Unexpected water uptake under drought conditions and thinning treatments in young and overstocked lodgepole pine (Pinus contorta) forests

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Abstract:

As drought and prolonged water stress become more prevalent in dry regions under climate change, understanding and preserving water resources has become the focal point of many conversations. Forest regeneration after deforestation or disturbance can lead to over-populated juvenile stands with high water demands and low water use efficiency. Forest thinning improves tree health, carbon storage, and water use while decreasing stand demands in arid and semi-arid regions. However, little is known about the impacts of over-population on seasonal variation in depth to water uptake nor the magnitude of the effect of growing season drought conditions on water availability, and existing reports are highly variable by climatic region, species, and thinning intensity. In this study, stable isotope ratios of hydrogen (δ²H) and oxygen (δ¹⁸O) in water collected from soil varying depths and from twigs of lodgepole pine (Pinus contorta) under different degrees of thinning (control: 27,000 stems per ha; moderately thinned: 4,500 stems per ha; heavily thinned: 1,100 stems per ha) over the growing season and analyzed using the MixSIAR Bayesian mixing model to calculate the relative contributions of different water sources in the Okanagan Valley in the interior of British Columbia, Canada. We found that lodgepole pine trees shift their depth to water uptake depending on water availability under drought conditions and rely more heavily on older precipitation events that percolate through the soil profile when shallow soil water becomes less accessible. Interestingly, forest thinning did not cause a significant change in depth to water uptake. Our results support other findings by indicating that although lodgepole pines are drought tolerant and have dimorphic root systems, they cannot shift from deep water sources when shallow water becomes more available at the end of the growing season.

Keywords: Pinus contorta; stable water isotopes; forest thinning; water use strategies; preferential water uptake; dual-isotope analysis; Bayesian isotope mixing model; soil water uptake; transpiration; the interior of British Columbia
1. Introduction

As forests recover after harvesting, carbon and water demands change, and future climate projections of increased drought severity will further complicate biogeochemical cycling and carbon-water trade-offs (Giles-Hansen et al., 2021; Wang et al., 2019). Regenerating stands add further stress on ecosystems; for example light competition in dense juvenile stands increases stand water demands by driving vertical growth and stand leaf area (Liu et al., 2011a). To mitigate this stress, management strategies such as systemic thinning of high-density juvenile stands promotes forest regeneration while decreasing competition and providing remaining vegetation with increased light availability, rooting space, nutrient access, and space for horizontal branch growth (Giuggiola et al., 2016). Over a variety of forest ecosystems, reductions in stand density have been shown to increase light availability, tree water use, carbon storage, and water use efficiency, an indication of improved tree health, and to decrease stand water use, reducing the intensity of water stress under drought conditions (Belmonte et al., 2022; Fernandes et al., 2016; Giuggiola et al., 2016; Liu et al., 2011b; Manrique-Alba et al., 2020; Molina & del Campo, 2012; Park et al., 2018; Sohn et al., 2012, 2016; Wang et al., 2019). Because the primary goal of forest thinning is to decrease stand water use and increase productivity, literature reporting the effects of this management strategy often focuses on changes in carbon storage, tree growth, transpiration, and water use efficiency (Giuggiola et al., 2016; Manrique-Alba et al., 2020; Park et al., 2018; Sohn et al., 2016). However, few studies have reported sources of water use and their shifting in association with thinning treatments in overstocked naturally-regenerating forests, particularly under drought conditions.

Quantifying stand water use is imperative to predicting the future of water availability in our ecosystems. However, various studies indicate that trees do not always use the most recent precipitation, and that vegetation can utilize different sources of water at different soil depths depending on availability or stress (Dawson & Pate, 1996; Grossiord et al., 2017; Wang et al., 2017). Many studies also report the depth of water uptake of various species and the relationship between co-existing species and shared water sources (Andrews et al., 2012; Brinkmann et al., 2019; Grossiord et al., 2017; Langs et al., 2020; Liu et al., 2015; Maier et al., 2019; Meinzer et al., 2007; Sánchez-Pérez et al., 2008; Szmyczak et al., 2020; Wang et al., 2017; Warren et al., 2005). In arid and semi-arid regions where water is the limiting factor, some species have adapted to derive water from various depths depending on seasonal water variability and have higher ecological plasticity and drought tolerance (Langs et al., 2020; Wang et al., 2017). Understanding where in the soil profile plants use water over prolonged dry periods and at different stand densities is essential in assessing the impact of forest thinning and the relative importance of different seasonal water sources under future climate conditions and shifts in water availability in arid regions (Evaristo et al., 2015; Prieto et al., 2012; Sohn et al., 2016).

Stable isotope ratios can be used as powerful natural tracers to identify distinct water sources such as rainfall, snow, groundwater, and stream flow (Brinkmann et al., 2018; Lin & da S. L. Sternberg, 1993; Sprenger et al., 2017; Stumpp et al., 2018). The isotopic signature of precipitation events is altered by elevation, temperature, and evaporative fractionation creating distinctive layers within the soil profile (Kleine et al., 2020; Sprenger et al., 2017; Stumpp et al., 2018). More specifically, soil water reflects precipitation events as they infiltrate through the soil layer with the influence of evaporative fractionation until mixing with older groundwater and depleted isotopes creating individualized isotopic signatures throughout the soil profile (Andrews...
The isotopic composition of plant water corresponds to the water uptake depth in the soil profile (Brinkmann et al., 2019; Langs et al., 2020; Meinzer et al., 2007; Stumpp et al., 2018; Wang et al., 2017). Due to these unique characteristics, stable water isotopes have been used by researchers to assess sources of water use by plants and their possible shifts under altered environmental conditions (Evaristo et al., 2015; Flanagan & Ehleringer, 1991; Meinzer et al., 2001; Stumpp et al., 2018).

Lodgepole pine (Pinus contorta Douglas) is an early successional montane conifer with a deep tap root, fine roots in shallow layers, and advantageous rooting system which allow this species to access water throughout the soil profile (Fahey & Knight, 1986; Halter & Chanway, 1993). Depending on the species, root structures have two components, lateral roots to increase their soil surface area and tap root to reach deeper soil water or groundwater when surface water is limited. Some species have also adapted to have dimorphic rooting habits, or the ability to access water from different depths in the soil profile depending on soil moisture content and water availability making them more resilient to water scarcity or prolonged drought conditions (Dawson & Pate, 1996; Meinzer et al., 2013). One study, comparing Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco and lodgepole pine in southern Alberta, found that lodgepole pines are able to minimize seasonal variations in stem water potential and that tap roots are deep enough to access groundwater (Andrews et al., 2012). This finding is consistent with other literature that lodgepole pines can access water from different depths depending on moisture availability and can access bound soil water when there is low water potential (Meinzer et al., 2007a; Warren et al., 2005). The literature indicates that lodgepole pines can access water from different soil layers even under extreme or prolonged drought conditions, but little is known about the shifting of water use under different stand densities as a result of thinning treatments and drought conditions.

In this study, we used the stable isotope ratios ($\delta^2$H and $\delta^{18}$O) of soil and xylem water to evaluate how overpopulated stands and thinned stands use water over the growing season to further our understanding of the ecosystem-level impacts of thinning as a management strategy. We hypothesized that lodgepole pine primarily relies on spring snowmelt but reductions in shallow source water during the growing season (along with the low soil water holding capacity) would drive lodgepole pines to utilize deeper sources of water. Prolonged aridity was expected to push trees to depend on different water sources towards the end of the growing season. We also hypothesized that overpopulated stands may be limited in their rooting depth and unable to access deep soil water under extremely dry conditions, and that thinning can effectively mitigate these stresses. Through a detailed partitioning of tree water sources, we can better understand how lodgepole pine uses water, estimate proportional dependence of lodgepole pine on specific source waters, and determine if thinning affects tree water use and uptake strategies under drought conditions.

2. Methods

2.1. Study Site
The study was conducted in the Upper Penticton Creek experimental watershed (UPC) northeast of Penticton in the interior of British Columbia, Canada (49°39'34" N, 119°24'34" W). The site elevation is approximately 1675 m a.s.l. with steep, rocky terrain and a southern aspect (Wang et al., 2019). The Luvisolic soils were formed from granite; the texture is coarse sandy-loam and is well drained with a low water holding capacity (Hope, 2011; Winkler et al., 2021; Winkler & Moore, 2006). The biogeoclimatic region is the Engelmann Spruce-Subalpine Fir zone with cold, snowy conditions from November to early June and seasonal drought conditions during the summer months, June to October (Coupe et al., 1991; Wang et al., 2019). This research site was initially established as a paired watershed experiment in the early 1980s to quantify the impact of forest harvesting on water resources (Creed et al., 2014; Moore & Wondzell, 2005; Winkler et al., 2021). The juvenile thinning experiment began in 2016 when 16-year-old, evenly aged, regenerating lodgepole pine stands were thinned to different densities than a control (C: 27,000 stem ha⁻¹, T1: 4,500 stems ha⁻¹, and T2: 1,100 stems ha⁻¹) where C represents the control stands, T1 represents the lightly thinned stands, and T2 represents the heavily thinned stands (Figure 1). The three treatments were repeated across three replicate blocks. Each block was 75 m long and 25 m in width with three 20 m² plots and 5 m between treatment plots. After the initial thinning, all debris was left on site. The first two years’ post-thinning results showed increased tree-level water use and decreased stand-level water use in the thinned stands (Wang et al., 2019). Wang et al. (2019) concluded that thinning positively influenced tree growth and water use and that moderate and heavy thinning are effective management strategies for drought mitigation of lodgepole pine in the UPC watershed.

Climate stations (HOBO weather station, Onset Computer, Bourne MA, USA) were deployed across Block 1 treatments and have measured meteorological data since 2016 (ambient temperature, relative humidity (rH), wind speed, precipitation, and solar radiation) in 10-minute intervals. From this, we calculated daily vapor pressure deficit (VPD) as well as daily and monthly potential evapotranspiration (PET) using temperature fluxes, relative humidity, and precipitation (Flint & Childs, 1991; Russell, 1960; Streck, 2003). Recorded historical precipitation (1997-2008) was acquired from a long-term climate station in a lodgepole pine forest in the 241 experimental watershed (climate station P7) (Moore et al., 2021).

Rainfall and temperature data from Block 1 was related to historical data to calculate the monthly dryness (PET/P), standardized precipitation index (SPI), and standardized precipitation evapotranspiration index (SPEI) (Beguería et al., 2014; Stagge et al., 2014; Wu et al., 2005).
the middle of the growing season in 2021, four soil moisture probes (HOBO TEROS 11 Soil Moisture/Temp Probes) were deployed in each treatment in Block 1 to measure changes in soil moisture and temperature at 5 cm and 35 cm at 15-minute increments (n=12).

2.2 Sample collection

We sampled three trees per treatment across the three blocks and three in the mature plot (n = 30) four times over the 2021 growing season in approximately six-week intervals (June 11-12, July 21-22, September 10-11, and October 7-8) around noon to capture peak transpiration time. We used a pole pruner to cut a mid-canopy branch in the live crown. We peeled the bark off branch segments with no needle coverage to remove outer bark and phloem, placed them into a glass tube, sealed it with Parafilm wrap, covered it in aluminum foil, and set them in a cooler until the end of the day when they were transferred to a freezer at -18°C. During the last two sampling periods, some trees had red needles, likely an indication of dryness or higher temperatures from an early growing season heat dome that began in June.

Soil samples were collected horizontally from 40 cm soil pits randomly dug across each treatment plot at 5 and 35 cm depths from the surface. Large rocks were removed from the profile. Soils were then sealed in freezer seal bags and frozen until cryogenic distillation for water extraction. In the middle of the field season, 1 m pits were dug to sample the vertical profile in 20 cm intervals in each treatment of Block 2. From the vertical pit, samples were collected in 20 cm increments to determine the depth of tree water access. After samples were collected, the larger rocks and soils were used to fill the pits.

Precipitation samples were collected when available during field collection days. Snow from a late spring event was collected on June 11\textsuperscript{th} and another snow event on October 11\textsuperscript{th}. A rain event was collected on September 10\textsuperscript{th}. Groundwater and stream samples were collected from the creek 241 watershed at the end of the growing season and beginning of the seasonal hydraulic recovery. Groundwater was collected using a hand pump. Groundwater and stream samples were collected at the end of the growing season as stream beds were dry and groundwater was inaccessible during the dry period. Once the well had been pumped and cleared, test tubes were rinsed with ground water three times before being filled. Precipitation, groundwater and stream samples were collected into test tubes, sealed with parafilm and foil, and stored in a fridge at 4\textdegree C.

2.3 Cryogenic extraction and isotopic analysis

Before extraction, samples were thawed, and weighed. For stable isotope analysis, water was extracted from stem and soil samples using cryogenic distillation (Orlowski et al., 2013; Pearcy et al., 2012). The test tube and branch sample segment of the line was immersed in liquid nitrogen for 10 minutes until frozen (Chillakuru, 2009). Soils were frozen for 45 minutes in a 500 mL round-bottom flask using a dry-ice and 95% ethanol mixture before pumping out the air. Frozen samples were pumped down to 60 mTorr, not disturbing the sample (Tsuruta et al., 2019). The vacuum-sealed extraction unit was detached from the pump and transferred to a boiling water bath; the extraction tube was submerged in liquid nitrogen. Branch samples were set to distill for 1 hour and soil samples for 2 hours or until the tubing was clear to ensure all
mobile and bound source water was extracted (Orlowski et al., 2013; Tsuruta et al., 2019; Vargas et al., 2017; West et al., 2006). Samples were also weighed after extraction and compared to oven dried samples to ensure distillation was complete. Water extracted from branch and soil samples accounted for 47.9±3.2% and 9±6% of mean sample weight.

All samples were pipetted and sealed into glass vials with screw tops and shipped to the University of California Davis Stable Isotope Facility (Davis, CA, USA) for analysis using headspace gas equilibration on a GasBench-II device (Thermo-Finnigan, Breman, Germany) for 18O and 2H analysis. Precision was less than or equal to 2.0‰ for δ2H and 0.2‰ for δ18O. Results were returned in the "delta" notation expressing the isotopic composition of each sample as a ratio in parts per million over to a standardized range of reference waters calibrated against IAEA reference waters and reported relative to VSMOW (Vienna-Standard Mean Ocean Water) where:

$$\delta(\%) = \left( \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right)$$

Sample extract was situated in an isotope biplot and compared to the global meteoric waterline (GMWL) along with a local meteoric waterline (OMWL) (δ2H = 6.6 (δ18O)-22.7) and local evaporative line (LEL) (δ2H = 5 (δ18O) - 48.4) calculated for the Okanagan Valley by Wassenaar et al. (2011).

To test the variance between thinning treatments, block replicates, dates collected, and soil depth, we first tested the normality of the subsets using the Shapiro-Wilk test and found that all subgroups were approximately normally distributed. Repeated measures ANOVAs were used to compare effects of date and treatment on δ2H and δ18O in branches, soils and groundwater to determine if changes in lodgepole pine uptake patterns occur over time, if soil signatures vary between different depths (0-100 cm and groundwater) and densities, and if thinning juvenile stands changes seasonal shifts. All statistical analysis was conducted in R Studio (version 1.3.1073) using the appropriate tests to determine site distinctions and seasonal variability in depth to uptake (RStudio Team, 2020).

2.4 MixSIAR model scenarios

Process-based models (PBM) with a Bayesian approach include integrating other processes or existing information as priors allowing for a more informed approach than a simple linear model (Ogle et al., 2014). To accurately partition potential lodgepole pine water sources, we used the MixSIAR modeling package, a Bayesian mixing model (BMM) based on the Markov Chain Monte Carlo method (MCMC) (Langs et al., 2020; Stock, 2013/2022, p. 201; Stock et al., 2018; Wang et al., 2017; Wang et al., 2019). The MixSIAR modeling package was selected over the previous iterations of dual-isotope BMM (SIAR and Simmr) and other partitioning models because of the accuracy in the analysis of covariates and the ability of the model to include source-specific uncertainties and discrimination factors (Stock et al., 2018; Wang et al., 2017). We partitioned potential water sources for five different scenarios using a combination of single and dual isotope approaches and different potential sources: scenario 1- single isotope δ18O two sources 5 cm and 35 cm depth; scenario 2-single-isotope δ2H two sources 5 cm and 35 cm depth; scenario 3- dual-isotope two sources 5 cm and 35 cm depth; scenario 4- dual isotope three sources 5 cm, 35 cm and 45-100 cm depth; scenario 5 – dual isotope three sources 5 cm, 35-100
cm and groundwater; and scenario 6 – dual isotope four sources 5 cm, 35 cm, 45-100 cm and groundwater. In scenarios using deep soil water (35-100 cm depths), the isotopic composition was calculated as a weighted average between seasonally collected soil water from depth 35 and average soil water at depths collected in 10 cm intervals during the early growing season (n=38 per season). There were no source concentration dependencies, and the discrimination was set to zero for both isotopes in the analysis. The run length of the Markov chain Monte Carlo (MCMC) was set to ‘normal’ (chain length = 100,000; burn =50,000; thin = 50; chains = 3). The Gelman-Rubin and Geweke diagnostic tests included in the model package were used to determine convergence (Gelman-Rubin score < 1.01). Scenarios that did not converge were run again with a longer runtime (chain length: 300,000; burn: 200,000; thin: 100; chains = 3). No priors were used, so each water source was considered equally (α = 1).

3. Results

3.1. Meteorological droughts

The ambient temperature peaked in the moderately thinned plot (T1) on June 29th with a maximum temperature of 36.3°C in an abnormally hot and dry summer. Relative humidity (rH) and subsequently vapor pressure deficit (VPD) recorded in T1 showed the most variability and highest evaporative capacity during July. Atmospheric water vapor was higher in late September and October when precipitation was more frequent, and the watershed began to exhibit traits of hydrologic recovery. One indication of increased water availability was an increase soil moisture at 5 cm and 35 cm depths and more groundwater recharge in October.

Rainfall events recorded at a nearby long-term research station between June to October from 1997-2008 represented approximately 30.1% of annual precipitation (Winkler et al., 2021). Over the 2021 study period, there was 147.8 mm of rainfall, while the mean summer rainfall from 1997 to 2008 was 232.5 mm, and most of the rainfall occurred in the early growing season. SPI and SPEI were significantly lower in 2021 than the mean historical range (Figure 2). Although there was precipitation and the beginning of hydraulic recovery in October, drought conditions persisted. Drought conditions of the study site reflected the drought conditions of the region as reported by the Agriculture and Agri-Food Canada from June to August 2021 in moving from severe (level 2 drought) to exceptional (level 4) before recovering in September (Canada, 2014: Figure 2 From left to right: dryness index (monthly PET using the Thornthwaite method divided by mean monthly precipitation), standard precipitation index (SPI) with a 3-month period, and standardized precipitation evapotranspiration index (SPEI) with a 3-month period.
3.2. Water Stable Isotopes

The biplot of sample isotopic composition shows the distribution and effect of fractionation on source water isotope ratios where the meteoric water line of samples collected during the 2021 field season produced a slope and intercept of 5.79 and -28.64 ($R^2=0.89$), respectively; the slope was less steep than the one reported by Wassenaar et al. (2011) (OMWL) while the intercept was slightly more negative (Figure 3). Precipitation samples collected during the field season fell along the OMWL (Wassenaar et al., 2011). The $\delta^{2}H$ and $\delta^{18}O$ of the June 11th rainfall event were -$127.5‰$ and -$13.03‰$, respectively. The September rainfall event was much more enriched with a $\delta^{2}H$ of -38.4‰ and $\delta^{18}O$ of -2.89 (Figure 3). The snowfall collected on October 7th more closely resembled the lighter, colder, June precipitation event.

![Figure 3 Isoscape biplot of $\delta^{18}O$ and $\delta^{2}H$ including precipitation, xylem, soil, stream, and groundwater from the 241-creek watershed thinning treatments during the 2021 growing season compared to the global meteoric waterline (GMWL), and local meteoric waterline for the Okanagan (OMWL) produced by Wassenaar et al. (2011).](https://agriculture.canada.ca/en/agricultural-production/weather/canadian-drought-monitor/drought-analysis).

3.2.1. Soil Moisture and Seasonal Water Composition
Soil moisture probes and percent soil water content from samples collected for isotopic analysis were compared between treatments and deployment depths. Water content of soil samples was highest in June (21.5% at 5 cm and 21.6% at 35 cm) because of high snow melt and early spring precipitation, while soils were driest in September (6.32% at 5 cm and 6.19% at 35 cm). Continuous soil moisture measurements showed that soil water began to increase in mid-September as precipitation became more frequent, daily solar radiation decreased, and water percolated into deeper soil layers. There were significant differences in the continuously measured soil moisture by depths, treatments, and month, respectively (5-35 cm) (Depth: F-value=3545.9, p <2e-16***). (Treatment: F-value = 1883.3, p<2e-16***). (Month: F-value=3359.8, p < 2e-16***), but soil water content of samples for isotopic analysis only varied significantly by month (August – October) (F-value = 22, p<5.4e-9***).

Soil isotopic results were broken into two datasets to analyze the variation in isotopic composition over time and between treatments, and then a profile of isotopic variance with depth was constructed. Soil water δ²H and δ¹⁸O varied significantly by depth (δ²H: p=2.57e-6***; δ¹⁸O: p = 2.45e-7***). δ²H significantly varied monthly except between July and September and September to October. δ¹⁸O also had significant change in water stable isotope composition by month except when directly comparing July to October and September to October, then there was no significant change in soil isotopic composition. Despite variability in continuous soil moisture by the treatments, there were no statistically significant distinctions in the isotopic composition δ²H or δ¹⁸O of soil water at either depth. Soil δ¹⁸O in June was -16.8±2.57‰, and δ²H was -136.7±13.6‰ at 5 cm; at 35 cm depth, δ¹⁸O was -19.2±1.52‰, and δ²H was -149.2±9.6‰. Both δ¹⁸O and δ²H increased more during the growing season at 5 cm depth and with more variability (Figure 4). In October, δ¹⁸O at the 5 cm depth decreased to -11.4±2.58‰, but δ¹⁸O at 35 cm as well as δ²H at 5 and 35 cm remained enriched at -15.8±2.02‰, -101.1±12.4‰, and -129.4±18.8‰, respectively. These results suggest that soil isotopic composition follows trends in precipitation samples, being most enriched in September, while the precipitation samples collected in June and October were much more depleted. Shallow soil water (depth 5 cm) varied more throughout the study than deeper soil water. In October, δ¹⁸O in shallow soils began decreasing again, indicating the addition of less enrichment as water availability began to increase.

Figure 4 Soil water δ²H (top) and δ¹⁸O (bottom) at 5 (left) and 35 cm (right) depths collected repeatedly over the growing season from each treatment and block.
Both δ2H and δ18O were higher in the shallow soils than deeper in the profile (Figure 4A and
4C). While there were significant differences in the δ2H and δ18O of soil water by month (δ2H:
p=2.72e-5**; δ18O: p =1.5e-5**), there was no significant difference between treatments.

Table 1 Depth profile of moisture content, δ18O, and δ2H including the mean and standard deviation across C, T1,
and T2 in Block 2 as well as groundwater (GW) samples collected at the end of the growing season.

<table>
<thead>
<tr>
<th>Depth</th>
<th>Treatment</th>
<th>Mean δ18O</th>
<th>Mean δ2H</th>
<th>SMC (%)</th>
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<tbody>
<tr>
<td>5</td>
<td>C</td>
<td>-17.23</td>
<td>-141.9</td>
<td>6.89</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>-10</td>
<td>-110.5</td>
<td>12.16</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>-9.66</td>
<td>-107.84</td>
<td>11.25</td>
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<td>C</td>
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<td>4.96</td>
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<tr>
<td></td>
<td>T2</td>
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<td>Groundwater</td>
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<td>-127.3</td>
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</table>
From the isotopic soil profile, there were three significant groupings of isotopic composition (p<0.05): shallow soil water (5-20cm), deep soil water (35-100cm), and groundwater. Mean groundwater collected at the end of the growing season most closely resembled spring and fall snowfall events. The mean δ¹⁸O of groundwater was -16.82±0.34‰ which resembles that in the soil profile but mean δ²H was slightly more depleted than soil water (n=4) (Table 1). This fractionation may be due to interactions with bound soil water and soils as the water infiltrates through the vadose zone. One extreme outlier of B1C at the 20 cm depth was removed; the high δ²H and δ¹⁸O values were likely due to contamination or incomplete cryogenic distillation. The more negative values for both δ¹⁸O and δ²H with soil depth indicate that snow melt is the main source of water to the deep unsaturated zone and that enriched summer precipitation is not infiltrating deeper soil layers (Figure 5).

3.2.2. Isotopic Variability in Branch Xylem Water

There were no significant differences in both δ¹⁸O and δ²H of xylem water across blocks and thinning treatments; there was, however, significant variation over time (δ¹⁸O: F=24.8*; δ²H: F = 146.6*). More specifically, δ¹⁸O and δ²H of xylem water varied by month for all months collected except for between June and September and July and September (Figure 6). Because the isotopic composition of xylem water showed significant change over the growing season but...
did not follow the same seasonal trends as soil water, the trees were likely changing their primary water source within the soil profile.

3.3. Partitioning xylem source water and seasonal fluxes using MixSIAR

With a “normal” runtime (chain length: 100,000; burn: 50,000; thin: 50; chains: 3), scenarios 1, 2 and 6 approached the Gelman-Rubin diagnostic, which indicates convergence when the variable is less than 1.05 (Table S2). Scenarios 4 and 6 were rerun with the run time set to “long” (chain length: 300,000; burn: 200,000; thin: 100; chains: 3). The Gelman-Rubin diagnostic variable for scenario 4 was 120, and scenario 6 was 17, meaning scenario 6 was closer to convergence (>1.05). Results of scenario 6 indicate that, in June, trees in each treatment acquired the most water from the 5 cm depth (C: 76%; T1: 77%; T2: 79%) (Figure 7). In July, shallow soil water was still the primary source for T1 and T2 at 47% and 61%, but C had 55% water from 45-100 cm deep and only 33% from 5 cm below the surface. By September, all treatments acquired less than 15% of tree water from shallow soil. Lodgepole pine water use in treatments 1 and 2 was composed of approximately 48% and 54% from around 35 cm, and 72% of water in control stand trees was from 35-100 cm. By October, although SPEI results indicate more moisture and less evaporative demand, scenario six indicated that all three treatments had most water uptake from below 45 cm in the soil profile (Figure 7). Results of the MixSIAR model support findings of branch water stable isotope trends over the growing season where the branch water started with a mean $\delta^{18}O$ and $\delta^2H$ of -16.9±0.89‰ and -134.37±3.8‰ in June and was slightly more enriched in July. There was a shift to a more depleted source in September. And, Lodgepole pine water was the most enriched with heavy isotopes in October, like shallow soil water, with a mean $\delta^{18}O$ and $\delta^2H$ of -12.9±1.76‰ and -103.77±7.0‰, respectively.

4. Discussion

4.1. Seasonal variability in soil water
Soil water showed mixed gradient of older, more depleted, water molecules deeper in the profile indicating that deep soil water mainly originates from spring snowmelt and that low intensity and less frequent summer precipitation events are evaporated out of the shallow soil layers before they can recharge the unsaturated zone. The muted enrichment of $\delta^{18}O$ around 35 cm depth in the soil indicates a mixing of the left-over enriched summer precipitation with older and lighter water. Our results did not indicate that differences in soil exposure canopy coverage were effective enough to significantly change the isotopic composition of soil water across treatments.

4.2. Seasonal lodgepole pine water use

Literature utilizing stable water isotopic analysis to determine plant preferential water uptake in arid regions indicates that vegetation can utilize precipitation despite the temporal origin (Andrews et al., 2012; Brinkmann et al., 2019; Ehleringer et al., 1991). Seasonal water availability depends on precipitation, soil water holding capacity and drainage, and evaporative loss (Gibson & Edwards, 2002; Kleine et al., 2020; Stumpf et al., 2018). Based on the seasonal shift in the isotopic composition of soil water 5 cm below the surface showed more enrichment over the growing season than around 35 cm below the surface due to more evaporative fractionation of the soil surface and a lack of heavy rainfall to drive precipitation deeper into the soil profile. However, variability in branch isotopic composition did not follow the same trends. Our results indicate that lodgepole pines access water from multiple depths in the soil profile. Regardless of depth and forest density, spring snowmelt is the main source for lodgepole pines as it infiltrates through the vadose zone.

The MixSIAR isotopic partitioning model results from each of the six scenarios also indicated a seasonal shift in uptake source. At the beginning of the growing season, when snow meltwater is more available at shallow depths and beginning to infiltrate through the soils, lodgepole pines obtain most of their water likely from snow melt in shallow soils with small contributions from other potential sources (< 25% of June water uptake in all treatments). The mean $\delta^{18}O$ and $\delta^2H$ of branch water from each treatment in September was more depleted than in July and a larger proportion of tree water was from 35-100 cm deep in the soil profile as shallow soils were dry from a lack of rainfall and surface soil evaporation. Local monitoring close to the study site indicated that the depth to groundwater stayed 6.5 m below the surface from August through the end of the study period. The continued use of deep soil water even during rewetting in late September and October suggests that the drought conditions may have led to fine root mortality or some other mechanistic restriction in the use of shallow soil water late in the growing season.

Our results indicate that lodgepole pine, like other pine species in arid regions, is flexible in its ability to access deep soil water and can change its depth to water uptake over time (Brinkmann et al., 2018; Grossiord et al., 2017; Kerhoulas et al., 2013; Kleine et al., 2020; Moreno-Gutiérrez et al., 2011; Simonin et al., 2006; Sohn et al., 2014; Wang et al., 2021). Our results of depth to water uptake by lodgepole pine support the reports of lodgepole pine’s seasonal shift in depth to water uptake in Alberta (Andrews et al., 2012). Tree species native to arid regions exhibit a variety of adaptations to long-term drought stress and decreased water availability in the soil profile such as deep tap roots, access to the water table, utilizing bound and mobile soil water, fine root mortality, and hydraulic redistribution in ecosystems with low water holding capacity (Amin et al., 2020; Brinkmann et al., 2018; Grossiord et al., 2017; Kerhoulas et al., 2013; Kleine et al., 2020; Sohn et al., 2014; Wang et al., 2021).
et al., 2020; Langs et al., 2020; Meinzer et al., 2007b; Prieto et al., 2012; Sohn et al., 2016; J. Wang et al., 2017, p. 201).

However, the literature is inconsistent across different biogeoclimatic regions and species regarding the effects of thinning on inter-tree competition or altered depth to water uptake with tree density (Kerhoulas et al., 2013; Moreno-Gutiérrez et al., 2011; Sohn et al., 2016; Wang et al., 2021). Our findings that there is no significant impact of forest thinning on depth to water uptake. Despite stem density, seasonal shifts in depth to water uptake support results of a study on the impacts of thinning intensity on 60-year-old Pinus halepensis Mill. in a semi-arid region of Spain which concluded that forest thinning reduced competition for water resources but did not alter water uptake patterns (Moreno-Gutiérrez et al., 2011). Another study on the impact of thinning Pinus ponderosa Dougl. on depth to water uptake concluded that water was consistently more isotopically enriched in low-density stands potentially due to prolonged evaporative fractionation in the soil profile, or that understory vegetation utilized more shallow water sources (Kerhoulas et al., 2013). The impact of forest thinning on stand and understory water use is highly variable and dependent on understory growth, canopy structure, water availability, when forest thinning is implemented, and the time since stem removal (Kerhoulas et al., 2013; Moreno-Gutiérrez et al., 2011; Sohn et al., 2016). More research is needed to discern if lodgepole pine relies more on mobile or bound soil water, the extent of lodgepole pine rooting zones, what biogeoclimatic factors cause seasonal shifts in water uptake, and if severe seasonal drought has a lasting effect on water uptake strategies during hydrologic recovery (Simonin et al., 2007; Vargas et al., 2017).

4.3. Impacts of the drought and implications for future climate conditions

The 2021 growing season was an abnormally hot and dry period for the interior of British Columbia with severe to exceptional drought conditions. Wang et al. (2019) found that thinning improved water-use efficiency, drought tolerance, and drought recovery by decreasing stand density and improving carbon storage. Our results support the finding that lodgepole pine trees can adjust to prolonged water scarcity, and that over-populated stands may be more resilient than the literature initially indicated. In fact, drought conditions over the study period likely intensified the change in xylem water isotopic composition over the growing season. However, the scope of this study did not include pre-drought seasonal water use patterns nor the impact of forest density on depth to water uptake during drought recovery. Because lodgepole pine depth to water uptake changes during prolonged dry growing season conditions, the trees are more reliant on winter snowpack and spring infiltration to recharge deeper source water below the evaporative front. One experiment on juniper (Juniperus monosperma (Engelm.) Sarg.) and pinion pine (Pinus edulis Engelm.) investigated the simultaneous stress of increased heat and decreased precipitation on depth to water uptake and found that extreme temperatures and decreased precipitation lead to less reversible embolism and more root death in surface soil levels preventing trees from accessing shallow water sources if precipitation becomes more available late in the growing season (Grossiord et al., 2017). It is becoming more imperative to understand the climatic drivers of lodgepole pine water use and access as mean annual temperatures continue to rise, the seasonal frequency and intensity of precipitation change, and drought conditions become more severe. This study indicates that severe seasonal dryness pushes lodgepole pines to rely more on snowmelt while losing function in shallow roots. Decreased...
winter snowpack could lead to water scarcity in the late growing season if lodgepole pines are unable to access water during the rewetting period post-summer drought.

5.1 Conclusions

Lodgepole pine, across all treatments, was able to shift from shallow soil water at the beginning of the growing season to deeper soil water as drought conditions progressed. The quick draining and sun-exposed soils do not retain small summer precipitation events, and as a result, either due to changes in water availability or limitations in rooting function, lodgepole pines shift to a more readily available source in the soil profile (Aranda et al., 2012; Prieto et al., 2012). Our findings support the literature that lodgepole pines are a drought-tolerant species with dimorphic rooting systems making them more advantageous in their ability access water from varying depths in the soil layer depending on water availability (Andrews et al., 2012; Liu et al., 2011). Despite the ecological plasticity under extreme heat and low summer precipitation conditions, there was no significant difference in depth to water use between the over-populated plots and thinned ones. Future climate projections indicate hotter growing seasons and less precipitation (Allen et al., 2010). Further investigation is needed to discern how lodgepole pines, under different stand densities, use water during prolonged drought and drought recovery periods (Grossiord et al., 2017; Navarro-Cerrillo et al., 2019; Simonin et al., 2007; Sohn et al., 2016). However, from our findings, during prolonged growing season, stand density does not alter tree depth to water uptake, nor seasonal shifts in water sources. Lodgepole pines indicate a strong level of drought tolerance and ability to access water under extreme heat conditions. If summer precipitation decreases, lodgepole pines have alternative strategies to access deeper soil water from spring snowmelt in the interior of British Columbia. However, if snowpack and spring snowmelt begin to decrease, lodgepole pines will need to acclimate to these hydrological shifts.

**Code and Data Availability:**

The codes of the data analysis and plotting are available at [https://github.com/emory-ce/LodgepolePineWaterUseStrategies2021](https://github.com/emory-ce/LodgepolePineWaterUseStrategies2021) and are available upon request (ece58@nau.edu)

**Author Contributions:**

EE conceived the idea as a part of their Master’s research with AW, and performed the extractions with RG. Analysis was primarily conducted by EE with guidance from AW and RG. All authors contributed to the manuscript.

**Competing Interests:**

None of the authors have competing interests.
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