tr>
<th>Tree</th>
<th>Diameter at breast height (cm)</th>
<th>Tree Height (m)</th>
<th>Canopy Width (m)</th>
<th>Associated crop root depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar one</td>
<td>43.0</td>
<td>18.0</td>
<td>3.4</td>
<td>7</td>
</tr>
<tr>
<td>Poplar two</td>
<td>42.0</td>
<td>16.8</td>
<td>3.7</td>
<td>9</td>
</tr>
<tr>
<td>Poplar three</td>
<td>45.7</td>
<td>21.8</td>
<td>6.1</td>
<td>6</td>
</tr>
<tr>
<td>Walnut one</td>
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</tr>
<tr>
<td>Walnut two</td>
<td>29.5</td>
<td>15.1</td>
<td>4.6</td>
<td>8</td>
</tr>
<tr>
<td>Walnut three</td>
<td>27.5</td>
<td>14.5</td>
<td>4.8</td>
<td>9</td>
</tr>
</tbody>
</table>
Figure 5: Mean (±S.E.) early and late season soil moisture content ($m_{\text{water}}/m_{\text{dry soil}} \times 100\%$) near poplar trees.

Figure 6: Mean (±S.E.) early and late season soil moisture content ($m_{\text{water}}/m_{\text{dry soil}} \times 100\%$) near walnut trees.
However, in late season, the maximum SMC for soil near walnut was 20.09% at 5 cm but the minimum SMC was 3.93% much deeper in the soil profile at 60 cm (Figure 6).

A distinct shift in soil moisture content is demonstrated between early and late season (Figure 5 and Figure 6). There is a 32% decrease in mean soil moisture content from early season to late season near poplar. There was a slightly lower reduction in the SMC near walnut with an 18% decrease in SMC from the early to the late season. Combining all samples for soil near both species, there is an overall reduction of 25% in SMC found in late season (August). See Appendix 1 for graphs showing soil moisture content near individual trees.

4.3 Isotopic composition of water from samples

The $\delta^{18}O$ in water extracted from soil samples taken 1 m from poplar trees in early season ranged from -13.24‰ to -6.66‰, with a mean of -10.54‰ (±1.98) (Table 2). The $\delta^{18}O$ values in soil water 1 m from poplar in late season ranged from -12.66‰ to -7.75‰, with a mean of -9.29‰ (±1.75) (Table 2). There was an increase of 12% in the mean $\delta^{18}O$ value of soil water near poplar in the late season. The values for $\delta^{18}O$ in soil water for walnut in early season ranged from -14.10‰ to -7.51‰ with a mean of -10.72‰ (±1.85) (Table 2). The values for $\delta^{18}O$ in soil water near walnut in late season ranged from -9.58‰ to -7.75‰, with a mean of -8.56‰ (±0.66) (Table 2). There was an increase of 20% in the mean value of $\delta^{18}O$ in soil water near walnut in the late season.

Soil water values of both $\delta^{18}O$ and $\delta D$ show a similar pattern of enrichment of the lighter isotope with depth. This pattern is consistent in gradient from a soil depth of 5 cm to 30 cm, and both graphs show the isotopes reaching a more constant value below 30 cm (Table 2).
**Table 2:** Isotopic composition in soil water samples near poplar and walnut trees during early season (maximum, minimum, mean and standard error of samples; n = 3).

<table>
<thead>
<tr>
<th>Tree Type</th>
<th>Depth (cm)</th>
<th>δ^{18}O (%)</th>
<th>δ^{18}O (%) Min.</th>
<th>Mean</th>
<th>S.E.</th>
<th>δD (%)</th>
<th>δD (%) Min.</th>
<th>Mean</th>
<th>S.E.</th>
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<td></td>
</tr>
<tr>
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<td>-11.12 &amp; -8.26</td>
<td>2.48</td>
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<td>-79.65</td>
<td>-60.67</td>
<td>17.75</td>
<td></td>
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<tr>
<td></td>
<td>20</td>
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<td>-10.37 &amp; -9.79</td>
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<td>-100.93</td>
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<tr>
<th>Tree Type</th>
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<th>Mean</th>
<th>S.E.</th>
<th>δD (%)</th>
<th>δD (%) Min.</th>
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<td>30</td>
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<td>-12.01 &amp; -11.55</td>
<td>0.65</td>
<td>-73.55</td>
<td>-91.35</td>
<td>-83.34</td>
<td>9.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>9.58</td>
<td>-13.24 &amp; -12.45</td>
<td>1.02</td>
<td>-74.24</td>
<td>-97.54</td>
<td>-88.22</td>
<td>12.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8.48</td>
<td>-12.74 &amp; -11.46</td>
<td>1.83</td>
<td>-59.03</td>
<td>-92.46</td>
<td>-80.85</td>
<td>18.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>8.75</td>
<td>-12.39 &amp; -11.48</td>
<td>1.03</td>
<td>-75.67</td>
<td>-87.16</td>
<td>-83.16</td>
<td>6.50</td>
<td></td>
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<tr>
<td></td>
<td>70</td>
<td>9.26</td>
<td>-11.72 &amp; -11.71</td>
<td>0.01</td>
<td>-78.13</td>
<td>-87.78</td>
<td>-82.95</td>
<td>6.82</td>
<td></td>
</tr>
<tr>
<td>Walnut</td>
<td>5</td>
<td>7.75</td>
<td>-8.35 &amp; -7.85</td>
<td>0.44</td>
<td>-50.39</td>
<td>-60.26</td>
<td>-55.55</td>
<td>4.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8.77</td>
<td>-9.87 &amp; -8.90</td>
<td>0.85</td>
<td>-57.91</td>
<td>-72.43</td>
<td>-63.64</td>
<td>7.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7.84</td>
<td>-11.34 &amp; -10.54</td>
<td>0.96</td>
<td>-75.52</td>
<td>-81.95</td>
<td>-77.73</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9.05</td>
<td>-12.08 &amp; -11.14</td>
<td>0.82</td>
<td>-73.08</td>
<td>-84.59</td>
<td>-78.26</td>
<td>5.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>9.58</td>
<td>-11.70 &amp; -11.04</td>
<td>0.57</td>
<td>-70.53</td>
<td>-80.4</td>
<td>-75.94</td>
<td>5.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8.48</td>
<td>-11.45 &amp; -10.65</td>
<td>0.69</td>
<td>-68.36</td>
<td>-76.93</td>
<td>-72.62</td>
<td>4.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>9.26</td>
<td>-14.10 &amp; -12.95</td>
<td>1.34</td>
<td>-82.49</td>
<td>-100.93</td>
<td>-92.44</td>
<td>9.30</td>
<td></td>
</tr>
</tbody>
</table>
As expected, there is a strong correlation between the values of δ^{18}O and δD for the poplar and walnut trees cores and soil samples in both early (r = 0.951; P < 0.0001; Figure 7) and late season (r = 0.962; P < 0.0001; Figure 8). The values for all of the xylem tissue samples can be seen to form a cluster close to the trend line.

The range of δ^{18}O xylem water values for poplar at the 0-5 cm radius of the tree in the early season was -10.78‰ to -9.34‰, with a mean of -9.90‰ (± 0.77) (Table 3). The range of δ^{18}O xylem water values for walnut at the same radius in the tree (0-5 cm) inearly season was -9.55‰ to -8.48‰, with a mean of -8.91‰ (± 0.57) (Table 3). The value for δ^{18}O in xylem water of poplar at the 0-5 cm radius for the late season was -9.63‰ whereas the value for δ^{18}O in xylem water of walnut at the 0-5 cm radius for the late season was -9.07‰ (Table 3).

### 4.3.1 Direct estimate of soil horizon-tree water source

A direct inference approach, through matching of the δ^{18}O value of the tree core with soil isotope values, can identify the dominant depth of water uptake (Appendix 1). Early season poplar has a mean core δ^{18}O value of -9.90‰ (±0.77) (Table 3). The corresponding early season soil depth for water acquisition is at 20 cm (Figure 9). Late season data with a poplar xylem water δ^{18}O value of -9.63‰, shows a shift to water acquisition lower in the soil profile to. For late season poplar, water acquisition is indicated to be between 40 and 70 cm soil depth (Table 4, Figure 9).

Early season walnut has a mean core δ^{18}O value of -8.91‰ (±0.57) (Table 3). The early season soil depth of water uptake is at 10 cm (Figure 10). Late season data with a walnut xylem water δ^{18}O value of -9.07‰ (Table 3), shows a water uptake zone shift similar to poplar, to water acquisition lower in the soil profile at a soil depth of 40 and 70 cm (Figure 10, Table 4).
Figure 7: Relationship of $\delta^D$ to $\delta^{18}O$ for early season poplar and walnut showing all soil and tree core water samples. Linear trend line includes all values, equation of line on graph. The correlation coefficient for all early season samples was $r = 0.951$, $P < 0.0001$.

Figure 8: Relationship of $\delta^D$ to $\delta^{18}O$ for late season poplar and walnut showing all soil and tree core water samples. Linear trend line includes all values, equation of line on graph. The correlation coefficient for all for all late season samples was $r = 0.962$, $P < 0.0001$. 
Table 3: Isotopic composition of tree core water samples during early and late season
(maximum, minimum and mean with standard error for early season samples; n = 3; late season samples; n = 1).

<table>
<thead>
<tr>
<th>Season</th>
<th>Tree Type</th>
<th>Radius (cm)</th>
<th>n</th>
<th>Max.</th>
<th>Min.</th>
<th>Mean</th>
<th>S.E.</th>
<th>Max.</th>
<th>Min.</th>
<th>Mean</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>Poplar</td>
<td>0-5</td>
<td>3</td>
<td>-9.34</td>
<td>-10.78</td>
<td>-9.90</td>
<td>0.77</td>
<td>-73.96</td>
<td>-80.22</td>
<td>-76.27</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-15</td>
<td>2</td>
<td>-9.04</td>
<td>-9.45</td>
<td>-9.24</td>
<td>0.29</td>
<td>-72.01</td>
<td>-73.89</td>
<td>-72.95</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>Walnut</td>
<td>0-5</td>
<td>3</td>
<td>-8.48</td>
<td>-9.55</td>
<td>-9.45</td>
<td>0.57</td>
<td>-68.68</td>
<td>-76.15</td>
<td>-72.13</td>
<td>3.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-15</td>
<td>2</td>
<td>-8.54</td>
<td>-8.55</td>
<td>-8.54</td>
<td>0.01</td>
<td>-75.67</td>
<td>-78.01</td>
<td>-76.84</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Late

<table>
<thead>
<tr>
<th>Tree Type</th>
<th>δ¹⁸O (‰)</th>
<th>δD (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>-9.63</td>
<td>-71.76</td>
</tr>
<tr>
<td>Walnut</td>
<td>-9.07</td>
<td>-75.42</td>
</tr>
</tbody>
</table>
**Figure 9:** Early and late season $\delta^{18}O$ values of soil water (0 - 70 cm at 10 cm intervals) and poplar tree xylem tissue. [Early season data (n = 3; (±S.E.); late season data, n = 1)].

**Figure 10:** Early and late season $\delta^{18}O$ values of soil water (0 - 70 cm at 10 cm intervals) and walnut tree xylem tissue. [Early season data (n = 3; (±S.E.); late season data, n = 1)].
Table 4: Direct matching results by species displaying early and late season data.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Season</th>
<th>Xylem $\delta^{18}$O (‰)</th>
<th>Standard Error</th>
<th>Matching Soil Depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>Early</td>
<td>-9.90</td>
<td>0.77</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>-9.63</td>
<td></td>
<td>40-70</td>
</tr>
<tr>
<td>Walnut</td>
<td>Early</td>
<td>-8.91</td>
<td>0.57</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>-9.07</td>
<td></td>
<td>40-70</td>
</tr>
</tbody>
</table>
4.3.2 Proportional contributions of soil water sources

Results for the multiple source mass-balance analysis are reported based on the frequency (as a percent of the total number of possible solutions) that a particular source (depth) contributes to a proportion of the solution. The proportion of the mixture that a particular depth contributes is reported in decimal increments from 0-1 in four categories (0 signifies no contribution; 0.01-0.25 means a 1-25% proportion of mixture; 0.26-0.5 means 26-50% proportion of mixture; 0.51-1 means 51-100% proportion). The last category, 0.51-1 is the most promising to examine as the highest frequency in this category would indicate a dominant soil depth for contributing water. Similarly, the 0 category indicates the frequency that a soil depth is not contributing to water acquisition.

**Poplar**

Analysis of mean early season poplar data of all eight soil depths shows that soil depths 5-20 cm most frequently produced solutions with >0.5 proportion of the mixture (Table 5). The 20 cm soil depth showed the highest frequency at 2.21% (Table 5). Solutions which required >0.25 proportion were predominately found in the 5 to 20 cm soil depths, with 5 cm (37%) and 10 cm (39%) having highest frequency of contribution (Table 5). Contributions from the 30-70 cm depths appear in a very large number of solutions at the 0.01 to 0.25 level in the solution, indicating that some water is required from these depths.

In late season, the region of maximum contribution shifted to lower in the soil profile. The soil depths from 30-70 cm most frequently produced solutions using >0.25 proportion 30 cm (14%), 40 cm (13%), 50 cm (20%) and 70 cm (25%) (Table 5). The 50 cm soil depth had the greatest contribution above 0.50 at 2.3% frequency. The 50 cm soil depth also had the greatest range of contribution from 0.1-0.90 (Table 5). See Appendix 2 (A-D)
Table 5: Mean poplar early season and late season multisource mass-balance frequency distribution at eight soil depths. The percent frequency of a solution for each soil depth is based on the proportional contribution of that soil depth. Data run at 1% intervals and ±0.1‰ tolerance.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Soil depth (cm)</th>
<th>Percent frequency (%)</th>
<th>Proportion of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00 to 0.25</td>
<td>0.26 to 0.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.01 to 0.25</td>
<td>0.26 to 0.5</td>
</tr>
<tr>
<td>Poplar mean early season</td>
<td>5</td>
<td>2.67</td>
<td>87.11</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.18</td>
<td>88.58</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.39</td>
<td>75.22</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8.48</td>
<td>36.14</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>10.05</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8.33</td>
<td>86.81</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>8.36</td>
<td>86.88</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>8.76</td>
<td>87.57</td>
</tr>
<tr>
<td>Poplar one late season</td>
<td>5</td>
<td>7.67</td>
<td>85.96</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7.43</td>
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<td>7.90</td>
<td>86.61</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.96</td>
<td>83.83</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>6.32</td>
<td>80.31</td>
</tr>
<tr>
<td></td>
<td>50</td>
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<td>74.59</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>7.59</td>
<td>85.70</td>
</tr>
</tbody>
</table>
|                       | 70             | 4.17                  | 70.92                 | 24.06                  | 0.86
**Table 6:** Mean walnut early season and late season multisource mass-balance frequency distribution at eight soil depths. The percent frequency of a solution for each soil depth is based on the proportional contribution of that soil depth. Data run at 1% intervals and ±0.1‰ tolerance.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Soil depth (cm)</th>
<th>Percent frequency (%)</th>
<th>Proportion of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01 to 0.25</td>
</tr>
<tr>
<td>Walnut mean early season</td>
<td>5</td>
<td>0.00</td>
<td>94.07</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.20</td>
<td>73.95</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12.86</td>
<td>86.64</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>15.53</td>
<td>84.41</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>15.09</td>
<td>84.83</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>13.35</td>
<td>86.29</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>23.18</td>
<td>76.82</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>22.15</td>
<td>77.85</td>
</tr>
<tr>
<td>Walnut one late season</td>
<td>5</td>
<td>16.92</td>
<td>83.07</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7.99</td>
<td>84.90</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>16.16</td>
<td>83.81</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>14.63</td>
<td>85.25</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.39</td>
<td>21.37</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10.60</td>
<td>87.44</td>
</tr>
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<td></td>
<td>60</td>
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<td>85.27</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>3.45</td>
<td>60.25</td>
</tr>
</tbody>
</table>
for histograms of multisource mass-balance analysis for individual poplar trees at eight levels.

When soil contributions are aggregated into 3 depths (5-10 cm, 20-30 cm and 40-70 cm) the analysis is simplified and the results are more prescriptive. For mean early season poplar the 5-10 cm soil depth shows a 9.00% contribution frequency at >0.50 of the mixture and the 20-30 cm region shows a 28.02% contribution frequency at >0.50 of the mixture (Table 7A). This indicates a strong contribution from the top 30 cm of soil. All solutions required some level of contribution from the 5-10 cm soil region (Table 7A, Figure 11A). The 40-70 cm soil region also contributes at a high frequency, but at lower levels in the mixture (Table 7A, Figure 11A).

For aggregate analysis of late season poplar the 5-10 cm soil region contributes at a high frequency, but at a low level in the mixture, 85.51% at the 0.1-0.25 mixture contribution level (Table 7B). The 20-30 cm region shows a 45.84% contribution frequency at >0.50 of the mixture (Table 7B). The 40-70 cm soil region also contributes at a high frequency, 34.54% contribution frequency at >0.50 of the mixture (Table 7B). This indicates a strong contribution from lower in the soil horizon. The histograms in 11A and 11B show a shift in the frequency of contribution of the 5-10 cm source to lower levels (<0.40) while the shift in the 40-70 cm soil zone contribution for late season has increased (> 0.95). See Appendix 3 (A-D) for histograms of multisource mass-balance analysis for individual poplar trees at three levels.

**Walnut**

Analysis of mean early season walnut data of all eight soil depths shows that soil depths 5 cm (78.68%) and 10 cm (2.77%) most frequently produced solutions with >0.5 proportion of the mixture (Table 6). Solutions which required >0.25 proportion were predominately found in
Table 7 A-D: Multisource mass-balance frequency distribution at three aggregate source levels for poplar and walnut early and late season. Data run at 1% with a tolerance of ±0.2‰.

<table>
<thead>
<tr>
<th>A</th>
<th>Soil Region Range (cm)</th>
<th>Frequency of Solutions (%)</th>
<th>Proportion of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01 to 0.25</td>
</tr>
<tr>
<td>Poplar mean early season</td>
<td>5-10</td>
<td>0.00</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>1.41</td>
<td>35.35</td>
</tr>
<tr>
<td></td>
<td>40-70</td>
<td>2.06</td>
<td>50.39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Soil Region Range (cm)</th>
<th>Frequency of Solutions (%)</th>
<th>Proportion of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01 to 0.25</td>
</tr>
<tr>
<td>Poplar late season</td>
<td>5-10</td>
<td>3.54</td>
<td>85.51</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>0.14</td>
<td>15.40</td>
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<td></td>
<td>40-70</td>
<td>1.25</td>
<td>32.18</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Soil Region Range (cm)</th>
<th>Frequency of Solutions (%)</th>
<th>Proportion of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01 to 0.25</td>
</tr>
<tr>
<td>Walnut mean early season</td>
<td>5-10</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
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<td></td>
<td>40-70</td>
<td>6.04</td>
<td>93.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>Soil Region Range (cm)</th>
<th>Frequency of Solutions (%)</th>
<th>Proportion of mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.01 to 0.25</td>
</tr>
<tr>
<td>Walnut late season</td>
<td>5-10</td>
<td>9.33</td>
<td>90.67</td>
</tr>
<tr>
<td></td>
<td>20-30</td>
<td>13.33</td>
<td>86.67</td>
</tr>
<tr>
<td></td>
<td>40-70</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
the 5 to 20 cm soil depths, with 5 cm (37%) and 10 cm (39%) being the highest (Table 6). Contributions from the 30-70 cm depths appear in a very large number of solutions at the 0.01 to 0.25 level in the solution, indicating that some water is required from these depths (Table 6).

In late season, the region of maximum contribution has shifted to lower in the soil profile. The soil depths 40 cm (20.08%) and 70 cm (9.36%) contribute >0.5 to the mixture (Table 6). These soil depths also contribute >0.25 proportion 40 cm (78.24%) and 70 cm (36.31%) (Table 6). There are high frequency contributions from soil depths 5-30 cm in 0.1-0.25 range. The most significant soil depth higher in the profile is 10 cm which contributes 7.11% frequency at the >0.25 proportion of the mixture (Table 6). See Appendix 2 (E-H) for histograms of multisource mass-balance analysis for individual walnut trees at eight levels.

When soil contributions are aggregated into 3 depths for mean early season walnut the 5-10 cm soil depth shows a 100% contribution frequency at >0.50 of the mixture (Table 7C, Figure 11C). This indicates a very strong contribution frequency from the top 10 cm of soil. The 20-30 cm region shows a 3.40% contribution frequency at >0.25 of the mixture (Table 7C). All solutions required some level of contribution from the 5-10 cm soil region (Table 7C). The 40-70 cm soil region also contributes at a high frequency, but at lower levels in the mixture (Table 7C).

In analysis of late season walnut data the 40-70 cm soil depth shows a 100% contribution frequency at >0.50 of the mixture (Table 7D, Figure 11D). This indicates a very strong
A: Early season mean poplar.  
B: Late season poplar.  

C: Early season mean walnut.  
D: Late season walnut.

Figure 11 A-D: Multisource mass-balance frequency analysis. Histograms of aggregated soil layer analysis (mean of 5-10 cm, 20-30 cm and 40-70 cm), combined early season poplar and walnut, and individual late season poplar and walnut trees. The percent frequency of a solution for each soil depth based on the proportional contribution of that soil depth. Data run at 1% intervals with a tolerance of ±0.2‰.
contribution frequency from the lower soil horizon. For late season walnut the 5-10 cm (90.67%) and 20-30 cm (86.67%) soil regions contributes at a high frequency for 0.1-0.25 mixture contribution level (Table 7D, Figure 11D). See Appendix 3 (E-H) for histograms of multisource mass-balance analysis for individual walnut trees at three levels.
This study examined soil water isotopic profiles and corresponding tree core xylem water isotopic signatures in a 30-year-old tree-based intercropping system during May, prior to the planting of an annual crop and in August, with the barley crop present. The purpose of this study was to identify the depth at which the trees were accessing water in both of these seasons in order to show how competition for water uptake may affect annual crops. This work is one of the first applications of oxygen isotopes in a temperate agroforestry setting, providing useful information for optimizing water efficiency in TBI systems.

5.1 Soil isotope analysis

Due to the preferential evaporation of lighter isotopes, the evaporative effect is expected to cause a clear trend of decreasing proportion of $^{18}$O to $^{16}$O measured in soil water as one moves down in the soil gradient (Fernandez et al., 2008; Nie et al., 2012). The poplar and walnut trees in this study were grown under similar edaphic and climatic conditions and the soil profiles near these trees displayed similar $\delta^{18}$O isotopic gradient slopes early in the season (Figure 9 and Figure 10). The soil 1 m from each trees showed the predicted pattern of decreasing proportion of $^{18}$O through the first 30 cm in the soil profile in both early and late season.

The slopes of the isotopic gradients may be due in part to infiltration rates or the degree of evaporative fractionation from the soil. TBI systems are a mixture of perennial and annual vegetation types. The early samples were taken in May prior to planting the no-till fields surrounding the trees and late season samples were collected in mid-August with the annual crop (barley) planted. The soil surrounding trees is affected by microclimate variations.
resulting from the perennial tree cover, and variable air temperature, wind speed, gust speed, humidity, dew point rainfall, soil-moisture content, total solar radiation, photosynthetically active radiation and soil temperature (Lin 2007; Karki and Goodma, 2013). It is important to consider the effect these factors have on infiltration rates of precipitation and evaporation rates. Furthermore, Gat (1996) suggests that the through-flow rain that falls under a tree is not as isotopically enriched in $^{18}$O as compared to the rain in the nearby cleared area. The precipitation that reaches the ground near trees therefore tends to be this heavier rainfall that is enriched in $^{16}$O, partially negating the evaporative effect under trees to some extent (Gat 1996; Thomas et al., 2013).

It was interesting to note that late season $\delta^{18}$O soil profiles were different than early season. For all early season soil water samples, the mean $\delta^{18}$O value was $-10.63\%$ (±1.90). For all late season soil water samples, the mean $\delta^{18}$O value was $-8.92\%$ (±1.33). It is possible that precipitation, which occurred during the summer period, presumably had a less negative $\delta^{18}$O value (Bertrand et al., 2012). Other soil water phenomenon may also be influencing soil water $\delta^{18}$O values. For instance, the principle of hydraulic lift may have affected the soil water flux throughout the growing season (Ludwig et al., 2004).

The less negative $\delta^{18}$O values in late season observed here for the most part support the literature. Other studies using isotopes as a tracing technique have found an enrichment of $^{18}$O in the soil later in the season. Bertrand et al. (2012) found that the upper soil levels from 0-40 cm had strongly depleted $^{18}$O isotopic signature in the spring and winter and $^{18}$O was enriched in the summer while the lower soil depth (40-80 cm) varied less and were closer to groundwater levels. They argue that the upper layers were heavily influenced by precipitation, while constantly being modified by evaporative enrichment (Bertrand et al., 2012). This variation has been noted in numerous studies and typically explained by
preferential flow paths between the surface and lower layers transporting the enriched water down (Bertrand et al., 2012; Li et al., 2006; Li et al., 2007). The general enrichment of $^{18}\text{O}$ in late season illustrated in my study may reflect evaporation which is reaching further down into the soil, or the effects of a heavy rain event.

5.2 Plant isotope analysis

In this study, the tree core samples were split into two equal sections of the core radius after the bark was removed. The first section was the sapwood 0-5 cm radius and the second section was the heartwood at a 10-15 cm radius. An evaluation of the $\delta^{18}\text{O}$ and the $\delta$D for both poplar and walnut demonstrated differences in the values at 0-5 cm radius when compared to the 10-15 cm radius for all test trees (Table 3). These results were expected based on earlier studies of the difference in $\delta^{18}\text{O}$ and $\delta$D between sapwood and heartwood. White et al. (1985) measured the $\delta$D of the heartwood and sapwood of Pinus strobus under conditions of moisture stress over several weeks, with various precipitation events. The mean $\delta$D of the heartwood did not change significantly throughout these events; 40 times the water flowed through sapwood than the heartwood in a 10 hour test period (White et al., 1985). Therefore, the $\delta$D value of the heartwood was not included in their water source model (White et al., 1985). Savard (2010) evaluated hydrogen, oxygen, nitrogen and carbon isotopes in trees rings in response to pollution. That study demonstrated that when tracing nitrogen, the highest concentrations were found at the boundary between the heartwood and the sapwood, thus demonstrating again a distinction between isotopic processes occurring in the sapwood and heartwood (Savard, 2010).

Sapwood is the active transport region of water and nutrients in the tree and therefore sapwood provides the best representation of recent water from the soil uptake zone (White, 1985; Naughton et al., 2006; Beauchamp, 2013). These discrepancies between the heartwood
and sapwood isotopic values in the tree tissue suggest an area of future work. What are the ratios of $\delta^{18}$O to $\delta$D in heartwood as compared to sapwood by species and climatic condition? What is the turnover rate of this stored water and to what extent is this stored water represented in the sapwood under dry season conditions?

5.3 Water acquisition - direct inference method

Direct matching only works in cases where the tree xylem is within the isotopic range of the soil profile and where there is not two different soil depths that could be a clear match (Asbjornsen et al., 2007; Wang et al., 2010; Isaac and Anglaaere, 2013). The lack of a distinct isotopic gradient for the soil near some of the individual trees means that making a direct inference that uses a vertical line match of the core value with the soil isotopic value will not always have a unique interpretation. See Appendices 1C, 1G and 1H for direct inference matching graphs for individual trees which did not provide a single unique solution.

The mean $\delta^{18}$O soil water near early season poplar trees exhibited a clear trend of decreasing $\delta^{18}$O for 5 cm to 40 cm (Figure 9). The mean early season tree xylem value of -9.90‰ (Table 3) was reached at 20 cm in the soil profile. This 20 cm soil depth is the only soil region with water near that value. In late season poplar xylem tissue was -9.63‰ the soil was close to that value from 40-70 cm, with 40 cm and 50 cm being particularly close (Figure 9).

Soil water $\delta^{18}$O values near walnut trees exhibited a trend of increasing negative $\delta^{18}$O values from 5 cm to 30 cm soil depth in early season (Figure 10). From 30 cm to 50 cm, soil water $\delta^{18}$O stabilization occurred, but at the 60 cm soil horizon there were significantly lower $\delta^{18}$O values measured (Figure 10). The soil water at 10 cm matches the mean tree xylem value of -8.91‰ (Table 3 and Figure 10). This is significantly higher in the soil profile than
determined for poplar. However, in early season both trees are taking water primarily for the top 20 cm of the soil profile. In late season walnut xylem tissue was -9.07‰ the soil was close to that value in the region of from 40-70 cm (Figure 10).

The direct inference approach, where the isotopic values of the soil water at the various layers are compared to the isotopic signature of the xylem water with the most similar value is chosen as the dominant uptake zone, has been utilized successfully in several studies (Fernandez et al., 2008; Isaac and Anglaere, 2013). In this study, the direct matching approach showed that water acquisition occurred at a soil depth for poplar that was just at 20 cm in the early season. In late season, for poplar, the main water acquisition zone had shifted lower in the soil profile to 40 cm to 70 cm (Figure 9). For walnut in the early season, water acquisition occurred at a soil depth near 10 cm. However, similar to poplar in late season, water acquisition shifts in walnut to a soil depth in the 40 to 70 cm range (Figure 10). These results suggest that in the early season, walnut trees were acquiring water from a soil horizon higher than the poplars. This trend is supported by previous research done at this same agroforestry site in Guelph. Gray (2000) showed that the rooting zone for poplar trees at 15 years was concentrated (56%) within 2 m of the tree and that these poplar trees had roots throughout the soil profile. Fine roots were at a minimum close to the soil surface and reached a maximum at a depth of 15 cm to 20 cm (Gray 2000; Thevathasan and Gordon. 2004). Coarse root imaging data using GPR (ground penetrating radar) conducted in 2012 demonstrated that for both poplar and walnut, the majority of the coarse roots were in the top 70 cm (Borden et al., 2013). Walnut trees generally have a deep taproot that can reach a 2 m depth with large lateral roots near the surface (Buck et al., 1999). My results indicate a 10 cm uptake zone for walnut in the early season, which is indicative of these feeder roots high in the soil profile.
5.4 Water acquisition - multisource mass-balance analysis

A multisource mass-balance assessment was used to provide a quantitative analysis of the possible mixtures composed of variable proportions of contributions of water from soil horizons throughout the rooting zone of the trees. Trees are known to obtain water from a range of depths depending on species, location and time of year (Dawson and Ehleringer, 1991; Yaseef et al., 2009; Miller 2010). Therefore, although the direct inference approach is a useful method to determine the dominant water acquisition zone of trees, water present in a tree will more likely be a mixture of the water obtained from multiple soil horizons.

Asbjornsen et al. (2007) highlight that there are three benefits of multisource mass-balance over direct matching: 1) observer error in terms of matching the data is minimized because the analysis is systematic, 2) the difficulty of dealing with very similar isotopic values at multiple depths is addressed, and 3) this approach provides a quantitative assessment of the likelihood of contributions from different depths.

If it is assumed that trees which have roots that extend through at least the top 1 m of soil obtain water throughout the range of the rooting zone, then the isotopic signature found in the xylem tissue would be a mixture of the proportional contributions taken up at each level. In this type of multisource model, a percentage of water is taken up over a range of depths and the final mixture reflects that proportional combination. As $\delta^{18}\text{O}$ was measured at eight soil depths in my study, there are many possible ways to combine portions of each level (with distinct isotopic signatures) to arrive at the isotopic signature of the final mixture (xylem water).

Through direct matching we show that the water uptake zone is concentrated at 20 cm in early season and in the range of 40 to 70 cm in the late season for poplar. Walnut had an early season uptake zone of around 10 cm and a late season shift to the 40-70 cm level with
the direct matching model. The multisource mass-balance analysis shows that while these levels may provide significant contributions to the mixture, some water from other levels in the soil profile was required for a large percentage of the possible solutions to the multisource mass-balance model. Used in conjunction with direct matching, as is currently the norm, there is higher accuracy in predicting uptake zones. By collapsing the data into different intervals as described below, it is possible to gain a better understand of the water uptake characteristics of poplar and walnut (Phillips et al., 2003).

One set of assessments was made for eight individual soil levels using increments of 1% of solution volume with a tolerance of ±0.1‰ of the target mixture. In another analysis three soil horizons were established by aggregating results (mean); I aggregated 5-10 cm, 20-30 cm, and 40-70 cm depths. This three-level assessment was also run at 1% solution increments, with the mixture tolerance broadened to ±0.2‰. The broader mixture tolerance better reflects the level of precision of the isotope analysis. This level of tolerance was not possible for the eight-level analysis as the number of possible solutions to the mixing problem would be too great. The soil regions were chosen based on the similarity of $\delta^{18}O$ values across the sampling sites. A record of the eight-soil depth and three-soil depth analyses appear in Table 5, Table 6 and Table 7 (A-D). Histograms for each individual analysis are provided in Appendix 2 and Appendix 3. From this analysis two conclusions can be made: 1) the eight-soil depth results coincide with the three-soil depth analysis, although the eight-soil depth analysis has a broader range of possible solutions; 2) the three-soil depth analysis shows a distinct region of soil which provides the major contribution to the mixture, but it also shows that there are contributions required from other soil levels. For these reasons I have chosen to discuss and evaluate the three-soil depth analyses in detail below. Also, instead of discussing data for individual trees for the early season data, I have chosen to
evaluate the early season means for poplar and walnut. This will allow an evaluation of the trends demonstrated by the two species. Late season data represents measurement of a single tree from each species.

**Early Season Poplar**

The “contribution” histograms for poplar show that a portion of tree xylem water must be derived from the 5-10 cm soil region, as this region is represented in all model solutions, contributing from 23 to 56% to the mixture (Figure 11A). This suggests that at least 44% of the mixture requires soil water from lower in the profile. Also, >50% contribution of water from the 20-30 cm soil region was present in 28% of possible solutions. This information supports the direct matching data, which identified the main uptake zone in the 20 to 30 cm range. The multisource mass-balance analysis demonstrates a clear water contribution from high in the soil profile, whereas this was not identified in the direct matching. However, combined with the direct matching information, it seems to be clear that a large portion of the xylem water is derived from the 20-30 cm soil region, but water is also acquired from other soil regions. Multisource mass-balance analysis demonstrates that there is a contribution from higher in the soil profile (5-10 cm) and there is a strong representation of possible solutions (48%) requiring water below 40 cm (at >25% proportion of the xylem water).

**Early Season Walnut**

The “contribution” histograms for walnut show that a significant portion of tree xylem water must be derived from the 5-10 cm soil region as this region is represented in all model solutions, contributing from 71 to 90% to the mixture (Figure 11B and Appendix 3F). This suggests that at least 10% of the mixture is from soil water lower in the profile. This correlates well with the direct matching result of 5 to 10 cm. It would appear that although 5-10 cm was the dominate source, the remaining portion of the mixture could be from either
the 20-30 cm or 40-70 cm regions, or a combination of both. A small number (frequency
3.4%) of possible solutions required more than a 25% contribution of soil water from the 20-
30 cm region. Unlike the direct matching analysis this result does indicate that that some
portion of the mixture was coming from lower than 10 cm in the soil profile. This analysis
also demonstrates that in early season, walnut acquires water from higher in the soil profile
than poplar.

**Late Season Poplar**

The “contribution” histograms for poplar show that from 0 to 35% of the mixture can come
from the 5-10 cm soil region. This indicates a much lower proportion of the xylem water
mixture is contributed from this soil region overall in late season (Figure 11C). The 20-30 cm
soil region contribution to the xylem water mixture is strongly represented (50%) in 46% of
possible solutions. However, the mean value for the 20-30 cm soil region (-10.11‰)
corresponds to a wide range (-7.75‰, at 20 cm and -12.46‰, at 30 cm). The values at 20 cm
or 30 cm are not close to the tree xylem water (-9.63‰). There was not a gradual change in
$\delta^{18}O$ in this region of the soil, and while the mean is closer to the xylem $\delta^{18}O$ value, the mean
value may misrepresent the actual soil water isotopic signature in this area; this points to a
limitation in the use of mean values for soil to consolidate possible sources. The direct
matching result for this set of isotopic data clearly shows a match for the soil regions from
40-70 cm. The possible “contribution” for the 40-70 cm region ranged from 0 to 100%
proportion of the mixture (Table 7C). Combined with the inference from direct matching, it
appears that the 40-70 cm range was the main water acquisition zone. There is a strong
representation of possible solutions (35%) from soil water in the 40-70 cm soil region (at
>50% proportion of the xylem water).
Late Season Walnut

The “contribution” histographs for walnut show that a portion of tree xylem water must be derived from the 40-70 cm soil region, as this region is represented in all model solutions, contributing 80 to 100% to the mixture (Table 7D and Figure 11D). Late season walnut has the clearest prediction of water acquisition zone. The xylem water mixture had at least a 50% contribution from the 40-70 cm soil region in 100% of possible solutions. At least half of the mixture was made up from water from soil in this region. The contribution ranges for the other two regions was much smaller, with the 5-10 cm depth contributing from 0-19% and the 20-30 cm region contributing from 0 to 13% proportion of the mixture (Table 7D). It is clear from this analysis and the direct matching graph that the 40-70 cm range was the most important for late season walnut.

5.4.1 Summary of multisource mass-balance analysis

Using the multisource mass-balance analysis, several interesting conclusion can be made. The first is the confirmation that poplar acquires soil water from a more broad range of soil horizons than walnut in the early season. Walnut xylem water signatures cannot be achieved without a contribution of 71 to 90% of water from the 5-10 cm depth. Although this region of the soil was also important for poplar, the preference was not as strong. In combining the layers one would expect to see a boost in the influence of the soil water from the 40-70 cm region as $\delta^{18}O$ was similar at each of these levels. When evaluating the eight levels independently the contribution of each is small. By combining these layers in the three-level analysis, a contribution from 40-70 cm was shown to be significant. Multisource mass-balance analysis demonstrated that for early season poplar and walnut these 40-70 cm areas were important and it was very likely some of the water came from this soil region for both
species. In analysis of the late season data, this method confirmed that water was being taken up predominantly from the 40-70 cm soil region in both species.

A combined approach using direct matching and multisource mass-balance analysis of data has been used in several studies to date (Asbjornsen et al., 2007, Wang et al., 2010, Zhang et al., 2011). The direct matching method can provide a great deal of success under conditions when the xylem water matches a water source uniquely represented in the soil profile. The multisource mass-balance mixing model can be used to account for the possibility that the xylem tissue water is a mixture of various soil water sources. Asbjornsen et al. (2007) highlights two reasons to use the multiple-source mass balance approach in conjunction with the direct matching; 1) to compare the two approaches and the utility of both, and 2) to determine the probability of the proportional contribution of water from different soil depths.

When using the direct matching inference method, one might conclude that poplar and walnut early in the season were acquiring water from 5-20 cm in the soil profile. However, the multisource mass-balance analysis shows that these trees are also acquiring water from lower in the profile. By showing that soil water is required in some proportion from more than one soil region, multisource mass-balance analysis demonstrates that suggesting a tree derives its water from a single level in the soil profile may be an over simplification of a complex process. Late season poplar and walnut both demonstrated a shift in water acquisition regions to lower in the soil profile, a finding supported by the multisource mass-balance analysis.

5.5 Seasonal effects on soil water
The seasonal difference in soil water isotopic profile may be due in part to differences in seasonal precipitation inputs (Tang and Feng, 2001). Tang and Feng (2001) found that soil
isotopic profile was influenced by precipitation in two ways when measuring the effect of soil hydrology on the isotopic composition of plant source water. First, in spring, the soil isotopic profile is still affected by winter precipitation, which is a gradual replacement process. Second, isotopic variability is less affected by precipitation below 20 cm and the effect decreases even further at 50 cm unless there is a significant precipitation event (Tang and Feng, 2001).

In this current study, there is a clear difference in the soil profile near both the poplar and the walnut trees in late season as described above. It would appear that the winter precipitation turnover effect would have had the largest impact on the soil profile in early season. Thus the soil water had an isotopic signature below 30 cm that was reflective of winter precipitation. The soil water from the surface to 30 cm reflected the combined effects of spring precipitation and the selective evaporation of lighter isotopes. In late season there may have been a significant summer precipitation event, what Tang and Feng label a “soil water reset” which exchanged the soil water from the winter equilibrium state, replacing it with summer precipitation (Tang and Feng, 2001).

Interestingly, despite the difference in soil water below 30 cm, there was very little difference between the plant xylem values measured in the early and late season. The δ18O value for the deeper soil water was closer to that of the xylem tissue for the late season measurement allowing both the direct matching inference and the multisource mass-balance analysis to suggest that water uptake was occurring deeper in the soil profile without there being a significant change in the xylem tissue isotopic value.
5.6 Species effects on water acquisition

Trees that are well adapted to living in moisture limited conditions have the following adaptations: maintenance of a low baseline osmotic potential, active solute accumulation (i.e., by lowering their osmotic potentials), and protoplasmic resistance (Gebre, 1998). These characteristics of poplar and walnut are important to consider. *P. deltoides* ‘*P. nigra* L. clones had higher full turgor pressure over the other species evaluated, making it a more drought resistant species. However, it should be noted that poplar in general has been noted to experience a high degree of cavitation, where xylem vessels are blocked by air bubbles reducing hydraulic conductivity and productivity (Plavcova et al., 2012; Schreiber et al., 2011). Black walnut is known to be a species that is sensitive to dry soil conditions. This species typically has a deep rooting system that requires soils that have high water holding capacity (Gauthier and Jacobs, 2011; Vahdati and Lotif, 2013). Black walnut trees are also susceptible to cavitation due to their semi-ring porous nature (Gauthier and Jacobs, 2011). Black walnuts are found to have the highest hydraulic conductivity in spring to remove any winter cavitation (Gauthier and Jacobs, 2011). It has been hypothesized that this may give black walnut a competitive advantage in TBI systems reducing competition with crops (Geyer and Ponder, 2013).

It was predicted that poplar and walnut trees would vary in their water acquisition zones. This was hypothesized because: 1) it has been shown that trees allocate root systems where there is a predictably constant source of water, and 2) this available source of water varies spatially and temporally between different plant functional types (Nie et al., 2012; Rossato et al., 2013). Plants also demonstrate a tremendous ability to adapt to changes in nutrient availability by changing the way they function, e.g., leaf and root shape (Rossato et al., 2013). Part of this species based root plasticity is demonstrated when trees modify water
uptake zones (Casper and Jackson, 1997; Kulmatiski et al., 2010). Numerous studies have demonstrated that tree roots have the ability to change where they access nutrients based on factors including above ground demand, or to minimize competition with competing plants (Hutching and John, 2004; Mulia et al., 2010). This work comprises one of the first empirically tested indicators of interspecific root plasticity by evaluating water uptake zones before and after interspecific competition with crops was introduced.
Overall, this study provides valuable information on the water uptake strategies of poplar and walnut trees in a temperate agroforestry setting. This work was completed at the Guelph Agroforestry Research Centre, at University of Guelph, Guelph, Ontario. In TBI systems numerous studies have demonstrated that annual crops such as maize take-up water in the top 20 cm region (Miller and Pallardy 2001; Wang et al., 2010; Zhang et al., 2011). This study did demonstrate that both poplar and walnut were taking some of their water from this region in the soil profile early in the season, demonstrating that these trees do have active fine roots in these areas. This would suggest that interspecific competition in these TBI systems may be an issue. However, the preference by both trees for water acquisition in the top 20 cm of the soil profile was only demonstrated in the early season, before the barley crop was planted. The uptake zone was much lower in the profile at 40-70 cm in the late season when the crop was present suggesting that competition for water resources in this TBI system intercropped with barley is limited late in the growing season.

6.1 Limitations
The direct inference method relies on the assumption that water is taken up predominantly from one soil level. This assumption is problematic because it has been demonstrated that: i) trees are capable of taking up water from multiple depths simultaneously; ii) they alter their uptake zone temporally based on availability; and, iii) they alter the isotopic profile in the soil by transporting soil water to different depths through hydraulic redistribution, a process whereby the xylem path creates a water potential gradient to relocate water from wet to dry areas in the soil (Burgess, 2011; Nie et al., 2012). There is also the concern that since the
isotopic profile below 40 cm depth often varies within a narrow range at a particular site, it may be a challenge to discriminate between water acquired at different soil depths below this level. Caution needs to be exercised when applying the multiple source mass-balance technique. When combining many sources, there are many possible mixing proportions, which could produce the resultant target mixture (Phillips and Gregg, 2003). The number of unique solutions for a particular model depends on several factors including the increment size, the tolerance level, the number of isotopes considered and the number of sources contributing to the mixture. The multisource mass-balance analysis does not provide one unique solution, but a range of possible solutions.

From previous work, it is evident that the isotopic profile of the soil is highly dependent on edaphic conditions; under different soil types, the rate of change of the isotopic profile may display a lack of gradient or irregularities in the slope of the gradient (Asbjornsen et al., 2007; Thomas et al., 2013). This study demonstrated that $\delta^{18}$O soil profiles change rapidly even under similar physical slope and climatic conditions. Therefore, the inferences made about the water uptake zones of poplar and walnut may not be representative in other edaphic conditions and sites.
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Appendix 1: Soil moisture and $\delta^{18}$O versus soil depth for individual trees. Direct matching line based on tree xylem isotopic value. Poplar early season A, B, C, poplar late season G. Walnut early season D, E, F, walnut late season H. Soil moisture ($m_{\text{water}}/m_{\text{dry soil}} \times 100\%$).
Appendix 2A: Histographs for poplar one early season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2B: Histograms for poplar two early season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2C: Histographs for poplar three early season (70 cm soil depth sample spoiled). The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2D: Histographs for poplar one late season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2E: Histographs for walnut one early season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2F:  Histographs for walnut two early season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2G: Histographs for walnut three early season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 2H: Histographs for walnut one late season. The percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. Data run at incremental contributions of 1% with a tolerance of ±0.1‰.
Appendix 3 (A-H)

Histograms of aggregated three layer analysis (mean of 5-10 cm, 20-30 cm and 40-70 cm) for poplar and walnut early season (one-three) and late season (poplar one and walnut one). Percent frequency a solution is found at each soil depth is based on the proportional contribution of that soil depth. The data was run at incremental contributions of 1% with a tolerance of ±0.2‰.
Appendix 3D: Walnut one early season

Appendix 3E: Walnut two early season

Appendix 3F: Walnut three early season
Appendix 3G: Poplar one late season

Appendix 3H: Walnut one late season