

# A $\delta^{18}\text{O}$ and $\delta^2\text{H}$ stable water isotope analysis of subalpine forest water sources under seasonal and hydrological stress in the Canadian Rocky Mountains

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## Abstract

Subalpine forests are hydrologically important to the function and health of mountain basins. Identifying the specific water sources and the proportions used by subalpine forests is necessary to understand potential impacts to these forests under a changing climate. The recent ‘Two Water Worlds’ hypothesis suggests that trees can favour tightly bound soil water instead of readily available free-flowing soil water. Little is known about the specific sources of water used by subalpine trees *Abies lasiocarpa* (Subalpine fir) and *Picea engelmannii* (Engelmann spruce) in the Canadian Rocky Mountains. In this study, stable water isotope ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) samples were obtained from Subalpine fir and Engelmann spruce trees at three points of the growing season in combination with water sources available at time of sampling (snow, bound soil water, saturated soil water, precipitation). Using the Bayesian Mixing Model, MixSIAR, relative source water proportions were calculated. In the drought summer examined, there was a net loss of water via evapotranspiration from the system. Results highlighted the importance of tightly bound soil water to subalpine forests, providing insights of future health under sustained years of drought and net loss in summer growing seasons. This work builds upon concepts from the ‘Two Water Worlds’ hypothesis, showing that subalpine trees can draw from different water sources depending on season and availability. In our case, water use was largely driven by a tension gradient within the soil allowing trees to utilize tightly bound soil water and saturated soil water at differing points of the growing season.

A  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stable water isotope analysis of subalpine forest water sources under seasonal and hydrological stress in the Canadian Rocky Mountains

**SHORT RUNNING: Isotopic analysis of subalpine forest water use in Canadian Rockies**

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Subalpine forests are hydrologically important to the function and health of mountain basins. Identifying the specific water sources and the proportions used by subalpine forests is necessary to understand potential impacts to these forests under a changing climate. The recent ‘Two Water Worlds’ hypothesis suggests

that trees can favour tightly bound soil water instead of readily available free-flowing soil water. Little is known about the specific sources of water used by subalpine trees *Abies lasiocarpa* (Subalpine fir) and *Picea engelmannii* (Engelmann spruce) in the Canadian Rocky Mountains. In this study, stable water isotope ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) samples were obtained from Subalpine fir and Engelmann spruce trees at three points of the growing season in combination with water sources available at time of sampling (snow, bound soil water, saturated soil water, precipitation). Using the Bayesian Mixing Model, MixSIAR, relative source water proportions were calculated. In the drought summer examined, there was a net loss of water via evapotranspiration from the system. Results highlighted the importance of tightly bound soil water to subalpine forests, providing insights of future health under sustained years of drought and net loss in summer growing seasons. This work builds upon concepts from the ‘Two Water Worlds’ hypothesis, showing that subalpine trees can draw from different water sources depending on season and availability. In our case, water use was largely driven by a tension gradient within the soil allowing trees to utilize tightly bound soil water and saturated soil water at differing points of the growing season.

**KEY WORDS:** subalpine forest; transpiration; evapotranspiration; forest health; water use; Canadian Rocky Mountains

## Introduction

Freshwater supplies in mountainous regions are at risk as snow and ice stores continue to decline under rising global temperatures, earlier winter snowmelt and changing regional climate regimes (Stewart, Cayan & Dettinger, 2005; Whitfield & Cannon, 2000; Rasouli, Pomeroy, Janowicz, Carey, & Williams, 2014). Subalpine forests are of particular importance due to their hydrological connectivity within basins, controlling groundwater baseflow fluctuations, influencing basin-wide evapotranspiration (ET) and altering snow redistribution and storage dynamics. A change in water availability to alpine vegetation could have a significant effect on the health of these forests, in turn impacting annual water budgets (Harder, Pomeroy, & Westbrook, 2015; Carroll, Knapp, & Martin, 2017; Kelsey, Redmon, Barger, & Neff, 2018). Quantifying and conceptualizing these changes is imperative to better inform downstream water management, in addition to providing better parameterization for climate models. Thus, understanding how subalpine forests are obtaining and using water during the short mountain region growing season must be improved.

Past research has conceptualized vegetation water use by a reservoir of simple well-mixed subsurface storage, which is refilled by growing season precipitation (P) and always available for transpiration (T) and growth (Wigmosta, Vail, & Lettenmaier, 1994; Lawrence *et al.*, 2011). However, recent research has proven water transport through soils to vegetation is more complicated, with preferential pathways via macropores and water storage occurring in the finer pore spaces (Barbeta & Peñuelas, 2017; Sprenger *et al.*, 2018; Allen, Kirchner, Braun, Siegwolf, & Goldsmith, 2019). Plant water use then, becomes a more dynamic system involving soil pore spaces, preferential pathways and rooting depth of vegetation (Brooks, Barnard, Coulombe, & McDonnell, 2010). In subalpine vegetation systems, this complexity intensifies with high heterogeneity and anisotropy within the subsurface soil and bedrock geology. Often, there are large differences in soil depths and storage capacities further limiting available water during the growing season, represented in surficial discontinuities and glacially formed landscape features (Hood & Hayashi, 2015; Christensen, Hyashi, & Bentley 2016; Harrington, Hyashi, & Krulyk, 2017). Rooting structures of subalpine forests are highly dependent on this subsurface structure, with limitations in rooting depth due to shallow soils or limited access to groundwater as a consequence of fractured bedrock. Many studies have explored the relationship of rooting depth and water uptake in generalized landscapes (Schenk & Jackson, 2002; West *et al.*, 2012; Fan, Miguez-Macho, Jobbágy, Jackson, & Otero-Casal, 2017; Dubbert, Caldeira, Dubert & Werner, 2019), but few have focused on these processes in subalpine landscapes at higher altitudes (Jia, Lie, Chen, & Yu, 2017).

With stable water isotopes ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ), it is possible to determine the origin of water held within the xylem of plants, which is later transpired (Bertrand, Masini, Goldscheider, & Meeks, 2014; Allen *et al.*, 2019). These tracers have been used to show that plants draw water from a variety of sources, visible

through the isotopic signatures unique to each source. Studies have shown that, despite being situated next to streams, many plants were using an unidentified source with isotopically different signatures than the ‘obvious’ source they were located next to (Brookset al. , 2010; Good, Noone, & Bowen, 2015; Bowling, Schuleze, & Hall, 2017). McDonnell (2014) described this as the ‘two water worlds’ hypothesis, where streams and trees appear to evaporate different pools of water back to the atmosphere. He called upon more research and isotope-based tracer studies to be completed in forested catchments in order to understand the tightly bound soil water and more mobile water as sources for vegetation.

In mountainous environments that have limited growing seasons and water availabilities, subalpine forests rely strongly upon soil moisture stores to sustain growth (Day, DeLucia, & Smith, 1990; Small & McConnell, 2008). Current research on dominant alpine forest species (i.e. *Abies lasiocarpa* (subalpine fir, hereafter fir) and *Picea engelmannii* (Engelmann spruce, hereafter spruce)) has focused on physiological and biological differences by elevation and climate (Sowell & Spomer, 1986; Sala, 2006; Kueppers et al. , 2017), and species management (Alexander, 1987). However, there are limited studies of ET and water use (Brodersen, 2006). Most Rocky Mountain forest research has been conducted in the United States, with limited research programs conducted in the Canadian Rocky Mountains, highlighting a gap in knowledge of these species in northern latitudes. Determining the water sources of these Canadian Rocky Mountain species is important to understanding tree growth and T throughout the shoulder and growing seasons to understand the hydrological connectivity of mountain basins (Kräuchi, Brang, & Schonenberger, 2000; Matyssek, Wieser, Patzner, Blaschke, & Haberle, 2009), and how these behaviours could be altered under long-term climate change.

This study aims to identify and characterize subalpine forest water use at an elevation of 2100 m in the Canadian Rocky Mountains by addressing three main objectives through a  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stable water isotope analysis on trees: I) determine subalpine forest source water during pre-, mid- and end- of the growing season; II) partition relative source water contributions of xylem water within fir and spruce using the Bayesian mixing model MixSIAR; and III) evaluate which source waters are most important for subalpine tree T, and how long-term climate pattern changes could affect forest health.

## 2 Materials and Methods

### 2.1 Study Site Description

The data for this study was collected at Fortress Mountain Snow Laboratory (FMSL) in the Kananaskis River Valley of the Canadian Rocky Mountains, Alberta. FMSL is part of the Canadian Rockies Hydrological Observatory (<https://research-groups.usask.ca/hydrology/science/research-facilities/crho.php>) of high elevation hydrometeorological stations and research basins and is near the long studied Marmot Creek Research Basin (Rothwell et al., 2016). A research campaign was conducted from May - September 2017, from the beginning of snowmelt until the end of the growing season in order to obtain hydrometric and isotopic data. Fortress Mountain has a highly variable climate depending on season, but typically has long cold winters and cool wet summers, falling in the humid continental category (Beckstead & Veldman, 1985; Alberta Environment and Sustainable Resource Development, 2014).

Two adjacent subalpine sites at Fortress Mountain were used in this study to accurately represent the diverse forest structure at this elevation, hereafter referred to as ‘Tower’ and ‘Powerline’. They are close in proximity (115 m apart) and sit on a low gradient rolling bench at elevations of 2079 m and 2083 m, respectively (Figure 1). The sites differ primarily by tree age and density, with Tower having the younger and higher population density, and Powerline the older and lower population density (Figure 3). The subalpine forest at this elevation consists of coexisting subalpine fir and Engelmann spruce tree species. Species proportions are similar between both sites, with subalpine fir being the dominant species and Engelmann spruce making up the remainder (71.9 % and 29.5 %, respectively). There are only coniferous tree species at this elevation, with the remaining 1.4 % of the population consisting of lodgepole pine (*Pinus contorta* ) and alpine larch (*Larix lyalli* ). A transect system with arms spanning approximately 50 m in each cardinal direction, and 10 sampling locations along each segment was established at each site (Figure 1) and used to collect forest

inventory characteristics (DBH, height, population density and leaf area index (LAI)).

## Hydrometric Data Collection

Shallow groundwater wells were installed at both study sites to understand early season snowmelt and the ephemeral water table it generates. A total of five wells were installed at each site (10 total) (Figure 1). Between Tower and Powerline, 6 auto-logging water level transducers (HOBO U20 Water Level Logger; OnSet, MA, USA) were distributed, with a barometric data logger (HOBO U20 Water Level Logger; OnSet, MA, USA) installed at Cutline for atmospheric water level compensation for all loggers. Atmospheric compensation was corrected using Onset HOBOWare Pro 3.7.12 data processing software.

The vadose zone was instrumented for characterization of moisture dynamics after precipitation (P) events (Figure 2). Three soil moisture stations were distributed among different cover types: an open tree canopy, a closed canopy, and a semi-open tree canopy with ample ground vegetation. Each station was instrumented with two CS650 Soil Moisture probes at a depth of 30 cm (Campbell Scientific Inc., UT, USA). Soil temperature (type K thermocouple; Omega, CT, USA) was also measured in the same pits at 5, 10, 15 and 30 cm depths. Both CS650's and thermocouples were logged on a CR1000 data logger (Campbell Scientific Inc., UT, USA), sampled every 30 seconds and averaged over 30 minutes. Soil tensiometers (2725ARL Jet Fill Tensiometer; Soil Moisture, CA, USA) were setup adjacent to the three existing soil moisture stations, each setup comprised of three tensiometers at depths of 10, 20 and 30 cm. Tensiometer data was collected by manual measurement 25 times from June 22<sup>nd</sup> to August 10<sup>th</sup>, during the snow-free growing season.

Basic meteorological (MET) data was collected by instrumentation mounted on a 15.5 m tower, approximately 1.5 m above the top of the forest canopy. MET data collected included net radiation via sensors at the top (15.5 m) and bottom (1.4 m) of the tower (NR Lite2; Kipp & Zonen, VA, USA), relative humidity at a height of 15 m (HMP 155; Vaisala, Finland), and wind speed and direction using a CSAT3 sonic anemometer. P was collected at the Powerline site using an Ott Pluvio<sup>2</sup> (Ott Hydromet, CO, USA) wired to a CR3000 data logger (Campbell Scientific Inc. UT, USA) with a wind protection shield, and was additionally corrected for wind undercatch.

## Isotope Sample Collection

All potential sources for tree water use, including snow, P, saturated and bound soil water, were sampled for analysis of  $\delta\text{O}^{18}$  and  $\delta\text{H}^2$ . There was no surface water at the study site to sample. Water samples were collected during pre-, mid- and post growing seasons from May-September in 2017.

Snowpack samples were collected for both the pre- and end- growing season sampling periods. In the pre-period, the spring snowpack was approximately 1.2 m deep at the time of sampling. Snowpack cores were taken with a 4.1 cm diameter PVC pipe, and melted at room temperature to ensure complete mixing and limited phase changes before being sub-sampled into 20 mL glass poly-seal sampling bottles. A total of 6 cores were taken on two different dates, May 23<sup>rd</sup> and June 3<sup>rd</sup>. Snow was additionally sampled during the end- growing season collection period due to an early season snowfall just prior to September 26<sup>th</sup>, the sampling date.

P (rain) was collected throughout the entire study period (May-September) after every major storm event when possible, and less frequently from July-September due to extremely dry conditions and minimal major P events. Samples were collected nine times, and were sub-sampled into 20 mL glass poly-seal containers. The P collector was built to collect and limit evaporation of samples between sampling periods. A plastic hose was watertight sealed to the bottom of a funnel, which was then sealed to the top of a water reservoir container. The hose was cut with enough length to coil on the bottom of the reservoir to ensure the water level of collected samples topped over the hose, limiting evaporation and phase changing of the sample. A Ping-Pong ball was placed in the top of the funnel to further limit evaporation.

Saturated soil (pore) water was sampled during the snowmelt period starting in May until the snowmelt groundwater table dissipated. The geology at this site consists of fractured bedrock and glacial till, and no

constant water table persists during the growing season (Christensen *et al.*, 2016). All saturated soil water samples were taken from 10 wells ranging from 71 - 132.5 cm in depth, which falls within the maximum rooting depth of *Abies lasiocarpa* and *Engelmann spruce*. Sampling procedure consisted of purging the entire well volume 3 times before sampling into a 20 mL glass poly-seal container. Water samples were bottled with minimal headspace and stored at room temperature (never refrigerated or frozen to limit phase changing) before processing. Saturated soil water was sampled a total of 13 times from 15 May to 19 June.

Bound soil water samples were taken at every tree sampled for stem water. Two samples were taken at each tree during every pre-, mid- and end- growing season sampling campaign totaling 48 overall samples. Samples were taken with a 1-inch soil auger at a depth of 35 - 45 cm and stored in a thick poly-plastic bag. Soil samples were refrigerated during storage and transportation to ensure no evaporation or phase change occurred before azeotropic distillation.

Eight trees were sampled for each pre-, mid- and end- growing season sampling period for a total of 24 tree stem water samples. Trees were sampled from the Tower and Powerline sites, which each contributed 4 samples each to a single sampling period. Each set of 4 trees was selected first by species and then by size class (Figure 1). At both Tower and Powerline, two fir and two spruce were sampled. The two trees from each species were sampled according to the forest survey conducted in 2015, ensuring trees from above and below the median species DBH and height were sampled. The same trees were sampled during each sampling event.

Tree water was sampled from fir and spruce trees by harvesting older growth (no current year growth) from stem sections by cutting branch sections from the top, middle and bottom of each tree. Small stem sections were snipped from the mid-point of the branches. Needles were stripped from the stem samples before being placed in 30 mL vial containers. As many stems as possible were stored in the containers to limit potential phase changing headspace until azeotropic distillation analysis occurred. For each tree, 10 vial containers were filled in order to obtain an adequate sample size for distillation. After sampling, samples were placed in the freezer for storage and transport until analysis in order to further limit evaporation or phase changes from occurring.

## Isotope Sample Processing and Distillation

All samples collected were submitted and processed by the Environmental Isotope Laboratory (EIL) at the University of Waterloo, Ontario. Snow, saturated soil water and P water samples were processed using the  $\delta\text{O}^{18}$  and  $\delta\text{H}^2$  LGR-OA-ICOS Laser System as described in the methods used by EIL, University of Waterloo (LGR, 2010; Penna *et al.*, 2012; Berman, Levin, Landais, & Owano, 2013). Soil and tree samples were first processed by azeotropic distillation (Dewar & McDonald, 1961; Revesz & Woods, 1990) using toluene to extract pure water from the sample. The bound soil water samples were then run on the LGR-OA-ICOS Laser System at Environmental Isotope Lab (EIL), University of Waterloo (2013), while tree water samples were run using the EA/HT Mircomass IsoPrime system as described in the methods by EIL, University of Waterloo (Drimmie, Shouaker-Stash, Walters, & Heemskerk, 2001; Morrison, 2001). The EA system was used over the LGR-OA-ICOS Laser System due to better accuracy and analysis of organically derived water contents (Drimmie *et al.*, 2001).

## Data Processing

### Isotopic Framework Development

An isotopic framework for the 2017 study year was developed to generate a local meteoric water line (LMWL) and local evaporation line (LEL) specific to the Fortress Mountain, Kananaskis, AB region (Table 1). This local framework was necessary to analyze the stable water isotope samples obtained. To properly develop a framework, past climate normals (relative humidity and temperature) for the study region were determined from long-term Environment Canada data along with records from Fortress Mountain since 2014 (Environment Canada, 2015). In addition to relative humidity and temperature, normal established individual

framework parameters were calculated specific to the Kananaskis region using Brock, Wolfe, and Edwards (2007) as a guide (refer for framework parameter terminology).

Once calculated, the LEL was determined as the intersection of the calculated  $\delta^*$ ,  $\delta_{ssl}$  and  $\delta_P$  points in  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  space. The LMWL for the framework was determined by plotting all sampled source water on the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  plot (P, saturated soil water, snow), then obtaining the  $R^2$  value generated by a linear regression. The LMWL generated had an  $R^2$  value of 0.99, and equation of  $\delta^2\text{H} = 7.78\delta^{18} + 0.17$  (Figure 4), which indicates a strong relationship adequate for LMWL representation of the Fortress Mountain, Kananaskis area. All calculated parameters, LEL, LMWL, Global Meteoric Water Line (GMWL) and processed isotope samples were subsequently plotted on a  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  plot.

### ***MixSIAR Bayesian Mixing Model***

In order to partition the relative source water contributions of sub-alpine forest xylem water the R package MixSIAR, a Bayesian mixing model (BMM) that runs the Markov Chain Monte Carlo (MCMC) method, was used. MixSIAR is the latest iteration of a series of mixing models (MixSIR, SIAR) designed to analyze biotracer and isotope data to determine relative proportions of a mixture and its sources (Stock & Semmens, 2016). MixSIAR successfully incorporates the uncertainties associated with stable water isotope compositions, multiple sources, error terms, priors and varying data structures allowing for analysis of covariates and multiple variables at once. For this study, the GUI version of MixSIAR was used within the R console (Stock & Semmens, 2016; R Core Team, 2019). MixSIAR was chosen over simple linear mixing models and previous versions of compartment mixing models due to its performance partitioning source water from multiple sources while including error terms and statistical checks for competency (Stock *et al.*, 2018; Wang, Lu, & Fu, 2019). Importantly, the BMM assumes uncertainty and variability associated with stable water isotope sources are normally distributed. Along with the residual error term, the model will find a solution even if it is nonsensical. This was avoided by ensuring the proper checks and  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  biplot analysis was followed as suggested within the MixSIAR GUI operation guidelines (Stock & Semmens, 2016).

Two separate BMM runs (with the same parameters) were completed, analyzing the covarying effects of: (1) time of season and species, and (2) time of season and tree age. Time of season was always considered a ‘random’ variable, while age and species were always considered ‘fixed’ (Semmens, Ward, Moore, & Darimont, 2009). Run lengths were set at a chain length of 1,000,000 iterations to ensure the Gelman-Rubin and Geweke statistical diagnostic checks were met and the MCMC chains had converged. The error structure used in the model was ‘residual \* process’, as listed in the model GUI and instructions (Parnell, Inger, Bearhop, & Jackson, 2010). Both tropic enrichment factor and concentration dependence were set to 0. A non-informative prior (uniform) distribution was chosen based on insights of the MCMC method and observations drawn from the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  biplot (Newsome, del Rio, Bearhop, & Phillips, 2007).

## **Results**

### **Isotopic Characteristics of Sub-alpine Trees and their Source Waters**

The isotopic compositions of tree source water (excluding bound soil water) varied extensively between P, snow and saturated soil water throughout the three seasonal sampling periods (pre-, mid-, end-) (Figure 5). The  $\delta^{18}\text{O}$  value of P ranged from -19.99 to -12.59 ( $\pm 1$  SD)  $\delta^2\text{H}$  value of P ranged from -150.44 to -96.49 ( $\pm 1$  SD)  $\delta^2\text{H}$  P values varied significantly over the growing season ( $p = 0.040$  for both when  $p < 0.05$  at 95% confidence). P isotopic signatures typically vary greatly, influenced by the temperature of P condensation, and the ratio of P being condensed to the P already condensed in the air mass. Successful creation of a LMWL specific to the Kananaskis region ( $R^2$  0.99, Figure 4) was dependent on the variation in P signatures over the growing season, from snow melt to the end of senescence, in order to provide confidence and a strong linear relationship. The  $\delta^{18}\text{O}$  value of snow water ranged from -23.52 to -21.79 ( $\pm 1$  SD) -22.48 ( $\pm 0.54$ ) ranged from -180.83 to -166.67 ( $\pm 1$  SD) (+/- 4.59) water values varied significantly between May, June and September ( $p = 0.021$  and  $p = 0.003$  when  $p < 0.05$  at 95% confidence, respectively). Like rain, snow

isotopic signatures also varied due to similar atmospheric processes governing particle formation. The  $\delta^{18}\text{O}$  value of saturated soil water ranged from -21.75 to -18.33 ( $\pm 1$  SD) 0.91) from -166.08 to -141.56 ( $\pm 1$  SD) water values did not vary significantly during the snowmelt period, while a water table was present and accessible to trees ( $p > 0.05$ ). There was no significant difference due to the saturated soil water pool being generated from the spring snowpack melt over a concentrated time period.

Bound soil water and tree xylem water were analyzed separately, as they both had potential for species and growing season variation and were sampled concurrently during each sampling period. The  $\delta^{18}\text{O}$  value of bound soil water ranged from -20.47 to -13.40 ( $\pm 1$  SD)  $\delta^2\text{H}$  value ranged from -158.44 to -133.63 ( $\pm 1$  SD) with a mean value of -146.30 ( $\pm 6.48$ ) significant difference in the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  isotopic signature of bound soil water samples of different tree species ( $p > 0.05$ ). There was, however, a significant difference in the  $\delta^{18}\text{O}$  values in bound soil water obtained over the growing season ( $p = 0.037$  when  $p < 0.05$ , 95% confidence interval). Small differences between  $\delta^2\text{H}$  in sampled bound soil water indicates minimal evaporative influence causing isotopic enrichment during the sampling process. However, the bound soil water line plotted below the LMWL ( $\delta^2\text{H} = 4.22 \delta^{18}\text{O} - 72.95$ ,  $R^2 = 0.81$ ) indicating bound soil water experienced evaporative enrichment naturally, before collection (Figure 6). Differences in  $\delta^{18}\text{O}$  values indicate replenishment from a variable source, P was consistently available throughout the growing season and was isotopically variable over the growing seasons. Limited availability of saturated soil water after June meant that this source was likely not the key influencer of  $\delta^{18}\text{O}$  variation.

The  $\delta^{18}\text{O}$  mean value of tree xylem water was -18.04 ( $\pm 1.27$ )  $\delta^2\text{H}$  value of tree xylem water ranged from -154.31 to -134.82 statistically significant difference in xylem water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values between species ( $p > 0.05$ ), nor in xylem water  $\delta^{18}\text{O}$  distribution between sampled growing season stages ( $p > 0.05$ ). However, there was a significant seasonal difference ( $p = 0.025$  when  $p < 0.05$ , 95% confidence interval) in  $\delta^2\text{H}$  values. Differences in  $\delta^2\text{H}$  between soil and xylem water are indicative of evaporative influence causing isotopic enrichment, where  $\delta^2\text{H}$  fractionation was displayed in 8 of 25 tree xylem water samples (Figure 6).  $\delta^2\text{H}$  fractionation in vegetation is calculated as  $\delta^2\text{H}_{\text{SOIL}} - \delta^2\text{H}_{\text{TREE}}$ . A positive difference indicates fractionation, where a negative, or 0 difference indicates no fractionation. Most vegetation are assumed to not  $\text{H}^2/\text{H}^1$  fractionate when using and transpiring water, although it has been observed in some cases (Dawson and Ehleringer, 1992; Evaristo *et al*, 2017). When partitioned over the growing season incorporating soil and groundwater,  $\delta^2\text{H}$  values of tree xylem water showed seasonal variation in sources used (Figure 7). Although a large overlap between bound soil water and saturated soil water signatures were observed ( $\delta^2\text{H}$  -158.44 to -143.56 between the three sampling periods. Pre- season trees appeared to use a mixture of sources, having access to the water table during spring snowmelt, while in the mid-season bound soil water was predominantly used, with some retention and storage of saturated soil water from the beginning of the growing season. End-season xylem water  $\delta^2\text{H}$  values again incorporated more saturated soil water, lessening dependencies on bound soil water only. The increase  $\delta^2\text{H}$  signatures similar to groundwater may be explained by an increase in P and snow near the end of the growing season, replenishing soil moisture and deep saturated soil water stores after a summer season of no recharge (Figure 2). Snow  $\delta^2\text{H}$  values during the end- period were -169.29, helping to support the recharge utilization theory. End- season was the only sampling period to observe a trend in tree age and water source, with smaller trees utilizing more bound soil water than larger trees.

## Quantifying Relative Source Water Partitioning

Results for the MixSIAR BMM are partitioned into growing season stage and species (spruce and fir), and growing season stage and tree age. Tree age is based on the size class separations chosen for initial tree xylem isotope sampling. The lower size class of both species is hereafter the ‘young’ category, and the upper size of both species the ‘old’ category. Growing season stage is partitioned by the three major sampling events of tree xylem and bound soil water, pre-, mid- and end-growing season. The MixSIAR R package is not able to run more than two constraining variables at one time, although specializing in multiple source partitioning, which is why runs of species and age were separated.

The growing season stage and species BMM analysis showed differences across time and within spruce and fir (Figure 8). Although both species had different source water proportions, they both followed similar water use and source trends. For firs, the proportions of water sources during the pre- period were predominantly bound soil water (50.1 %, SD  $\pm$  20.5 %) and saturated soil water (36.5 %, SD  $\pm$  23.0 %) (Figure 8a). This shifted to mainly bound soil water (71.0 %, SD  $\pm$  17.6 %) in the mid-period, with only 11.3 % from saturated soil water (SD  $\pm$  11.5 %). During this period firs only used approximately 13.4 % of water from P (SD  $\pm$  7.9 %). During the end- period bound and saturated soil water were the dominant fir source with 57.6 % (SD  $\pm$  20.7 %) and 28.5 % (SD  $\pm$  20.6 %), respectively (Figure 8a). In spruce, the largest proportion of source water was during the pre- period was saturated soil water (69.3 %, SD  $\pm$  8.4 %), which transitioned to bound soil water (79.2 %, SD  $\pm$  14.4 %) in the mid-period (Figure 8b), and remain the primary source in the end- period at 74.7% (SD  $\pm$  15.1 %) (Figure 8b).

Fir trees used the highest proportion of saturated soil water in the pre-period, utilizing increased water tables due to spring snowmelt (Figure 8). Bound soil water and P proportions were highest in the mid-period, due to tree reliance on soil moisture as the water table receded and was eventually no longer available. What little P fell recharged shallow soil moisture layers, becoming available for the trees. There was a slight increase in proportion of saturated soil water used, due to late growing season P recharging deeper soil moisture stores (Figure 8). As highlighted earlier, the  $\delta^2\text{H}$  signatures of late growing season snow likely influenced the increase in saturated soil water signatures observed in xylem water. With similar source usage throughout the growing season as fir, spruce relied upon bound soil water more heavily with a minimum difference of 8.2 % at the mid- sampling points and a maximum spread of 19.2 % at the pre-growing season sampling (Figure 8). Spruce relied less upon initial saturated soil water stores and end of season recharge with differences of 16.0 % and 13.8 %, respectively (Figure 8).

The BMM analysis of growing season stage and ages showed minimal water source differences across time, but similarities in behaviour between age groups (Figure 9). Slight differences between young and old were noticeable in the overall source partition spreads, in the mid- and end- period specifically. In the young group, pre-season water used was primarily bound (76 %, SD  $\pm$  16.9 %), followed by saturated soil water (15.0 %, SD  $\pm$  14.4 %) (Figure 8a). In the mid-period, dominant proportions were bound soil water (76.4 %, SD  $\pm$  13.5 %) and P (11.5 %, SD  $\pm$  6.0 %) (Figure 9a). Finally, the end-period water was primarily bound (78.4, % SD  $\pm$  14.7 %) and saturated soil water (12.1 %, SD  $\pm$  11.0 %) (Figure 9a). For the older tree group, pre-period proportions were dominated by 61.8 % (SD  $\pm$  21.4 %) bound soil water and 24.1 % (SD  $\pm$  19.2 %) saturated soil water (Figure 9b). In the mid-period, water was primarily from 58.7 % (SD  $\pm$  18.2 %) bound soil water, 20.7 % P (SD  $\pm$  8.3 %), and 15.3 % (SD  $\pm$  15.2 %) saturated soil water (Figure 9b). Lastly, end-period proportions were largely 60.8 % (SD  $\pm$  20.7 %) bound soil water and 23.2% (SD  $\pm$  18.8 %) saturated soil water (Figure 9b).

The young group, showed a very slight increase in bound soil water use over the growing season (2.4 %), while relying upon this source for over 75 % of its water needs (Figure 9). Interestingly, despite an available water table at the beginning of the growing season, this only slightly influenced the saturated soil water source portion compared to the other growing season stages (15 % in the pre- stage and 8.6 % during mid-), but most reliance remained on bound soil water (Figure 9). This may be explained by less established rooting systems of younger trees, which do not have as much access to the water table as their larger counterparts. Like the young group, the old tree group had a steady proportion of bound soil water use throughout the growing season, although of a smaller magnitude (16.5 % less on average) (Figure 9). In this age group, saturated soil water reliance was 7.8 % more on average, with higher values at the start and end of the season, during snowmelt and end of growing season recharge (Figure 8). Both age groups utilized P more during the mid- period, while there was no water table present, with older trees using twice as much P as younger trees during this period (20.7 % vs 11.5 %) (Figure 9). Generally, older trees used more saturated soil water when it was available than did younger trees, but both relied mostly upon soil moisture.

The MixSIAR BMM also generated source water proportions for the entire season, with runs also split into species and age-based groups (Figure 10). For spruce, season long proportions were: 15.4 % (SD  $\pm$  14.1



%) saturated soil water, 15.7 % (SD  $\pm$  15.4 %) P, 8.8 % (SD  $\pm$  12.5 %) snow and 60.1 % (SD  $\pm$  21.8 %) bound soil water (Figure 10a). For fir, 26.5 % (SD  $\pm$  16.9 %) saturated soil water, 16.3 % (SD  $\pm$  12.9 %) P, 11.3 % (SD  $\pm$  11.8 %) snow and 45.9 % (SD  $\pm$  18.6 %) bound soil water (Figure 10a). Overall, fir had a larger reliance on saturated soil water with almost 10 % more coming from this source. Bound soil water was equally important to both species, being the highest proportion of sources, but spruce placed a higher reliance upon this source by approximately 15 % (Figure 10). Both species used P and snow sources similarly. When examining season long trends in water source among tree age, the proportions for the young group were: 19.3 % (SD  $\pm$  15.1 %) saturated soil water, 15.2 % (SD  $\pm$  13.5 %) P, 10.8 % (SD  $\pm$  11.9 %) snow and 54.8 % (SD  $\pm$  21.5 %) bound soil water (Figure 10b). For the old group, 28.4 % (SD  $\pm$  21.6 %) saturated soil water, 21.6 % (SD  $\pm$  13.8 %) P and 36.2 % (SD  $\pm$  20.8 %) bound soil water (Figure 10b). Older trees were less reliant upon bound soil water, with a proportion of only 36.2 %, a 18.6 % difference between the young group (Figure 10). Older trees appear to pull similarly from many different sources, while younger trees are much more reliant on soil moisture stores.

## Discussion

### Effectiveness of MixSIAR BMM

With the emergence of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  use in vegetation water sourcing, there are many active discussions regarding collection and processing of organic materials (soil and tree xylem water) (Orlowski, Pratt, & McDonnell, 2016; Barbeta, Ogee, & Peñuelas, 2018; Millar, Pratt, Schneider, & McDonnell, 2018) in addition to presentation of results and data analysis related to partitioning models (Barbeta & Penuelas; Wang *et al.*, 2019). With stable water isotopes, care must be taken to prevent evaporation and subsequent phase changing of sampled organic materials in order to prevent isotopic enrichment and fractionation viewable in  $\delta^2\text{H}$  results. It is generally believed that trees do not fractionate water during uptake (Dawson & Ehleringer, 1992), although it has been observed in trees within a few studies (Lin & da Sternberg, 1993; Newsome *et al.*, 2007). Magnitude of isotopic separation between  $\text{H}^2/\text{H}^1$  can be tested in comparison to bound soil water isotopic signatures (Ellsworth & Williams, 2007). Eight (of 25) subalpine trees sampled in this study displayed potential  $\text{H}^2/\text{H}^1$  fractionation, when compared to bound soil water values. This discrepancy could be a product of sampling difficulty, with tree stem segments experiencing some evaporation or phase changing before analysis. Primarily,  $\text{H}^2/\text{H}^1$  fractionation is most important when considering linear mixing models. Fractionation can cause discrepancies when only considering  $\delta^2\text{H}$  in calculations, skewing relative source water proportions incorrectly. As addressed by Evaristo, McDonnell, and Clemens (2017), when using two isotopes together in analysis, such as a BMM, the potential fractionation of  $\delta^2\text{H}$  does not hinder outputs. The authors further suggest that if uncertainties exist in sources and mixtures (xylem water), the BMM approach is the most appropriate method. With this in mind, for this study the MixSIAR BMM was the most suitable method to capture relative source contributions with multiple source inputs and one mixture (xylem water) and was effective in representing source water throughout the growing season.

### Relative Source Water Partitioning

Stable water isotope identification of source water in vegetation is becoming a more commonly utilized method in ecohydrology (Goldsmith *et al.*, 2012; Bertrand *et al.*, 2014; Barbeta & Peñuelas, 2017; Liu, Yu, & Jia, 2017; Allen *et al.*, 2019). In this study,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stable water isotopes were used in order to help determine subalpine tree source water during three points in the growing season (pre-, mid-, end-), and to partition the relative contributions of sources to tree xylem composition. Through creation of an isotopic framework and generation of the LMWL and LEL for the FMSL area,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stable water isotopes provided an understanding of subalpine forest water sources. The isoplot produced in this study, in addition to the BMM source water-partitioning model, identified subalpine tree source water for three periods of the growing season. The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  isoplot in addition to the seasonal  $\delta^2\text{H}$  xylem water plot highlight that overall, bound soil water is the most utilized resource. Saturated soil water is also an important resource, utilized primarily during the beginning of growing season snowmelt period. The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  isoplot and

the seasonal  $\delta^2\text{H}$  xylem water plot were used to gain a special understanding of isotopic signatures within the system, where the BMM source water- partitioning model generates and calculates the proportion of each source utilized. Both methods are important for a complete understanding of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in the subalpine forest examined.

Recently, several studies have analyzed the efficiency and adequacy of using more complex mixing models, in particular the MixSIAR BMM package, to answer questions similar to the aim of this study (Rothfuss & Javaux, 2017; Wang *et al.* , 2019). Wang *et al.* (2019) found that MixSIAR and SIAR had better source water appointment performances due to inclusion of error terms and uncertainties associated with varying isotopic compositions. Evaristo *et al.* (2017) discussed uncertainties in different models and their proportion predictions. They highlighted in a source water study like theirs, the BMM approach may prove useful to quantify source water appointment, especially between the vadose and saturated zones. BMM results in this study indicate that between both tree species and age classes, subalpine trees switched reliance on source water throughout the growing season, mainly between the two subsurface compartments (saturated/unsaturated). Numerous studies have sought to understand physiological characteristics behind tree water use in co-occurring fir and spruce subalpine forests (Kaufmann, 1982; Boyce & Saunders, 2000; Sala, 2006; Andrus, Harvyem Rodman, Hart & Veblen, 2018; Davis & Gedalof, 2018), but limited studies have considered season long source water identification in Rocky Mountain regions.

### Species Differences and Water Use Behaviours

Saturated soil water supplied via snowmelt is an important source, primarily at the beginning of the growing season. This source replenishes early season bound soil water supplies by increasing soil moisture levels via an increased water table. Saturated soil water levels then recede below a tree-accessible level after the snowmelt period concludes. Towards the end of the growing season, nearing senescence, both fir and spruce show increases in saturated soil water isotope signatures, likely due to increased P levels slowly increasing soil moisture stores and recharging saturated soil water at the end of the growing season in 2017. Saturated soil water signatures differ from P due to the extended residence time within the soil, allowing for fractionation. Unlike the drought conditions of the summer, in which P evaporated and transpired within a few days, increased amounts of P at the end of the growing season allow for recharge in the saturated zone. End of season snowfall also influenced source water proportions, appearing isotopically distinct in tree xylem water. Bound soil water was the most important water source for both subalpine tree species, regardless of age class, although there were distinct differences when examining species and tree age relating to source water proportion. Overall, spruce were more reliant on bound soil water, with fir trees using less bound soil water and utilizing more saturated soil water. Interestingly, subalpine fir show greater levels of drought stress while spruce are more resilient (Veblen, 1986; Kelsey *et al.* , 2018). Observing seasonal water source partitioning of fir, it appears that in the mid- season they retain, or store, more saturated soil water than their counterparts. This highlights differences in physiology and rooting structures between fir and spruce, with fir rooting deeper and relying less upon vadose zone water compared to spruce. Fir and spruce rooting systems vary extensively with dependencies on subsurface geology and soil structure. Both species in subalpine environments more commonly have shallow rooting systems, with deeper taproots more commonly in well-draining deep soils (Alexander, 1987). Is it possible that with fir being more abundant at the study location (70 % of population), their rooting systems could be more established and reach deeper depths due to successional dominance.

The summer growing season studied experienced drought with no saturated soil water recharge during July and August. Soil moisture values are half of the values observed the year before at the end of the growing season. Despite small differences in source water spread, limitations in bound soil water could affect both fir and spruce tree T, and long-term health if there are successive summer drought seasons (Pataki, Oren, & Smith, 2000; Adams *et al.* , 2009; Matyssek *et al.*, 2009; Tague, Keyn, & Christensen, 2009; Shafer, Bartlein, Gray & Peltier, 2015). Based on BMM results showing spruce placing more reliance on bound soil water, spruce may show effects of drought stress before fir, despite spruce having been observed to be a more resilient species in past studies (Veblen, 1986). As indicated previously, this may be site specific and

based on the succession of species proportion, with fir being the dominant species at this study location. Results also highlighted different water source proportions with tree age, regardless of species. Older trees, overall, tend to use more saturated soil water compared to younger. A direct relationship is observed with rooting depth and age, with younger spruce and fir typically displaying lateral root spread and weak taproots (Alexander, 1987). These differing behaviours may provide more physiological resilience to drought for older subalpine trees, allowing them to use an additional water source. Age and maturity also corresponds to increased storage, allowing trees contain source signatures obtained earlier, throughout the length of the growing season.

The three sampling periods were able to highlight the progression of water uses; snowmelt finished and soil moisture stores lessened toward senescence during the dry summer of 2017. The percentages developed also indicate relative importance of these sources to the tree species examined. Mid-summer, 71% and 79.5% of total xylem water for fir and spruce, respectively, contained bound soil water signatures. Recent studies examining the constraints on seedling and sapling growth in the Rocky Mountain identified soil moisture as an important limiting factor in successful establishment (Andrus *et al.*, 2018). In particular, Davis and Gedalof (2018), found treeline advance and establishment of co-occurring fir and spruce is unlikely considering earlier snowmelt and longer snow-free growing seasons depleting soil moisture are expected to become more frequent (Fang and Pomeroy, 2020). Hydrological conditions of the growing season examined in this study were representative of summer drought conditions, with no summer P recharging subsurface storage. Only the first 30 cm of soil received percolated water before being utilized by vegetation or evaporated within a few days (Figure 2). Results showed younger trees relied upon soil moisture stores more than their counterparts, who incorporated saturated soil water more into their overall source use. These results, in conjunction with research indicating poor success of sapling establishment and forest health with lower soil moisture reliability (Cul & Smith, 1991; Harpold *et al.*, 2014; Lazarus, Castanha, Germino, Kueppers, & Moyes, 2017; Andrus *et al.*, 2018; Davis & Gedalof, 2018), could indicate younger trees show more signs of stress in addition to potential die back if drought conditions become more frequent in subsequent summer growing seasons (Adams *et al.*, 2009). The ability of older trees to spread their water source allocations differently, likely due to more established rooting structures, may give them an edge in resilience to less than favourable growing season conditions under a changing climate. A growing season experiencing an extended snowmelt period due to a large winter snowpack may help established subalpine forests experience less drought stress by providing an extended saturated soil water source for longer, despite drought conditions occurring later in the summer, such as in 2017. This was evident in the MixSIAR model results, with saturated soil water signatures being maintained throughout the entire growing season despite the saturated soil water depletion after snowmelt (after the pre- sampling period).

## Conclusions

The aim of this study was to understand and partition subalpine forest water sources during a growing season experiencing a drought period in the Canadian Rockies. Development of an isotopic framework, LMWL, and LEL were integral to the successful identification of tree water sources. The LMWL was generated with an  $R^2$  value of 0.99, indicating a successful relationship and potential for use in future  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stable water isotopes studies occurring on the eastern slopes of the Rocky Mountains. Subsequent use of the MixSIAR BMM with source and xylem water samples provided an understanding of water use behaviour at three points throughout a growing season at 2100 m of elevation.

Tree species and age both displayed differences in water source allocations, although all categories placed a higher reliance on bound soil water. Saturated soil water supplied by snowmelt was highlighted as an important source during the beginning of the growing season, and closer to the end of the growing season with increased P in the fall. Results highlighted the importance of soil moisture reserves to the health of subalpine forests, and shed light upon future behaviours should years of successive drought periods occur. These findings support established literature that have indicated future success of co-occurring fir and spruce forests could be limited should soil moisture stores decrease. This moisture limitation has the ability to limit

tree line advance to higher elevations, which has the potential to further limit forest population growth as valley bottoms experience die backs with faster rates of change due to higher baseline temperatures than the alpine. Successionally, sub-alpine forests could be nudged off balance as older trees, able to utilize deeper groundwater, will have a higher survival rate compared to their counterparts who may experience increased levels of drought stress. Although soil moisture was determined to be the most important source, extended snowmelt periods supplied by large winter snowpacks could offset summer droughts by supplying subalpine forests with an additional source for a limited period of time. Extended growing seasons with rising temperatures could cancel this effect, as trees will need to rely on soil moisture stores for longer periods of time. A multi-year  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stable water isotope study could help provide further understanding of these co-occurring subalpine forests under hydrologically variable mountain growing seasons. Thus, this work suggests that two water worlds do not exist as permanent distinct, isolated sources of water in this environment, but that moisture in natural vegetated systems is really just a gradient between water held under high (soil moisture) to low tension (saturated soil water) and so the water worlds are more endpoints on a gradient than distinctive reservoirs. Results here show that trees can draw on both endpoints depending on season and availability or antecedent and climatic conditions.

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## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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