~~Unexpected water uptake under drought conditions and thinning treatments in young and overstocked lodgepole pine (*Pinus contorta*) forests~~Seasonal shifts in depth to water uptake by young thinned and overstocked lodgepole pine (*Pinus contorta*) forests under drought conditions in the Okanagan Valley, British Columbia, Canada

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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~~ a focal point ~~of~~ for ~~many conversations~~ maintaining forest health. Forest regeneration after ~~deforestation~~ forest ~~lost~~ loss or disturbance can lead to over-~~populated~~ stocked juvenile stands with high water demands and low water-~~-~~use efficiency. Forest thinning is a common practice with the goal of improvingimproving~~improv~~ing~~es~~ 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 stand~~over-population~~stand density on seasonal variation in depth to water uptake nor the magnitude of the effect of growing season drought conditions on water availability..~~, and e~~Existing reports are highly variable by climatic region, species, and thinning intensity. In this study, stable isotope ratios of ~~hydrogen~~ deuterium (δ^2H) and oxygen (δ^{18}O) in water collected from soil varying depths and from ~~twigs~~ branches 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~~ were 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 under drought conditions the lodgepole pine trees shifted their depth to water uptake through~~depending~~ through~~out~~ the growing season (June to October),) ~~on water availability~~, ~~under drought conditions and~~ to rely more heavily on older precipitation events that percolatedpercolated~~percolate~~ through the soil profile when shallow soil water ~~becomes~~ became less accessible. Decreased forest density subsequent to forest thinning did not cause a significant difference in isotopic composition of branch water, but did cause changes in the timing and relative proportion of water utilized from different depths. Thinned lodgepole pines stands were able to maintain water uptake from 35 cm below the soil profile whereas the overstocked stands relied on a larger proportion of deep soil water and groundwater towards the end of the growing season.~~Interestingly, to 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~~ did not shift back from deep water sources ~~when~~ to shallow soil water when soil water availability increased following

Commented [EE1]: R2 - title of the manuscript could be a bit more specific, so that it matches the story of abstract, and the main findings of the manuscript. For instance, it's not 100% clear what "unexpected" means throughout the manuscript and whether it's related to the effect of soil water availability or thinning

Commented [EE2]: General Comment: R2 - d2H and d18O can be higher or lower, but not depleted or enriched. However, e.g., soil water can be 18O-depleted or 2H-enriched.

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precipitation events at the end of the growing season. ~~due to increased precipitation events~~. ~~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

Commented [EE4]: R1- They cannot shift from deep water sources when shallow water becomes more available at the end of the growing season? Or perhaps they don't need to shift to shallow water because deep water is plentiful and sufficient to meet their transpiration demand (which is presumably lower in October than in midsummer)? Or perhaps these late-season rain events are insufficient to recharge the topsoil layer meaningfully (i.e. the soil matric potential in upper layers may still remain lower than that in deeper layers in October despite these rain events)?

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). Overpopulated ~~Regenerating~~ regenerating stands can add further stress on ecosystems; for example, light competition in dense juvenile stands increases stand water demands by driving vertical growth and canopycanopy~~stand leaf area~~canopy cover (Liu et al., 2011a). To mitigate this stress, management strategies such as systemic thinning of high-density juvenile stands have been shown to promotepromote~~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~~ 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, papers~~literature~~ reporting the effects of this management strategy often focus~~focuses~~ on changes in carbon storage, tree growth, transpiration, and ~~water~~ 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 for vegetation water uptake and ~~their shifting~~ shifts in depth to water uptake in association with thinning treatments in overstocked ~~naturally regenerating~~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; Szymczak et al., 2020; Wang et al., 2017; Warren et al., 2005). In ~~water~~ water-limited regions such as arid and semi-arid landscapeslandscapes~~regions~~ landscapes~~where water is the limiting factor~~, some species have adapted to derive water from various depths over time depending on seasonal water variability, ~~and~~ indicating ~~have~~ higher ecological plasticity and drought tolerance (Langs et al., 2020; Wang et al., 2017). Understanding where in the soil profile plants ~~use~~ obtain 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 duringduring~~under future climate conditions~~ ~~and~~during shifts in water availability in arid regions and ~~in~~ under future climate conditions (Evaristo et al., 2015; Prieto et al., 2012; Sohn et al., 2016). The implications of depth to water uptake and seasonal changes in water utilization, in conjunction with ~~water~~ water-use efficiency, can emphasize the importance of the timing and volume of precipitation events and primary contributors to vegetation water use.

Stable isotope ratios can be used as powerful natural tracers to identify distinct water sources such as rainfall, ~~,~~ snow, and 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 soil water and ~~older~~ groundwater and ~~depleted isotopes~~ creating individualized water isotopic signatures throughout the soil profile (Andrews & Science, 2009; Brinkmann et al., 2018; Dawson & Pate, 1996; Sprenger et al., 2017; Stumpp et al., 2018). The isotopic composition of plant water can correspondcorrespond~~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 usedused~~d~~~~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 soil layers, and an adventitious ~~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 main components~~,~~; namely, lateral roots to exploit~~increase their~~exploit soil near the surface, ~~area~~ and, in species with dimorphic root systems, ~~tap root~~ sinker roots or a well-developed tap root- to reach deeper soil water or groundwater when surface water is limited. Species with~~Some s~~Species~~species have also adapted to have~~dimorphic ~~dimorphic~~ rooting dimorphic rooting systems cancan~~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). Wang et al. (2019) studied the short-term effects of thinning overstocked juvenile (16- year-old) lodgepole pine stands in the Upper Penticton Creek Watershed, British Columbia, Canada, and found a significant positive relationship between growth and water use from decreased stand density and that the heavily thinned treatments showed the most drought resistance. Andrews et al. (2012) compared water uptake strategies between ~~One study, comparing~~ Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and ~~lodgepole~~ lodgepole pine in southern Alberta, and found that lodgepole pines are able to minimize seasonal variations in stem water potential and that tap roots are deep enough to access groundwater. These finding~~(Andrews et al., 2012). This~~ These ~~finding is~~ are consistent with other literature reporting that decreased stem density can improve ~~lodgepole~~ pinelodgepole ~~pines~~ ~~water~~ water-use efficiency and that conifer treeslodgepole 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 therefore 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 ~~will~~ build on the research from Wang et al. (2019) which looked at the effects of thinning on ~~water~~ water-use efficiency during a droughtdroughtdrough and non-drought year

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Commented [RG6]: Access to bound water is a separate matter that should be broken out and developed within a separate sentence.

Commented [RG7]: Meinzer et al 2007 studied Douglas-fir and hemlock, not lodgepole pine. Also, note that this paper is listed twice in the literature cited, once as 2007a and then again as 2007b.
Similarly, Warren et al studied Ponderosa pine and Douglas-fir, but not lodgepole pine.
Did either one of these studies consider bound water? I don't get that impression.

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Commented [EE8]: Insert results of Wang et al 2019 on water use efficiency

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by analyzinganalyzinganalyzing~~used~~ the stable isotope ratios (δ^2H and δ^{18}O) of soil and xylem water to evaluate ~~how~~ at what depths ~~overpopulated~~ overstocked ~~stands~~ and thinned stands ~~use~~ access water over ~~the~~ a 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 trees~~lodgepole pines~~trees to utilize deeper sources of water as the ~~growing~~ season progressed. ~~progresses~~progressed~~. Prolonged aridity was expected to push drive trees to accessdepend on~~access ~~different water sources deeper in the soil profile towards the end of the growing season.~~ We also ~~hypothesis~~ hypothesized that decreased stand density (thinning) ~~will~~ would increase shallow soil evaporation due to decreased canopy cover, ~~and~~ but also decrease competitivespatial limitations in tree rooting zones so that at lower densities ~~lodgepole pine~~ trees could better~~access mid-level soil water sooner, but are able to~~ maintain mid-level soil water uptake. due to a more expansive rooting network. ~~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.

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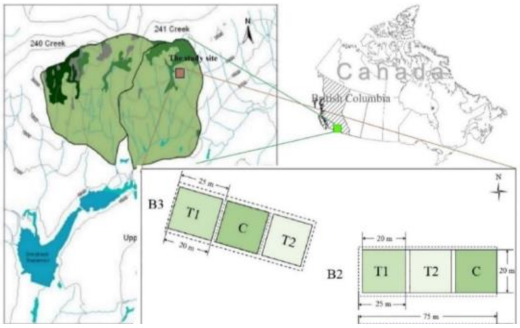
Commented [EE11]: R3 - why would they be limited in depth? Would not the horizontal extend be limited in an overstocked forest plot?

2. Methods

2.1. Study site~~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~~Luvisolic~~ soils were formed from granite; the texture is coarsecoarse~~co~~a~~urse~~ 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).



~~*Figure 1 Watershed location and treatment plots of moderately thinned (T1), heavily thinned (T2), and the controlled (C) over-populated stands across the three replicate blocks (Wang et al., 2019)*~~

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 (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~~ moderately 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 m^2 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). In 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. Climate and soil moisture monitoring

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 these~~this~~these data, 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).

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2.333~~2~~ Sample collection

We sampled three trees per treatment across the three blocks and three in ~~the~~ an adjacent mature plot south of the study site (n = 3333333~~30~~33) 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 (Table 1). 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~~ 10 mL glass tubes that were then ~~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.

Table 1 Overview of the branch, soil, and precipitation samples collected over the four sampling periods during the 2021 growing season with an additional campaign to collect groundwater and stream water.

Sampling Period			1	2	3	NA	4
Sampling Date			June 11-12	July 21-22	September 10-11	October 1	October 9
Branches (n)			33	33	33	0	33
Sample Type	Soils	*5*	9	9	9	0	9
		20	0	6	0	0	0
		35	9	9	9	0	9
		40	0	6	0	0	0
		60	0	6	0	0	0
		80	0	6	0	0	0
		100	0	6	0	0	0
	Precipitation	Rain	1	0	1	0	0
		Snow	1	0	0	0	1
	Stream		0	0	0	8	0
	Groundwater		0	0	0	6	0

Soil samples were collected horizontally from 40 cm soil pits randomly dug ~~across~~ within each treatment plot at 5 and 35 cm depths from the surface from June to October of 2021. Large rocks were removed from the profile. We conducted a soil ribbon field tests to ensure that clay composition was less than 10% (soil ribbons were less than 20 mm in length). Soils were taken directly from the pit, then sealed in freezer seal bags and frozen until cryogenic distillation for water extraction. In JulyIn July~~In the middle of the field season~~July, 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. We assumed that the isotopic signature of soil water below 40 cm would be similar throughout the growing season and would be representative of deep soil water. Soil samples were stored in a freezer at -18˚C until cryogenically distilled.

Precipitation samples were collected ~~when available during~~ cumulatively over individual field collection days where precipitation was present (Table 1). Snow from a late spring event was

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Commented [EE14]: ADD Section 2.2. Meteorological Data and 2.3. Continuous soil moisture monitoring

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Commented [EE15]: R3- "in the middle of the growing season…" when is that exactly, please provide the dates to when you did things.

Commented [RG16]: When were the first pits dug? Before July presumably.

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Commented [EE18]: Indicate briefly how close the soil pits were to the "sample" trees? What was the exact sampling period for the vertical 1 m pit samples. Where the soil sample taken directly after digging the pits?

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collected on June 11th to represent snow water isotopic composition during the sublimation and melt period of early 2021. Another. ~~and another~~ snow event was collected on October 11th during an active snowfall. A rain event was collected on September 10th. Groundwater and stream samples were collected from the creek 241 watershed ininin~~at the end of the growing season and~~in early October 2021 at the beginning of the seasonal hydraulic recovery period (Table 1). 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, 10 mL glass test tubes were rinsed with ground water three times before being filled. Precipitation, groundwater and stream samples were collected into 10 mL glass test tubes, sealed with ~~parafilm~~ Parafilm® and foil, and stored in a fridge at 4ºC.

2.~~3~~ 4 Cryogenic extraction and isotopic analysis

Before extraction, branch ~~sampled~~ samples remained sealed and were weighed in the glass test tubes used for field collection. Branches remained in the test tubes until cryogenic distillation was complete to ensure that any liquid water lost from the branch to the test tube was contained in the extract. Soils s~~s~~amples were mixed in the Ziploc®Ziplock bag, ~~thawed, and~~ weighed, and transferred to a glass round bottom flask. 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). Soil sample size for extractionextractionextract was roughly determined based on the expected moisture of the frozen sample and soil moisture readings from continuous measurements in the field. 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). As reviewed by Allen & Kirchner (2022), the cryogenic vacuum distillation of water from plant tissuesAll branch and soils can bias measurements of δ^2H, the amount depending on species and soil type. In contrast, bias in δ^{18}O values is close to zero (Allen & Kirchner, 2022). Reported biases in δ^2H average about -6.1‰ for xylem water and -4‰ for watersamples were extracted from sandy soils, such as the soils sampled here, which are of using the same cryogenic distillation unit, so extraction bias is similar magnitude. Furthermore, allamongst samples. Bias in hydrogen isotopic composition with cryogenic distillation is found to be approximately 4‰ (Allen & Kirchner, 2022). All sources we identified hadreported to have a difference in δ^2H greater than 4‰ (with the minimum distance being 14‰ between groundwater samples and deep soil water), minimizing any major effects on partitioning calculations.).

The volume of branchb~~B~~ranch water extractedextractedextract ranged from 1 to 3 mL depending on the size of the branch sample. Total extracted water varied dependent on the mass of the initial sample. The volume of soil water extract ranged from 1mL to 7 mL depending on the size

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Commented [RG21]: Just last fall?

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Commented [EE22]: R3 - "Precipitation samples were collected when available during field collection days." What does this mean? Is this then a cumulated precipitation sample? "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." When was this? Please provide the dates. Also see above, fill a table with this information so that the reader knows when you have which data available. From how this reads you have three snow/rain samples and samples groundwater once?

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Commented [EE23]: R2 - The process of sampling handling of soil and plant samples between sampling in the field and before CVD extraction is not clear. For instance, were the samples transferred into a new tube or glass vial before CVD? What is a test tube?

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Commented [EE24]: R2 - CVD extraction can affect isotope values of the extracted water (Chen et al, 2020, PNAS, Barbeta et al 2022, New Phytologist). Given the growing number of papers stating a bias for d2H values of woody material, I was wondering whether such a bias could affect the conclusion of the manuscript. This is because d2H values are used in the MixSIAR model to estimate root wat ...

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of the sample prepared for extraction. 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 ± standard deviation.

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 ^{18}O and ^{2}H analysis using headspace gas equilibration on a GasBench-II interfaced to a Delta Plus XL isotope-ratio mass spectrometerspectrometer~~device~~ (Thermo-Finnigan, Bremen~~Breman~~Bremen, Germany) normalized to a range of secondary reference waters calibrated against three IAEA standard waters. waterss. ~~for ^{18}O and ^{2}H analysis.~~ Precision was less than or equal to 2.0‰ for δ^2H and 0.2‰ for δ^{18}O. Results were returned in the "delta" notation expressing the isotopic composition of each sample as a ratio in parts per thousand,~~million~~ thousand ~~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 water line (GMWL) along with a local meteoric water line for the Okanagan Valley (OMWL) (δ^2H = 6.6 (δ^{18}O) - ~~)~~22.7) and local evaporative line (LEL) (δ^2H = 5 (δ^{18}O) - 48.4) calculated for the Okanagan Valley by Wassenaar et al. (2011). The LEL is a linear regression that indicates the departure of water sources from the OMWL to indicate the degree of evaporative processes fractionating the isotopic composition of water sources or variance in the isotopic composition of seasonal precipitation events.

One extreme outlier of B1C at the 20 cm depth was removed before analysis; the high δ^2H and δ^{18}O values were likely due to contamination or incomplete cryogenic distillation. 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 δ^2H and δ^{18}O in branches, soils and groundwater to determine if changes in lodgepole pine uptake patterns occurredoccurred~~occur~~ over time, if soil signatures ~~vary~~ varied between different depths (0-100 cm and groundwater) and densities, and if thinning juvenile stands ~~changes~~ changed 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~~ ~~p~~previous~~previous~~ iterations of the 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

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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 $\delta^{18}O$ two sources 5 cm and 35 cm depth; scenario 2 – ~~-~~single-isotope $\delta^{2}H$ 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 202202~~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).

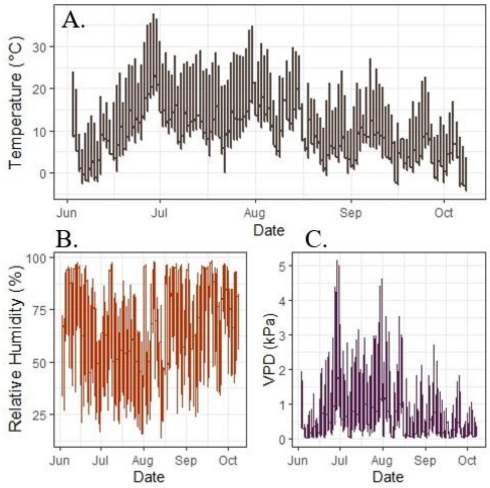

Figure 2 15-minute measurements of A. atmospheric temperature (˚C), B. Relative humidity (%), and C. vapor pressure deficit (VPD) (kPa).

Figure 5

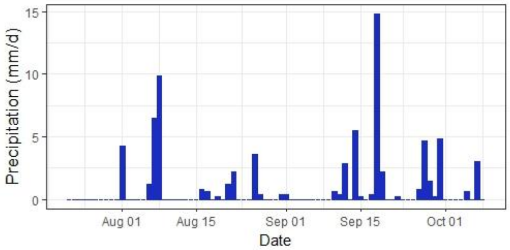

Figure 4 Rainfall (mm/d) from July 22 to October 8, 2021.

3. Results

3.1. ~~Meteorological droughts~~ Climate and soil water content

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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 (Figure 2). 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 (Figure 3). One indication of increased water availability was increased~~an~~ increased~~increase~~ soil moisture at 5 cm and 35 cm depths and more groundwater recharge in October (Figure 4). There was 17.5 mm of precipitation from September 16th to 18th that infiltrated to at least 35 cm below the soil surface along with subsequent rainfall events that likely infiltrated past the 35 cm sample depth changing the isotopic composition of deep soil water from what was measured during the deep pit sampling in July.

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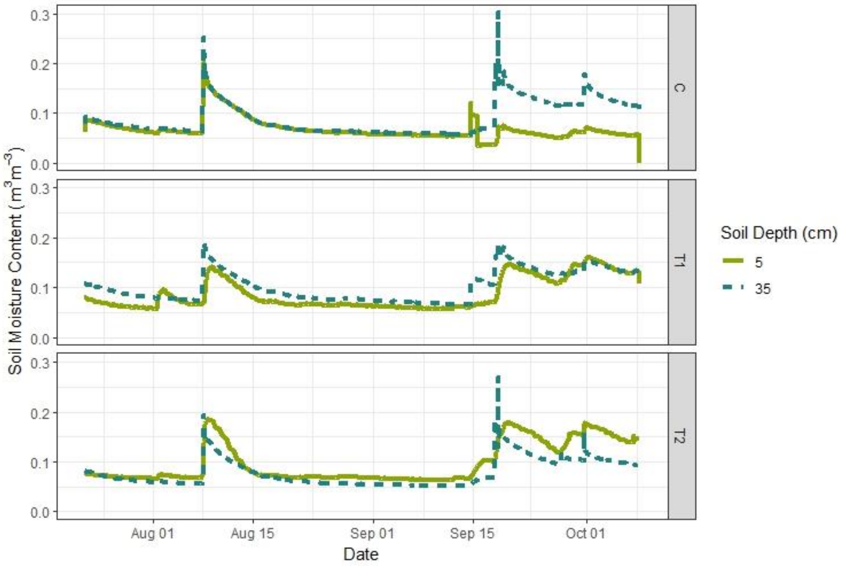

Figure 7 Average in-situ continuous measurements (15-minute interval) of soil water content (m^3/m^3) from the control, moderately thinned, and heavily thinned stands in Block 1.

~~fFigure 3 Rainfall (mm/d) from July 22 to October 8, 2021.~~

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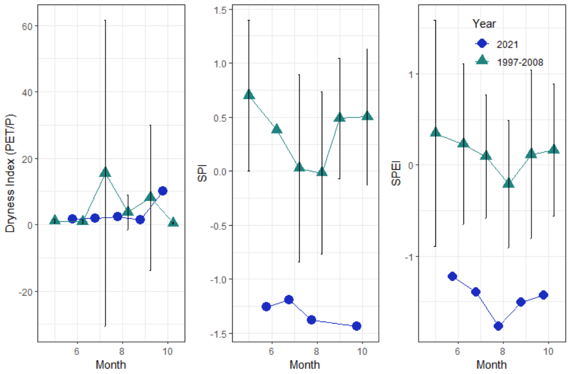
Rainfall events recorded at a nearby long-term research station between June to October from 1997-2008 represented approximately 30.1% of annual precipitation (Winkler et al., 2021). Over the 2021 study period, there was 147.8 mm of rainfall, while the mean summer rainfall from 1997 to 2008 was 232.5 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 5555~~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: https://agriculture.canada.ca/en/agricultural-production/weather/canadian-drought-monitor/drought-analysis).



~~*Figure 5 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.*~~

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3.2. Water stable isotopes~~Stable Isotopes~~

The biplot of sample isotopic composition shows the distribution and effect of isotopic 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 (R^2=0.89) and -28.64, respectively (R^2=0.89)~~. Field collected samples were compared to the Okanagan Meteoric Water Line (OMWL) (Wassenaar et al., 2011).).~~, respectively; the ~~The slopes for branch and soil water were~~ was~~were~~ ~~less steep than the ~~one reported by Wassenaar et al. (2011) (~~OMWL, and ~~) while, but ~~ ~~the intercept was slightly~~and the intercepts more negative, ~~for the OWML ~~indicating that ~~there is was higher effects of greater~~of evaporative fractionation contributed to the isotopic composition of these pools at the UPC. ~~that than the general multi-year trends trend for of the Okanagan Valley and that meteorological variables such as temperature or relative humidity are, on average, greater for the general Okanagan Valley region in comparison to UPC ~~(Figure 6666~~3~~). Soil samples seemed to follow the LEL produced by Wassenaar et al. (2011) for the region indicating similar evaporative fractionation effects. Branch water more closely following the OMWL than soils, ~~likely ~~suggesting that~~due to part of the samples using water in the soils~~

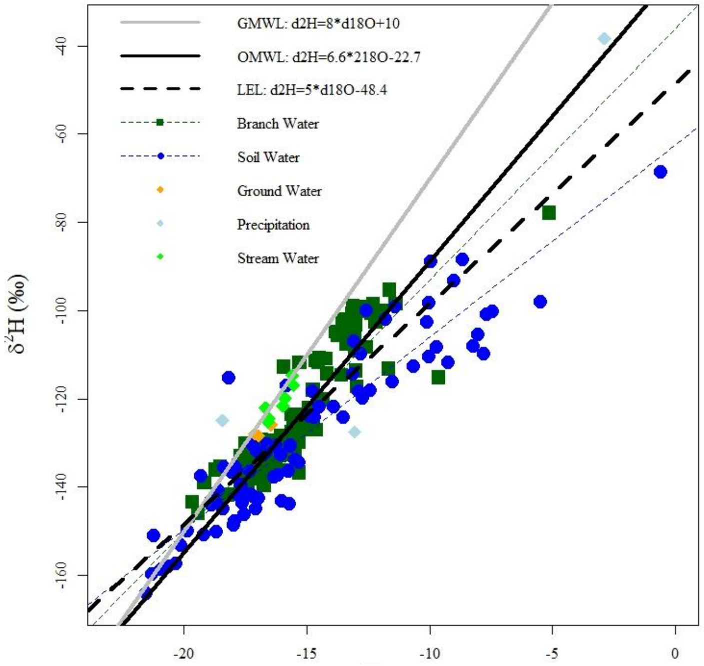

Figure 10 Biplot of δ^{18}O and δ^{2}H including all samples collected over the study period from branches, soils, streams, groundwater, and precipitation events outlined in Table 1 along with the global meteoric water line (GMWL), Okanagan Meteoric Water line (OMWL) and the Local Evaporative Line (LEL) developed by Wassnaar et al. (2011), and linear regressions for branch water and soil water.

season compared to the global meteoric waterline (GMWL), and local meteoric waterline for the Okanagan (OMWL) produced by Wassenaar et al. (2011).

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Commented [EE33]: R2- better explain data used for lines (slope and intercept), description of branch, soil, ground, and STREAM water, LEL represents?
ZOOM in/ make points bigger/ alter colors

Commented [RG34]: I've cut this way back because I don't think much of it is valid. In fact, what I've left also isn't 100% solid because the branch and soil water samples were collected in just one year, whereas the OMWL was presumably constructed over several years, and certainly did not include 2021, which may have yielded a different line because of different meteorological conditions. Still, you indicate later that precip events fell on the OMWL, so presumably we can accept it and can conclude that further evaporative fractionation contributed to the isotopic composition of the branch and soil water pools. As you note, this effect is likely to be depth dependent (i.e., we would not expect much departure from the OMWL for deep water acquired in the wet or winter seasons, at lower d18O and lower d2H values). This should manifest in some curvature in the branch and soil water lines, which I think I can see in Figure 6 (especially for soil). In other words, the soil values approach (and then pass) the LEL when they are high, but remain consistent with the OMWL when they are low. Not many branch samples depart from the OMWL, but a few do.

Note that extraction bias in H abut not O values (see below) can also cause the biplot slope to change, leading to the conclusion that there has been more evaporative fractionation than actually occurred. This concern is nicely detailed in Allen and Kirchner 2022.

~~that experienced evaporative fractionation, and the other~~that most samples consisted ofof~~using~~ water that was accessed from~~ are ~~deeper in the soil profile and had infiltrated past the evaporative front. Precipitation samples collected during the field season fell along the OMWL (Wassenaar et al., 2011). The δ^{2}H and δ^{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 δ^{2}H of -38.4‰ and δ^{18}O of -2.89 (Figure 666~~3~~). The snowfall collected on October 7th more closely resembled the lighter, colder, June precipitation event.

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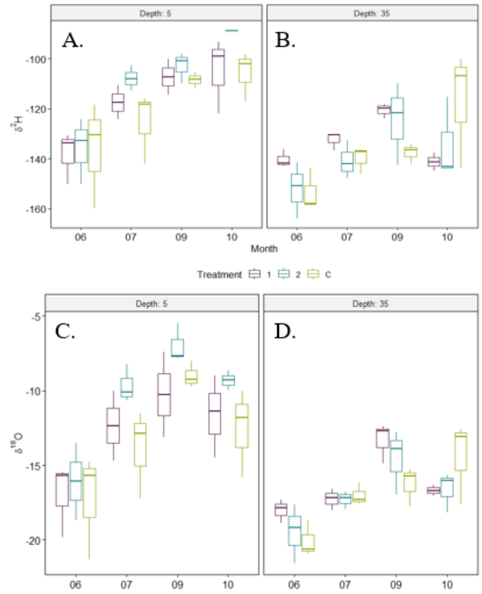

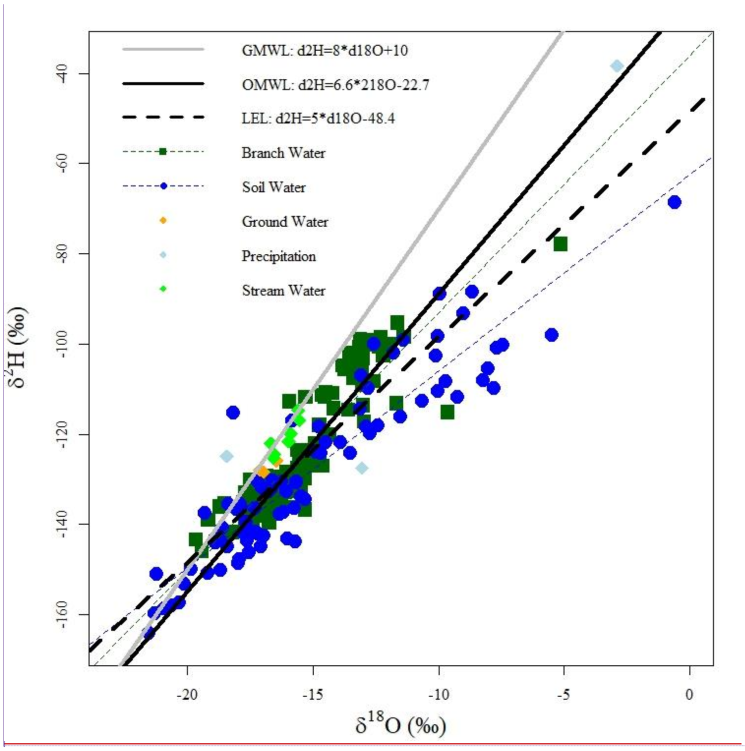

Commented [RG36]: For Fig 7 – The x-axis title (month) and legend are under panels A & B, but not C & D. They are obviously the same, but general convention would be to but these under C & D (and not under A & B).

3.2.1. Soil moisture~~Moisture~~ and seasonal water composition~~Seasonal Water Composition~~

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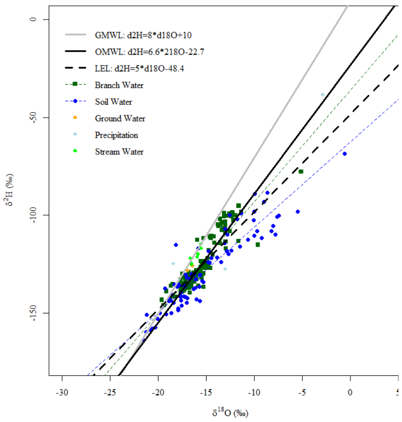

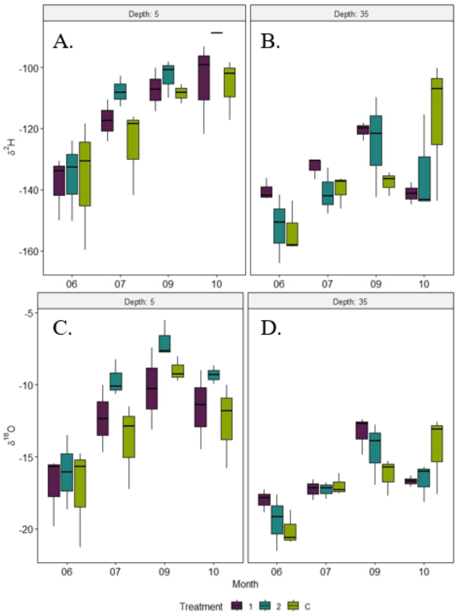

Figure 7 Soil water δ^2H (top) and δ^{18}O (bottom) at 5 (left) and 35 cm (right) depths collected repeatedly over the growing season from each treatment and block.

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***) (((~~***)~~(Figure 4), but soil water content of samples for isotopic analysis only varied significantly by month (August – October) (F= ~~value =~~ 22, p< ~~<~~5.4e-9***).

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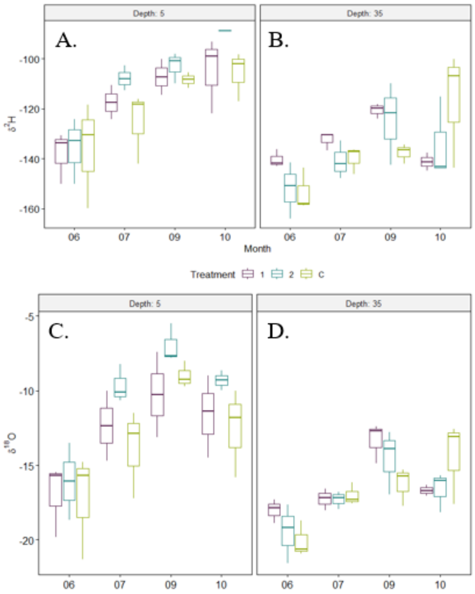
Soil isotopic results were broken into two datasets to analyze the variation in isotopic composition over time and between treatments, and then a profile of isotopic variance with depth was constructed. Soil water δ^{2}H and δ^{18}O varied significantly by depth (δ^{2}H: p=2.57e-6***; δ^{18}O: p =~~-~~2.45e-7***), being~~.~~ higher in the shallow soils than deeper in the profile (Figure 4A and 4C).).~~***).~~ δ^{2}H ~~significantly~~ varied significantly across monthsmonths~~ly~~ (p=2.72e-5**), but not not~~monthly except~~ between July and September and September ~~to~~ and October. δ^{18}O also varied~~had significant change in water stable isotope composition~~varied significantly ~~by~~ across months (p=1.5e-5**)~~month~~ except when directly comparing July to October and September to October.~~, then there was no significant change in soil isotopic composition.~~ Despite treatment differences ~~variability~~ in ~~continuous~~ soil moisture ~~by the treatments~~ (Figure 4),),~~,~~ there were no statistically significant treatment differencesdifferences~~distinctions~~ in the isotopic composition ~~δ^{2}H or δ^{18}O~~ of soil water at either depth. In June, the mean soil waterwater~~Soil~~ δ^{18}O at 5 cm ~~in June~~ was -16.8±2.57‰ while~~,‰, and~~ the δ^{2}H was -136.7±13.6‰;~~‰ at 5 cm;~~ at 35 cm~~ depth~~, the δ^{18}O was -19.2±1.52‰~~‰,~~ and δ^{2}H was -149.2±9.6‰. Both δ^{18}O and δ^{2}H increased more during the growing season at 5 cm ~~depth~~ than at 35 cm, and with more variability (Figure ~~7).4~~7 In September, δ^{18}O and δ^{2}H at 5 cm were -8.75‰ and -106.23 and at 35 cm were -14.71‰ and -127.64 respectively suggesting that soil isotopic composition nearer the soil surface follows trends in precipitation samples, being most enriched with O^{18}. By October, δ^{18}O and δ^{2}H at 5 cm reflected more recent precipitation events indicating that water availability in shallow soils began to increase..~~). In By October, δ^{18}O and δ^{2}H at the 5 cm depth decreased increased by 5.4‰ and 35.6‰ to -11.4±2.58‰ and -101.1±12.4‰, whereas‰, but δ^{18}O at 35 cm they were increased by 1.0‰ and 19.8‰ as well as δ^{2}H at 5 and 35 cm remained enriched at to -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 5cm) varied more throughout the study than deeper soil water. In October, δ^{18}O in shallow soils began decreasing again, indicating the addition of less enrichment as water availability began to increase.~~



~~Figure 74 Soil water δ^{2}H (top) and δ^{18}O (bottom) at 5 (left) and 35 cm (right) depths collected repeatedly over the growing season from each treatment and block.~~

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Commented [RG40]: I think it would be better to use September here, as that is the month when samples were most enriched. October data, at least for 18O at 5cm, appear to reflect some influence of renewed precipitation, as you note in the next couple of sentences.

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~~Both δ^2H and δ^{18}O were higher in the shallow soils than deeper in the profile (Figure 4A and 4C). While there were significant differences in the δ^2H and δ^{18}O of soil water by month (δ^2H: $p = 2.72e-5^{**}$; δ^{18}O: $p = 1.5e-5^{**}$), there was no significant difference between treatments.~~

~~Table 21 Depth profile of moisture content, δ^{18}O~~(‰)~~, and δ^2H~~(‰), and soil water content (SMC)(%) ~~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.~~

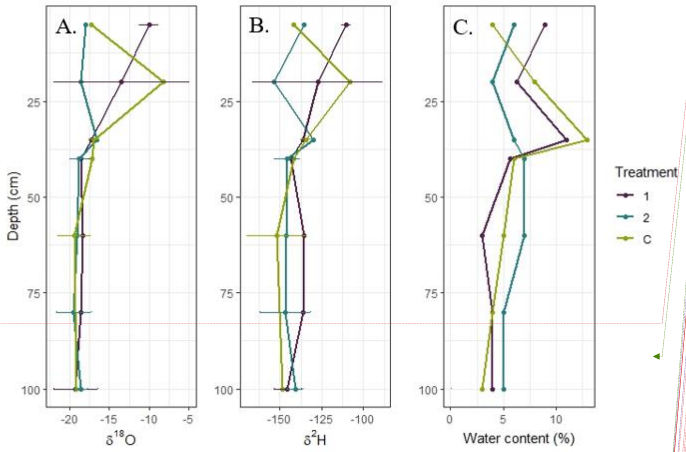

Figure 8 Vertical isotopic profiles and soil water content from treatments in Block 2 and samples collected in mid-July.

Table ~~2~~1 ~~Table 21~~ Depth profile of ~~moisture content,~~ δ^{18}O (‰), ~~and~~ δ^2H (‰), and gravimetric moisture content (GMC) (%) 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.

~~DEPTH~~ (CM)	~~TREATMENT~~	~~MEAN~~ Δ2H (‰)~~Δ18O~~	~~MEAN~~ Δ18O (‰)~~Δ2H~~	MEAN GMC~~SMC~~ SWC ~~(%)~~
~~5~~	~~C~~	~~-17.23~~	~~-141.9~~	~~6.89~~ ±3.76
5	~~T1~~	-110.5~~3210~~	-10.0~~059110.5~~	9%~~12.16~~±7.96
5	~~T2~~	-108.~~054~~1~~9.66~~	-8.2~~1823107.84~~	8%~~11.25~~±6.35
5~~20~~	~~C~~	-141.9~~10.61~~	-17.2~~31368.7~~	4%~~7.95~~±
20	~~T1~~	-148.~~562~~6~~17.96~~	- ~~17~~18.~~9595~~0~~148.55~~	5%~~4.96~~±0.63
20	~~T2~~	-130.~~465~~5~~16.24~~	-16.2~~377130.5~~	~~13~~%~~.12~~
20~~40~~	~~C~~	-68.~~667~~1~~116.62~~	-0.6~~0798132.1~~	8%~~9.35~~
40	~~T1~~	-144.3~~2718.7~~	-18.3~~314144.53~~	~~4~~%~~.84~~
40	~~T2~~	-144.9~~2518.15~~	-19.~~0584~~1~~141.5~~	~~6~~%~~.41~~
40~~60~~	~~C~~	-132.1~~2618.36~~	-16.6~~196137.5~~	9%~~3~~
60	~~T1~~	-131.8~~1317.04~~	-17.0~~421131.8~~	~~7~~%~~.25~~
60	~~T2~~	-157.3~~2220.32~~	-20.3~~188157.33~~	7%~~6.94~~
60~~80~~	~~C~~	-135.5~~3619.31~~	-18.~~3561~~4~~137.5~~	3%~~4.48~~
80	~~T1~~	-135.~~466~~5~~17.89~~	-17.~~8848~~9~~135.45~~	~~4~~%~~.44~~
80	~~T2~~	-153.~~095~~1~~20.11~~	-20.1~~059153.1~~	~~5~~%~~.3~~
80~~100~~	~~C~~	-137.~~493~~5~~19.31~~	-19.3~~051151.1~~	4%~~2.91~~
100	~~T1~~	-139.~~526~~5~~17.64~~	-17.6~~406139.5~~	5%~~4.56~~

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100	~~T2~~	-138.4~~0818.66~~	-18.~~175~~2~~141.45~~	~~5~~%~~.08~~
100~~GROUNDWATER~~	C-~~16.8~~	-151.1~~48~~	-21.2042	~~-127.3~~%

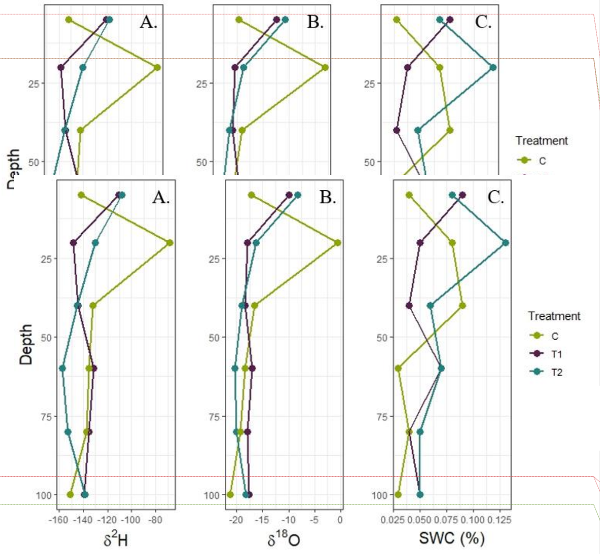

Figure ~~5~~ ~~8~~9 Vertical isotopic profiles and soil water content from treatments in Block 2 and samples collected in mid-July.

From the isotopic soil profile, there were three significant groupings of isotopic composition ($p<0.05$): shallow soil water (5-20 cmcm~~20cm~~), deep soil water (35-100 cmcm~~100cm~~), and groundwater. Mean groundwater collected at the end of the growing season most closely resembled spring and fall snowfall events. The mean δ^{18}O of groundwater was -16.82±0.34‰,~~‰~~ which resembles that in the soil profile, but mean δ^2H was slightly higher~~larger~~ higher~~more~~ ~~depleted~~larger than soil water (n=4~~)~~ ~~(Table 1~~). This isotope fractionation may be due to interactions with bound soil water and soils as the water infiltrates through the vadose zone, but the spread of values as potential uptake sources was greater than any predicted bias from cryogenic vacuum extraction therefor groundwater was included in the model as a isotopically distinct potential source for lodgepole pine water use (Allen & Kirchner, 2022; Vargas et al., 2017).

~~One extreme outlier of B1C at the 20 cm depth was removed; the high δ^2H and δ^{18}O values were likely due to contamination or incomplete cryogenic distillation.~~ The more negative values for both δ^{18}O and δ^2H 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 8~~5~~8).

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Commented [EE48]: Revise figure to have surface soils measurements and SE bars

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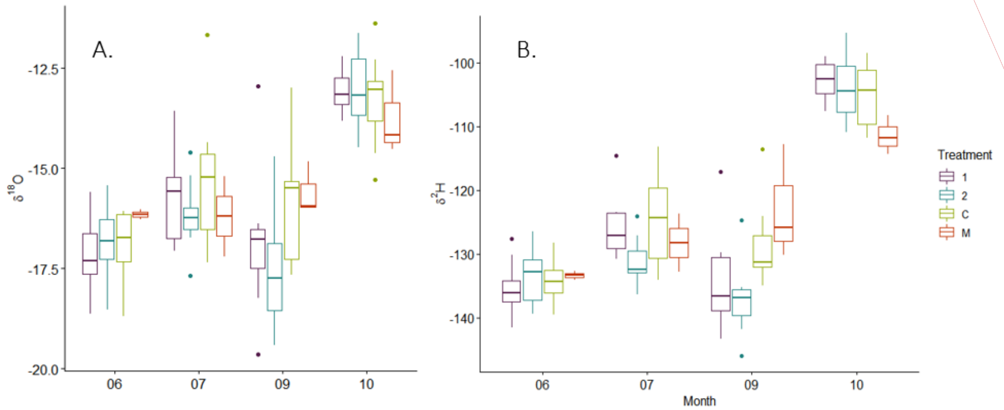
3.2.2. Isotopic variability~~Variability~~ in branch xylem water~~Branch Xylem Water~~



~~Figure 6 9 Branch A. δ^{18}O and B. δ^{2}H by month and treatments (control (C), lightly thinned (T1), and heavily thinned (T2) along with a mature (M) plot.~~

Branch xylem for each treatment across the three blocks and the adjacent mature stand were compared for each sampling period. All treatments closely resembled the mature stand in both ~~the~~ δ^{18}O and δ^{2}H ~~composition~~. There were no statistically significant differences in both δ^{18}O and δ^{2}H of xylem water across ~~blocks and~~ thinning treatments; there was, however, significant variation over time (δ^{18}O: F=24.8*; δ^{2}H: F=~~ = ~~146.6*). More specifically, δ^{18}O and δ^{2}H of xylem water varied by month for all months collected except for between June and September and July and September (Figure 9~~6~~9). 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.

Commented [RG57]: Figure B has an x-axis title, but A doesn't.

Commented [EE58]: R3 - Define M treatment; figure caption incomplete; what do the boxes show? Enlarge axis and figure text

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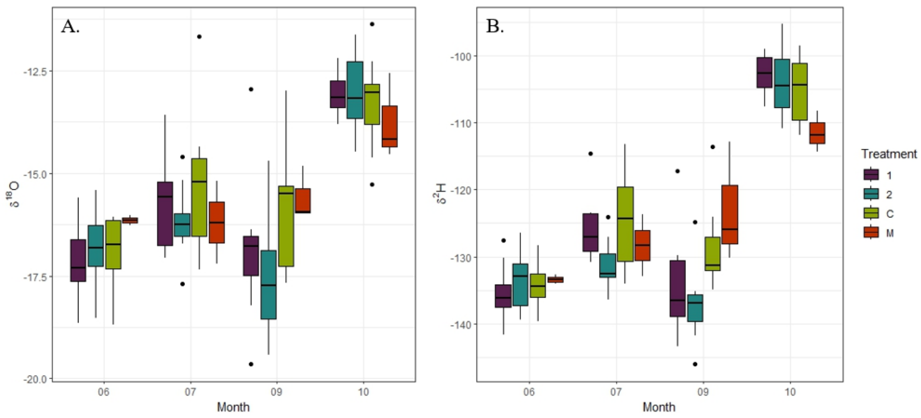
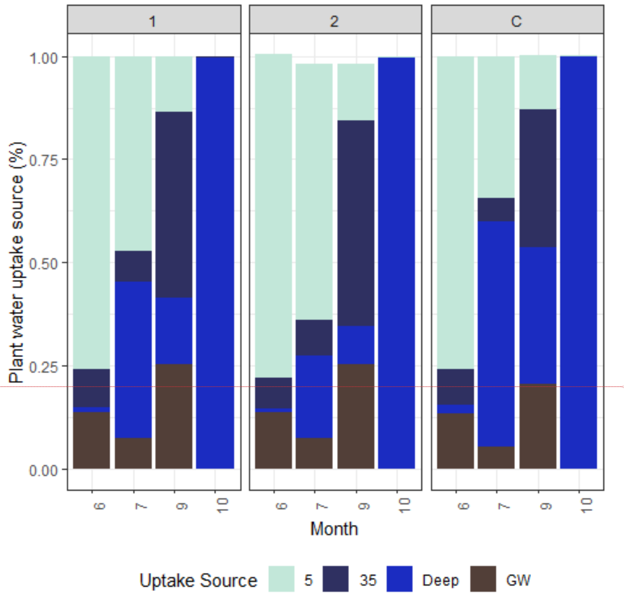


Figure 9 Branch A. $\delta^{18}O$ and B. $\delta^{2}H$ by month and treatments (control (C), lightly thinned (T1), heavily thinned (T2) , and mature (M)).

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 for 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 10~~7~~10). 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

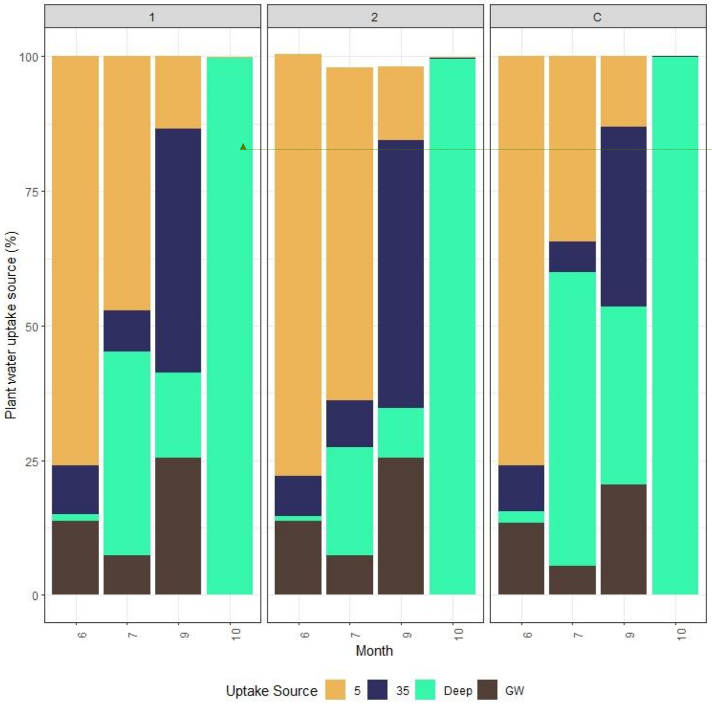


Commented [EE59]: R3 - incorrect y-axis description

Commented [EE60]: R2 - In contrast to 5 and 35 cm soil samples, "deep" soil water has been sampled only once per growing season. Therefore, the deep soil samples lack a temporal component, right? Is the potential variation in deep soil water isotope values with time irrelevant for the study conclusion? If yes, can the authors back this up for the experimental site? Would it make sense to consider precipitation (e.g. modelled precipitation) as an additional source or do the authors assume that the 5 cm soil samples reflect isotope variation in precipitation?

treatments 1 and 2 was composed of approximately 48% and 54% from around 35 cm, ~~and~~ while 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 10~~7~~10). 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 δ^{18}O and δ^{2}H values of -16.9±0.89‰ and -134.37±3.8‰ in June, becoming ~~and was~~ slightly more enriched in July. There was a shift to a ~~more depleted~~ source with a higher concentration of lighter isotopes in September. Branch~~And, Lodgepole pine~~Branch water was ~~the~~ most enriched with heavy isotopes in October, like shallow soil water, with ~~a~~ mean δ^{18}O and δ^{2}H of -12.9±1.76‰ and -103.8~~77~~8±7.0‰, respectively. However, the MixSIAR model does not account for potential changes in the isotopic composition of water from precipitation events from mid-September to mid-October.. ~~The stable isotopic composition of branch water~~

The branch water in October was more enriched in heavy oxygen isotopes for each treatment than soil water at a depth of 35 cm and was more isotopically similar to soil water at 5 cm.



~~ources of water~~
~~th long~~

Figure 10 Partitioned relative contribution of different sources of water in the soil profile by the MixSIAR model of scenario 6 with long runtime.

Commented [EE61]: R3 - LL419-420: is that not a contradiction of what the MixSiar model shows in fig. 7? What does that mean?

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Deuterium also followed a similar trend~~. It is likely that the isotopic profile of deep soil water sampled in July skewed the results. It is plausible that lodgepole pinesthe trees began to rely on shallow soil water towards the end of the growing season when soil water content increased, . Further research is needed with more intensive sampling of deep soil water during the hydrological recharge period at the end of the growing season and beginning of senescence.~~

4. Discussion

4.1. Seasonal variability in soil water

Deep ~~Soil~~ 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 during the summer months. LowLow~~and that l~~L~~ow~~ intensity and less frequent summer precipitation events are evaporated out of the shallow soil layers andand~~before they~~and do not infiltrate past the evaporative front to ~~can~~ recharge the unsaturated zone or groundwater. Although there was not a statistically significant difference in the depth to water uptake by thinning treatments, there~~decreased stem density did show~~there was increased evaporative enrichment, or a higher concentration of oxygen-18, in the shallow soils of the heavily thinned stand (Figure 4.C.). The muted enrichment of δ^{18}O around 35 cm depth in the soil indicates a mixing of the left-over ~~enriched~~ summer precipitation with ~~greater heavier isotopes with~~ older and lighter water. Our results ~~did~~ do not indicate that differences in soil exposure canopy coverage were effective enough to significantly ~~change~~ affect the isotopic composition of soil water ~~across treatments~~ below 5 cm in ~~soil~~ depth.

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; Stumpp et al., 2018). Based on the seasonal shift in the isotopic composition, ~~of~~ soil water at ~~a~~ depth of 5 cm ~~below the surface~~ was more enriched with heavier isotopes ~~showed more enrichment~~ over the growing season than ~~around~~ at 35 cm ~~below the surface~~ due to more evaporative isotopic fractionation ~~of~~ near the soil surface and a lack of ~~heavy~~ rainfall intense enough to drive precipitation deeper into the soil profile before September 16, 2021 (Figure 3). The effect of evaporative enrichmentenrich~~e~~ment of the near surface soil water was most obviousobvious~~prevalent~~ in July and September in the heavily thinned stand (T2). 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 thethe~~uptake source~~the depth to water uptake of lodgepole pine, regardless of changes in stem density, over the growing season. At the beginning of the growing season, when snow meltwater is more available at shallow depths and beginning to infiltrate through the soils,

Commented [RG62]: This is more like discussion than results.

Commented [EE63]: R3 - I think the discussion is too speculative and will be automatically improve once you can either provide flux data or direct the findings toward a different research question.

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Commented [EE64]: R3 - how do you then explain the differences in soil water signatures shown in figure 4C in 07 and 09, where the blue (T2 treatment heavy thinning) boxes show a clear enrichment at the 5cm depth?

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Commented [RG65]: It might be interesting to know which points on the biplot represent these conditions.

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Commented [EE66]: R2 - Does the soil evaporative effect, regardless of transpiration, increase with increasing thinning?

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Commented [EE67]: More enrichment of what?

Commented [RG68]: Curvature in the soil water biplot line supports this contention, but it must also be the case that summer precipitation (unlike the winter precip at greater depth) is also more enriched. In other words, it's not all about evaporative enrichment.

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Commented [EE69]: R3 - because this is the biggest seasonal water influx?? Relate to overall precip and summer precip?

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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). Then, in July, the trees in the control treatment were using less shallow soil water (34.3% of plant water uptake from 5 cm below the soil profile) whereas the moderately thinned and heavily thinned plots maintained a greater proportion of shallow water uptake (47.1% and 61.5% respectively). The mean δ^{18}O and δ^{2}H of branch water from each treatment in September had a higher concentration of lighter stable water isotopes ~~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. By September, the control stand was more dependent on deeper soil water and groundwater with only 33.4% of plant water uptake originating from 35 cm in the soil profile, whereas where as both thinning treatments maintained more than 45% of water uptake from 35 cm in the soil profile. In October, all treatments were completely dependent on deep soil water, but it is likely that the isotopic profile of deep soil water sampled in July skewed the results. It is plausible that the trees began to rely on shallow soil water towards the end of the growing season when soil water content increased. Further research is needed with more intensive sampling of deep soil water during the hydrological recharge period at the end of the growing season and beginning of senescence.

Local monitoring close to the study site indicated that the depth to groundwater stayed at least 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 suppressed top soil water uptake, but that deeper soil ~~water~~ was sufficiently saturated to sustain root water uptake and tree function enough to limit groundwater uptake to less than 30% for all treatments until the beginning of fall precipitation events recharging the saturated zone. ~~. However,~~ ~~It is likely that the isotopic profile of deep soil water sampled in July skewed the results. It is plausible that the trees began to rely on shallow soil water towards the end of the growing season when soil water content increased. Further research is needed with more intensive sampling of deep soil water during the hydrological recharge period at the end of the growing season and beginning of senescence.~~

~~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 depending on water availability (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 seasonal changes in depth to water uptake by lodgepole pine support the findings of Andrews et al. (2012) on changes in lodgepole pine depth to water uptake in Alberta.(2012) on changes in lodgepole pine depth to water uptake in Alberta~~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; Langs et al., 2020; Meinzer et al., 2007b; Prieto et al., 2012; Sohn et al., 2016; J. Wang et al., 2017, p. 201).

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Commented [RG70]: If the groundwater was this deep (6500 cm), is it possible that the trees may not have had any direct access to it at all? Or did you mean to say that it stayed below 65 cm?

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Commented [RG71]: This is more like discussion than results.

Commented [EE72]: Several studies have shown that trees that have access to relatively shallow groundwater (6.5 m deep at the study site) can support the integrity and functionality of their shallow fine roots and associated mycorrhizal fungi in dry topsoil layers during prolonged drought through internal hydraulic redistribution (Bauerle et al 2016; Querejeta et al 2007), so the assumption made in L454-455 and L508 is perhaps speculative and questionable, in the absence of any direct measurement of root function. Are there any other plausible explanations for the apparent inability of the lodgepole pines to use recent rainwater during the late growing season? Perhaps those late season rainfall events were of insufficient magnitude to recharge the topsoil layer in a physiologically meaningful way?

The~~However, t~~The~~the~~ literature is inconsistent across different biogeoclimatic regions and species with regards to ~~regarding~~ the effects of thinning on stand dynamics that influence inter-tree competition for water resources or ~~altered~~ changes in depth to water uptake.~~ with tree density~~ (Kerhoulas et al., 2013; Moreno-Gutiérrez et al., 2011; Sohn et al., 2016; Wang et al., 2021). We~~Our findings that there is~~We found no significant impact of forest thinning on depth to water uptake. However~~Despite stem density~~However, our observation of ~~,~~ 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 decern if lodgepole pine relies more on mobile or bound soil water, the extent of lodgepole pine rooting zones, what biogeochemical 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).

Commented [EE73]: R2 - Sentence not clear, rephrase

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 has 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 reversable 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. Our results are inconclusive in determining the depth to water uptake in September and October because of limited deep soil water ~~Decreased~~ measurements. However, increased annual temperatures and more variable precipitation patterns as a part of climate change projections are predicted to drive decreases in winter snowpack and could drive lodgepole pine stands, regardless of stem density, to rely on groundwater influencing water availability and depth to groundwater. These projections could lead to prolonged inter-annual water scarcity alongalong~~in the~~along with seasonal water scarcity during 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 access from shallow soil water at the beginning of the growing season to deeper soil water as drought conditions intensified.~~progressed~~intensified~~.~~ The quick-~~-~~draining and sun-exposed soils of the UPC do not retain small summer precipitation events, and these patterns are intensified in the shallow soil layer of the heavily thinned stand because decreased canopy cover can be directly related to increased soil evaporation. ~~as~~ 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 allowing~~making them more advantageous in their ability~~allowing them to 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 statistically significant ~~difference~~ variance in depth to water use between the over-populated plots and thinned ones. Both thinned and unthinned lodgepole pine stands were able to access shallow soil water during the early months (June and July), then switched to deeper soil water and a larger proportion of groundwater during September. Although there was not a statistically significant difference in isotopic composition of branch water for the different treatments, our results indicate that decreased stem density may lead to the prolonged use of soil water 35 cm below the surface during prolonged dry periods which would decrease the dependency of lodgepole pine on deep soil water or ground water.

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). From our findings, stand density did not prevent lodgepole pines from accessing soil water from various depths, but decreased stem density did result in lodgepole pines using soil water higher in the soil profile for longer under extremely dry conditions.~~However, from our findings, during prolonged growing season, stand density does~~ did ~~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 pine~~pines~~ in the interior of British Columbia can~~have alternative strategies to~~can access deeper soil water from spring snowmelt.~~ in the interior of British Columbia.~~ However, if snowpack and spring snowmelt begin to decrease, lodgepole pine~~pines will~~ may need to acclimate to these hydrological shifts.

Commented [EE74]: do you mean that pines are unable to access water made available by late-season rainfalls during the rewetting period?

Commented [EE75]: R3 - Subsequently the conclusion you draw that forest thinning does not significantly influence the change in water uptake depth is misleading and at least incomplete because likely the transpiration in the control plot is so low that no significant amounts of water do leave the system.

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Commented [EE76]: R1 - I think that the strong assertions made in L520-522 and 526-528 of the Conclusions section (and similar statements in the Abstract) should be reconsidered and rewritten after careful examination of the issues raised above.

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Code and Data Availability:

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

Field Code Changed

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.

Acknowledgements:

This study was funded by the Ministry of Forests, Lands, Natural Resource Operations and Rural Development. Field work was done with the assistance of Fiona Moodie. Cryogenic distillation was conducted at the University of British Columbia. Samples were sent to the Stable Isotope Facility at University of California, DavisDavi~~iv~~s~~Daivs~~.

Financial Support:

This research was funded by the Ministry of Forests, Lands, Natural ~~resource~~ Resource Operations and ~~rural~~ Rural Development (grant number: RE21NOR-029)

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Commented [EE78]: Some of the references mentioned in the text appear to refer to wet riparian habitats and tree species, and I thus wonder whether they are really directly relevant to this particular study which has a strong focus on dry interior forests of the semiarid Okanagan Valley (e.g. Gibson &Edwards 2002; Liu et al 2015; Maier et al 2019; Sanchez-Perez et al 2008). It might be more appropriate to replace these references by others referring to semiarid pine forests more closely resembling your study system.

Commented [EE79]: R1 - BAUERLE, T.L., RICHARDS, J.H., SMART, D.R. and EISSENSTAT, D.M. (2008), Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil. Plant, Cell & Environment, 31: 177-186. Querejeta JI, Egerton-Warburton LM, Allen MF (2007) Hydraulic lift may buffer rhizosphere hyphae against the negative effects of severe soil drying in a California Oak savanna. *Soil Biology and Biochemistry*, 39, 409–417.

Commented [EE80]: Allen, Scott T., and James W. Kirchner. 2022. 'Potential Effects of Cryogenic Extraction Biases on Plant Water Source Partitioning Inferred from Xylem-Water Isotope Ratios'. *Hydrological Processes* 36 (2): e14483. https://doi.org/10.1002/hyp.14483.

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