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Using stable isotopes to quantify water sources for trees and shrubs in a riparian cottonwood ecosystem in flood and drought years

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Abstract

Riparian cottonwood forests in dry regions of western North America do not typically receive sufficient growing season precipitation to completely support their relatively high transpiration requirements. Water used in transpiration by riparian ecosystems must include alluvial groundwater or water stored in the potentially large reservoir of the unsaturated soil zone. We used the stable oxygen and hydrogen isotope composition of stem xylem water to evaluate water sources used by the dominant riparian cottonwood (*Populus* spp.) trees and shrubs (*Shepherdia argentea* and *Symphoricarpos occidentalis*) in Lethbridge, Alberta, during 3 years of contrasting environmental conditions. Cottonwoods did not exclusively take up alluvial groundwater but made extensive use of water sourced from the unsaturated soil zone. The oxygen and hydrogen isotope compositions of cottonwood stem water did not strongly overlap with those of alluvial groundwater, which were closely associated with the local meteoric water line. Instead, cottonwood stem water $\delta^{18}O$ and $\delta^{2}H$ values were located below the local meteoric water line, forming a line with a low slope that was indicative of water exposed to evaporative enrichment of heavy isotopes. In addition, cottonwood xylem water isotope compositions had negative values of deuterium excess (*d*-excess) and line-conditioned (deuterium) excess (*lc*-excess), both of which provided evidence that water taken up by the cottonwoods had been exposed to fractionation during evaporation. The shrub species had lower values of *d*-excess and *lc*-excess than had the cottonwood trees due to shallower rooting depths, and the *d*-excess values declined during the growing season, as shallow soil water that was taken up by the plants was exposed to increasing, cumulative evaporative enrichment. The apparent differences in functional rooting pattern between cottonwoods and the shrub species, strongly influenced the ratio of net photosynthesis to stomatal conductance (intrinsic water-use efficiency), as shown by variation among species in the $\delta^{13}C$ values of leaf tissue.

KEYWORDS
hydrogen and oxygen isotopes, *Populus* sp, *Shepherdia argentea*, *Symphoricarpos occidentalis*

1 | INTRODUCTION

Rivers that flow out of mountainous landscapes create important links with their adjacent terrestrial ecosystems (Hauer et al., 2016). In semi-arid regions, water flows laterally out of the river channels to feed the alluvial groundwater beneath the adjacent terrestrial floodplain. This creates a variety of habitats that have important ecological connectivity with the river even though they can extend some distance laterally away from the river channel. Hauer et al. (2016) have highlighted the diverse range of ecological processes in these systems that promote biodiversity across a full spectrum of ecological interactions involving microbes to large vertebrate animals. Riparian forest trees, in particular, are strongly influenced by their associated river and its flow dynamics (Snyder & Williams, 2000; Rood, Braatne, & Hughes, 2003; Rood, Gourley, et al., 2003; Scott, Shuttleworth, Goodrich, & Maddock, 2000; Scott et al., 2003; Scott et al., 2004). In many areas of North America, riparian forests have declined in response to water diversion associated with dams that withdraw river water for agricultural crop irrigation, municipal and industrial needs, and therefore, restrict water supply to the riparian trees (Rood, Mahoney, Reid, & Zilm, 1995; Rood, Gourley, et al., 2003; Rood et al., 2005; Rood et al., 2008; Scott, Shafroth, & Auble, 1999; Schindler & Donahue, 2006). In order to conserve riparian forest ecosystems, protect their biodiversity, and maintain the many valued ecosystem services they provide (Hauer et al., 2016; Naiman, Décamps, & McClain, 2005), we must manage dam operations so that river flows are regulated at levels sufficient to support riparian ecosystems. An important research objective becomes, therefore, to determine the amount of river water (alluvial groundwater) that is required to meet the normal evapotranspiration requirements of healthy riparian forest ecosystems.

In theory, riparian forest trees could access water from at least three potential water sources: (a) alluvial groundwater taken up by deep roots at the capillary fringe, just above the saturated soil water zone; (b) recent summer precipitation inputs to the shallow soil layers taken up by roots active near the soil surface; and (c) water accumulated throughout the nonsaturated soil zone extending from the soil surface to the capillary fringe. Access to this third source would require active, functional roots throughout the nonsaturated soil zone. The large unsaturated soil zone could accumulate and mix precipitation inputs over time, including melt water from snow, and water input from other sources, such as runoff from plateaus above the river channel and water from periodic over-bank river flooding (Penna et al., 2018; Sprenger, Leistert, Gimbel, & Weiler, 2016). The amount of river water (alluvial groundwater) that was used by riparian trees to support transpiration would depend on the amounts of water taken up from other sources, which in turn would depend on water potential gradients within the soil and the plant's functional rooting pattern (Bowling, Schulze, & Hall, 2016; Penna et al., 2018). It is clear that cottonwood riparian forests in semiarid western regions of North America do not receive sufficient growing season precipitation to completely support the relatively high transpiration requirements of these forests (Flanagan, Orchard, Logie, Coburn, & Rood, 2017; Scott

et al., 2000; Yang, Rood, & Flanagan, 2019). For example, cumulative evapotranspiration in cottonwood riparian forests can exceed precipitation inputs substantially during the growing season (Figure 1). This indicates that a variable, but often substantial, portion of the water used in transpiration in riparian forest ecosystems must be supplied by alluvial groundwater or water stored in the potentially large reservoir of the unsaturated soil zone. Stable isotope analyses offer potential to gain some insights into the relative amounts of water sourced from alluvial groundwater and the unsaturated soil zone by riparian forest plant species (Penna et al., 2018).

Soil water uptake by plant roots predominantly occurs via bulk flow without significant isotopic fractionation, so the stable isotope composition of water in xylem reflects that of water taken up by roots (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002; Penna et al., 2018). There is potential, therefore, to compare the isotope composition of plant xylem water with that of possible water sources to identify and possibly quantify the relative uptake of different water sources (Ogle, Tucker, & Cable, 2014). In many temperate, continental locations, the isotopic composition of river water (alluvial groundwater) is very different from that of summer precipitation, allowing groundwater uptake to be determined by analysing the stable isotope composition of water extracted from plant stems (Dawson et al., 2002; Penna et al., 2018). This can occur despite the fact that river water, groundwater, and precipitation tend to plot along the same local meteoric water line (LMWL), in a graph of the relationship between the oxygen (x-axis) and hydrogen (y-axis) stable isotope composition of the waters (Sprenger et al., 2016). By contrast, water in the nonsaturated soil zone tends to have stable oxygen and hydrogen isotope compositions that do not overlap with the LMWL but instead plot below the LMWL (Sprenger et al., 2016). The unsaturated soil zone would integrate water with a range of stable isotope compositions that reflected the timing and amount of different precipitation
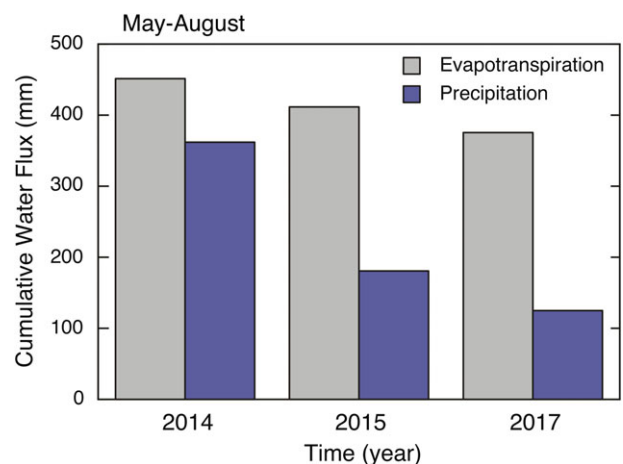

FIGURE 1 Comparison of cumulative evapotranspiration and precipitation in a riparian cottonwood forest during May–August in three different years with contrasting environmental conditions. Evapotranspiration was measured using the eddy covariance technique at the same location used in this study, as previously reported by Flanagan et al. (2017) and Yang et al. (2019). No evapotranspiration measurements were conducted during 2016

events during the year and also include water from surface runoff, throughflow, and stemflow (Allen, Brooks, Keim, Bond, & McDonnell, 2014; Allen, Keim, Barnard, McDonnell, & Brooks, 2017; Sprenger et al., 2016; Sprenger et al., 2018). Water in the unsaturated soil would be exposed to evaporation during transit to the soil and while in the shallow soil layers, and thus, its stable isotope composition would be altered by fractionation processes that occur during evaporation. As a consequence, the isotopic composition of water in the unsaturated soil zone would be heterogeneous spatially and temporally but would likely be consistently different from the stable isotope composition of local groundwater samples (Penna et al., 2018; Sprenger et al., 2016; Sprenger et al., 2018). This creates a scenario where deep-rooted, pheatophyte plants in riparian forests could have stem water stable isotope compositions that reflected predominant groundwater use, if that was their main water source. For example, a riparian cottonwood tree (*Populus fremontii*) and a willow shrub species (*Salix gooddingii*) in Arizona were shown to make use of groundwater throughout the growing season at perennial and ephemeral streams (Busch, Ingraham, & Smith, 1992). Alternatively, if the riparian plants predominantly made use of water from the heterogeneous, unsaturated soil zone, their stem water isotopic compositions would differ markedly from groundwater isotopic compositions. Australian *Eucalyptus* trees in riparian habitats have been shown to take up a mixture of groundwater, shallow soil water from recent precipitation inputs, and stream water on the basis of stable isotope measurements (Dawson & Pate, 1996; Mensforth, Thorburn, Tyerman, & Walker, 1994; Thorburn & Walker, 1994). More generally, Evaristo, Jasechko, and McDonnell (2015) have shown that plants sampled across a variety of biomes typically show stem water with oxygen and hydrogen stable isotope compositions that differ from groundwater values (with values that plot below the LMWL), suggesting that the water taken up by plants has been exposed to isotopic enrichment during evaporation, consistent with expectations for water in the unsaturated soil zone (although other interpretations have been presented for these observations; Penna et al., 2018). In addition, a meta-analysis conducted by Evaristo and McDonnell (2017) concluded that plants in most ecosystems have limited groundwater use.

In this study we characterized water use by a riparian forest along the Oldman River in southern Alberta. The main objective was to test if the xylem water extracted from the dominant cottonwood (*Populus* spp.) trees in the riparian forest was (a) primarily sourced from the alluvial groundwater supplied by the adjacent river or whether it was also (b) obtained from the unsaturated soil zone. In the case of water use from the unsaturated soil zone, cottonwood xylem water stable isotope compositions should differ from the LMWL and have negative values of deuterium excess (*d*-excess) and line-conditioned (deuterium) excess (*lc*-excess), both of which provide evidence of the extent that soil water had been exposed to fractionation during evaporation (Landwehr & Coplen, 2014; Sprenger et al., 2016; Sprenger et al., 2018). In addition, we compared seasonal variation in the stable isotope composition of cottonwood xylem water with that of two smaller stature shrubs (silver buffaloberry, *Shepherdia argentea*; and snowberry, *Symphoricarpos occidentalis*) that were also present in

our riparian forest. We predicted that the two shrub species would have lower (more negative) values of *d*-excess and *lc*-excess than would the cottonwood trees due to shallower rooting depths and that *d*-excess and *lc*-excess values would decline during the growing season, as shallow soil water that was taken up by the plants was exposed to increasing, cumulative evaporative enrichment.

2 | METHODS

2.1 | Study site description

We studied a riparian cottonwood forest within the Helen Schuler Nature Reserve (HSNR) in Lethbridge, Alberta, Canada (49.702°N, 112.863°W, elevation 928 m). This study site has been described in detail in our previous publications (Flanagan et al., 2017; Rood et al., 2013; Yang et al., 2019), and so we provide below only a short summary of the site characteristics. The average annual precipitation and temperature in Lethbridge during 1981–2010 were 380.2 mm and 5.9 °C, respectively (www.climate.weather.gc.ca/climate_normals/).

The forest consisted of cottonwood trees including individuals from primarily two species, narrowleaf cottonwood (*Populus angustifolia* James) and prairie cottonwood (*P. deltoides* Bartr. Ex Marsh), along with their hybrids, which are referred to as lanceleaf cottonwoods (*P. x acuminata* Rydb.; Gom & Rood, 1999; Rood et al., 2013; Zanewich, Pearce, & Rood, 2018). Some balsam poplar (*P. balsamifera* L.) trees were also present. The sparse understory had a few major shrub species including silver buffaloberry (*Shepherdia argentea* (Pursh) Nutt.), snowberry (*Symphoricarpos occidentalis* Hook.), wolf willow (*Elaeagnus commutata* Bernh. Ex Rydb.), and wild rose (*Rosa acicularis* Lindl.). The herbaceous plant community was dominated by a range of grass species. The tree canopy and understory plants contributed approximately equally to the ecosystem leaf area index, which was measured at 1.8 ± 0.2 m^2/m^2 in 2014 (Flanagan et al., 2017). The tree density was relatively low (276 ± 300 trees/ha, mean $\pm$ SD), with average tree height and tree diameter (at 1.35 m above ground) of 18 ± 5 m and 37 ± 15 cm, respectively (Flanagan et al., 2017).

2.2 | Meteorological measurements and river flows

We characterized the major environmental conditions during the growing season (May–October) using the following measurements and calculations: daily-integrated photosynthetically active photon flux density, daily average air temperature, daily average vapour pressure deficit (VPD), integrated soil moisture content in the top 250-cm depth of the soil, and cumulative growing season precipitation input. The instruments used to measure these environmental conditions were described in Flanagan et al. (2017). To help illustrate seasonal variation in air temperature, we also calculated a lagged air temperature average ("acclimation temperature" *sensu* Makela, Hari, Berninger, Hanninen, & Nikinmaa, 2004; and Kolari, Lappalainen, Hanninen, & Hari, 2007; evaluated using a 200-hr time constant as described by Flanagan et al., 2017). Volumetric soil moisture content was calculated

on the basis of a calibration equation used to convert the temperature-corrected period measurements of the soil water content reflectometer measurements. Volumetric soil water content values at soil depths of 100, 150, 200, and 250 cm were integrated vertically to estimate the total moisture content in the upper 2.5 m of soil. We recorded the maximum integrated soil water content (1,577 mm) during the flood conditions that were apparent in June 2014.

We obtained daily average flow rates for the Oldman River from the Water Survey of Environment Canada (http://wateroffice. ec.gc.ca), as recorded at Lethbridge (water ID station 05AD007).

2.3 | Stable isotope measurements

Samples were collected during April–October in 2014–2016 to characterize the stable isotope composition of Lethbridge precipitation, the Oldman River water, and HSNR alluvial groundwater. Precipitation was collected weekly, or more frequently during large rain storms, whereas river water and groundwater samples were collected at approximately 2-week intervals. Precipitation, river water, and groundwater samples were sealed in 30-ml glass vials with lined caps and stored in a cool, dark cabinet, or fridge (4 °C) until analysis.

A plastic container attached inside a modified tipping bucket rain gauge was used to collect precipitation for stable isotope analyses, an apparatus very similar to that described by Gröning et al. (2012). The rain gauge housing served as protection from the wind as well as a screen from solar radiation to prevent evaporation from the sample collection container. The precipitation collection device was located on the roof of the Alberta Water and Environmental Sciences Building at the University of Lethbridge, approximately 2 km away from the HSNR study site.

River water samples were collected from below the water surface in the centre of the Oldman River. This was achieved by lowering a weighted collection vessel from a pedestrian bridge located above the river, near the southern end of the HSNR. Groundwater was collected from two groundwater wells in the HSNR made from 2.5-cm-diameter vertical pipes that penetrated the saturated groundwater table beneath the surface (approximately 2.5-m depth). In order to minimize fractionation caused by evaporation, any standing water in the wells was pumped out and discarded, and the wells were allowed to recharge before collecting a groundwater sample. Collected river and groundwater samples were filtered for debris and sediment using a Buchner funnel, lined with 1.1-μm filter papers (Whatman International Ltd, Maidstone England), that was connected to a vacuum pump.

Woody plant samples were collected so that water in xylem tissue could be extracted via cryogenic vacuum extraction (apparatus described by Ehleringer, Roden, & Dawson, 2000; using methods described by West, Patrickson, & Ehleringer, 2006; samples were extracted for 1.5 hr) and compared with the environmental water samples described above. In 2014, samples of cottonwood trees (*Populus* spp.) were collected every 2 weeks from June 4 (Day 155) to October 2 (Day 275). For the majority of the sample dates in 2014, six replicates were collected (on two dates, only four samples were collected), each originating from a separate, randomly selected tree that was located centrally within the HSNR forest. In 2015, three sets of paired cottonwood trees (one male and one female within 5 m of each other) were repeatedly sampled at approximately 2-week intervals during June–August (Days 152–243). The three sets of paired trees were located near the centre of the HSNR site along the east–west axis and spaced approximately equally across the HSNR area along the north–south axis of the site. A similar procedure was used in 2015 for collecting samples from three sets of paired silver buffaloberry (*Shepherdia argentea*) shrubs (one male and one female). We included both males and females for *Populus* and *Shepherdia* plants in order to sample the biological diversity present, but the sample sizes were minimal and we did not anticipate (or observe) differences between male and female stem water isotopic compositions in either plant genus. In 2016, we also collected stem samples of snowberry (*Symphoricarpos occidentalis*), in addition to the cottonwood and silver buffaloberry stem samples. Two or three branch samples (approximately 5 cm long, 0.5-cm diameter) were cut from an individual tree or shrub, and the green bark was quickly peeled off and discarded before sealing the woody tissue in a glass vial. The sample vials were placed inside an insulated container on an ice pack in the field until they were returned to the lab and stored in a freezer.

All water samples were analysed for the stable oxygen ($^{18}O/^{16}O$) and hydrogen ($^2H/^1H$) isotope ratio, with the isotope ratios expressed using delta notation:

$$\delta^{18}O \text{ or } \delta^2H = \left(\frac{R_{Sample}}{R_{Standard}}\right) - 1,$$

where R is the $^{18}O/^{16}O$ or $^2H/^1H$ ratio of a sample and a known international standard (Vienna Standard Mean Ocean Water), respectively. Measurements are conveniently expressed in per mille (‰). Prior to analysis, plant water samples were equilibrated with activated charcoal to help remove any plant organic chemical extracted along with the water. All isotope measurements were conducted at the University of Calgary Isotope Science Laboratory (www.ucalgary.ca/uofcisl/) primarily by isotope ratio infrared spectroscopy using a liquid water isotope analyser (DLT-100 v.2, Los Gatos Research Inc., Mountain View, CA, USA) equipped with an autosampler (CTC LC PAL, LEAP Technologies, Carrboro, NC, USA). Some water samples were analysed for $\delta^{18}O$ by gas isotope ratio mass spectrometry (Delta V with Gas Bench, ThermoFinnigan, Bremen, Germany) using pure CO_2 that had been equilibrated with a water sample. During this study, we also measured the $\delta^{18}O$ and δ^2H values of four working standards (water samples) that had been previously calibrated against international reference materials and had the following assigned (informal) isotopic compositions ($\delta^{18}O$, δ^2H [‰], respectively): VIC: −10.64, −76.5; LMX: −14.38, −105.6; BCGW: −17.48, −131.8; RMSW: −19.25, −147.7. Repeated measurements (n = 14 or 15) of the working standards during the course of this study indicated that both the precision (±1 SD) and accuracy of the measurements were better than 1.1‰ for δ^2H and 0.25‰ for $\delta^{18}O$ for all the working standards.

Deuterium excess (d-excess = $\delta^2H - 8\,\delta^{18}O$; Dansgaard, 1964) values and the line-conditioned excess values (lc-excess = $\delta^2H - $ (slope $\cdot\ \delta^{18}O$) – intercept, where slope and intercept represent values from the LMWL for a given year; Landwehr & Coplen, 2014; Sprenger et al., 2016) were calculated for all measurements of plant stem water isotope composition. We assume on the basis of evidence discussed below that d-excess and lc-excess values could be used as a proxy for plant uptake of shallow soil water that had been exposed to fractionation during evaporation. Lower values of d-excess and lc-excess occur in water samples that have been exposed to greater evaporative enrichment (Peng, Mayer, Harris, & Krouse, 2004; Sprenger et al., 2016; Sprenger et al., 2018), and lower d-excess values in stem water have previously been shown to occur in plants with shallower rooting depths (Dawson & Simonin, 2011; Matheny et al., 2017; Simonin et al., 2014; West et al., 2012). We predicted that the stem water of the two shrub species would have lower values of d-excess and lc-excess than would the cottonwood trees and that d-excess and lc-excess values would decline during the growing season in all of the plants. Average height of the plants sampled for stem water isotopic composition differed as follows: *Symphoricarpos occidentalis*, 0.8 ± 0.08 m; *Sherpherdia argentea*, 3.1 ± 0.4 m; *Populus* sp., 18 ± 5 m, and we expected that rooting depth could be at least partially correlated with height among these plant types. Excavation at the study site during installation of soil moisture sensors confirmed that *Populus* sp. trees had roots that extended 250 cm deep and reached the capillary fringe just above the level of alluvial groundwater in autumn of 2013, a time when the river discharge was low. This rooting depth for *Populus* sp. trees was consistent with observations published by Rood, Bigelow, and Hall (2011) in several other study areas. A two-way repeated-measures analysis of variance (ANOVA) was conducted, to test for significant differences among plant types across the repeated stem water d-excess and lc-excess measurements made during the 2016 growing season (time [sample date] was the repeated factor), using Matlab software (R2018b, The Mathworks Inc., Natick, MA, USA). The lc-excess measurements from stem water in *Populus* and *Shepherdia* were also averaged across the different sample dates for the separate study years (2014–2016 for *Populus* and 2015–2016 for *Shepherdia*). A one-way ANOVA (*Populus*) or Kruskal–Wallis (*Shepherdia*) test was used to test for significant differences among years using Matlab software.

On August 5, 2016, we collected several leaves from across the canopy of the same plants sampled for stem water isotope ratios for each of three plant types: *Populus* sp., *Sherpherdia argentea*, and *Symphoricarpos occidentalis*. Leaves from an individual plant canopy were combined and dried at 60 °C. After drying, the plant tissue was ground using a ball mill (Retsch MM200, Haan, Germany). We analysed the $^{13}C/^{12}C$ carbon isotope composition (expressed using delta notation, $\delta^{13}C_{PDB}$, ‰) of leaf tissue using a coupled elemental analyser (Costech 4010) and gas isotope ratio mass spectrometer (Finnigan Mat Delta+XL) at the University of Calgary Isotope Science Laboratory. The precision of the $\delta^{13}C$ measurements was 0.2‰ based on the SD of repeated analyses of international and internal laboratory standards. A one-way ANOVA was used to test for significant

differences among plant types for leaf $\delta^{13}C$ measurements using Matlab.

3 | RESULTS

3.1 | Seasonal and interannual variation in environmental conditions

The daily average air temperature increased from lows in early May (Day 120) to midseason peaks in early July (approximately Day 190; Figure 2d–f). The low air temperatures in May occurred as the growing season was initiated, despite the relatively high daily integrated solar radiation at that time (photosynthetically active photon flux density; Figure 2a–c). The seasonal pattern of change in air temperature was quite similar among all three study years, except that June 2014 (Days 156–170) was quite cool because of high rain input during that time and a second, unusually cold spell occurred in early September 2014 (approximately Day 253; Figure 2d–f). Daily average VPD was very variable, and the highest daily average VPD values occurred in 2015, the year with the lowest growing season precipitation (Figure 2g–i).

The Oldman River flooded during 2014, the study year with the highest cumulative precipitation (374 mm) during May–October (growing season). The long-term average (± SD) May–October precipitation for Lethbridge was 268 ± 92 mm. Growing season cumulative precipitation was lowest in 2015 (192 mm), and in 2016 (273 mm), it was very close to the long-term average (Figure 3). The contrasting patterns of precipitation that occurred among study years resulted in correlated seasonal and interannual differences in Oldman River discharge (Figure 4) and integrated soil moisture content (Figure 5). Flooding of the Oldman River resulted in soil moisture remaining high throughout the growing season in 2014, but soil moisture did progressively decline throughout the study to low values in 2016 that were approximately 30% of the 2014 maximum (Figure 5).

3.2 | Stable isotope analyses of environmental waters and plant stem water

A strong linear relationship was observed between the oxygen ($\delta^{18}O$ values) and hydrogen (δ^2H values) isotope compositions of precipitation samples collected during April–October (Figure 6), which defined a LMWL. River water and alluvial groundwater samples had $\delta^{18}O$ and δ^2H values that overlapped the LMWL (Figure 6).

The oxygen and hydrogen isotope compositions of plant stem waters are illustrated in relation to the isotope composition of precipitation, river water, and alluvial groundwater that were measured during the same time periods for plant sampling; 2014–2016 for *Populus* (Figure 7a), 2015–2016 for *Shepherdia* (Figure 7b), and 2016 for *Symphoricarpos* (Figure 7c). In addition, the plant stem water values are plotted on altered x-axis and y-axis scales in Figure 8 to allow a closer comparison of the plant water isotopic compositions to those of the river and alluvial groundwater and the LMWL for the relevant time of sampling. For all three plant genera, linear regressions
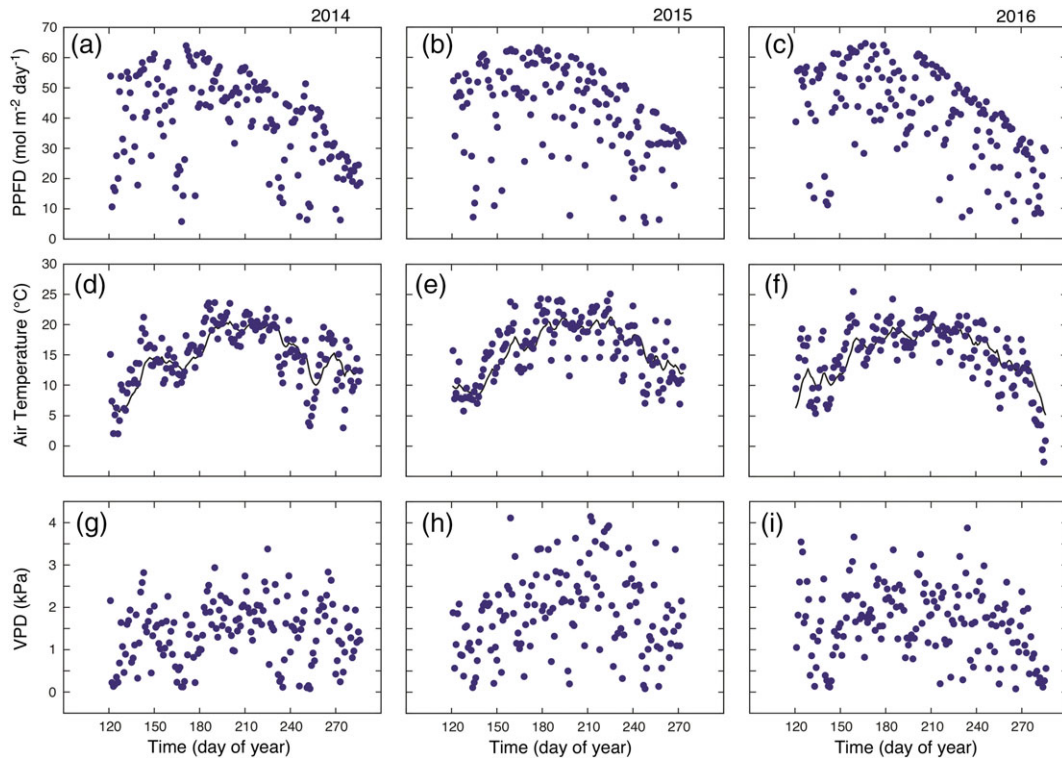
FIGURE 2   Seasonal variation in (a–c) daily-integrated photosynthetically active photon flux density (PPFD), (d–f) daily average air temperature (1 m), and (g–i) daily average vapour pressure deficit (VPD) during 2014–2016 in Lethbridge, Alberta. The solid black line in (d)–(f) shows the acclimation temperature (a lagged, daily average temperature calculation using a 200-hr time constant)
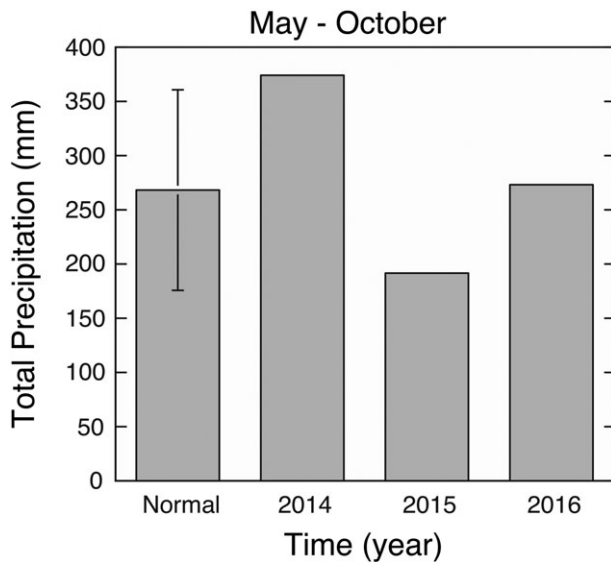

FIGURE 3   Total precipitation during May–October recorded during 2014–2016 in Lethbridge, Alberta. Also shown is the climate normal precipitation (30-year average ± SD, 1981–2010)

between the $\delta^{18}O$ and $\delta^{2}H$ values of stem water samples had relatively low slopes (ranging from 3.9 to 4.6), and most of the stem water isotopic compositions plotted below the LMWL lines, suggesting that the stem water had been exposed to some evaporative enrichment (Figures 7 and 8). As expected, the small stature *Symphoricarpos* shrub had stem water isotope compositions that did not overlap with those

of either the river or alluvial groundwater values (Figures 7c and 8c). The stem water $\delta^{18}O$ and $\delta^{2}H$ values for *Shepherida* also showed very limited overlap with river and alluvial groundwater values (Figures 7b and 8b). Although stem water samples from *Populus* had $\delta^{18}O$ and $\delta^{2}H$ values that showed some overlap with river and alluvial groundwater, it was noteworthy that the vast majority of stem water samples had isotopic compositions that were quite distinct from the alluvial groundwater or river water samples (Figures 7a and 8a).

The average *d*-excess and *lc*-excess values for stem water samples declined over time during the 2016 growing season in all plant types and were consistently lower in the two shrub species than in the *Populus* trees (Figure 9a,b). Plant type and time (sample date was the repeated factor) were statistically significant effects on the basis of a repeated-measures two-way ANOVA for both *d*-excess and *lc*-excess values. For *d*-excess values, plant effect, $F_{(2, 52)} = 20.09$, $p < .0001$, and time effect, $F_{(4, 52)} = 14.55$, $p < .0001$, whereas their interaction was not a significant factor, interaction, $F_{(8, 52)} = 1.72$, $p = .1157$. For *lc*-excess values, plant effect, $F_{(2, 52)} = 19.92$, $p < .0001$, and time effect, $F_{(4, 52)} = 12.93$, $p < .0001$, whereas their interaction was not a significant factor, interaction, $F_{(8, 52)} = 1.42$, $p = .2098$.

The slope of the regression line between the oxygen and hydrogen isotope compositions of stem water differed among *Populus* samples collected in the three study years, with the slopes of these regression lines increasing progressively from 2014 to 2016 (Table 1). In addition, the *lc*-excess values for *Populus* stem water samples, which were averaged for a given year's growing season, increased
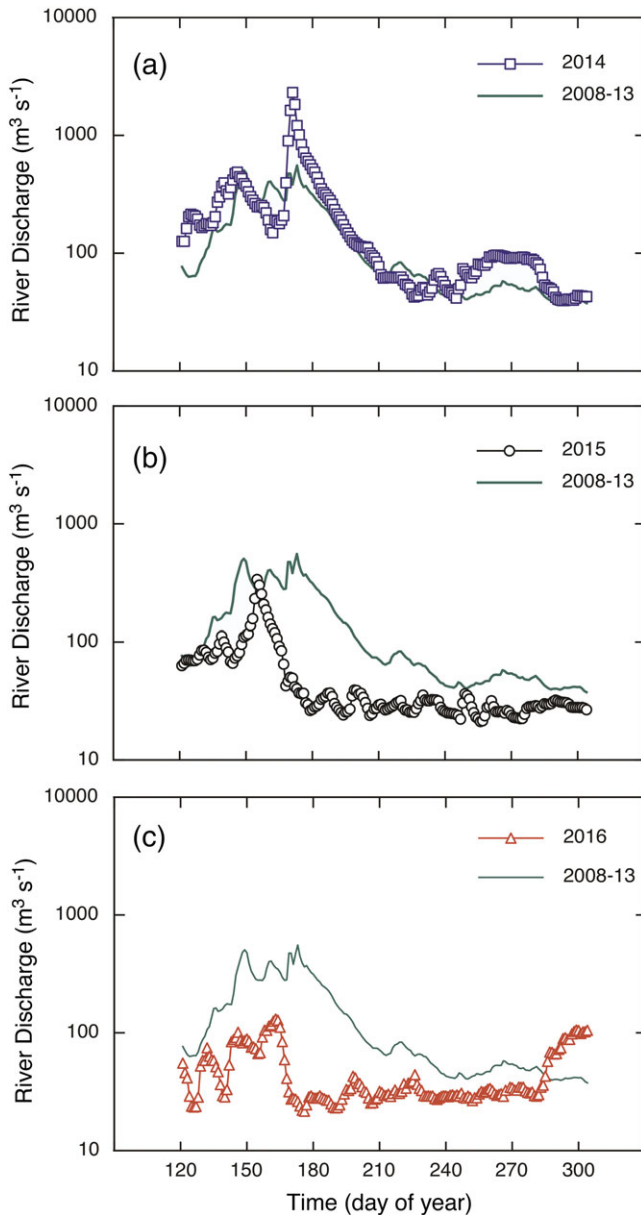
FIGURE 4 Seasonal variation in daily average discharge of the Oldman River in (a) 2014, (b) 2015, and (c) 2016, compared with average river discharge rates measured during 2008–2013 in Lethbridge, Alberta
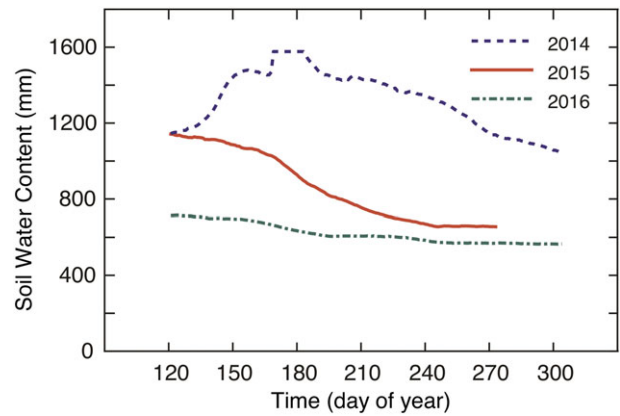

FIGURE 5 Seasonal variation in integrated soil moisture content in the top 250-cm depth during 2014–2016 in Lethbridge, Alberta. Dotted blue line, 2014; orange solid line, 2015; green dash-dotted line, 2016
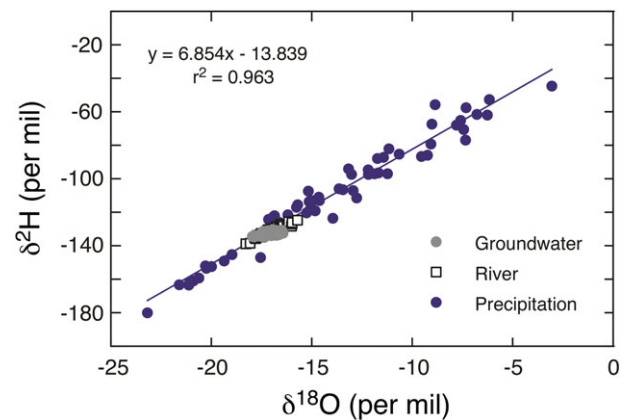

FIGURE 6 Comparison of the oxygen (δ^{18}O, ‰) and hydrogen (δ^2H, ‰) isotope compositions of precipitation (solid blue circles), Oldman River water (open black squares), and alluvial groundwater (solid grey circles) collected in Lethbridge, Alberta. All samples were collected between the months of April and October, 2014–2016. Precipitation samples were collected for specific rain events during the spring and summer seasons (see Section 2 for details). The equation shown represents a linear regression fitted to all the precipitation data, 2014–2016 ($y = 6.854x − 13.839$, $r^2 = 0.963$)

significantly from 2014 through to 2016 (Figure 10a), one-way ANOVA, $F_{(2, 158)} = 31.36$, $p < .001$, with all years having significantly different values based on Tukey–Kramer multiple comparison tests. The *lc*-excess values for *Shepherdia* stem water samples, which were averaged during a given year's growing season, were also significantly higher in 2016 than in 2015 (Figure 10b), Kruskal–Wallis test, $\chi^2_{(1, 135)} = 7.79$, $p = .0052$.

3.3 | Stable carbon isotope analyses of leaf tissue

Plant type was a significant source of variation among leaf tissue δ^{13}C values (Figure 10c), one-way ANOVA, $F_{(2, 13)} = 21.62$, $p < .001$.

Tukey–Kramer multiple comparison tests indicated that the average δ^{13}C values of both shrub species were significantly higher than the value for the *Populus* trees but that there was no significant difference between the δ^{13}C values for the two shrub species (Figure 10c).

4 | DISCUSSION

Our results indicated that the riparian cottonwood trees did not exclusively take up alluvial groundwater but made extensive use of water sourced from the unsaturated soil zone. For example, the oxygen and hydrogen isotope compositions of cottonwood stem water did not strongly overlap with those of alluvial groundwater, which were
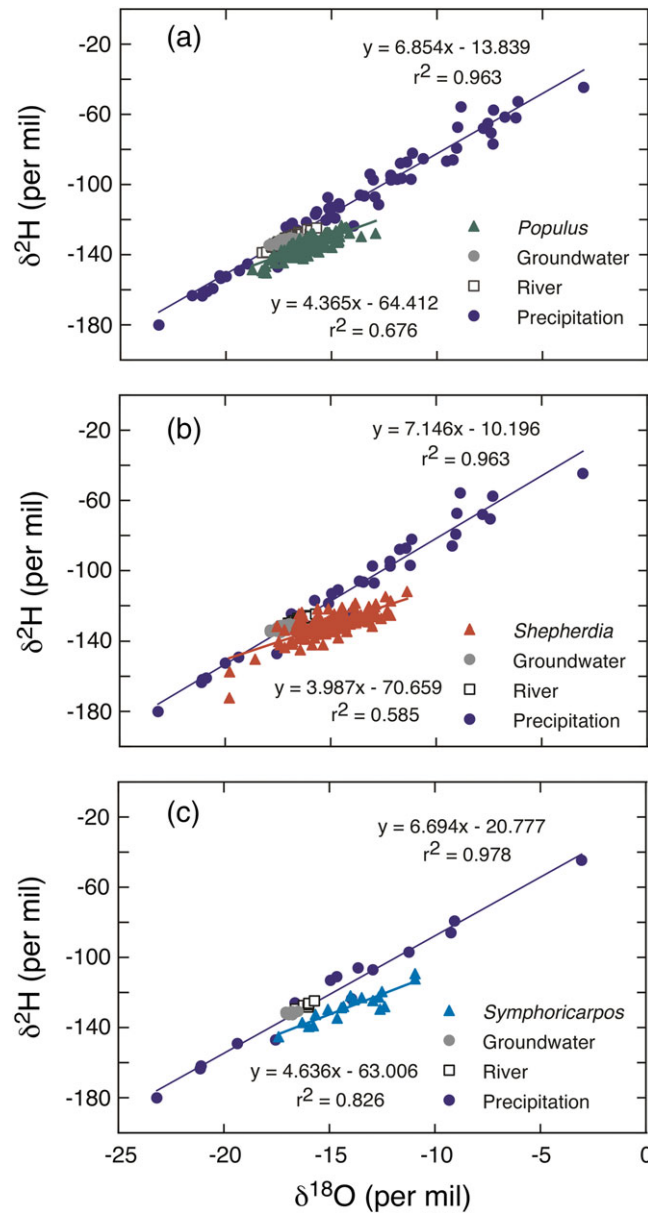
FIGURE 7 Comparison of the oxygen ($\delta^{18}O$, ‰) and hydrogen ($\delta^{2}H$, ‰) isotope compositions of precipitation (blue circles), Oldman River water (open squares), and alluvial groundwater (solid grey circles), and water extracted from plants stems. (a) Environmental waters and stem water from cottonwood trees (*Populus* sp., solid green triangles) collected during 2014–2016. These are the same data as shown in Figure 6, except with the cottonwood tree stem water data added. The top equation shown represents a linear regression fitted to all the precipitation data ($y = 6.854x − 13.839$, $r^2 = 0.963$), and the bottom equation represents a linear regression fitted to all the *Populus* stem water data ($y = 4.365x − 64.412$, $r^2 = 0.676$). (b) Environmental waters and stem water of silver buffaloberry (*Shepherdia argentea*, solid orange triangles) collected during 2015–2016. The top equation shown represents a linear regression fitted to the precipitation data from 2015–2016 ($y = 7.146x − 10.196$, $r^2 = 0.963$), and the bottom equation represents a linear regression fitted to all the *Shepherdia* stem water data ($y = 3.987x − 70.659$, $r^2 = 0.585$). (c) Environmental waters and stem water of snowberry (*Symphoricarpos occidentalis*, solid light blue triangles) collected during 2016. The top equation shown represents a linear regression fitted to the precipitation data from 2016 ($y = 6.694x − 20.777$, $r^2 = 0.978$), and the bottom equation represents a linear regression fitted to all the *Symphoricarpos* stem water data ($y = 4.636x − 63.006$, $r^2 = 0.826$)

closely associated with the LMWL (Figures 7 and 8). Instead, cottonwood stem water $\delta^{18}O$ and $\delta^{2}H$ values were predominantly located in dual isotope plots below the LMWL, forming a separate line with a slope that was indicative of water that had been exposed to evaporative enrichment of heavy isotopes. In addition, cottonwood xylem water stable isotope compositions had negative values of *d*-excess

and *lc*-excess (Figures 9 and 10a), both of which provide evidence that water taken up by the cottonwood trees had been exposed to fractionation during evaporation, which would be expected for water in the unsaturated soil zone (Landwehr & Coplen, 2014; Sprenger et al., 2016; Sprenger et al., 2018). Finally, seasonal variation occurred in the *d*-excess/*lc*-excess values of cottonwood stem water, which
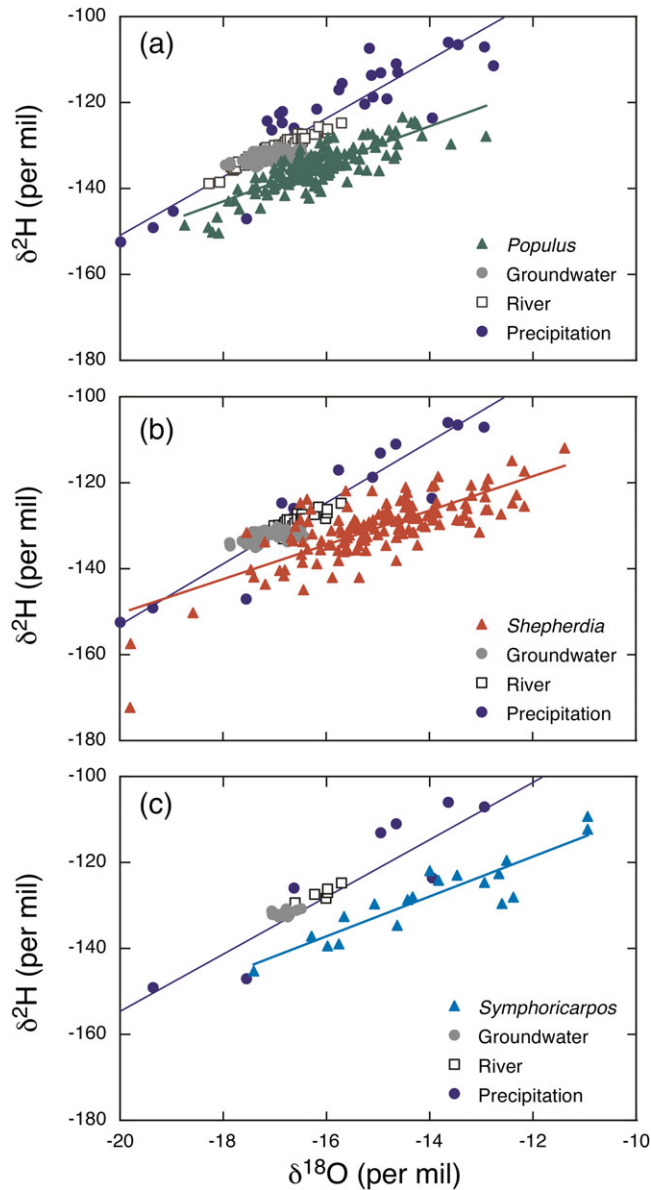
FIGURE 8 Same data and figure format as those shown in Figure 7a–c, except that the scaling of both the x-axis and the y-axis is altered to better allow a close comparison between the stem water data and the ground water and river water data. (a) Data for 2014–2016 including *Populus* stem water; (b) 2015–2016 data including *Shepherdia* stem water; (c) 2016 data including *Symphoricarpos* stem water

FIGURE 9 Seasonal variation in the average (± SE) (a) deuterium excess (*d*-excess) and (b) line-conditioned excess (*lc*-excess) calculated for plant stem water values sampled during 2016: cottonwood trees (*Populus sp.*, solid green squares), silver buffaloberry (*Shepherdia argentea*, open orange triangles), snowberry (*Symphoricarpos occidentalis*, solid light blue circles)

TABLE 1 Comparison of linear regression parameters for the relationship between the oxygen (δ^{18}O values, x-axis) and hydrogen (δ^{2}H values, y-axis) isotope composition of stem water extracted from cottonwood (*Populus* sp.) trees in Lethbridge, Alberta

Variable	2014	2015	2016
slope	3.273	4.760	5.069
intercept	−81.295	−57.492	−53.894
r^2	0.439	0.687	0.774
n	37	82	42

Note. The r^2 value is the coefficient of determination for each regression equation, and *n* is the number of stem water samples analysed.

would be expected for soil water exposed to cumulative seasonal evaporation effects in the shallow soil layers (Figure 9).

The results of this study were consistent with a previous water source study of riparian cottonwood trees in southern Arizona (Sonoran Desert) that made use of stable isotope analyses of tree stem water and two- and three-component source mixing models (Snyder & Williams, 2000). Some shallow soil water use was observed in *Populus fremontii* along the San Pedro River in southern Arizona, an area that receives significant summer precipitation input from monsoon rain, typically in late July through September (Snyder & Williams, 2000). The extent of cottonwood tree water use from the unsaturated
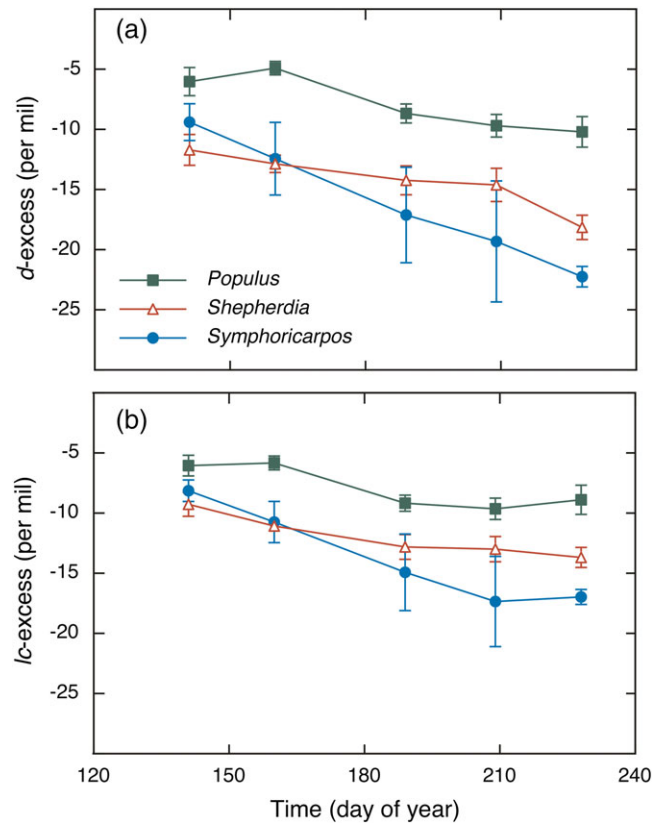
soil zone was dependent on whether the floodplain groundwater was perennial, intermittent, or ephemeral, factors that resulted in associated seasonal changes in the groundwater depth during the active growing season (Snyder & Williams, 2000). The magnitude of shallow soil water use in *P. fremontii* at the ephemeral site increased from less than 10% to approximately 30% as depth to groundwater increased, and was also influenced by the timing of significant input of monsoon rain water in the later growing season (Snyder & Williams, 2000). By contrast, *P. fremontii* trees along the Bill Williams and Lower Colorado
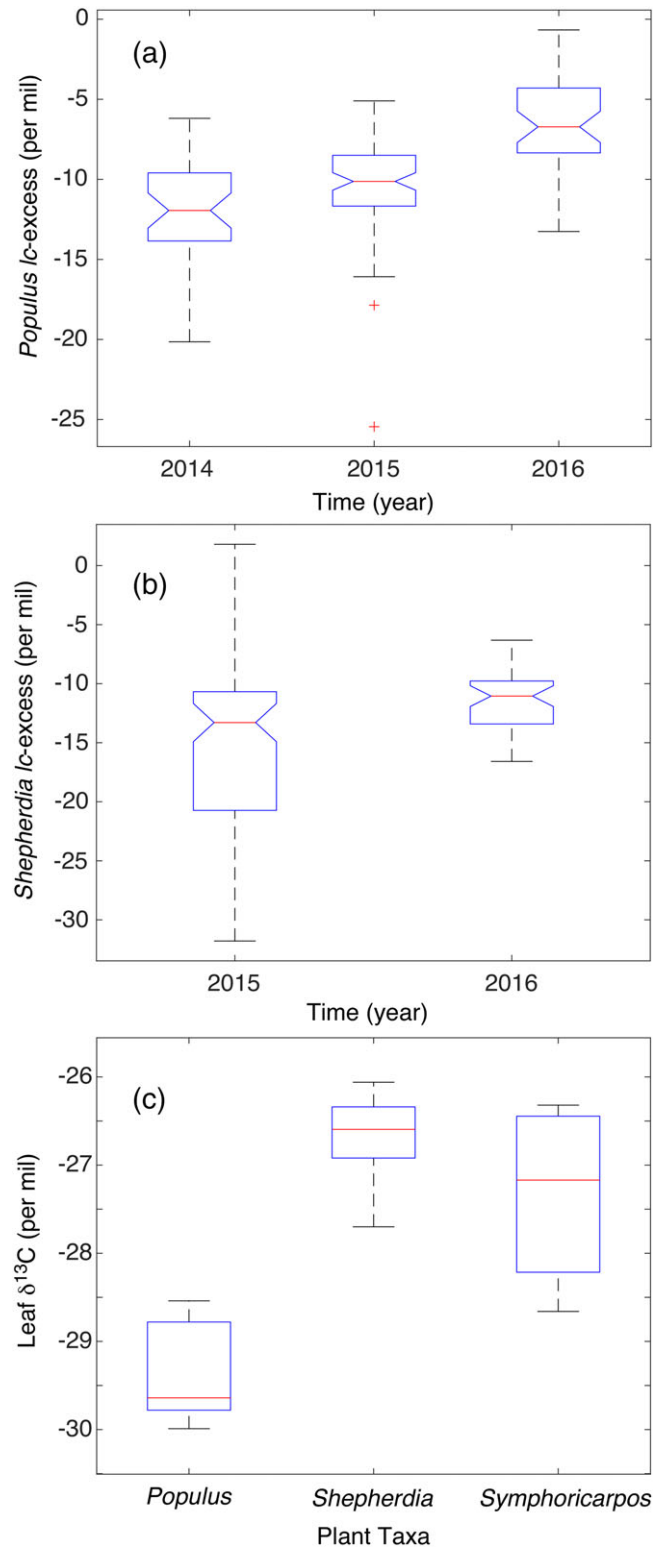
FIGURE 10 (a) Box plots for the line-conditioned excess (*lc*-excess) values calculated from stem water stable isotope compositions measured for cottonwood trees (*Populus* sp.) during the growing seasons of 2014–2016. The red horizontal line and notch in the box represents the median, whereas the top and bottom of the box represent the 25th and 75th percentiles, respectively. The whiskers represent the smallest and largest values, excluding extreme values (greater than 1.5 times the interquartile range), which are indicated as red crosses. (b) Box plots for the line-conditioned excess (*lc*-excess) values calculated from stem water stable isotope compositions measured for silver buffaloberry (*Shepherdia argentea*) during the growing seasons of 2015 and 2016. (c) Box plots for the leaf tissue carbon isotope ($^{13}C/^{12}C$) composition ($\delta^{13}C$ values) for the cottonwood (*Populus* sp.), silver buffaloberry (*Shepherdia argentea*), and snowberry (*Symphoricarpos occidentalis*) plants sampled during 2016

rivers in western Arizona only made use of groundwater and did not take up shallow soil water throughout the growing season, but very little summer precipitation input occurs in this region of the Mojave Desert (Busch et al., 1992). In our study region of southern Alberta, almost 70% of the annual precipitation occurs during May–October, with approximately 40% of the annual precipitation received in the three months (June–August) that define the major period of the growing season for local cottonwood trees. With such significant summer precipitation input, and with litter decomposition and soil nutrients likely most readily available in shallow soil layers, it is expected that cottonwood trees would allocate significant root production in the shallow soil zone to access these water and nutrient resources in our study region.

Our measurements of stem water stable isotope composition did indicate that cottonwood trees have a different functional rooting pattern compared with two understory shrub species that were also part of the riparian forest plant community. *Sherpherdia argentea* and *Symphoricarpos occidentalis* had significantly lower (more negative) values of *d*-excess and *lc*-excess than had the cottonwood trees (Figure 9), which we suggest was due to shallower functional rooting depths in these shrub species. In addition, the *d*-excess and *lc*-excess values for the two shrub species declined to lower values than observed for the cottonwood trees during the growing season (Figure 9), a result that we suggest was caused by acquisition of shallow soil water that had been exposed to increasing, cumulative evaporative enrichment over the course of the growing season. A stronger reliance on shallow soil water by the two shrub species was consistent with a higher proportion of roots in the shallow soil layers, compared with the cottonwood trees.

We observed significant interannual variation in the *lc*-excess values for stem water extracted from *Populus* trees and *Sherpherdia argentea* shrubs (Figure 10a,b). This was caused by the strong differences in growing season precipitation recorded among study years (Figure 3) and the fact that over-bank flooding of the Oldman River occurred during 2014. During June 2014, the flood waters reached heights of approximately 2 m above ground in the riparian forest, at positions that were located the greatest lateral distance away from the river (Figure 11). Standing water remained in the forest for several weeks after the flood water retreated in late June 2014 (Figure 4). This standing water contributed to high soil water contents in the shallow soil layers that persisted throughout the 2014 growing season (Figure 5). There was likely strong evaporation that occurred from the shallow soil layers throughout July and August of 2014, which contributed to the lowest values of *lc*-excess in cottonwood stem water during that year (Figure 10a). The subsequent differences in stem water *lc*-excess in cottonwood during 2015 and 2016 were associated with the contrasting precipitation inputs received in those two years (Figure 3). The lower precipitation inputs and generally higher VPD conditions in 2015 provided the opportunity for greater soil evaporation and lower *lc*-excess values in soil water and stem water during 2015 compared with 2016. Our observations of differences in stem water *lc*-excess between 2015 and 2016 were consistent with differences observed by Sprenger et al. (2018) among contrasting sampling

FIGURE 11 Picture of the Oldman River Valley and the Helen Schuler Nature Centre building in Lethbridge, Alberta, on June 20, 2014, during the flood conditions

locations where differences in precipitation inputs influenced soil evaporation and the shallow soil water *lc*-excess values observed among wet and dry locations.

The apparent differences in functional rooting pattern between cottonwood trees and the two shrub species strongly influenced leaf photosynthetic gas exchange characteristics such as the ratio of photosynthetic capacity to stomatal conductance (intrinsic water-use efficiency), which in turn affects the $\delta^{13}C$ values of leaf tissue (Farquhar, Ehleringer, & Hubick, 1989). The cottonwood trees, which have roots deep enough to make use of the alluvial groundwater, had significantly lower leaf $\delta^{13}C$ values (higher stomatal conductance in relation to photosynthetic capacity) than had the two shrub species (Figure 10c). The greater reliance by the shrub species on shallow soil water would result in those species experiencing drier conditions and having lower ratios of stomatal conductance to photosynthetic capacity and higher intrinsic water-use efficiency than the cottonwood trees (Farquhar et al., 1989).

Soil water undergoes isotopic changes across space (laterally and vertically with soil depth) and time, leading to large variation in the stable isotope composition of water in the shallow and deep layers of the unsaturated soil zone (Sprenger et al., 2016; Sprenger et al., 2018; Penna et al., 2018). It is very difficult, therefore, to characterize the stable isotope composition of this rapidly varying soil water pool for use in mixing models, to evaluate quantitatively the proportional use of soil water, groundwater, and recent precipitation. In addition, there is no standard method that can be applied to extract water from soil, and different methods (e.g., cryogenic vacuum extraction and soil lysimeters) can collect water with very different isotopic characteristics (Orlowski, Winkler, McDonnell, & Breuer, 2018; Orlowski, Breuer, et al., 2018; Penna et al., 2018). Due to these complexities, we did not collect soil water for isotope analysis and we also did not attempt quantitative calculations of the proportions of possible source waters taken up by the plant species in this study based on stem water stable isotope compositions.

Access to water in the large storage volume of the unsaturated soil zone, in addition to water supplied from the alluvial groundwater,

allows riparian cottonwood trees to have high and relatively consistent evapotranspiration rates, despite widely varying environmental conditions (Figure 1; Yang et al., 2019). We have previously estimated that the total water used in the growing season by evapotranspiration in this cottonwood forest during a dry year (2017) was supplied approximately equally from (a) alluvial groundwater, (b) growing season precipitation, and (c) other water stored in the unsaturated soil zone (Yang et al., 2019). Water in the unsaturated soil zone can be replenished by over-bank flooding of the adjacent river (Figure 5). However, the relatively low frequency of such flooding events (approximately 5- to 10-year return interval; Smith, 1979) means that precipitation inputs provide the main annual input of water to the unsaturated soil zone in this region. Only approximately 30% of maximum water holding capacity of the unsaturated soil remained 2 years after a major flood (Figure 5), and normal growing season precipitation inputs (268 ± 92 mm) are not sufficient to fill the remaining soil volume with water. This means that riparian cottonwood trees are reliant on water supplied by the alluvial groundwater in order to survive in the semiarid region of southern Alberta.

These analyses support the suggestion by Sperry and Love (2015) that riparian cottonwood forests should be much more sensitive to reduction in supply of alluvial groundwater than exposure to lower precipitation inputs during the summer growing season.

Model calculations indicate that climate change and early snowmelt will result in low river discharge during the late summer in the South Saskatchewan River basin of southern Alberta, a basin that includes the Oldman River (Shepherd, Gill, & Rood, 2010; St-Jacques, Lapp, Zhao, Barrow, & Sauchyn, 2013). Recent observational studies have already reported declines in summer river discharge within this basin (Rood et al., 2008; St-Jacques, Sauchyn, & Zhao, 2010). A continuation of such trends may result in river flow rates that are unable to support the high evapotranspiration rates of riparian forests along the Oldman River. This could lead to water stress-induced declines in cottonwood trees, similar to past events that occurred when a dam severely limited river discharge rates in the nearby St. Mary River (Rood et al., 1995). As such, climate change poses a threat to cottonwood riparian forests in this region and the important ecosystem services that these ecosystems provide (Hauer et al., 2016; Naiman et al., 2005).

5 | CONCLUSIONS

We characterized water use by plants in a riparian forest. Our analyses indicated that cottonwood trees in southern Alberta take up water from the unsaturated soil zone, in addition to water supplied from the alluvial groundwater. The use of water from shallow soil depths supplements water taken up from the alluvial groundwater and would help to keep shallow roots active in soil layers where most decomposition and nutrient availability likely occurs. However, it is clear that normal growing season precipitation inputs are insufficient to fill the large soil volume above the capillary fringe and to completely support the high evapotranspiration rates of this riparian

cottonwood ecosystem. Therefore, cottonwood trees are reliant on water supplied by the alluvial groundwater in order to survive in the semiarid region of southern Alberta. Our analyses also indicated that *Shepherdia* and *Symphoricarpos* shrubs had shallower functional rooting depths and made greater use of shallow soil water than did the cottonwood trees. The lower ratios of stomatal conductance to photosynthetic rate and higher intrinsic water-use efficiency observed for the shrub species compared with the cottonwood trees were consistent with greater reliance by the shrub species on shallow soil water and their likely overall drier soil conditions. Precipitation inputs therefore play an important role in supporting the understory plant community in this semiarid ecosystem.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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