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Research paper

# Conifer water-use patterns across temporal and topographic gradients in the southern Sierra Nevada

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**Climate change is increasing the severity and duration of drought events experienced by forest ecosystems. Because water is essential for tree physiological processes, the ability of trees to survive prolonged droughts will largely depend on whether they have access to reliable water sources. While many woody plant species exhibit the ability to shift water sources between different depths of soil and rock water in response to changes in climate and water availability, it is unclear if Sierra Nevada conifers exhibit this plasticity. Here we analysed the $\delta^{18}$O and $\delta^{13}$C values of annual tree rings to determine the water-use patterns of large Sierra Nevada conifers during the 2012–16 California drought and 4 years before this drought event (2004–07). We analysed four species (*Pinus jeffreyi* Grev. & Balf. (Jeffrey pine), *Pinus lambertiana* Dougl. (sugar pine), *Abies concolor* (Gord. & Glend.) Lindl. Ex Hilderbr (white fir) and *Calocedrus decurrens* (Torr.) Florin (incense-cedar)) across a range of topographic positions to investigate differences in water-use patterns by species and position on the landscape. We found no significant differences in $\delta^{18}$O and $\delta^{13}$C values for the pre-drought and drought periods. This stability in $\delta^{18}$O values suggests that trees did not shift their water-use patterns in response to the 2012–16 drought. We did find species-specific differences in water-use patterns, with incense-cedar exhibiting more depleted $\delta^{18}$O values than all other species. We also found trends that suggest the water source used by a tree may depend on topographic and growing environment attributes such as topographic wetness and the surrounding basal area. Overall, our results suggest that the water source used by trees varies by the species and topographic position, but that Sierra Nevada conifers do not switch their water-use patterns in response to the drought. This lack of plasticity could make Sierra Nevada conifers particularly vulnerable to drought mortality as their historically reliable water sources begin to dry out with climate change.**

***Keywords:*** carbon isotopes, climate change, drought, oxygen isotopes, rock moisture, soil moisture, tree ring cellulose, water source.

## Introduction

Access to a reliable water source has an integral role in tree function and survival. Water is a critical component of tree physiological processes, with trees that experience water stress exhibiting lower stomatal conductance (Irvine et al. 1998, Panek and Goldstein 2001), reduced photosynthetic and growth rates (Grieu et al. 1988, DeLucia and Heckathorn 1989, Adams and Kolb 2005, Truettner et al. 2018), and increased risk of hydraulic failure (Brodribb and Cochard 2009, Anderegg and Anderegg 2013). As droughts increase in frequency, severity and duration globally (Allen et al. 2015), access to a reliable water source such as deep soil water or water stored in fractured bedrock (i.e. hydraulic refugia) may dictate how trees respond to periods of water stress (McDowell et al. 2019). For example, studies in the southwestern USA have indicated that trees with access to deeper, more reliable water sources experience lower rates of mortality during drought periods (Grossiord et al. 2017, McDowell et al. 2019). While the effect of water availability on tree function and survival is well studied, whether the water source used by trees varies through time and across topographic gradients remains poorly understood. Understanding variability in tree water-use patterns may provide insight into how forests will respond to increasing water stress under climate change.

Many woody plants exhibit the ability to switch water sources in response to changing soil water availability (Dawson and Pate 1996, Snyder and Williams 2000, Filella and Peñuelas, 2003, Guo et al. 2018), whereas other species seem to consistently rely on deep water sources (Plamboeck et al. 2008, Anderegg et al. 2013, Kerhoulas et al. 2013, Nardini et al. 2016). In semi-arid and Mediterranean forest types, some conifer species exhibit seasonal plasticity in their water-use patterns, with trees transitioning to the use of deeper water sources during summer dry periods (Barbeta et al. 2015, Voltas et al. 2015, Grossiord et al. 2017). Additionally, there is evidence that trees can switch the primary water source they use in response to drought stress or climate variability (Barbeta et al. 2015, Grossiord et al. 2017, Berkelhammer et al. 2020), but this transition may depend on trees being able to shift water uptake among existing roots (Mackay et al. 2019). Rooting depth and primary water source can also vary because of species-specific differences in the rooting structure (Canadell et al. 1996, Fan et al. 2017) and ability to penetrate bedrock (Zwieniecki and Newton 1996, Schwinning, 2010), as well as topographic variation in the soil structure and depth to bedrock (Nicoll et al. 2006, Meyer et al. 2007, Klos et al. 2018, Rempe and Dietrich 2018). Consequently, variability in tree water-use patterns may not be ubiquitous across all trees and the ability to switch water sources in response to decreasing water availability likely depends on whether a tree's roots have access to multiple water sources.

In mountain ecosystems, the water sources available to trees are typically divided into three categories: soil water, rock moisture and water stored in bedrock fissures (Klos et al. 2018, Rempe and Dietrich 2018). Rock moisture, or the water stored in weathered and decomposed bedrock, may be close to or exceed the soil moisture, providing a reliable water source for trees in montane ecosystems (Witty et al. 2003, Rempe and Dietrich 2018). In the Sierra Nevada, deep soil water (soil depths greater than 50 cm) and rock moisture serve as the primary water sources for conifers (Plamboeck et al. 2008, Klos et al. 2018, McCormick et al. 2021), recharging from winter snowmelt and providing reliable water for trees during summer droughts and dry years (Klos et al. 2018).

While deep soil water and rock moisture have historically been reliable water sources for Sierra Nevada conifers, increasing drought severity and duration with climate change may cause these deep-water reserves to dry out. For example, from 2012 to 2016, the Sierra Nevada experienced a severe drought, with the driest 12-month period in California recorded history occurring during this drought event (Swain et al. 2014). This drought was characterized by precipitation deficits and high temperatures during both the wet and dry seasons (AghaKouchak et al. 2014, Williams et al. 2015). During the 2012–16 California drought, tree die-off was linked to deep soil drying and an overdraft of soil moisture, suggesting that drying of once-reliable water sources contributed to mortality during this drought event (Goulden and Bales 2019). Consequently, the ability to switch water sources during periods of drought and use even deeper reserves of rock moisture or hydraulic refugia may ameliorate the water stress experienced by trees, but it is unclear if Sierra Nevada conifers exhibit this plasticity. Additionally, given that the depth of the rock moisture layer varies with topography (Klos et al. 2018, Rempe and Dietrich 2018), the ability of a tree to access deeper or alternative water sources may depend on where a tree has established on the landscape.

Stable isotope analysis of oxygen in tree ring cellulose provides a useful tool for assessing differences in tree water-use patterns through time, with the $\delta^{18}$O values in tree ring cellulose providing a signature of the water sources used by the tree throughout the year (Roden et al. 2000). In the Sierra Nevada, soil water exhibits an isotopic gradient with soil depth, with shallower soil water having relatively enriched $\delta^{18}$O values compared to deeper soil water because of evaporation. As a result, differences in the $\delta^{18}$O values of annual tree ring cellulose can be used to attribute the depths of water used by a tree during a given year. However, $\delta^{18}$O values in tree ring cellulose provide a mixed signal of transpiration and the $\delta^{18}$O values of the water sources used by the tree (Roden et al. 2000). Coupling $\delta^{18}$O values in tree ring cellulose with $\delta^{13}$C, a proxy measure for transpiration, can determine whether changes in $\delta^{18}$O values are because of differences in water source or changes in transpiration.

Here we analysed the $\delta^{18}$O and $\delta^{13}$C values of tree rings to assess conifer water-use patterns across a range of topographic positions during the 2012–16 California drought and a 4-year period prior to the drought (2004–07). We assessed water-use patterns for the largest size class of trees (diameter at breast height (DBH) >75 cm) which experienced high rates of mortality during the 2012–16 drought (Fettig et al. 2019, Steel et al. 2021) and had well developed root systems prior to this drought event. The four focal species of our study are commonly found in mixed-conifer forests of the southern Sierra Nevada and included Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), sugar pine (*Pinus lambertiana* Dougl.), white fir (*Abies concolor* (Gord. & Glend.) Lindl. Ex Hildebr) and incense-cedar (*Calocedrus decurrens* (Torr.) Florin). We sought to answer the following questions: (i) Do Sierra Nevada conifers exhibit differences in their water-use patterns during periods of drought versus non-drought? (ii) Do water-use patterns vary among species and with topographic position? We hypothesized that trees would have more depleted (lower) $\delta^{18}$O values during the 2012–16 drought period compared with during four non-drought years

(2004–07) because of drying of the upper soil layers during the prolonged drought period, resulting in the use of deeper water sources. Further, we hypothesized that there would be differences in the $\delta^{18}$O values of the four species because of variability in the maximum rooting depths of these different species (Stone and Kalisz 1991). Finally, we hypothesized that trees growing at higher positions on steeper slopes would have more depleted (lower) $\delta^{18}$O values than trees growing at lower positions on shallower slopes because of lower water availability at these sites, resulting in an increased use of deeper water sources.

## Materials and methods

### *Study site*

We conducted this study at the Teakettle Experimental Forest, located in the Sierra National Forest approximately 80 km east of Fresno, CA on the north fork of the Kings River. Elevation at the site ranges from 1900 to 2600 m. Common soils are well-drained Dystric and Lithic Xeropsamments of loamy sand to sandy loam textures derived from granitic rock and exposed granitic rock is also common throughout the study area (USDA Forest Service and Soil Conservation Service, 1993). Additionally, soil and saprolite thickness at the site varies from 5 to 137 cm and 1 to 208 cm, respectively (Meyer et al. 2007). $\delta^{18}$O values of soil water at the site exhibit a strong vertical gradient because of evaporative enrichment, with relatively enriched $\delta^{18}$O values in the upper soil layers (−4 to −6‰ from 1- to 10-cm soil depth) and relatively depleted $\delta^{18}$O values deeper in the soil profile (−11 to 12‰ from 20- to 30-cm soil depth, Plamboeck et al. 2008). The $\delta^{18}$O values of well water at the site range from −12 to −13‰.

The dominant tree species that comprise the mixed-conifer forest type at Teakettle are sugar pine, Jeffrey Pine, white fir and incense-cedar. Red fir (*Abies magnifica* A. Murr.) and California black oak (*Quercus kelloggii* Newberry) are also found at the site but at lower densities (North et al. 2007). Before 2000, the site had no prior history of logging or known stand-replacing disturbance. For a complete site description, see North et al. (2002). The climate at Teakettle is Mediterranean, typical of the western Sierra Nevada, with mean annual precipitation of 125 cm that falls predominantly as snow between November and April (North et al. 2002). From 2012 to 2016, this area experienced a severe drought event characterized by precipitation deficits and high temperatures (AghaKouchak et al. 2014, Williams et al. 2015). The combined effect of the drought and an endemic bark beetle outbreak resulted in widespread tree mortality at Teakettle and across the Sierra Nevada (Fettig et al. 2019, Goodwin et al. 2020, Steel et al. 2021).

### *Sampling design*

Within the mixed-conifer forest type at Teakettle, 18 permanent 4-hectare treatment units were established in 1998 as part of the Teakettle Ecosystem Experiment. Using a full-factorial design, treatments consisted of two levels of prescribed burning (Burn and Unburned) and three levels of thinning (no thin, understory thin and overstory thin) for a total of six treatments. Each treatment was replicated across three treatment units. The treatments that included thinning and burning were thinned in 2000 and burned in 2001 and the thin-only treatments were thinned in 2001. The first prescribed burn was applied in late-October 2001 after the first major fall rain. For a complete description of the treatments, see Goodwin et al. (2020). Prior to treatment implementation, trees within the 18 treatment units were mapped, tagged and measured. All trees were subsequently measured in 2004, 2011 and 2017.

During the summer of 2017, tree cores were collected using a 12-mm diameter increment borer following the stratified random sampling design outlined in Zald et al. (2022). These tree cores were collected from all six treatment combinations, the four dominant species (sugar pine, Jeffrey pine, white fir and incense-cedar), three diameter classes (10–25, 25–55 and >55 cm) and two local competition classes (high versus low competition).

### *Tree core processing*

Each tree core was taped onto a wooden mounting stick until dry and then glued and sanded with progressively finer grit sandpaper. Using either a high-resolution flatbed scanner with WinDENDRO software (Régent Instruments, Québec, Canada) or a stereo boom microscope and Velmex Unislide TA tree ring measuring system (Velmex, Bloomfield, NY, USA), tree ring widths were measured to the nearest 0.001 mm. Each tree ring series was cross dated to ensure correct calendar year assignment of ring-widths using the dplR package (Bunn et al. 2021). Following dating, annual rings from each core were excised with a razor blade for the years 1992–96, 2003–07 and 2012–16. Earlywood and latewood were extracted together for each year. These specific years were extracted as part of the sampling design for a larger carbon isotope study.

### *Tree ring selection*

For our study, we used a subset of the 12-mm diameter tree core data collected for the larger carbon isotope study and selected tree cores from trees >75-cm DBH ($n = 59$). For the oxygen isotope analysis, trees were selected based on whether they had enough available material (1 mg) in each annual ring for the pre-drought (2004–07) and drought (2013–16) periods following material excision for the carbon isotope study. While the drought began in the winter of 2011–12, we selected 2013 as the first annual ring for the drought period to account for potential lagged effects in tree response to drought. To keep the length of the pre-drought period the same as the drought period, we used 2004 as the first annual ring for the pre-drought period. Trees had to have at least 3 years of sufficient material for the pre-drought (2004–07) and drought (2013–16) periods. Requiring trees to have material for all 4 years of a given period would have resulted in an insufficient sample size because of limited material availability. For the oxygen isotope analysis, this resulted in a total of 38 trees with samples for both the pre-drought and drought periods (13 sugar pine, 8 Jeffrey pine, 11 white fir and 6 incense-cedar). We had an additional seven trees (two sugar pine, two Jeffrey pine, one white fir and two incense-cedar) with samples for the pre-drought period only. These trees were used in analyses that used only pre-drought years.

### *Isotopic analysis*

For carbon isotope analysis, approximately 1 mg of equal parts earlywood and latewood were excised from each annual ring. Care was taken to avoid sampling approximately the first third of the earlywood to avoid potential influence from the previous year's photosynthate. The excised whole wood material for each sample was then packed into a tin capsule and analysed using a Costech Elemental Analyzer connected to a Delta V Plus Mass Spectrometer via a Conflo IV Interface at the University of New Mexico Center for Stable Isotopes. Whole wood was used for the carbon isotope study instead of alpha cellulose because of the large number of tree rings analysed for this study. Additionally, a growing body of data suggests that both alpha cellulose and whole wood exhibit the signal of environmental effects, such as drought stress, in their $\delta^{13}$C values (Weigt et al. 2015). Finally, we did not homogenize the whole wood prior to carbon isotope analysis. We homogenized a subset of samples ($n = 17$ annual rings from all species across a range of wet to dry years) to assess differences in the $\delta^{13}$C values of homogenized versus whole wood samples and found negligible differences ($R^2 = 0.92$; $T = 0.69$, $P = 0.50$).

For the $\delta^{18}$O analysis, we then extracted alpha cellulose from the remaining selected tree ring material following methods outlined in Leavitt and Danzer (1993). Each annual ring was packed into a fiber filter bag (ANKOM Technology, Macedon, NY, USA) and heat sealed closed. To remove secondary compounds such as lipids and resins, we placed filter bags in a Soxhlet apparatus to reflux in a 2:1 mix of toluene:ethanol for 12 h, dried them for ∼36 h, refluxed them for another 12 h in pure ethanol and then dried them again for ∼36 h. To remove soluble sugars, we boiled the sample bags in deionized (DI) water for 3 h. We removed lignin using a sodium chlorite:glacial acetic acid solution that was replaced every 3–4 h over a 4-day period. We then rinsed samples with DI water over a 4-h period. Finally, to remove hemicellulose, we submerged samples in 17% sodium hydroxide for 2 h and then rinsed repeatedly with DI water. We then submerged samples in 10% glacial acetic acid for 1 h and again rinsed extensively with DI water. We then dried the alpha cellulose samples at 70 °C for 48 h. To homogenize the earlywood and latewood component of each sample, we placed each sample in a 2-ml Eppendorf safe-lock tube with a 6.35-mm diameter chrome steel ball (BioSpec Products Inc, Bartlesville, OK, USA). We homogenized the samples using a Qiagen Retsch TissueLyser Bead Mill (Boston Industries, Walpole, MA, USA) for 2 min at a frequency of 30 vibrations/s. For $\delta^{18}$O analysis, we weighed 0.3 mg of each sample and loaded the material into a silver capsule. We analysed the samples using a Costech Elemental Analyzer connected to a Delta V Plus Mass Spectrometer via a Conflo IV Interface at the University of New Mexico Center for Stable Isotopes.

### *Statistical analyses*

We conducted all statistical analyses in R Version 4.0.4 (R Core Team, 2021). To determine whether differences in $\delta^{18}$O values for each tree were because of changes in the water source or differences in transpiration, we used the corresponding carbon isotope data for each tree ring to conduct a linear regression with $\delta^{18}$O values for each tree with year as a predictor variable. Both the $\delta^{18}$O and $\delta^{13}$C values of cellulose become relatively enriched with decreasing transpiration (Farquhar et al. 2007), and as a result, a positive linear relationship between the $\delta^{18}$O and $\delta^{13}$C values in our trees would indicate that variability in $\delta^{18}$O values was likely because of differences in transpiration rather than water source. Additionally, we compared the AICc value of the linear regression with that of a linear mixed-effects model to determine whether using tree as a random effect improved the model. For the $\delta^{13}$C analyses, we did not correct for the Suess effect, given the relatively short period we were investigating (2004–16). Further, because our tree cores were collected from all six treatment types at Teakettle, we used analysis of variance (ANOVA) to assess whether $\delta^{18}$O values differed by treatment. Treatment did not have a significant effect on $\delta^{18}$O values and was removed from further analyses.

To evaluate changes in water-use patterns through time, we used ANOVA to assess differences in both $\delta^{18}$O and $\delta^{13}$C values by year as well as by period (pre-drought versus drought). For analyses looking at the two time periods, we averaged the annual $\delta^{18}$O and $\delta^{13}$C values for each tree within each of these time periods. To evaluate species-specific differences in water-use patterns, we used ANOVA to assess differences in $\delta^{18}$O values by species and period as well as the interaction between species and period. Because the interaction between species and period was not significant ($P = 0.81$), we also used ANOVA to assess differences between species for the pre-drought and drought periods, independently. Additionally, we used ANOVA to assess whether the variability (standard deviation) in $\delta^{18}$O values for all years differed among species to determine if some species were more plastic in their water-use patterns. We also used ANOVA to assess differences in $\delta^{13}$C values by species and period, including the interaction between the species and period. Prior to running all ANOVAs, we tested for normality using the Shapiro–Wilk test and for homoscedasticity using Levene's test. All ANOVA's met these assumptions at the

$P = 0.05$ level. For each significant ANOVA, we used Tukey's post-hoc analysis to conduct pairwise comparisons.

To analyze the relationship between water-use patterns and topographic position, we assigned elevation, slope, aspect and topographic wetness index values to each tree. We used lidar data that were collected in October 2010 by Watershed Sciences Inc. (Portland, OR, USA) as part of a larger acquisition for the USDA Forest Service. For a complete description of how the lidar data were collected, see Zald et al. (2022). We then clipped the contractor provided 1-m resolution digital terrain model (DTM) to the extent of the Teakettle Experimental Forest. The clipped DTM was used to create slope and aspect layers with a 5-m resolution and a topographic wetness layer with a 10-m resolution using the physically based basin contribution model (Beven and Kirkby 1979). We then assigned elevation, slope, aspect and topographic wetness index values to each tree by creating a 10-m buffer around each tree and taking the mean of all pixels within the buffer. Additionally, we calculated a neighborhood competition metric (hereafter neighborhood basal area) to assess the effect of local competition on water-use patterns. The neighborhood basal area was calculated using the 2011 tree census data. We summed the basal area of all trees greater than 5-cm DBH within a 10-m radius of each tree. Neighborhood basal area calculations were performed in QGIS (QGIS.org, 2022).

We used linear regression to assess the relationship between the mean pre-drought and drought $\delta^{18}$O and $\delta^{13}$C values for each tree with elevation, slope, topographic wetness index and period (pre-drought versus drought) as predictor variables. We omitted aspect from the analysis because of 84% of the trees having a northernly aspect. We also used a quadratic regression to assess the relationship between the mean $\delta^{18}$O values for each period and topographic wetness index because of the curved nature of the residuals. Additionally, we used linear regression to assess the relationship between the neighborhood basal area and mean pre-drought and drought $\delta^{18}$O values and $\delta^{13}$C values for each tree. We also performed a correlation test between the neighborhood basal area, elevation, slope and topographic wetness index using Pearson's correlation coefficient. The neighborhood basal area was correlated with both slope ($r = 0.25$) and topographic wetness index ($r = 0.47$) and was therefore not included as a predictor variable in the topographic models. We also assessed the relationship between the neighborhood basal area and topographic wetness index using linear regression. Finally, we used a linear mixed-effects model to assess the interactive effects of species, topography and period on $\delta^{18}$O values, with species, elevation, slope and period as fixed effects and tree as a random effect using the lmer function in the lme4 package (Bates et al. 2015). We compared the AICc value of the linear mixed-effects model with a linear regression model to determine whether including tree as a random effect improved the model. For all regression models, we used the Shapiro–Wilks test to assess normality, a non-constant error variance test to assess constant variance, and the Durbin–Watson test to test for autocorrelation. All assumptions were met at the $P = 0.05$ level, except for the assumption of normality for basal area and elevation. As a result, we log-transformed these variables before running linear regressions.

## Results

The cellulose $\delta^{18}$O values for each tree and year did not exhibit a strong linear relationship with corresponding $\delta^{13}$C values ($P = 0.31$, $R^2 = 0.01$). The linear mixed-effects model that included tree as a random variable had a lower AICc value ($\Delta$AIC = 217) than the linear regression that did not include tree, but still did not show a significant relationship between the $\delta^{18}$O and $\delta^{13}$C values ($P = 0.40$). The lack of a positive linear relationship between the $\delta^{18}$O and $\delta^{13}$C values for each tree suggests that the cellulose $\delta^{18}$O values are reflective of the water source and not differences in transpiration. Tree ring cellulose $\delta^{18}$O values ranged from 25.2 to 36.2‰. Using the isotope exchange model for cellulose synthesis presented in Roden et al. (2000), these cellulose $\delta^{18}$O values correspond to source water $\delta^{18}$O values of −20 to −8‰.

We did not find evidence that trees began using different water sources in response to the 2012–16 drought, with statistically similar mean $\delta^{18}$O values for the pre-drought and drought periods ($P = 0.76$, Figure 1a) when averaging across species. Further, we found only slight statistical differences in tree ring cellulose $\delta^{18}$O values among all investigated years ($P = 0.07$, Figure 1b). Pre-drought years 2005 and 2007 were the only years where trees had $\delta^{18}$O values that were significantly different from each other ($P = 0.04$), with 2007 exhibiting more enriched (higher) $\delta^{18}$O values than those found in 2005.

Investigating individual species, we found species-specific differences in $\delta^{18}$O values (Figure 2). When considering all investigated years, incense-cedar had significantly more depleted (lower) $\delta^{18}$O values than white fir ($P = 0.001$), Jeffrey pine ($P < 0.0001$) and sugar pine ($P < 0.0001$). White fir also had more depleted $\delta^{18}$O values than both Jeffrey ($P = 0.003$) and sugar pine ($P = 0.10$), whereas both pine species exhibited similar $\delta^{18}$O values to one another ($P = 0.41$). During the drought period, species-specific differences in $\delta^{18}$O values became less pronounced, with the only significant difference being more depleted values in incense-cedar compared with Jeffrey pine ($P = 0.03$). Additionally, the variability in $\delta^{18}$O values (standard deviation) across all investigated years did not differ by species, suggesting none of the four species was more plastic in their water-use patterns over this time frame ($P = 0.48$, Figure 2).

When investigating differences in $\delta^{13}$C values, we found slight differences in $\delta^{13}$C values between species ($P = 0.08$), but no significant differences between the pre-drought and drought periods ($P = 0.24$, Figure 3) or when considering each

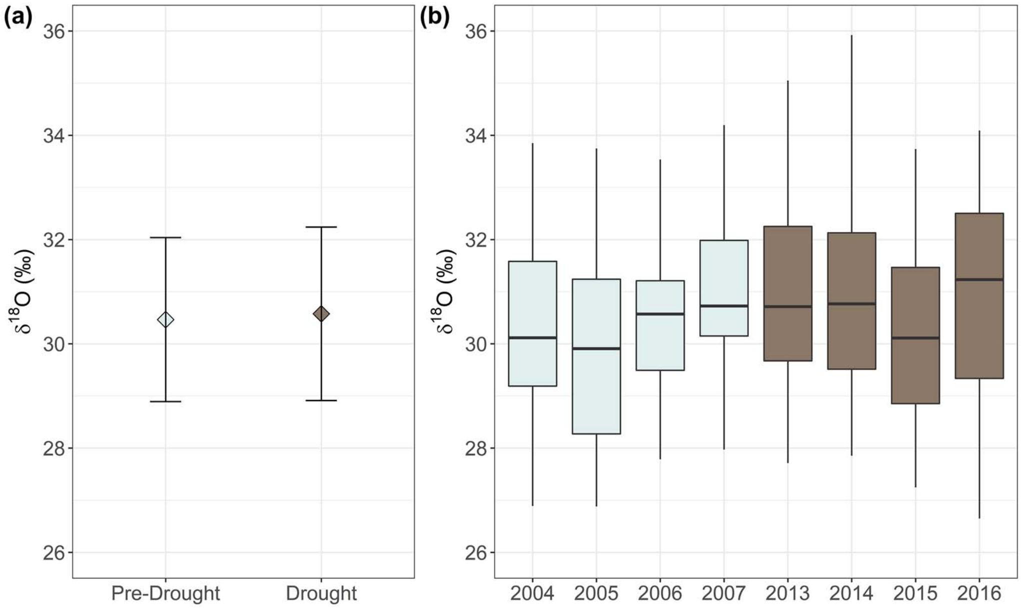

Figure 1. (a) Mean (± SD) cellulose $\delta^{18}$O values for the pre-drought and drought periods. $\delta^{18}$O values were not significantly different among years ($P = 0.07$) or the two time periods ($P = 0.80$). (b) Cellulose $\delta^{18}$O values of all trees for the four pre-drought years (2004–07, gray) and the four drought years (2013–16, brown). Data pooled from four conifer species at the Teakettle Experimental Forest in the southern Sierra Nevada.

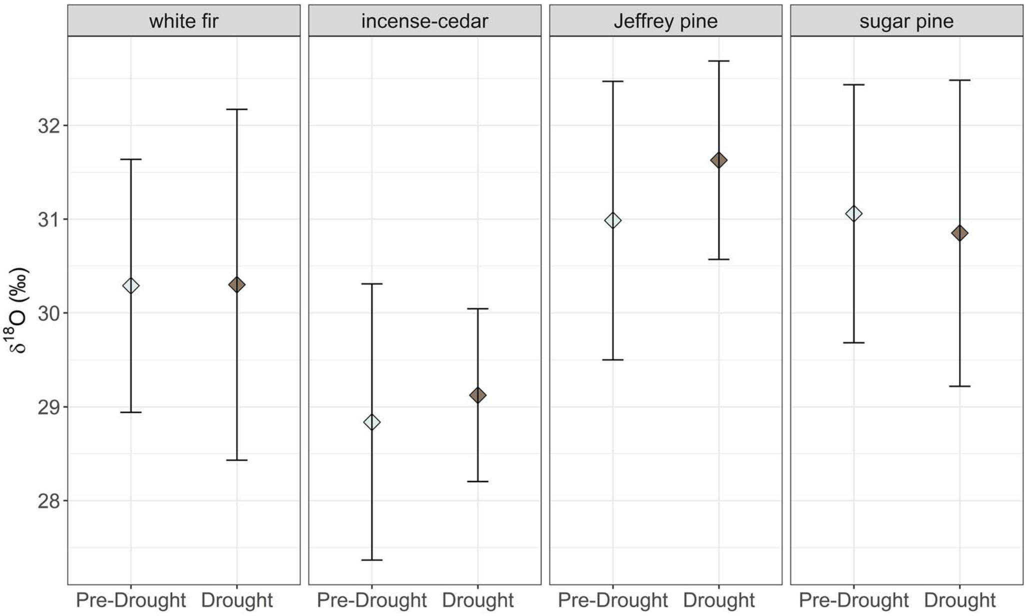

Figure 2. Mean (± SD) cellulose $\delta^{18}$O values by the species and time period. The pre-drought period (gray) includes years 2004–07 and the drought period (brown) includes years 2013–16. Data are from conifers growing at the Teakettle Experimental Forest in the southern Sierra Nevada.

investigated year ($P = 0.18$). When considering all investigated years, white fir had slightly more depleted (lower) $\delta^{13}$C values than incense-cedar ($P = 0.001$) and sugar pine ($P < 0.0001$). The species-specific differences in $\delta^{13}$C values do not mirror the species differences for $\delta^{18}$O values, further suggesting that differences in $\delta^{18}$O values are reflective of water source and not transpiration. Finally, we did not find a significant interaction between species and period ($P = 0.49$), with similar $\delta^{13}$C values during both the pre-drought and drought periods for all four species.

Topography had a minimal influence on tree ring cellulose $\delta^{18}$O values in the species we investigated. Topographic attributes for all trees ranged from 1985 to 2225 m for elevation, 3.8 to 22.6° for slope and values of 4.8 to 11.3 for topographic wetness. We did not find significant linear relationships between $\delta^{18}$O values and elevation ($P = 0.79$, $R^2 = 0.01$), slope ($P = 0.98$, $R^2 = 0.002$) or topographic wetness index ($P = 0.91$, $R^2 = 0.01$). We did however find a significant quadratic relationship between $\delta^{18}$O values and topographic wetness index ($P = 0.02$, $R^2 = 0.16$), with more

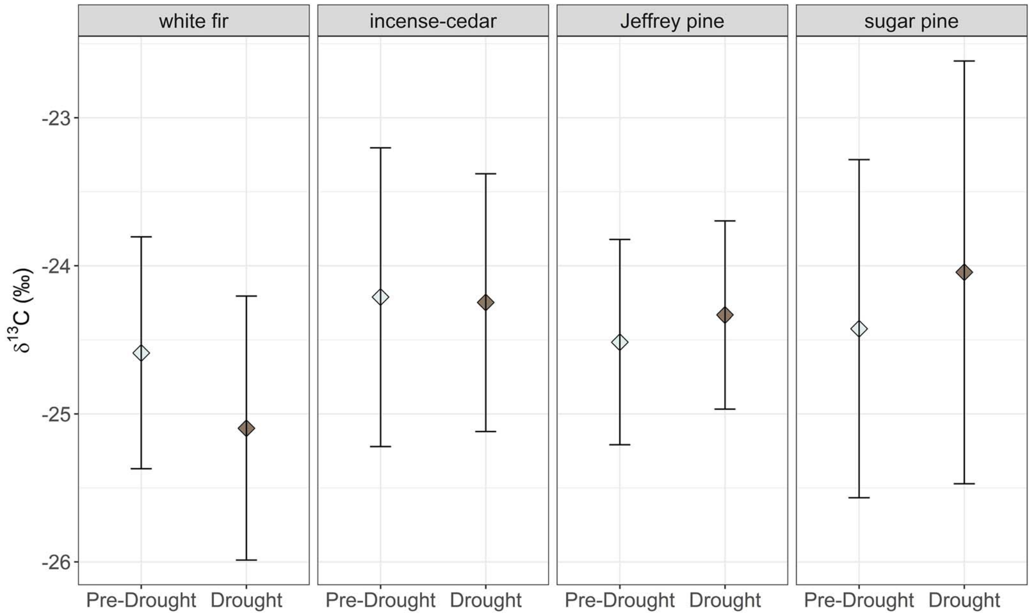

Figure 3. Mean (± SD) $\delta^{13}$C values by species and time period. The pre-drought period (gray) includes years 2004–07 and the drought period (brown) includes years 2013–16. Data are from conifers growing at the Teakettle Experimental Forest in the southern Sierra Nevada.

depleted (lower) $\delta^{18}$O values being associated with trees that had both the driest and the wettest topographic wetness index values. This relationship was consistent for both the pre-drought and drought periods (Figure 4), with no significant interaction between the topographic wetness index and period ($P = 0.78$). We also found a significant linear relationship between $\delta^{18}$O values and neighborhood basal area ($P = 0.002$, $R^2 = 0.23$, Figure 5), with $\delta^{18}$O values becoming more depleted as neighborhood basal area increased. Additionally, neighborhood basal area had a significant linear relationship with the topographic wetness index ($P = 0.02$, $R^2 = 0.13$), with higher neighborhood basal area values associated with wetter topographic wetness index values. The linear mixed-effects model that included slope, elevation and period as fixed effects and tree as a random effect had a lower AICc value ($\Delta$AIC = 27.5) than the linear regression model that included slope, elevation and period without the addition of tree as a random effect. However, species was the only significant predictor in the linear mixed effects model ($P = 0.005$). Finally, no significant linear relationships were found between the average $\delta^{13}$C values for each period and elevation ($P = 0.99$, $R^2 = 0.001$), slope ($P = 0.99$, $R^2 = 0.001$), topographic wetness index ($P = 0.84$, $R^2 = 0.01$) or basal area ($P = 0.98$, $R^2 = 0.002$).

## Discussion

As droughts become more severe, frequent and prolonged in forest ecosystems around the globe, the ability of trees to survive these drought events will largely depend on whether they have access to a reliable water source (Mackay et al. 2019, Brodribb et al. 2020). While woody species in other semi-arid and Mediterranean ecosystems exhibit the ability to switch their primary water source in response to climatic variation (Barbeta et al. 2015, Berkelhammer et al. 2020, Grossiord et al. 2017), our results suggest that large Sierra Nevada conifers may not exhibit this plasticity. We did not find a linear relationship between the $\delta^{18}$O and $\delta^{13}$C values for the trees we investigated, suggesting the $\delta^{18}$O values of the cellulose we analysed were indicative of water source rather than differences in transpiration. As a result, the similarity in the $\delta^{18}$O signature of tree ring cellulose produced both before and during the 2012–16 California drought period may indicate that the large conifer trees we sampled used similar water sources during both time periods and did not switch to using a different water source during this prolonged drought event (Figure 1). However, we did find significant differences in $\delta^{18}$O values that suggest that water source depth and therefore access to deep soil water or rock moisture may vary among species and topographic positions (Figures 2, 4 and 5).

The reliance of Sierra Nevada conifers on a primary water source is consistent with other water-use studies on conifer species of the western USA. For example, studies in southwestern ponderosa pine forests have found that ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws) uses predominantly winter precipitation despite precipitation inputs from monsoonal rains (Kerhoulas et al. 2013, Kerhoulas et al. 2017). Similarly, Guo et al. (2018) found that the majority of conifer species in mixed-conifer and spruce-fir forests of Arizona did not switch their water-use patterns following monsoonal rains. Coupled with these findings, our results further suggest that large montane conifers in the western USA do not switch their water-use patterns in response to changes in water availability and exhibit

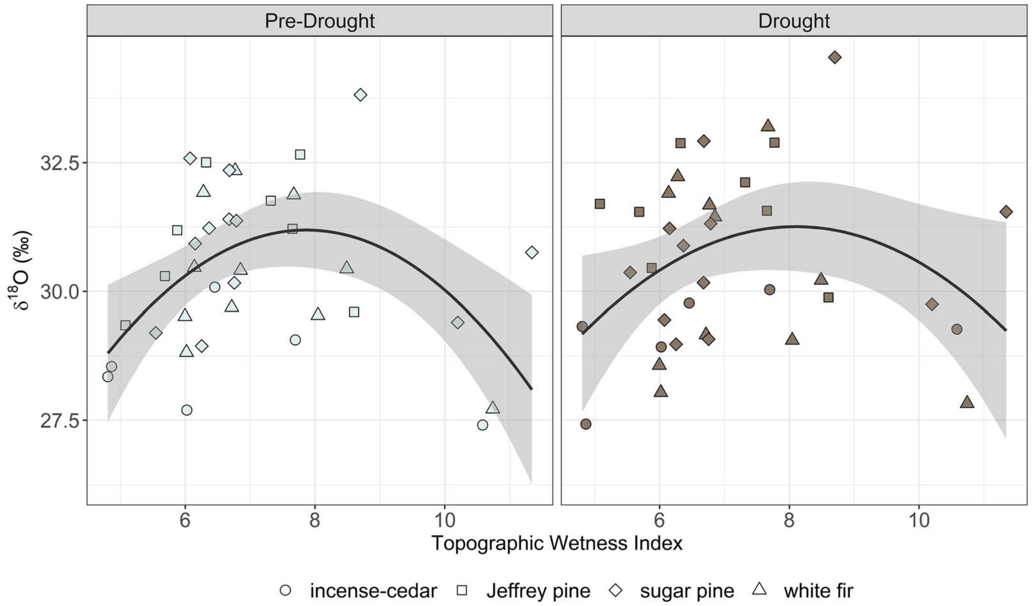

Figure 4. Quadratic relationship between the topographic wetness index for each tree and their mean cellulose $\delta^{18}$O value for the pre-drought period (2004–07, gray) and the drought period (2013–16, brown). The gray band represents the 95% confidence interval. $\delta^{18}$O values showed a significant quadratic relationship with the topographic wetness index ($P = 0.019$, $R^2 = 0.16$) and no significant interaction between the topographic wetness index and period ($P = 0.78$). Point shapes denote the four species (incense-cedar, Jeffrey pine, sugar pine and white fir) growing at the Teakettle Experimental Forest in the southern Sierra Nevada.

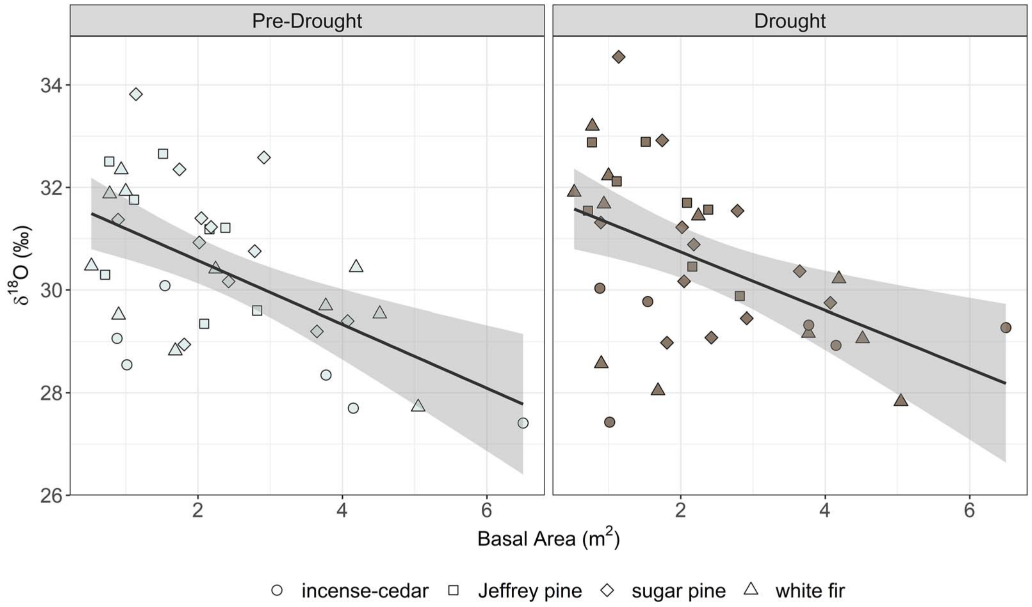

Figure 5. Linear relationship between the neighborhood basal area (m$^2$) for each tree and their mean cellulose $\delta^{18}$O values for the pre-drought period (2004–07, gray) and the drought period (2013–16, brown). The gray band represents the 95% confidence interval. $\delta^{18}$O values showed a significant linear relationship with the neighborhood basal area ($P = 0.00015$, $R^2 = 0.23$) and no significant interaction between basal area and period ($P = 0.73$). Point shapes denote the four species (incense-cedar, Jeffrey pine, sugar pine and white fir) growing at the Teakettle Experimental Forest in the southern Sierra Nevada.

the same annual $\delta^{18}$O signature from year to year. However, the significantly more depleted $\delta^{18}$O values we found for incense-cedar suggest that incense-cedar may be using more water from deeper or less evaporated water reserves than other species (Figure 2). The use of a different water source by incense-cedar may have contributed to the low mortality rates experienced by large trees of this species during the 2012–16 drought (Steel et al. 2021). The relatively more enriched $\delta^{18}$O values we found for both pine species suggest that these species may use more shallow water than both incense-cedar and white fir.

One hypothesis for this finding is that lateral root branching may allow pine species to access shallow soil water in addition to deeper water sources. The species-specific differences in $\delta^{18}$O values we detected may demonstrate that diversity in this mixed-conifer ecosystem increases the niche partitioning of soil water. However, while we did find species-specific differences in cellulose $\delta^{18}$O values, the standard deviation for those values across all investigated years did not differ among species, suggesting that all four species do not exhibit temporal plasticity in their water-use patterns (Figure 2).

While the greatest differences in $\delta^{18}$O values were observed among species, we also found trends suggesting that the primary water source used by trees can vary with topographic position. The quadratic relationship between topographic wetness index and $\delta^{18}$O values confirmed our expectation that trees in dry positions would use deeper water reserves, but also suggests that trees in wet locations use relatively deep water (Figure 4). Given that the neighborhood basal area was higher at wetter sites and trees with the highest neighborhood basal area values exhibited the most depleted $\delta^{18}$O values (Figure 5), this unexpected finding for wet locations could be because of greater competition for water at wetter sites. The more depleted $\delta^{18}$O values found in trees at wetter sites could also be because of these wetter areas being convergence zones where ground water and rock moisture are closer to the surface. Further investigation involving soil water samples at varying depths and throughout the growing season is needed to better understand the quadratic relationship between topographic wetness index and $\delta^{18}$O values. While there is evidence that water-use patterns vary with topographic position, the lack of an interaction between topography and period suggests that the lack of plasticity in the water-use patterns of these species may be due to root access. Despite drier conditions during the drought period, these trees exhibited the same $\delta^{18}$O values as the pre-drought period regardless of topographic position (Figure 4), suggesting no significant change in the annual signature of the water source they were using.

While the $\delta^{18}$O values of tree ring cellulose provide insight to the $\delta^{18}$O values of the water source used by a tree, there are limitations to this type of analysis. For example, we do not know how much $\delta^{18}$O values vary with soil depth or between different forms of hydraulic refugia (e.g. deep rock moisture versus rock fissures). It is possible that the trees we sampled used progressively deeper water throughout the drought period and we were not able to detect this shift in the $\delta^{18}$O signature of alpha cellulose because of isotopic similarity of the deepest water sources. Therefore, we cannot conclusively say that the trees in our study did not switch to using different water sources during the drought and can only identify that there are no significant differences in the average annual $\delta^{18}$O signature of their primary water sources between the pre-drought and drought periods. Future research looking at $\delta^{2}$H or $\delta^{17}$O in tree ring cellulose would be better able to detect these differences. Additionally, future research should consider investigating the isotopic signature of soil and xylem water throughout the growing season to detect changes in tree water-use patterns over the course of a year during drought versus non-drought periods.

Our study is also limited by a relatively small sample size for each species (*n* ranging from 8 to 15) and to a single geographic area. Thus, the lack of temporal and strong topographic trends may be because of the concentrated nature of our samples in the context of the potential range of variability that occurs across the landscape. Because our study focused on large trees that survived the drought, the water-use patterns we identified may also be a result of this sampling scheme. Trees that died during the drought may have relied on shallower water sources, but by only sampling survivor trees, we did not capture this discrepancy. Further, the absence of a significant difference between the $\delta^{13}$C values during these two time periods could indicate that the drought did not cause enough stress in these trees to result in them shifting their water source. Alternatively, the similarity in $\delta^{13}$C values during these two time periods could indicate that these montane tree species operate more anisohydrically and did not reduce stomatal conductance in response to the drought despite experiencing water stress. Future research incorporating $\delta^{18}$O and $\delta^{13}$C values of tree ring cellulose from trees that died during the 2012–16 drought would likely provide insights into water-use patterns associated with drought mortality. Furthermore, because these large, and often older, trees likely have extensive root systems capable of accessing reliable water sources during drought, future investigations should include smaller and younger trees (e.g. seedlings and saplings) to further assess climate-driven changes in water-use patterns (Plamboeck et al. 2008, Kerhoulas et al. 2013).

Overall, our results indicate that while there are species-specific differences in the isotopic signatures of the water source used by large Sierra Nevada conifers, these species do not exhibit temporal plasticity to switch their water source patterns in response to climate variability. Given that this ecosystem annually receives the majority of precipitation as winter snowfall and is then followed by a summer drought period, snowmelt stored in deep soil and rock moisture has historically been a reliable water source for Sierra Nevada conifers (Klos et al. 2018, McCormick et al. 2021). The reliability of these water sources through time may have reduced the need for trees in this region to use multiple water sources. However, as droughts become hotter, drier and longer with climate change, the likelihood that deep soil water and rock moisture begin to dry out increases as increasing vapor pressure deficits and reduced precipitation inputs overdraft the amount of water stored in these reserves (Diffenbaugh et al. 2015, Goulden and Bales 2019). Consequently, the inability of Sierra Nevada conifers to switch their water source in response to the 2012–16 drought may have contributed to the widespread mortality in these forests, as the once-reliable reserves of deep soil water and rock moisture dried out during this prolonged drought period (Goulden and Bales 2019). While trees of certain species may be able to access hydraulic refugia more readily than others, the lack of plasticity to change water source in response to decreased water availability may contribute to widespread mortality in Sierra Nevada forests as climate change dries out these once-reliable water reserves.

## Data availability statement

Supporting code and datasets are available at https://doi:10.5061/dryad.6t1g1jx22.

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## Conflict of interest

Authors declare no conflicts of interest.

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## Authors' contributions

M.J.G., L.P.K. and M.D.H. conceptualized and designed the experiment. L.P.K. and H.S.J.Z. oversaw data collection and tree core processing. M.J.G. conducted all laboratory work. M.J.G. and M.D.H. conducted data analysis. M.J.G., L.P.K., H.S.J.Z., M.P.N. and M.D.H. interpreted results. M.J.G. and M.D.H. wrote the original draft; L.P.K., H.S.J.Z. and M.P.N. contributed to revisions and edits.

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