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# Stable isotopes of water reveal differences in plant – soil water relationships across northern environments

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## Abstract

We compared stable isotopes of water in plant stem (xylem) water and soil collected over a complete growing season from five well-known long-term study sites in northern/cold regions. These spanned a decreasing temperature gradient from Bruntland Burn (Scotland), Dorset (Canadian Shield), Dry Creek (USA), Krycklan (Sweden), to Wolf Creek (northern Canada). Xylem water was isotopically depleted compared to soil waters, most notably for deuterium. The degree to which potential soil water sources could explain the isotopic composition of xylem water was assessed quantitatively using overlapping polygons to enclose respective data sets when plotted in dual isotope space. At most sites isotopes in xylem water from angiosperms showed a strong overlap with soil water; this was not the case for gymnosperms. In most cases, xylem water composition on a given sampling day could be better explained if soil water composition was considered over longer antecedent periods spanning many months. Xylem water at most sites was usually most dissimilar to soil water in drier summer months, although sites differed in the sequence of change. Open questions remain on why a significant proportion of isotopically depleted water in plant xylem cannot be explained by soil water sources, particularly for gymnosperms. It is recommended that future research focuses on the potential for fractionation to affect water uptake at the soil-root interface, both through effects of exchange between the vapour and liquid phases of soil water and the effects of mycorrhizal interactions. Additionally, in cold regions, evaporation and diffusion of xylem water in winter may be an important process.

**KEYWORDS**
cold regions, critical zone, northern environments, stable isotopes, soil isotopes, xylem isotopes

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# 1 | INTRODUCTION

The growth of the "critical zone" paradigm has added impetus to closer investigation of soil–plant-atmosphere interactions in ecohydrology (Grant & Dietrich, 2017). This follows from work emphasizing the importance of vegetation in regulating the global terrestrial hydrological cycle, with transpiration being the dominant "green water" flux to the atmosphere compared to evaporation from soils and canopy interception in most environments (Good et al., 2015; Jasechko et al., 2013). More locally, the role vegetation plays in partitioning precipitation into such "green water" fluxes and alternative "blue water" fluxes to groundwater and streamflow has increased interest in the feedbacks between vegetation growth and soil development in different geographical environments (Brooks, 2015; Brantley et al., 2017). The emerging consequences of climatic warming to changes in vegetation characteristics and the implications of land use alterations add further momentum to the need to understand where plants get their water from, and how water is partitioned and recycled in soil-plant systems (Ellison et al., 2017; Guswa et al., 2020).

Stable isotopes in soil water and plant stem water (usually assumed to be xylem water) have been invaluable tools in elucidating ecohydrological interactions over the past decade (Penna et al., 2018). Earlier work by Ehleringer and Dawson (1992) and Ehleringer and Dawson (1992) explained the isotope content of xylem water in trees in terms of potential plant water sources. Building on that, Brooks et al. (2010) showed that the isotope characteristics of xylem water did not always correspond to bulk soil water sources as plant xylem water was fractionated and offset relative to the global meteoric water line (GMWL) compared to mobile soil water, groundwater and stream flow signatures. This led to the "Two Water Worlds" hypothesis which speculated that plant water was drawn from a "pool" of water that was "ecohydrologically separated" from the sources of groundwater recharge and stream flow (McDonnell, 2014). Research at some sites has found similar patterns of ecohydrologic separation (e.g., Goldsmith et al., 2012; Sullivan et al., 2016) and suggested it may be a ubiquitous characteristic of plant-water systems (Evaristo et al., 2015). Others have found that differences between plant water and mobile water may be limited only to drier periods (e.g., Hervé-Fernández et al., 2016; McCutcheon et al., 2017; Zhao et al., 2016), or may be less evident in some soil-vegetation systems (Geris et al., 2015). Direct hypothesis testing of potential processes that may explain the difference between the isotopic composition of xylem water and that of potential water sources has been advanced by detailed experiments in controlled environments, often involving the use of Bayesian mixing models which assume all potential plant water sources have been sampled (e.g., Stock et al., 2018). However, as field data become increasingly available from critical zone studies, more exploratory, inferential approaches can be insightful in terms of quantifying the degree to which xylem water isotopes can or cannot be attributed to measured soil water sources (Amin et al., 2020).

As this research field has progressed, it has become apparent that extraction of soil and plant waters for isotope analysis is beset with a number of methodological issues (e.g., Marshall et al., 2020; West et al., 2011). Soil waters held under different tensions may have different isotopic characteristics: for example, freely moving (low tension) water sampled by suction lysimeters often shows a much less marked evaporative fractionation signal than bulk soil waters dominated by less mobile (high tension) storage extracted by cryogenic or equilibration methods (Sprenger, Tetzlaff, Buttle, Laudon, Leistert, et al., 2018; Vargas et al., 2017). Such differences between extraction techniques may be exacerbated by soil characteristics, such as texture and organic content, which may in turn affect the degree to which water held under different tensions can mix (Orlowski, Breuer, et al., 2018; Sprenger et al., 2016). Similarly, sampling xylem and its resulting isotopic composition has been shown to be affected by methodology. It is usually assumed that methods such as cryogenic extraction isolate water held in xylem, when in fact water stored in other cells may be mobilized to "contaminate" the results (Barbeta et al., 2020; Zhou et al., 2016).

Interpretation of plant-soil water relationships can also be complicated by processes in plants and soils that alter isotopic compositions independently. For example, the spatio-temporal isotopic composition of soil water can change dramatically in relation to precipitation inputs, evaporative losses, internal redistribution and phase changes between liquid and gaseous phases (Sprenger, Tetzlaff, Buttle, Carey, et al., 2018). Moreover, there is increasing evidence that plant physiological mechanisms may affect water cycling and the composition of xylem water (e.g., Dubbert et al., 2019; Martín-Gómez et al., 2017). These include effects of mychorrizal interactions in plant roots that may result in exchange and fractionation of water entering the xylem stream (Poca et al., 2019). Research also indicates that as flow in xylem slows, diffusion and fractionation can occur (Martín-Gómez et al., 2017), which may involve exchange with phloem cells (Bertrand et al., 2014; Cernusak et al., 2002). Finally, there is increasing evidence that water storage and release from non-xylem cells may sustain transpiration during dry periods or early in the day (Dubbert & Werner, 2019), also affecting xylem composition. Thus, there is a need to understand the different timescales involved in uptake processes in the rooting zone, residence times and mixing of water in different vegetation covers (Knighton et al., 2020). There is also evidence of differences between how such factors affect water movement in angiosperms and gymnosperms, as well as species-specific differences (Amin et al., 2020; Evaristo et al., 2016). Clearly, these methodological issues will take some time to address; in the interim there is a need for cautious interpretation of emerging data from critical zone studies in order to improve our understanding.

A striking feature of isotopic studies of soil-vegetation systems is a bias to lower and temperate latitudes, with northern latitudes and cold environments being under-represented (Evaristo et al., 2015). Yet, northern environments present particular challenges and opportunities to further advance the growing body of knowledge about plant-soil water interactions. For example, the coupled seasonality of precipitation magnitude and vegetative water demand can be complicated by the seasonality of the precipitation phase. Cold season precipitation that accumulates as snow can replenish soil water in the spring and be available to plants months after deposition (Allen et al., 2019). Despite the lack of studies, these areas are experiencing some of the most rapid changes in climate and, as a result, vegetation (Myers-Smith et al., 2019; Myneni et al., 1997). The effects of climatic

warming on patterns of snowpack accumulation and melt can have particularly marked consequences for soil water replenishment and plant water availability, particularly at the start of the growing season (Barnett et al., 2005; Carey et al., 2013; T. J. Smith et al., 2011). Despite the importance of northern environments, remoteness and harshness of environmental conditions result in logistical problems that constrain lengthy field studies and data collection (Tetzlaff et al., 2015).

This study seeks to contribute to the growing body of knowledge about plant-soil water interactions by expanding the geographical representation of sites in cold northern environments. We report the findings of a coordinated project on xylem water isotopic data collection in the dominant soil – vegetation systems of five long-term experimental sites. Isotopic characteristics of soil water have previously been reported for all five sites; this used a comparative approach with, as far as possible, common sampling methods across the sites for a 12 month period (see Sprenger, Tetzlaff, Buttle, Carey, et al., 2018; Sprenger, Tetzlaff, Buttle, Laudon, & Soulsby, 2018, for details). Here, we present xylem water isotopic composition data collected using common methods over the same time period encompassing the complete growing season, and then relate findings to soil water isotopic compositions. This inter-site comparison provides a meta-analysis aimed at answering the following research questions:

1. What is the temporal trajectory of xylem water isotopic composition during the growing season for common plant species across northern environments?

2. Does the relationship between the isotopic composition of xylem water and soil water differ between plant species and environments?

3. Can any differences between the isotopic compositions of xylem and soil water be explained in terms of current process knowledge and methodological issues?

Following on from question 3, we discuss the open research questions that need to be addressed to gain a more comprehensive understanding of the isotope systematics of plant-water interactions in northern/cold environments.

## 2 | DATA AND METHODS

### 2.1 | Study sites

The study was conducted at five long-term experimental catchments across the boreal or mountainous regions of the northern latitudes (Figure 1 and Table 1). The catchments were part of the VeWa project funded by the European Research Council investigating vegetation effects on water mixing and partitioning in high-latitude ecosystems (Tetzlaff et al., 2015). Previous inter-comparison work on this project has examined such issues as changing seasonality of vegetation-hydrology interactions (Wang et al., 2019), soil water storage and mixing (Sprenger, Tetzlaff, Buttle, Carey, et al., 2018), water ages
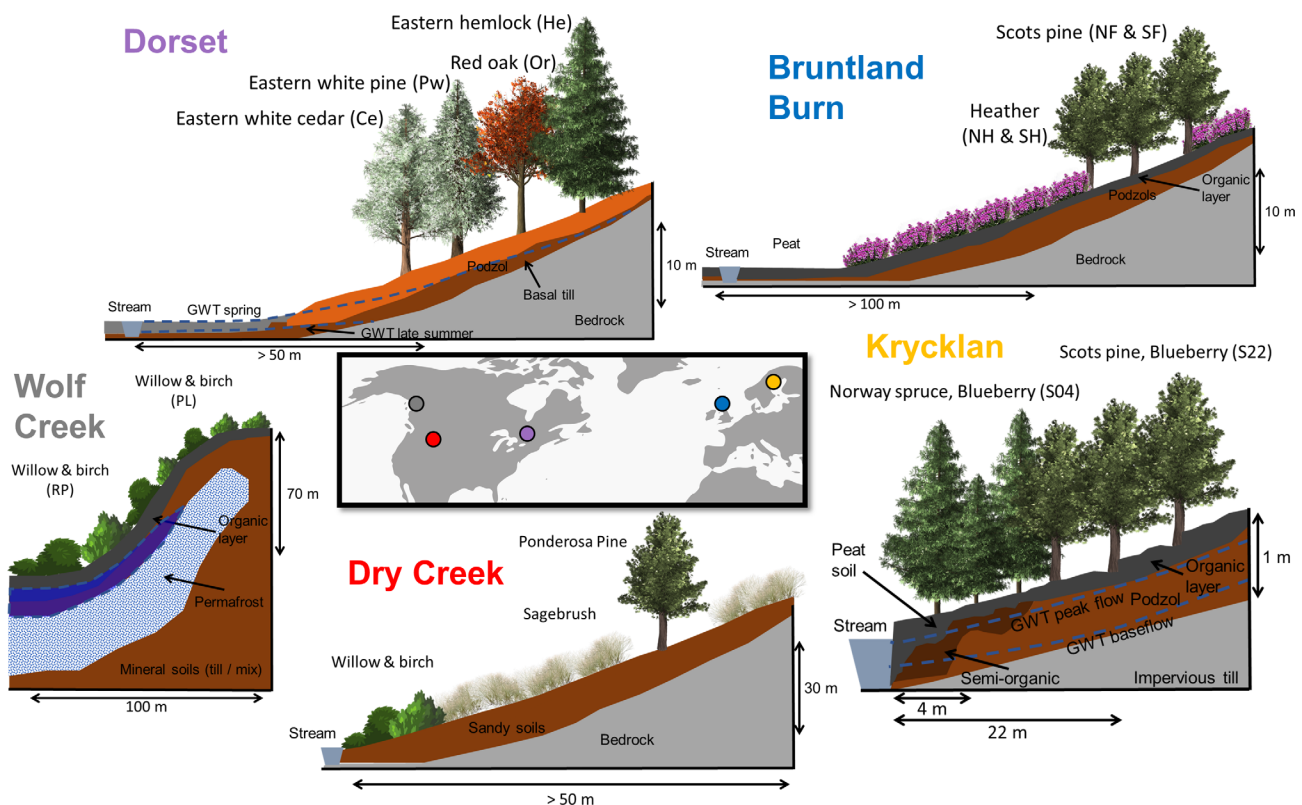


**FIGURE 1**  Map with the location of studied catchments and conceptual graphs showing the individual sampling locations at each catchment

**TABLE 1** Study sites, sampled vegetation and soil, number of sampling campaigns (*n*), period of vegetation sampling and mean deuterium compositions of the precipitation (P)

| Catchment | Site ID | Sampled vegetation (*Angiosperms; #Gymnosperms) | Sampled soil | Max. soil sampling depth | n | Sampling periods | P δ²H [‰] |
|---|---|---|---|---|---|---|---|
| Bruntland Burn | NF | Erica species (*Calluna vulgaris*)* | Loamy sand, OM = 5–20% | -20 | 7 | 2015-09-29 to 2016-09-23 | −52.8 ± 25.0 |
| | NH | Scots pine (*Pinus sylvestris*)# | | | | | |
| | SF | Erica species (*C. vulgaris*) | | | | | |
| | SH | Scots pine (*P. sylvestris*)# | | | | | |
| Dorset | Or | Red oak (*Quercus rubra*)* | Sandy loam, OM = 4% | −50 | 6 | 2015-10-26 to 2016-11-02 | −76.7 ± 26.3 |
| | He | Eastern hemlock (*Tsuga canadensis*)# | | | | 2015-10-29 to 2016-11-04 | |
| | Ce | Eastern white cedar (*Thuja occidentalis*)# | | | | 2015-11-03 to 2016-11-04 | |
| | Pw | Eastern white pine (*Pinus strobus*)# | | | | 2015-10-27 to 2016-11-02 | |
| Dry Creek | LG | Sagebrush (*Artemisea tridentata*) Bitterbrush (*Prushia tridentata*) Chokecherry (*Ericameria nauseosa*) Yellow willow (*Salix lucida*)* Water Birch (*Betula accidentalis*)* | Loam to sandy loam | −70 | 9 | 2011-06-29 to 2012-09-13 | −105.2 ± 25.0 |
| | TL | Douglas fir (*Pseudotsuga menziesii*) Pondersa pine (*Pinus ponderosa*) Douglas fir (*P. menziesii*) Pondersa pine (*P. ponderosa*) Sagebrush (*A. tridentata*) | | −70 | 6 | 2011-08-11 to 2012-09-07 | −113.8 ± 25.7 |
| | BSG | Douglas fir (*P. menziesii*) Pondersa pine (*P. ponderosa*) | | −70 | 6 | 2011-08-11 to 2012-09-07 | −107.6 ± 27.6 |
| Krycklan | S04 | Norway spruce (*Picea abies*)# and Blueberry* | Sand, OM = 80% | −30 | 7 | 2015-09-22 to 2016-09-20 | −102.8 ± 32.5 |
| | S22 | Scots pine (*P. sylvestris*)# and Blueberry* | Sand, OM = 5% | | | | |
| Wolf Creek | PL | Birch (*Betulaceae nana*)* and Willow (*Salix spec.*)* | Silty sand | −85 | 5 | 2015-09-17 to 2016-08-12 | −143.8 |
| | RP | Willow (*Salix spec.*)* and Birch (*B. nana*)* | | −40 | | 2016-05-11 to 2016-09-19 | |

(Sprenger, Tetzlaff, Buttle, Laudon, & Soulsby, 2018) and modelling the interactions between water storage, fluxes and ages (Piovano et al., 2020).

The sites cover a broad hydro-meteorological gradient. Bruntland Burn (BB) in the Scottish Highlands, UK (57°2′ N 3°7′ W) has a temperate/boreal humid climate with cool summers. At Dorset (D) in south-central Ontario, Canada (45° 12′ N 78° 49′ W), the climate is cold and humid with warm summers. Dry Creek (DC), Idaho, USA (43° 42′ N 116° 10′ W) represents a cold arid montane climate with dry summers. Krycklan (K) in northern Sweden (64° 14′ N 19° 46′ E) is characterized by a cold and humid climate with relatively cool summers. At Wolf Creek (WC) in Yukon Territory, Canada (60° 32′ N 135° 18′ W) the climate is cold with dry and warm summers (Table 2).

At each site, two to four representative landscape units with characteristic soil-vegetation types were investigated with regard to the isotopic composition of precipitation, soil water, and plant xylem water. Dominant plant cover and soil characteristics of the sites are

listed in Table 1 and shown schematically in Figure 1. Angiosperms and gymnosperms were sampled at all sites with the exception of WC, where only angiosperms were sampled.

At Bruntland Burn, study sites were dominated either by Scots pine (*Pinus sylvestris*) (sites NF and SF) or Ericacae species (e.g., *Calluna vulgaris*) (sites NH and SH). Dominant vegetation at the Dorset sites was either coniferous trees (Eastern hemlock (*Tsuga canadensis*), Eastern white cedar (*Thuja occidentalis*), Eastern white pine (*Pinus strobus*) at sites He, Ce, Pw, respectively) or deciduous red oaks (*Quercus rubra*) (site Or). At Dry Creek, tree-dominated high elevation locations included Douglas fir (*Pseudotsuga menziesii*) and Ponderosa pine (*Pinus ponderosa*). Mid-elevation sites had a mixture of similar trees plus shrubs including Sagebrush (*Artemisia tridentata*). Low elevation sites had no trees, but a variety of additional shrubs including Bitterbrush (*Purshia tridentata*), Chokecherry (*Ericameria nauseosa*), Yellow willow (*Salix lucida*) and Water birch (*Betula occidentalis*) (as reported in

**TABLE 2**  Growing season and annual average climate conditions of precipitation, air temperature, and relative humidity of each study site

| | | Growing season months | | | | | | | | Annual average |
|---|---|---|---|---|---|---|---|---|---|---|
| | | March | April | May | June | July | August | September | October | |
| **Bruntland Burn** | Precipitation (mm) | 48.3 | 52.3 | 43.7 | 81.6 | 95.5 | 102.0 | 35.0 | 87.9 | 1001.0 |
| | Temperature (°C) | 3.9 | 5.1 | 8.4 | 11.3 | 12.9 | 11.9 | 10.9 | 8.3 | 6.6 |
| | Relative humidity (%) | 77.8 | 75.4 | 72.9 | 77.7 | 77.2 | 79.3 | 81.8 | 82.1 | 79.0 |
| **Dorset** | Precipitation (mm) | 64.8 | 83.2 | 86.2 | 94.6 | 46.0 | 88.2 | 89.9 | 102.5 | 1020.0 |
| | Temperature (°C) | −2.0 | 4.0 | 12.2 | 16.5 | 18.9 | 18.1 | 13.6 | 7.1 | 4.8 |
| | Relative humidity (%) | 71.6 | 70.1 | 69.1 | 75.4 | 71.0 | 78.1 | 81.7 | 83.1 | 78.0 |
| **Dry Creek** | Precipitation (mm) | 51.6 | 40.6 | 39.8 | 22.0 | 3.0 | 7.9 | 13.5 | 28.7 | 653.0 |
| | Temperature (°C) | 5.3 | 8.5 | 13.3 | 18.0 | 24.5 | 23.0 | 17.7 | 10.5 | 9.0 |
| | Relative humidity (%) | 58.9 | 53.6 | 51.2 | 45.6 | 28.8 | 29.7 | 36.0 | 51.8 | 54.0 |
| **Krycklan** | Precipitation (mm) | 27.9 | 28.3 | 41.3 | 55.2 | 98.1 | 82.8 | 67.3 | 56.8 | 622.0 |
| | Temperature (°C) | −3.5 | 1.8 | 7.6 | 11.9 | 15.3 | 13.1 | 8.6 | 2.1 | 1.8 |
| | Relative humidity (%) | 78.4 | 72.5 | 68.6 | 69.2 | 75.3 | 82.5 | 85.5 | 90.3 | 82.0 |
| **Wolf Creek** | Precipitation (mm) | 30.5 | 27.1 | 13.9 | 57.1 | 54.6 | 50.8 | 22.0 | 25.8 | 471.0 |
| | Temperature (°C) | −8.8 | −2.5 | 5.1 | 8.6 | 10.4 | 9.2 | 4.1 | −3.2 | −2.0 |
| | Relative humidity (%) | 67.3 | 62.5 | 57.4 | 60.4 | 64.7 | 68.6 | 74.2 | 79.1 | 70.0 |

McCutcheon et al., 2017). At Krycklan, Norway spruce (*Picea abies*) and Blueberry (*Vaccinium*) were present at site S04 about 4 m away from a stream, while Scots pine and Blueberry were the dominant species at the upslope site S22 about 22 m from the stream. The Wolf Creek sites, RP in the riparian zone and PL located on a relatively dry plateau, were vegetated by birch (*Betula nana*) and willow shrubs (*Salix sp*.).

Prevailing soil textures at the sites varied from loam to silty sands (Table 1). Soil characteristics are described in detail by Sprenger, Tetzlaff, Buttle, Carey, et al. (2018). Briefly, these are podzolic soils at Bruntland Burn, Dorset and Krycklan, loamy sand at Dry Creek, and Wolf Creek had considerable amounts of organic matter in the upper soil layers. At Dry Creek, shrub and tree roots extend through the soil column, which ranges from ~10 to ~120 cm thick. Ponderosa pine roots may extend into fractured bedrock. The rooting depths are limited to the upper 15 cm for the heather sites at Bruntland Burn and to 50 cm depth for trees at Krycklan and Dorset. Rooting depths at Wolf Creek and Bruntland Burn are largely within the top 30 cm with smaller fractions to 50 cm.

## 2.2 | Methods

### 2.2.1 | Sampling

At each site, plants and surrounding soils were sampled concurrently for isotope analysis following a common sampling protocol (see full details in Sprenger, Tetzlaff, Buttle, Carey, et al., 2018; Sprenger, Tetzlaff, Buttle, Laudon, & Soulsby, 2018). Depending on the nature of the soil cover, the maximum depth of sampling varied from −20 cm at BB to −70 cm at Dry Creek (Table 1). Sampling took place at 5 cm

intervals for Bruntlad Burn, Dorset and Krycklan with two to five replicates for each depth. At Dry Creek, sampling was done at −10, −25, −45 and −70 cm with two to four replicates. Sampling depths at Wolf Creek varied between −2 and −40 cm with one to three replicates. Daily soil moisture data based on continuous soil moisture measurements at 10 or 15 cm soil depth were available for each soil water sampling location at Bruntland Burn, Dry CReek, Krycklan and Wolf Creek. Only weekly manual soil moisture measurements were available for Dorset, and daily soil moisture data were derived from soil physical modelling (Sprenger, Tetzlaff, Buttle, Carey, et al., 2018). The volumetric soil moisture (VSM, $cm^3$ $cm^{-3}$) data were used to assess the hydrologic state (e.g., wetness) on the sampling days.

Plant samples from trees with a diameter >30 cm (species listed in Table 1) were taken horizontally with increment borers at breast height (~1.2 m). Retrieved plant xylem cores were directly placed in vials without bark and phloem. Shrub vegetation (willow and birch at Wolf Creek, heather at Bruntland Burn, blueberry at Krycklan and sagebrush, bitterbrush and chokecherry at Dry Creek) was sampled by clipping branches. These were immediately placed in vials after the bark was chipped off (at Wolf Creek and Bruntland Burn) or left on (Dry Creek). All vials were directly sealed with parafilm and immediately frozen until extraction was conducted at Boise State University, Boise, Idaho, USA. There were five replicates for each species and day at the sites in Bruntland Burn, Krycklan, Dorset. At Wolf Creek, the number of replicates varied between two and five and there were always four replicates for each sampling campaign at the Dry Creek sites. In total, 1160 xylem water samples were collected; 831 for angiosperms and 329 for gymnosperms (see Table 3). Dates of sample events varied at each site, but included the end of the growing season/senescence, pre-leaf out the following year, post leaf out, peak growing season and senescence (See Figure S1).

| | Bruntland Burn | Dorset | Dry Creek | Krycklan | Wolf Creek |
|---|---|---|---|---|---|
| Angiosperm | 66 | 29 | 227 | 105 | 404 |
| Gymnosperm | 90 | 90 | 80 | 69 | N/A |
| 0–10 cm | 593 | 247 | 169 | 126 | 150 |
| 10–20 cm | 518 | 203 | 33 | 126 | 111 |
| 20–30 cm | N/A | 148 | 137 | 61 | 66 |
| 30–40 cm | N/A | 74 | 16 | N/A | 48 |
| >40 cm | N/A | 64 | 221 | N/A | 76 |

**TABLE 3** Number of total samples (all years) at each site for each vegetation type and at each soil depth (10 cm increment to 40 cm)

Precipitation was sampled daily or on an event basis at Bruntland Burn and Krycklan. Daily to fortnightly precipitation sampling was conducted at Dorset, Dry Creek and Wolf Creek. Melt water was sampled from lysimeters at Krycklan, Dorset, Dry Creek and Wolf Creek during several snow melt events, while snowfall seldom occurred over the study year at Bruntland Burn (Ala-aho et al., 2017). Various measures were taken to prevent evaporation of collected precipitation, including paraffin oil and water locks prior to transfer to the laboratory. The long-term groundwater signal was assessed at all sites, apart from Dorset, using several sampling campaigns of springs and wells tapping the saturated zone over the last few years (e.g., McCutcheon et al., 2017; Scheliga et al., 2018). There were no nearby wells from which to sample the regional groundwater at Dorset, which is found well below the surface in the granitic gneiss and amphibolite bedrock.

### 2.2.2 | Laboratory

Water samples were analyzed for their stable isotopic compositions ($^2$H and $^{18}$O) using Los Gatos DLT-100 laser isotope analysers for Dorset and Wolf Creek, a Los Gatos Liquid Water Isotope Analyzer (LWIA) for Bruntland Burn and Dry Creek, and a Picarro L2130-I for Krycklan. The precision of the liquid water stable isotope analysis is reported to be better than ±0.1 ‰ for $\delta^{18}$O and ±0.4 ‰ for $\delta^2$H. All isotope data are given in delta-notation (Coplen, 2011) in reference to the VSMOW.

At all sites – apart from Dry Creek – direct water-vapor equilibration analysis was used to sample the bulk soil water isotopic composition from the soil (Wassenaar et al., 2008). The accuracy of the direct water-vapor equilibration method was ±0.3 ‰ for $\delta^{18}$O and ± 1.1 ‰ for $\delta^2$H. For a detailed description of the procedure, we refer to Sprenger, Tetzlaff, Buttle, Laudon, Leistert, et al. (2018). Bulk soil water isotopic compositions at DC were sampled using cryogenic extraction at 100°C under vacuum of <30 millitorr over 40 min, as described by McCutcheon et al. (2017). We are aware that different methods of soil water extraction have been a major focus of research in the past few years, with no definitive agreement on a standard method (e.g., Araguás-Araguás et al., 1995; Orlowski et al., 2016). While differences between cryogenic extraction and the direct water-vapor method have been reported in laboratory experiments (Orlowski et al., 2016), previous work by the authors

has found the direct equilibrium method to be a reliable method for extracting bulk soil water from sandy soils (Sprenger, Tetzlaff, Buttle, Laudon, Leistert, et al., 2018; Sprenger, Tetzlaff, Buttle, Carey, et al., 2018) giving similar results to cryogenic extraction (Sprenger et al., 2015).

Xylem water isotopic compositions for all sites were sampled using cryogenic extraction at 100°C under vacuum of <30 millitorr over 60 min (McCutcheon et al., 2017). The accuracy of such analyses is given as ±0.15 ‰ for $\delta^{18}$O and ±0.69 ‰ for $\delta^2$H (West et al., 2006). To avoid analytical bias arising from alcohol contamination (Martín-Gómez et al., 2015), we analyzed extracted waters using a ThermoFisher TC/EA coupled with Thermo Delta V Plus mass spectrometer at Boise State University Stable Isotope Laboratory. Column and GC temperatures were set at 1250 and 90 °C, respectively, He flush rate was 90 ml/min, and sample injection volume was ∼0.2 µl. To avoid magnet jump instabilities, $\delta^2$H and $\delta^{18}$O were measured separately. Samples were standardized against reference waters from Los Gatos Research; typical reproducibilities were $\delta^{18}$O ∼ ±0.3‰ (2σ) and $\delta^2$H ∼ ±1.7‰ (2σ).

### 2.2.3 | Data Analysis

*Source water apportionment of plant xylem*: To quantify the potential source of vegetation water from different soil depths and over a range of time periods, a modification of the ellipsoid method (Amin et al., 2020) was utilized for the gymnosperms and angiosperms at soil depths in 10 cm increments up to 40 cm. All soil samples deeper than 40 cm were lumped together. The 40 cm cut off was chosen due to fewer sites sampling below 40 cm and a large decrease in the temporal resolution of sampling which could otherwise skew results. Due to soil water fractionation resulting in deviation from the local meteoric water line, the data are not well represented in an ellipsoid shape such as that employed in Amin et al. (2020). Therefore a minimum polygon area was used to encompass the data points.

For each vegetation type and soil depth sample in dual-isotope space, a minimum boundary polygon (Matlab boundary function) was drawn to encompass the sample population by minimizing the radius that encompasses all points. Outliers were removed according to 99% confidence intervals of $\delta^2$H and $\delta^{18}$O. The overlap of each vegetation type and soil depth was determined by:

$$\text{Overlap}\,(\%) = \frac{n_{source}}{n}, \qquad (1)$$

where $n_{source}$ is the number of vegetation samples within the both vegetation and soil depth boundary, and $n$ is the number of vegetation samples that are within the vegetation boundary (Amin et al., 2020).

To assess how the source of soil water for vegetation water may temporally change, the boundary method was applied using different window sizes to average the duration of sampling of the potential source water. Vegetation samples were grouped into individual months. Soil water prior to the day of sampling was grouped using moving monthly windows (backwards windows of 0–11 months) using soil water data for each calendar month as being more generally representative of the typical seasonal cycles of soil water data (cf. Tetzlaff et al., 2014). Since sampling was generally conducted monthly, a backwards window of 0 months shows the overlap of soil and vegetation on the same sampling day. The overlap of each monthly vegetation boundary was evaluated against the backwards windows months for bulk soil (e.g., June Angiosperms compared with soil for a 3 month [March – June] backward window):

$$Month_{Start} = \begin{cases} Month_{curr} - Window & Window < Month_{curr} \\ Month_{curr} - Window + 12 & Window > Month_{curr} \end{cases}, \qquad (2)$$

where $Month_{start}$ is the starting month of the backward window (prior to the current vegetation sampling month), $Month_{curr}$ is the current vegetation sampling month, and $Window$ is the backwards window size. For the samples at the beginning of the study period, subsequent samples from the same month are used and assumed to be representative of the seasonal cycles of soil waters.

*Line conditioned excess*: We used the line-conditioned excess (offset from the local meteoric water line, LMWL, Landwehr & Coplen, 2006) to evaluate soil and xylem linkages between sites and their relationships with catchment characteristics. The line-conditioned excess is defined as:

$$lc - excess = \delta^2 H - a * \delta^{18} O - b, \qquad (3)$$

where $a$ and $b$ are the slope and intercept of the LMWL, respectively. For lc-excess, values less than 0 ‰ indicate that samples plot below the LMWL in the dual isotope space.

*Soil water excess*: To investigate soil and xylem water isotopic compositions and possible linkages with each other, we used a soil water line conditioned excess (sw-excess), as suggested by Barbeta et al. (2019) and analogous to the definition of line-conditioned excess from the LMWL by Landwehr and Coplen (2006). For each sampling day, we derived the regression line of the soil water stable isotope data ($\delta_{sw}$) in dual isotope space (often referred to as "soil water line"). This regression line is then defined by its slope $m_{sw}$ and the intercept with the $\delta^2$H-axis $b_{sw}$:

$$\delta_{sw}{}^2 H = m_{sw} * \delta_{sw}{}^{18} O + b_{sw}, \qquad (4)$$

The soil water line excess is then defined as:

$$sw - excess = \delta^2 H - m_{sw} * \delta^{18} O - b_{sw}. \qquad (5)$$

Based on Equation (5), we derived the sw-excess of xylem isotope data. For sw-excess less than 0 ‰, the xylem data plot below the soil water line of the corresponding soil water isotopes sampled on the same day. Thus, the sw-excess can serve as an indicator for deuterium fractionation between the uptake time at the root-soil interface and the measured xylem water. We acknowledge that the soil water line is not necessarily solely a product of evaporative enrichment and that seasonal variability of the stable isotope compositions of the precipitation can affect how much soil waters deviate from the LMWL (Benettin et al., 2018). However, the process of how the "soil water line" developed is not important here, since we used the regression to describe the isotopic compositions of potential water sources for vegetation at the time of sampling.

The influence of site characteristics on soil and xylem isotopic samples was evaluated using Spearman rank correlation. Site characteristics, mean annual temperature (MAT, °C), elevation (m a.s.l.), aridity index (AI, ratio of annual precipitation to potential evaporation), annual precipitation (mm/year), and latitude (°), were correlated to xylem and soil water $\delta^2$H (‰), $\delta^{18}$O (‰), and the corresponding sw-excess (‰), and lc-excess (‰) to a significance level of 0.001. Isotopic compositions of all soil depths, vegetation species and sampling times were bulked for each site to assess an overall trend of soils and vegetation in relation to climate indices.

Statistical analysis of isotopes in precipitation, bulk soil water, angiosperms, and gymnosperms was conducted at each site using the Wilcoxon signed-rank test (Gibbons & Chakraborti, 2011) to test the statistical similarities of median values of the datasets. This allowed for a two-sided probability test without the assumption of normality. The datasets were tested to the 95% confidence limit using all available data (i.e., soil water was not characterized and tested independently for each depth).

## 3 | RESULTS

### 3.1 | Xylem water isotope composition

Plant water and soil water data from the five sites are plotted in Figure 2. For both soils and xylem, the sites occupied partially overlapping regions showing a general gradient from highly isotopically depleted (lower portion of the GMWL) at Wolf Creek, the coldest of our sites in Canada, to the more isotopically enriched (upper portion of the GMWL) waters at Bruntland Burn at the temperate/boreal transition in Scotland. For each site there was a substantial range of variability in soil and xylem water isotope composition over the course of the sampling year. Most soil and xylem samples plotted below the GMWL, although xylem waters were generally more $^2$H-depleted at each site, which was also evident from the lc-excess data (Tables 4a). Samples from Dry Creek and, in particular, Wolf Creek showed the greatest divergence from the GMWL. These two sites slightly obscured an otherwise clear relationship between plotting position along the GMWL and the mean annual temperature gradient
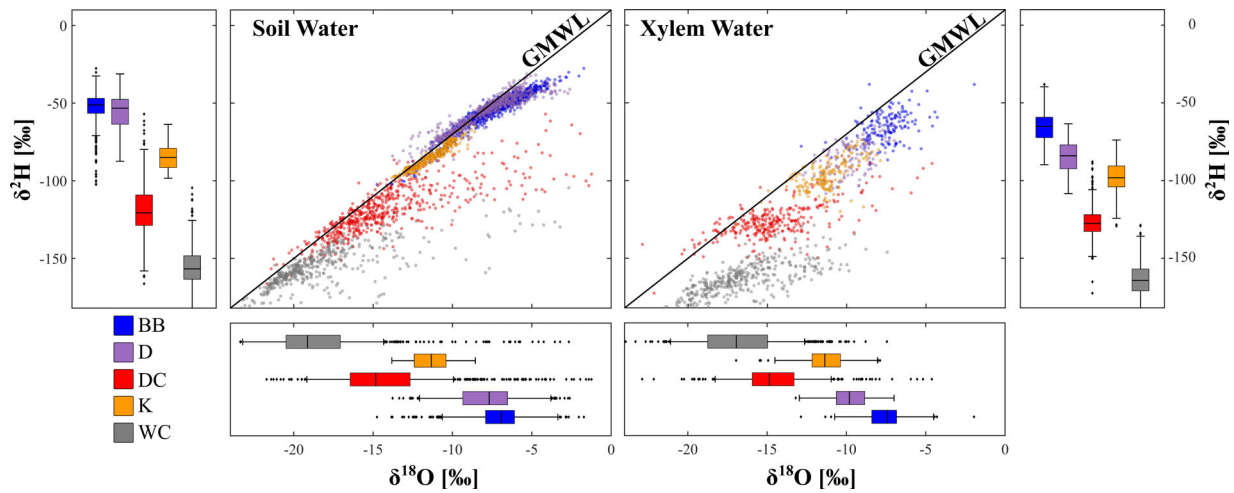
**FIGURE 2** Dual isotope plots and bar plots showing all soil water and xylem water for the Bruntland Burn (BB), Dorset (D), Dry Creek (DC), Krycklan (K), and Wolf Creek (WC) catchments bulked over time (colour code). Boxplots show the median (black line in box), the interquartile range (extent of the box), range (whiskers), and outliers (black points)

**TABLE 4a** Median lc-excess (‰) (number of samples) of soil and plant xylem water for different soil depths and vegetation groups at each study site

| | Study Site | | | | |
|---|---|---|---|---|---|
| | Bruntland Burn | Dorset | Dry Creek | Krycklan | Wolf Creek |
| Xylem water | | | | | |
| Angiosperms | −6.37 (66) | −9.12 (29) | −17.78 (227) | −11.18 (105) | −25.12 (181) |
| Gymnosperms | −16.09 (90) | −22.11 (90) | −6.57 (80) | −18.70 (69) | N/A |
| Soil water | | | | | |
| 0–10 cm | −5.43 (593) | −5.24 (247) | −11.62 (169) | −1.81 (126) | −7.51 (150) |
| 10–20 cm | −1.20 (518) | −3.27 (203) | −7.31 (33) | −1.14 (126) | −6.11 (111) |
| 20–30 cm | N/A | −3.28 (148) | −5.79 (137) | −1.41 (61) | −4.53 (66) |
| 30–40 cm | N/A | −2.42 (74) | 0.78 (16) | N/A | −2.07 (48) |
| >40 cm | N/A | −2.92 (64) | −4.96 (221) | N/A | −2.61 (76) |

**TABLE 4b** Median sw-excess (‰) (number of samples) of soil and plant xylem water for different soil depths and vegetation groups at each study site

| | Study Site | | | | |
|---|---|---|---|---|---|
| | Bruntland Burn | Dorset | Dry Creek | Krycklan | Wolf Creek |
| Xylem water | | | | | |
| Angiosperms | −6.07 (66) | −8.66 (29) | −4.61 (227) | −10.01 (105) | −15.49 (181) |
| Gymnosperms | −14.36 (90) | −22.88 (90) | −1.74 (80) | −17.24 (69) | N/A |
| Soil Water | | | | | |
| 0–10 cm | −0.09 (593) | −0.19 (247) | −0.45 (169) | −0.19 (126) | 0.20 (150) |
| 10–20 cm | 0.16 (518) | 0.83 (203) | 0.11 (33) | 0.16 (126) | −0.09 (111) |
| 20–30 cm | N/A | −0.05 (148) | 0.17 (137) | −0.03 (61) | −0.45 (66) |
| 30–40 cm | N/A | −0.29 (74) | 2.66 (16) | N/A | 0.40 (48) |
| >40 cm | N/A | −1.12 (64) | −0.37 (221) | N/A | 2.48 (76) |

through Krycklan, Dorset and Bruntland Burn. Despite this, the isotopic ratios of $\delta^2H$ and $\delta^{18}O$ in soils and xylem water correlate positively with air temperature, annual precipitation and aridity index, and negatively with elevation and to a lesser extent latitude (Table 5).

At all sites, substantial isotopic differences were apparent between xylem and soil water isotopes, and between angiosperms and gymnosperms (Table 6). Gymnosperms generally plotted further from the GMWL (Figure 3 and Tables 4a and 4b). Soil waters at each

**TABLE 5** Spearman rank correlation between soil and plant xylem samples ($\delta^2H$, $\delta^{18}O$, sw-excess, and lc-excess), and site characteristics: Mean annual temperature (MAT), elevation, aridity index, annual precipitation, and latitude (number of xylem samples, $n_{veg}$ = 1207, and number of soil samples, $n_{soil}$ = 3190)

| | Plant Xylem samples | | | | Soil samples | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | $\delta^2H$ (‰) | $\delta^{18}O$ (‰) | sw-excess (‰) | lc-excess (‰) | $\delta^{18}O$ (‰) | $\delta^2H$ (‰) | sw-excess (‰) | lc-excess (‰) |
| MAT (°C) | 0.53 | 0.48 | 0.25 | −0.18 | 0.43 | 0.45 | 0.00* | 0.01* |
| Elevation (m a.s.l.) | −0.87 | −0.75 | 0.13 | 0.12 | −0.78 | −0.90 | 0.00* | −0.32 |
| Aridity Index | 0.65 | 0.55 | −0.14 | 0.01* | 0.68 | 0.75 | 0.00* | 0.26 |
| Precipitation (annual) | 0.86 | 0.75 | −0.06* | −0.16 | 0.82 | 0.91 | 0.00* | 0.22 |
| Latitude (°) | −0.16 | −0.16 | −0.13 | 0.17 | −0.08 | −0.09 | −0.00* | 0.13 |

*Note*: All correlations except those denoted with * were significant at $p$ = 0.001

**TABLE 6** Datasets with statistical differences between precipitation, soils, angiosperms and gymnosperms $\delta^2H$ and $\delta^{18}O$ at each study site, Dorset (D), Krycklan (K), Bruntland Burn (BB), Wolf Creek (WC) and Dry Creek (DC)

| $\delta^2H$ | | | |
| --- | --- | --- | --- |
| | Soils | Angiosperms | Gymnosperms |
| Precipitation | D, WC, DC | D, K, BB, WC, DC | D, K, BB, DC |
| Soils | - | D, K, BB, WC, DC | D, K, BB, DC |
| Angiosperms | - | - | K, BB, DC |
| $\delta^{18}O$ | | | |
| | Soils | Angiosperms | Gymnosperms |
| Precipitation | D, BB | WC, DC | D, WC, DC |
| Soils | - | D, BB, WC, DC | D, BB |
| Angiosperms | - | - | D, DC |

site generally tracked precipitation and snowmelt inputs being more $^2H$- and $^{18}O$-depleted in winter/spring and more enriched in summer; evidence of evaporative fractionation was also most evident in the more $^2H$- and $^{18}O$-enriched summer soil water samples. The soil water data are shown relative to the sampling dates for each site in Figures S2–S6; also see Sprenger, Tetzlaff, Buttle, Carey, et al. (2018), Sprenger, Tetzlaff, Buttle, Laudon, and Soulsby (2018) for more detail. Soil water $\delta^2H$ data were significantly different from precipitation at Dry Creek, Dorset and Wolf Creek, while soil water $\delta^{18}O$ differed from precipitation at Bruntland Burn and Dorset (Table 6).

Bruntland Burn, Krycklan and Dorset showed the greatest visual deviation of xylem $\delta^2H$ samples from soil water, while the most southern site, Dry Creek, and the most northern site, Wolf Creek, showed smaller differences between the xylem and soil water isotopes for $\delta^2H$ (Figure 3). However, at all sites the $\delta^2H$ characteristics of both angiosperms and gymnosperms were significantly different from soil water (and precipitation). Angiosperm xylem water $\delta^{18}O$ at all sites, apart from Krycklan, was significantly different from soil water $\delta^{18}O$; whereas significant differences for gymnosperms were apparent only for Dorset and Bruntland Burn. Xylem water isotopic characteristics differed between angiosperms and gymnosperms at some sites. For $\delta^2H$, they were significantly different for Krycklan, Bruntland Burn

and Dry Creek, while for $\delta^{18}O$ they were different for Dorset and Dry Creek (Table 6).

Snowmelt (at Dorset, Dry Creek, Krycklan and Wolf Creek) plotted on the LMWL and was more depleted for $^{18}O$ than almost all measured soil and xylem waters, although a substantial number of xylem samples at Dry Creek, Krycklan and Wolf Creek were more depleted in $^2H$ but plotted off the LMWL (Figure 3). Similarly, at the four sites where groundwater samples were collected, the mean isotopic composition of groundwater fell on the LMWL but plotted towards the more depleted end of the range of soil water samples. This reflects the generally higher recharge of groundwater by depleted water following the spring melt at Dry Creek, Krycklan and Wolf Creek (Ala-Aho et al., 2017; Piovano et al., 2019); and during winter rainfall at Bruntland Burn (Scheliga et al., 2018). Isotopic composition of groundwater at all sites showed limited temporal variation, indicating the volume of annual recharge is small relative to groundwater storage. Groundwater was generally more strongly depleted in $^{18}O$ than xylem waters for both angiosperms and gymnosperms, although at each site a substantial proportion of xylem samples were more depleted in $^2H$.

## 3.2 | Inferred contributions of soil water to xylem water

The minimum boundary polygon analysis quantifies the degree to which xylem water for both angiosperms and gymnosperms overlaps bulk soil water sources at different depths. The use of the spatially bulked data for soils and vegetation at each site was necessary to provide a sufficient number of samples for the development of encompassing polygons. This may lead to larger estimated polygon areas and a greater estimated overlap of soil and xylem water, although the effect is much less marked than for the ellipse method of Amin et al. (2020). This may provide insight into the sources of xylem water, although the proportion that cannot be ascribed to soil water sources is equally informative regarding the need to hypothesize and identify other causal reasons. Distinct inter-site differences emerged in terms of the overall overlap of xylem and soil water isotopic composition (Figure 4a). For Bruntland Burn, soil water had a 77% overlap for angiosperms, but only 6% for Gymnosperms. At Dorset, like
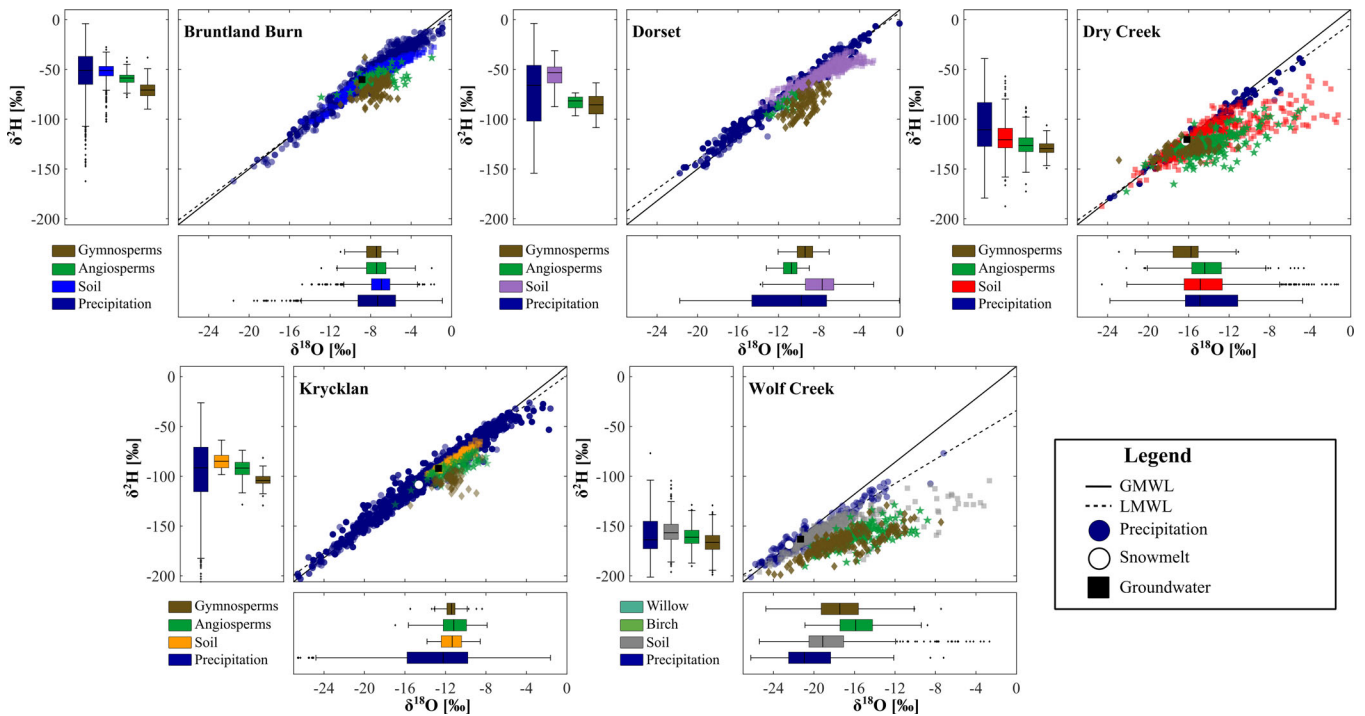
**FIGURE 3** Dual isotope plot of stable isotopic compositions of precipitation (blue circles), soil water (squares with site specific colours), and xylem water of Angiosperms (dark green stars) and Gymnosperms (light green diamonds). Note that for Wolf Creek, the vegetation is separated between Birch and Willow, since no Gymnosperms were sampled. Boxplots show the median (black line in box), the interquartile range (extent of the box) and range (whiskers). Outliers are shown as black points. All data are bulked over several sampling campaigns at up to four locations within each long-term experimental site. See supplementary material for individual sampling campaigns
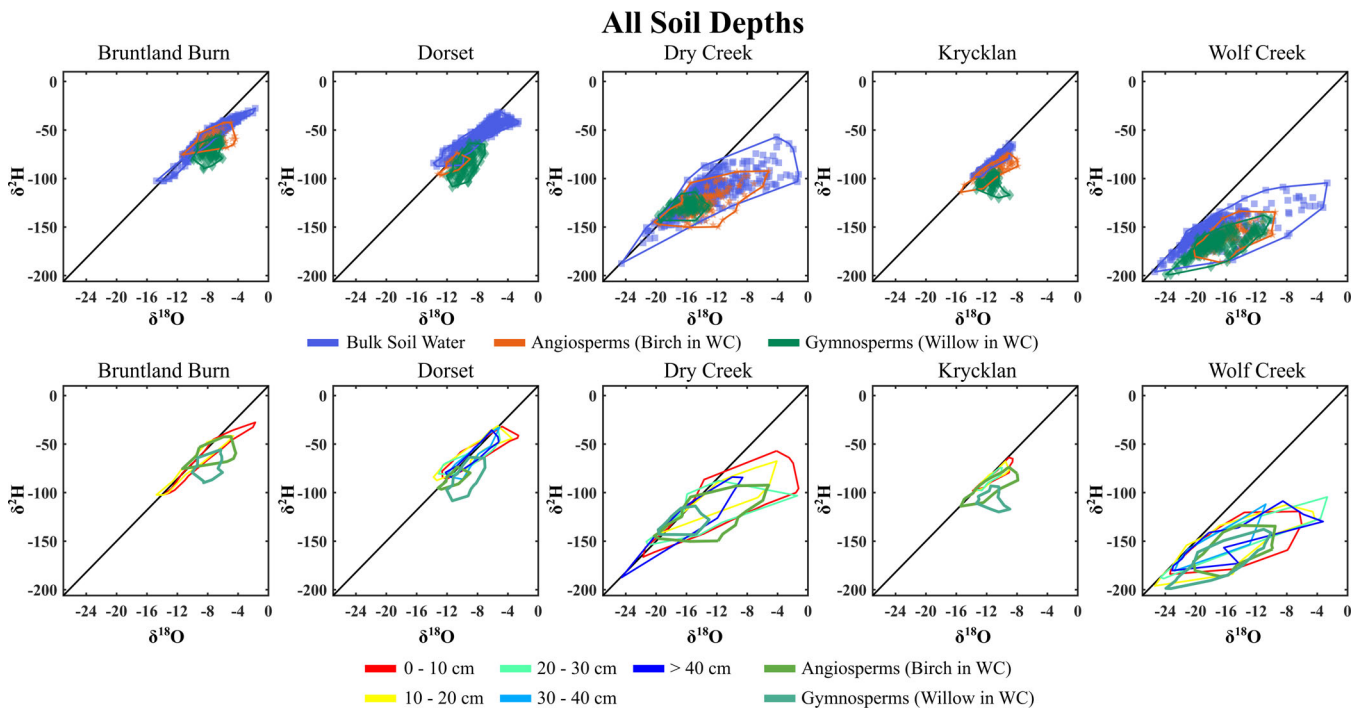


**FIGURE 4** Minimum boundary polygon for each bulked soil and vegetation type, and soil depth and vegetation type. All polygons are estimated with data bulked over all time at each site

Bruntland Burn, angiosperms showed a much higher (59%) degree of overlap than gymnosperms (18%). At Dry Creek, almost all xylem water in both angiosperms (96%) and gymnosperms (93%) overlapped soil water at almost all profile depths. Of all sites, Krycklan had the lowest degree of overlap with only 27% for angiosperms (which are all shrubs) and 0% of gymnosperms (Scots pine and Norwegian Fir trees). Finally, while Wolf Creek had only angiosperms present as willow and birch shrubs, a 99% overlap between xylem water and soil water was evident.

The depth dependent overlap of xylem and soil water isotopic composition (Figure 4b) showed differences between depths, with higher overlap tending to be in shallow soil depths for most sites. For Bruntland Burn, there was 72% and 55% overlap between angiosperms and soil water at 0–10 cm and 10–20 cm depths, respectively, but only 9% and 3% for gymnosperms. Dorset was the only site with the greatest overlap occurring in deeper soil, with overlaps of 34%, 28%, 24%, 59% and 31% for 0–10, 10–20, 20–30, 30–40 and >40 cm, respectively. The gymnosperms at Dorset had a similar deviation to that of angiosperms, with much smaller overlaps of 4%, 7%, 8%, 18% and 7%, for 0–10, 10–20, 20–30, 30–40 and >40 cm, respectively. Depth-dependent overlap of soil and angiosperms at Dry Creek was high through all soil layers with the greatest overlap in the near-surface soils (93%, 57%, 91%, 64% and 74% for 0–10, 10–20, 20–30, 30–40 and >40 cm, respectively). Gymnosperms at Dry Creek had a similarly high overlap of 78%, 55%, 86%, 72% and 86% for 0–10, 10–20, 20–30, 30–40 and >40 cm, respectively. At Krycklan, the upper two soil depths (0–10 and 10–20 cm) had approximately the same overlap for angiosperms (25% and 23%), with a moderate decrease to 12% in the 20–30 cm soils. None of the soil water at any depth overlapped the gymnosperm samples. Wolf Creek angiosperms showed a high overlap in the upper two soil depths (95% for both 0–10 and 10–20 cm) with a more substantial decrease in deeper soils

(59%, 45% and 66% in the 20–30, 30–40 and >40 cm soils, respectively).

## 3.3 | Effects of seasonality

### 3.3.1 | Seasonal differences in overlap of soil and xylem waters

The general patterns of the pooled data sets for the entire study year mask differences in the degree to which seasonal variations in the isotopic composition of xylem water can be ascribed to soil water data collected on the same day or integrated over increasing monthly time windows to capture antecedent conditions. However, as described in Section 2.2, soil water boundary polygons for increased averaging periods (1, 2 months, etc.) can also be calculated to estimate the overlap relative to xylem. The bulk soil overlaps are summarized for angiosperms (Figure 5a) and gymnosperms (Figure 5b). Depth dependent overlaps are shown in Figures S7 and S8, respectively. At Bruntland Burn, a longer time window (e.g., the preceding 3 or 6 months) of soil water isotopes explained a greater degree of variation in xylem water isotopic composition for angiosperms (Figure 5a). Bulked soil water samples collected on the same day provided 80% and 87% of overlap in spring and autumn, respectively, but only 4% in summer. Increasing this window to 3 months increased overlap to 90%, 38% and 87% in spring, summer and autumn, respectively. The spring and summer bulked soil and xylem water overlap increased to 100% and 58%, respectively, with a 6 month window. For gymnosperms, same day sampling provided no overlap in spring and summer, and only 7% in autumn (Figure 5b). For a 3 month window, overlap increased to 20% in spring, but only 3% in summer and 7% in autumn. For a 6 month window, the autumn overlap increased to 13%.
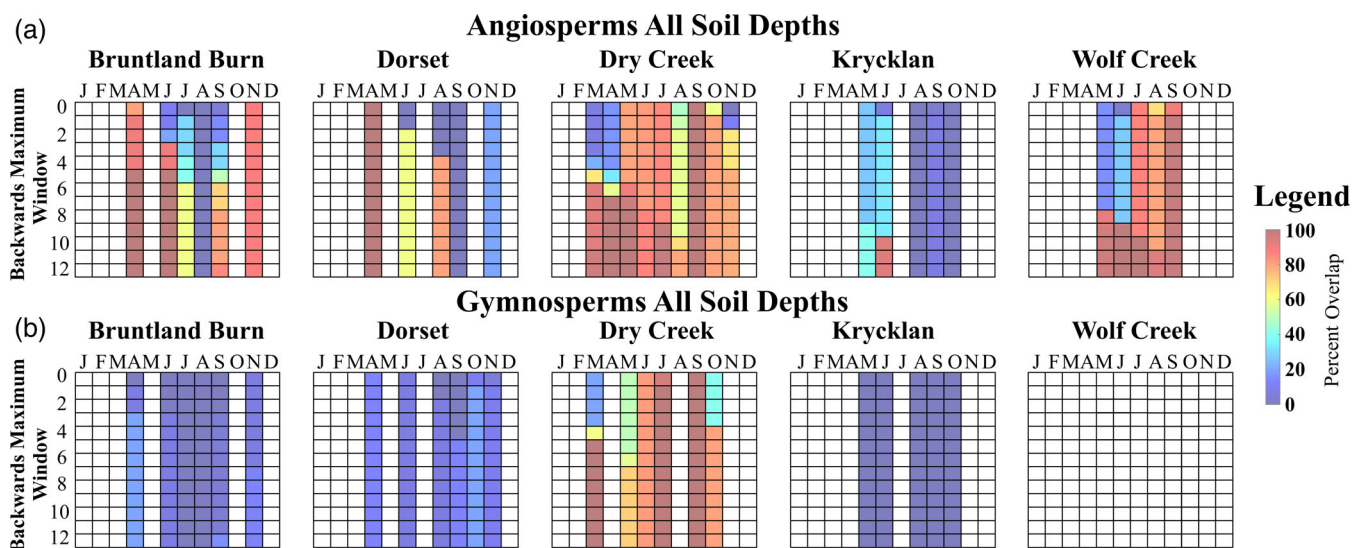


**FIGURE 5** Cumulative percentage of (a) angiosperm and (b) gymnosperms xylem isotopic composition minimum boundary polygon overlapped by soil isotopes for different backwards moving windows (which are months). X-axis is the months of sampling. Backwards window indicates maximum potential window (may not include samples). White squares show no data or insufficient data

There were marked seasonal differences between angiosperms and gymnosperms at Dorset. For angiosperms, bulked soil and xylem water overlapped for same day sampling 100% in spring, 0% in summer and 20% in autumn (Figure 5a). This increased to 20% in summer for a 3 month averaging window and 47% in summer for a 6 month average. The overlaps were much lower for gymnosperms; same day sampling showed bulked soil and xylem water overlaps of only 13% in spring, 2% in summer and 7% in autumn (Figure 5b). The respective increases were to 13%, 4% and 15% using a 3 month window; and 13%, 9% and 15% using a 6 month window.

For Dry Creek angiosperms, same day bulked soil water sampling provided 34% overlap with xylem water in spring, 78% in summer and 30% in autumn. For a 3 month sampling window, overlaps increased to 34% in spring, 81% in summer, 73% in autumn; and for a 6 month window respective overlaps were 80%, 81% and 86% (Figure 5a). This implies xylem water in angiosperms, especially in spring (but also autumn), is reflecting bulked soil water integrated over longer periods, including the previous growing seasons. Similar patterns were evident for gymnosperms at Dry Creek, with same day samples overlapping with xylem water by 35% in spring, 92% in summer, 40% in autumn. Overlaps using a 6 month window were 78%, 93% and 80%, respectively. The 3 month window values were intermediate (Figure 5b).

Of all sites, the vegetation at Krycklan showed the least overlap with bulked soil water, and this changed little with sampling period (Figure 5a). Same day sampling for angiosperms showed only 27%, 3% and 0% overlap for spring, summer and autumn. Values increased slightly for bulked soil sampling over the preceding 3 months to 27%, 13% and 0% for the three seasons, but remained constant for the 6 month window (27%, 13% and 0% for spring, summer and autumn, respectively). There was no overlap with any time window for gymnosperms (Figure 5b).

Only angiosperms were sampled at Wolf Creek, and the severe winter conditions allowed analysis only for summer and autumn. A 52% overlap was evident in summer and 89% in autumn for same day sampling. This increased to 64% and 97% for 3 and 6 month windows, respectively (Figure 5a).

### 3.3.2 | Degree of fractionation in xylem waters compared to soils

Unsurprisingly, sw-excess values of individual soil water samples plotted around 0 ‰ throughout the year (Figure 6). This gave confidence that the sw-excess is an appropriate metric to describe the potential water source, since individual soil water samples deviated relatively little from the regression through all soil water samples. Plant sw-excess was usually <0 ‰, indicating that xylem water was generally more depleted in $^2H$ compared to soil water. At Bruntland Burn, Dorset and Krycklan, sw-excess was more negative for gymnosperms than for angiosperms. The deviation from sw-excess of 0 ‰ occurred generally under lower soil moisture conditions.

At Bruntland Burn, angiosperms had a similar sw-excess to soils in most sampling periods, apart from the start of the study period in

October 2015 and the following summer, when it dropped in July, August and September before recovering in September 2016. Differences for gymnosperms were more pronounced and only close to the soils in winter and early spring. Similar patterns were evident for Dorset, although the differences from sw-excess were greater for both plant groups. In general, summer saw the greatest isotopic difference between xylem and soil waters. For Krycklan, angiosperms occasionally showed sw-excess closer to 0 ‰, although the timing was generally limited to early summer after snowmelt. Gymnosperms were closer to soils at this time too, although both plant groups deviated from the soils with the approach of autumn. The S4 site at Krycklan also had the wettest soil conditions. At Dry Creek, differences between angiosperms and gymnosperms were less pronounced and gymnosperm values were usually less negative than for angiosperms. Both gymnosperms and angiosperms periodically reached sw-excess of 0 ‰, although the timing was not as consistent as at the other sites. It was striking that the Dry Creek site with the greatest similarity between soil and xylem waters was also the driest, with the lowest soil water content. At Wolf Creek, angiosperm sw-excess was usually close to the soil water sw-excess with the exception of May 2016, which was at the end of winter when shrubs were not active.

## 4 | DISCUSSION

### 4.1 | Xylem waters

The xylem waters sampled in this study provided a series of snap-shots of plant water over the course of the growing season at five northern experimental catchments. This resulted in an unusually rich comparative data set allowing a meta-analysis of inter- and intra-site (dis)similarities. Some clear findings emerged from this inter-comparison, though there remain many unanswered questions. The close link to soil water at each site was apparent from the similar positions of xylem water when plotted in dual isotope space (Figure 2). However, for most sites, much of the xylem water tracked towards lower $\delta^2H$ and $\delta^{18}O$ plotting below the meteoric water line and below the soil water samples. The sw-excess was shown to be a helpful metric to describe the dynamics of the deuterium offset of xylem waters compared to soil water. For some sites, there was much less or no overlap for gymnosperms (e.g., White cedar at Dorset) or some angiosperms (*Vaccinium* at Krycklan). The results also showed seasonal variations in xylem composition (and correspondence to soil water) at most sites, although this differed (see below). The plotting positions of xylem water from angiosperms and gymnosperms were quite distinct at some sites, despite some overlap. Apart from Dry Creek, gymnosperms at most sites were more offset from both the LMWL and soil waters compared to the angiosperms.

### 4.2 | Evidence for soil water sources

The operationally-defined boundary polygon analysis provided an objective way of comparing the distribution of the soil and xylem data

**FIGURE 6** Soil moisture (lines) and SW-excess for soil waters (brown squares), Angiosperms (stars), Gymnosperms (diamonds) at the five VeWa sites. The large markers represent the mean values for one specific sampling location at that sampling day and the small half-transparent markers represent the original data
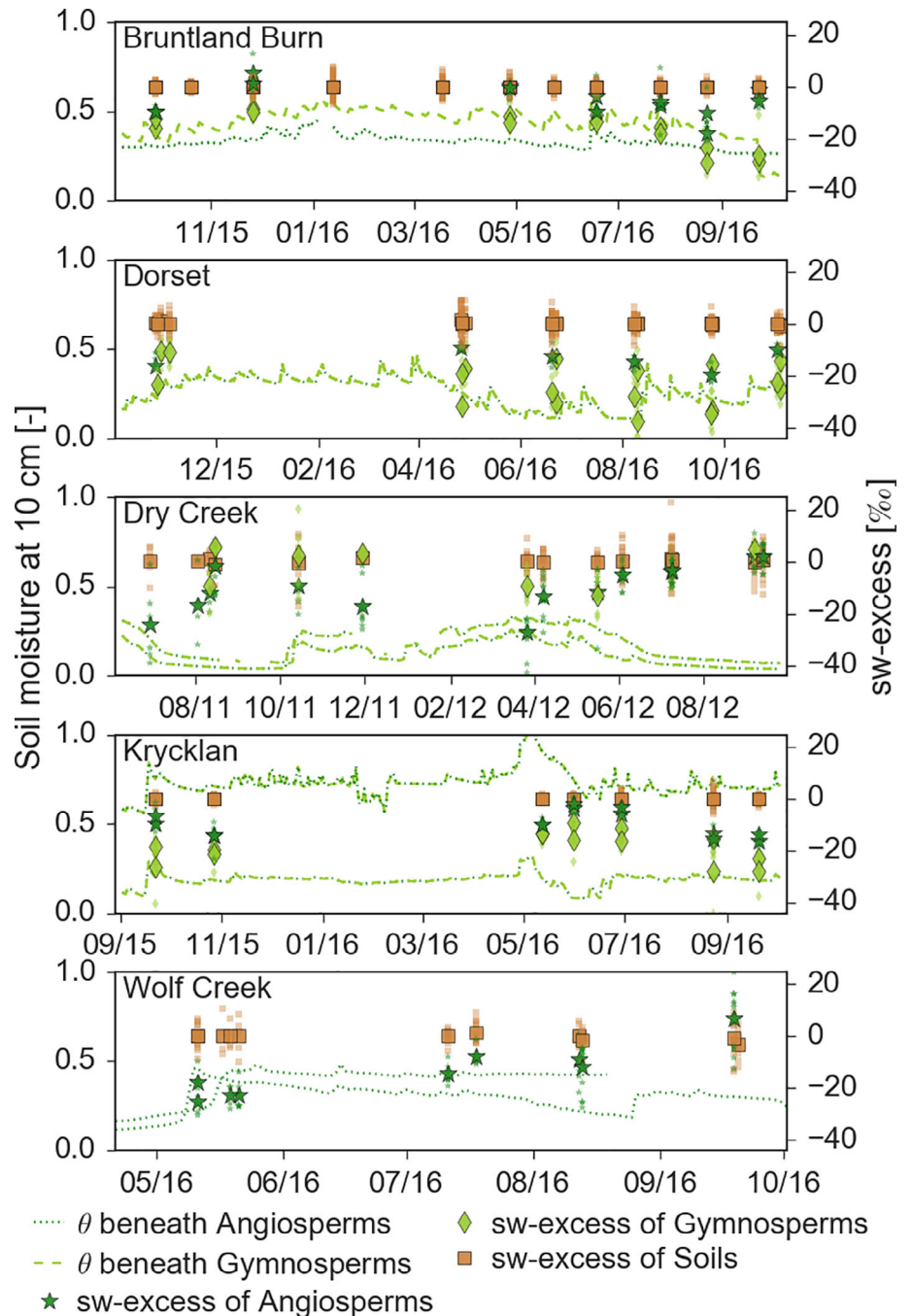


from the five sites (Figure 4). It is notable that the sites with greatest general overlap between all sampled angiosperm xylem waters and soil waters are characterized by smaller shrubs and trees (e.g., *C. vulgaris* at Bruntland Burn, *Betula* and *Salix spp*. at Dry Creek and Wolf Creek). That said, larger trees (*Q. rubra*) at Dorset also showed quite a high degree of overlap, especially for more depleted, potentially snowmelt-recharged water sources earlier in the growing season. In contrast, *Vaccinium* at Krycklan showed little overlap. However, the physiology of smaller plants, with shorter rooting systems, lower internal storage and more rapid water throughput rates may at least partly explain the greater coherence between xylem water and soil water. Indeed, previous ecohydrological modelling experiments at

Bruntland Burn by Kuppel et al. (2018) and calibrated only on hydrometric data, found quite good agreement between simulated and observed soil water and xylem $\delta^2H$ values in angiosperm (*Calluna*) using the spatial distributed EcH$_2$O-iso model. Conversely, the same model failed to simulate the xylem isotopes in gymnosperms (*P. sylvestris*).

The polygon analysis at most sites also seemed to indicate that overlaps between soil and xylem waters reflected integrating effects of water sources across the rooting zone, which at most sites was relatively shallow (Figure 4b). This is consistent with the conclusions of Amin et al. (2020) for northern sites in their global meta-analysis that found isotopic evidence that cold region plant water was sourced

from shallower depths compared to more temperate and arid regions. Given the groundwater isotope data available at all sites apart from Dorset, there is little evidence that deeper water sources can help explain the xylem samples not potentially related to soil water sources (Figure 3). Furthermore, at Dorset the thin (up to 0.5 m thick) soil cover overlies what seems to be relatively unfractured bedrock. It is possible that some trees have roots that are tapping water held in fractures, but given the geology it is unlikely that there is sufficient storage to sustain a significant fraction of evapotranspiration.

## 4.3 | Seasonality of potential soil water sources

It is clear that some of the observed changes in xylem water throughout the growing season are related to phenological changes (Figure 6). This temporal correspondence partly reflects the "switching on" of plants in the spring as photosynthesis and transpiration increase (Wang et al., 2019) as well as the availability and isotopic composition of soil water. Previous work by Sprenger, Tetzlaff, Buttle, Carey, et al. (2018) showed that variations in soil water isotopic composition at the study sites were mainly driven by precipitation and snowmelt over the preceding weeks, although there was also an effect of evaporation on kinetic fractionation of isotope ratios during summer. These dependencies highlight the importance of precipitation frequency and intensity, infiltration, soil wetness and the mixing interactions that govern soil water residence time distributions (A. A. Smith et al., 2020; Sprenger & Allen, 2020). The way in which processes and interactions relate to plant demand highlights the importance of the temporal integration of root uptake and water transport into the main plant stems. The non-stationary travel times from uptake to transpiration may average many months (Brinkmann et al., 2018), with tailing in the travel time distribution potentially a result of plant-stored water contributing to transpiration under dry conditions and possible mixing of xylem water with other plant water (Knighton et al., 2020).

The temporal trajectory of the xylem waters varied relative to soil water through the growing season, but this differed between angiosperms and gymnosperms. Also, inter-site contrasts between the angiosperm and gymnosperm differences were apparent: For Bruntland Burn, soil and xylem water signals were most similar in spring, deviated more strongly in summer and then returned to greater overlap in autumn for angiosperms. However, this was not the case for gymnosperms which showed dissimilarity throughout the year. For angiosperms at Dorset, there was a degree of overlap to start with, but depletion increased through summer and then closed again in autumn. In contrast, gymnosperm xylem waters became more $^2$H- and $^{18}$O-enriched. At Dry Creek, there was a large difference through the autumn and winter for both angiosperms and gymnosperms until spring, but compositions became increasingly similar in summer. At Krycklan, angiosperms were most similar in the spring and early summer, but became increasingly different as the summer progressed. At Wolf Creek, there was an offset at the beginning of spring but samples then increasingly converged. This post-winter offset, also evident at Dry Creek, may relate to desiccation and/or diffusion within

the plant during the biologically inactive period (McCutcheon et al., 2017).

Inclusion of longer antecedent periods for soil isotope data generally improved overlaps within the boundary polygons for most sites, especially for angiosperms. The "sampling window" over which soil water may have been a source for plant uptake and contributed to xylem water in the trunk at breast height is unknown, and is likely to be non-stationary given seasonal variations in soil moisture and plant physiology. However, the greater overlaps for the longer antecedent period would support the hypothesis that xylem water at any point in time represents an integrated sample of soil water accumulating over preceding months, rather than soil water on the sampling day which will be most influenced by the most recent rainfall. In this sense, the results are similar to those of Allen et al. (2019) who demonstrated that trees throughout Switzerland predominantly use soil water derived from winter precipitation for summer transpiration. In our study, however, findings across sites and plant species were not consistent. Regardless, results from both studies suggest that caution should be used when constructing conceptual models of how plants access soil water based on synoptic, space-based sampling.

Our phenologically-timed sampling strategy, particularly at such high latitude sites, is novel. However, more frequent sampling would likely be advantageous providing more nuanced insights into the phenological controls and short-term dynamics of xylem isotopes, particularly in relation to short term soil moisture dynamics and periods of higher atmospheric moisture demand (e.g., De Deurwaerder et al., 2020). Nevertheless, higher-frequency sampling will still likely show that the xylem samples indicate stronger fractionation which has been widely shown for many vegetation types around the world (Evaristo et al., 2015; and discussion by Barbeta et al., 2019). This focuses attention on potentially fractionating processes linked to small-scale interactions at the root-soil pore interface, especially close to the soil surface where most fine roots are present and where labile nutrients are also highest in acidic, organic soils. However, methodological issues may at least partly explain some of the difference. These are discussed in the following section.

## 4.4 | Inter-site comparison anomalies

Dry Creek stands out as an anomalous site in many results, most of which can be explained by its warm, dry conditions and high seasonality. Wolf Creek, however, the coldest site, shares similar results. The two sites obscure an otherwise clear relationship between plotting position along the GMWL and the mean annual temperature (Table 4a), they show the most overlap between xylem and soil water isotopes in bulk and at various depths (Figure 4), and they have the highest negative lc-excess values for both xylem and soil water (Table 4b). They also have the lowest May–August relative humidity at 38% and 63%, as well as precipitation at 19 mm and 44 mm, for Dry Creek and Wolf Creek, respectively (Table 2). The relatively dry conditions shared by both sites expose soil waters to sustained evaporative environments, which may cause hydro-patterning of roots

(Sprenger & Allen, 2020). Roots grow where water is available, which tends to be in less conductive pores where water has longer residence times and likely more isotopic fractionation due to evaporation. This evaporatively-enriched soil water also has limited potential for mixing with isotopically-different incoming precipitation that would alter its isotopic composition, partly because the growing-season precipitation at these sites is low. Accordingly, plant roots in dry environments have fewer soil water source options, so xylem water and bulk soil water will trend towards similar isotopic compositions.

## 4.5 | Open questions

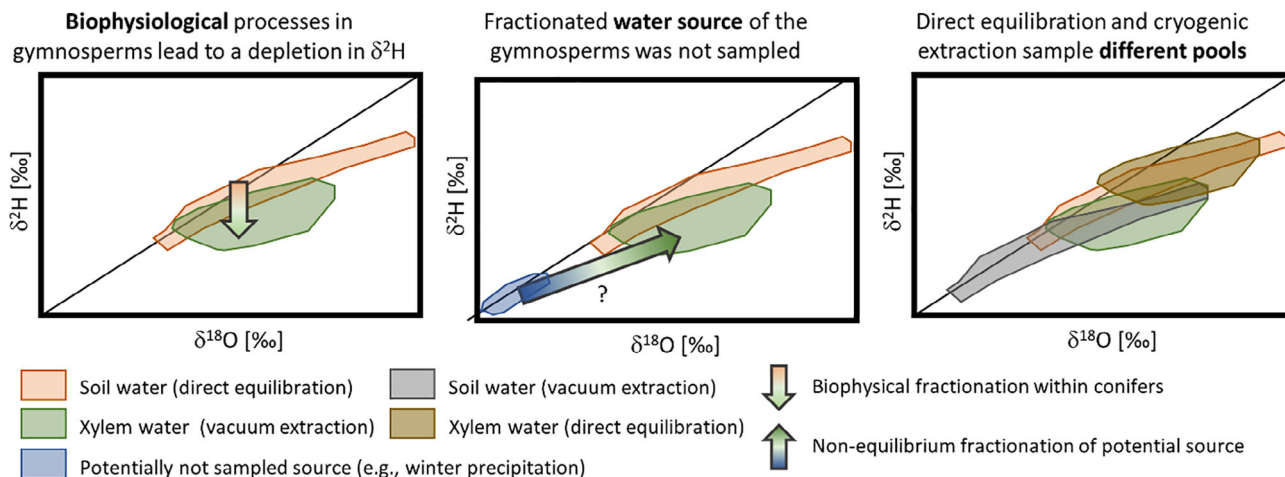Despite our unique data set and our observations, several open questions remain:

1. Biophysical processes: Recent research shows that various complex bio-physical processes in the soil–plant-atmosphere continuum may help explain why xylem water at the VeWa sites cannot be fully explained by soil water sources (Figure 7). As noted above, one possibility is that exchange between the soil liquid and vapour phase is complex and may affect root water uptake. This may be either through roots being able to access a fractionated vapour phase and/or condensation onto soil surfaces from the soil atmosphere increasing the likelihood that plants take up water depleted in heavier isotopes, especially deuterium. Both recent field (Oerter & Bowen, 2019) and modelling (Sprenger, Tetzlaff, Buttle, Laudon, Leistert, et al., 2018) studies have highlighted the plausibility of such mechanisms, but mechanistic studies to test such a hypothesis are limited and urgently needed.

Similarly, the complex interactions in the symbiotic relationship between mycorrhiza and plant roots cause uptake of more $^2$H- and $^{18}$O-depleted water compared to bulk soil water. In particular, widespread arbuscular mycorrhizal fungi which penetrate the cortical cells in the roots of vascular plants may be an effective mechanism that can facilitate fractionation of root water uptake (Poca et al., 2019). This occurs as part of the complex symbiosis of nutrient exchange

that also affects plant-water relationships and is focused in the upper soil horizons. Such mycorrhizal interactions are particularly important in nutrient-poor minerogenic northern soils, and may have strong effects at sites like Bruntland Burn, Dorset and Krycklan. Again, more specific process-based studies are required to test this hypothesis in contrasting soil-plant systems.

Finally, diffusion and evaporation through bark may be important biophysical processes, especially during winter when there is no transpiration (Gessler et al., 2014). This is potentially a factor in northern regions where winter conditions preclude transpiration but can expose vegetation to arid conditions with high wind speeds and low humidity at sites like Dry Creek and Wolf Creek (McCutcheon et al., 2017). Isotope transport through bark may explain why the gymnosperms at Dry Creek showed much greater overlap with the isotopic composition of soil water sampled over a range of antecedent intervals in spring (Figure 5b) compared with Bruntland Burn, Dorset, and Krycklan where there was very little overlap. However, this inter-site difference was less pronounced for angiosperms (Figure 5a).

2. Extraction of vegetation and soil water: We do not fully know what kind of vegetation water is mobilized by the cryogenic extraction, although it is usually assumed to characterize xylem water. However, it is likely that some of the water that gets extracted is part of live cells subject to potentially fractionating biophysical processes that are independent of the hydrological cycle. Zhao et al. (2016) saw large differences between xylem sap, extracted with a syringe, and twig water extracted via cryogenic extraction with the former being more enriched in $^2$H compared to the latter. In such cases, differences in the ratio of cell water to xylem water, which would depend on soil wetness, could have an effect on the differences between the isotopic composition of plant water and cryogenically extracted water (xylem + cell water). Barbeta et al. (2020) support this interpretation and call for more specific characterization of what is assumed to be extracted xylem water. Very recent experimental work by Chen et al. (2020) showed that cryogenic extraction can enhance deuterium exchange with organically bound water and contribute to the deuterium depletion. Moreover, they showed the effect can be greatest under more



**Biophysiological** processes in gymnosperms lead to a depletion in $\delta^2$H

Fractionated **water source** of the gymnosperms was not sampled

Direct equilibration and cryogenic extraction sample **different pools**

Soil water (direct equilibration)

Xylem water (vacuum extraction)

Potentially not sampled source (e.g., winter precipitation)

Soil water (vacuum extraction)

Xylem water (direct equilibration)

Biophysical fractionation within conifers

Non-equilibrium fractionation of potential source

**FIGURE 7** Potential explanations for the deuterium-offset observed between soil and xylem water stable isotopes

moisture-limited conditions which may explain the tendency for more negative sw-excess values as sites become drier. Physiological and biochemical differences between angiosperms and gymnosperms may also contribute to differences in extraction effects (see below).

As with vegetation water extraction, differences from contrasting soil extraction techniques (e.g., cryogenic and equilibration) may explain some of the mis-match between observed xylem water and soil sources. For example, the similarities between soil and xylem water at Dry Creek involved cryogenic extraction of soils, whereas all other sites used equilibration. However, at Bruntland Burn cryogenic and equilibration methods gave similar results for peaty soils, and reasonable agreement with xylem water (Geris et al., 2017). Extraction focusing on small-scale moisture isotope dynamics at the root – soil interface may be needed, including scalable methods to explore the phase change/mycorrhizal mechanisms suggested above. Our findings, based on bulk soil field measurements, underline the major difficulties associated with relating potential water sources to plant water stable isotope compositions. Even under controlled laboratory conditions, Orlowski, Winkler, et al. (2018) could not confidently link relate the soil water to root crown isotopic compositions, but reported similar $^2H$ depletion as we found in Dandelions growing on sandy soils.

c. Differences between angiosperms versus gymnosperms: A clear finding of our study is that the extracted xylem waters of angiosperms and gymnosperms have a very different isotopic composition at most sites, with gymnosperms generally showing a greater degree of fractionation. In this regard, several hypotheses could be tested. Firstly, root networks and root-mycorrhizal networks of different species may be able to access different pore sizes. For example, gymnosperms may have greater potential to mobilize water that has undergone some fractionation during the interactions among water, gas, and solid phases of the soil. Secondly, storage and mixing of water within plant tissues may be greater in softwood gymnosperms, as suggested in recent modelling work (Knighton et al., 2020). The generally slower metabolism and transpiration rates for gymnosperms might exacerbate this mechanism. Such differences may also contribute to what water is extracted in the laboratory. Interestingly, Amin et al. (2020) showed little difference between angiosperms and gymnosperm xylem waters for cold and temperate environments in their meta-analysis, whereas angiosperms in arid regions were offset in $\delta^2H$ compared to gymnosperms.

## 5 | CONCLUSIONS

We sampled xylem water in conjunction with soil water at five well-instrumented sites across northern cold landscapes. At all sites except Krycklan, water sources of angiosperms could be associated with soil water. At all sites except Dry Creek, the sources of water uptake by gymnosperms were much less easily explained. Whereas the isotopic composition of xylem water for angiosperms generally overlapped that of soil water for a range of antecedent periods, overlap did not occur for gymnosperms (with the exception of Dry Creek). This suggests that the xylem water of angiosperms was influenced by the isotopic composition of water retained in the soil weeks or months prior to plant sampling, whereas gymnosperms generally did not exhibit such a memory effect. The isotopic offset between soil and xylem samples was generally greatest during the growing season for the wetter sites (Krycklan, Dorset and Bruntland). However, at the drier two sites (Dry Creek and Wolf Creek) xylem and soil water isotopes tended to be similar, showing the effects of evaporation. We attribute this dry site anomaly to the relatively rare occurrence of mobile water during the growing season. There simply are not many choices of water sources form plants in dry areas, so soil water and xylem water trend towards similarity, and typically have a strong evaporation signal. Our study also raised questions that will need to be addressed in future research: Which biophysical processes at the root – soil interface contribute to isotopic fractionation in uptake that affects the composition of xylem water? What are the internal dynamics of water storage, mixing and release within vegetation and how does this relate to the degree of synchronicity between phenology and soil water availability? What reservoirs are sampled during cryogenic extraction – only xylem water or does this include water from other plant cells? And finally, why are angiosperms and gymnosperms at the same sites so isotopically different? Addressing some or all of these questions will contribute to our understanding of soil–plant-atmosphere interactions in northern landscapes.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.