TEMPORAL DYNAMICS OF TREE SOURCE WATER IN SKY ISLAND ECOSYSTEMS WITH EPHEMERAL SNOW PACK: A CASE STUDY USING *PSEUDOTSUGA MEZIESII* (DOUGLAS FIR)

by

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# TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................ 6

ABSTRACT ................................................................................................................... 7

1. INTRODUCTION ..................................................................................................... 8
   1.1 The Problem ........................................................................................................ 8
   1.2 The Soil-Plant-Atmosphere Continuum (SPAC) .................................................. 10
   1.3 Stable Isotope Tracers ......................................................................................... 12
   1.4 Stable Isotope Analysis ....................................................................................... 13
   1.5 Objectives .......................................................................................................... 15
   1.6 Study Site ............................................................................................................ 16
   1.7 Structure of the Following Chapters ................................................................. 18

2. PRESENT STUDY ..................................................................................................... 19
   2.1 Abstract of Appendix A: Temporal dynamics of tree source water in sky island ecosystems with ephemeral snow pack: A case study using Pseudotsuga meziesii (Douglas Fir) . 19
   2.2 Appendix B: Performance of induction module cavity ring-down spectroscopy (IM-CRDS) for measuring delta O-18 and delta H-2 values of soil, stem, and leaf waters .......... 19
   2.3 Appendix C: Field Protocol ................................................................................ 21
   2.4 Appendix D: Lab Protocol ................................................................................... 21
   2.5 Appendix E: Supplemental Data ......................................................................... 21
   2.6 Summary of Results .......................................................................................... 22
   2.7 Future Research Opportunities .......................................................................... 22

3. REFERENCES .......................................................................................................... 24
APPENDIX A: TEMPORAL DYNAMICS OF TREE SOURCE WATER IN SKY ISLAND ECOSYSTEMS WITH EPHEMERAL SNOW PACK: A CASE STUDY USING PSEUDOTSUGA MEZIESII (DOUGLAS FIR) .......................................................... 31

APPENDIX B: PERFORMANCE OF INDUCTION MODULE CAVITY RING-DOWN SPECTROSCOPY (IM-CRDS) FOR MEASURING DELTA O-18 AND DELTA H-2 VALUES OF SOIL, STEM, AND LEAF WATERS ................................................................. 66

APPENDIX C: FIELD PROTOCOLS ................................................................. 104

APPENDIX D: LAB PROTOCOLS ................................................................. 114

APPENDIX E: SUPPLEMENTAL DATA .......................................................... 123
LIST OF FIGURES

Figure 1. Field site map
ABSTRACT
Throughout the world, water is the limiting resource for both natural and human development. A primary source of water throughout the world comes from seasonal snowpack and snowmelt which largely occurs in the mountains. Our results show that the precipitation that falls in the Santa Catalina Mountains has seasonal isotopic variations, with summer monsoon being more enriched than that of winter precipitation. The isotopic signature of soil moisture has a seasonal trend determined by a combination of seasonal precipitation and dynamic soil moisture flow, this also results in a slight seasonal variation of the isotopic signature of groundwater. The Douglas Fir (Pseudotsuga mezesii) utilizes water from the shallow soils (0 – 20 cm), deep soils (30 – 100 cm), and groundwater. The main source of water for the Douglas Fir throughout most of the year is the shallow soils, but during the dry pre-winter season the source of water switches to groundwater due to a drying soil profile. Hydraulic redistribution during the dry pre-winter season likely causes wetting in the shallow soils. If climate change predictions are correct a shift in precipitation could alter the source water of the Douglas Fir such that they may become increasingly water stressed. If Douglas Fir is unable to survive under this increased water stress, it will have important implications for water resources in the region.
1. **INTRODUCTION**

1.1 **The Problem**

Water is limiting to natural development in arid and semi-arid regions throughout the world. A primary source of water throughout the world comes from seasonal snowpack and snowmelt which largely occurs in the mountains (Brown-Mitic et al., 2007; Earman, Campbell, Phillips, & Newman, 2006; A. Harpold et al., 2012; A. A. Harpold et al., 2015; Luca, 2018; Nelson, Kurc, John, Minor, & Barron-Gafford, 2014). Seasonal snowpack has been defined as snowpack that has a predictable melt timing and volume of melt water and ephemeral snowpack is snowpack that lasts for less than 60 days and produces less/earlier snowmelt than seasonal snowpack (Petersky & Harpold, 2018). Global climate change models predict that many regions around the world will become drier and hotter, have reduced winter snowpack, and have an earlier onset of spring melt (Barnett, Adam, & Lettenmaier, 2005; Beniston, 2003; Grundstein & Mote, 2010; Luca, 2018; López-Moreno, Goyette, & Beniston, 2009; Räisänen, 2008; Schmucki, Marty, Fierz, Weingartner, & Lehning, 2017; U.S. Global Change Research Program, 2017; Vicuña, Garreaud, & McPhee, 2011; You et al., 2017). These changes will have significant implications for the ecohydrological cycle of regions of the world that are reliant upon seasonal snowpack/melt. There are numerous studies that have shown that these changes have already had an adverse effect on regional water resources and are predicted to continue to do so (Grundstein & Mote, 2010; Hennessy, 2008; Rocca, Brown, Macdonald, & Carrico, 2014; Schmucki et al., 2017; U.S. Global Change Research Program, 2017). However, given the complexity of ecohydrological interactions there are effects that these climate changes will have on regions that have not been studied. For instance, as the global average temperature continues to increase many areas that in the past experienced seasonal snowpack may experience a shift to ephemeral snowpack in the future (Barnett et al., 2005; Luca, 2018; López-Moreno et al., 2009; Petersky & Harpold, 2018; Schmucki et al., 2017; Vicuña et al., 2011). This shift from seasonal to ephemeral snowpack, potentially, has significant implications for ecohydrological processes within a variety of ecosystems.

In many watersheds there are multiple ecosystems, such as pinon-juniper woodlands, lower/upper montane forests, subalpine forests, and riparian corridors, which rely on the seasonal snowpack and snowmelt occurring in the mountains (Archer & Predick, 2008; Brown-Mitic et al., 2007; A. A. Harpold et al., 2015; Luca, 2018; Nelson et al., 2014; Rocca et al., 2014). These ecosystems
are home to numerous species of flora which utilize snowmelt for the ecohydrological process of transpiration (Brown-Mitic et al., 2007; Gergel, Nijssen, Abatzoglou, Lettenmaier, & Stumbaugh, 2017; Grundstein & Mote, 2010; A. Harpold et al., 2012; A. A. Harpold et al., 2015; Luca, 2018; Nelson et al., 2014; Pederson et al., 2011). Transpiration is the process by which water is absorbed by a plants roots, translocated through the vascular system to the stomata on the stems/leaves from which it is evaporated back to the atmosphere (Dingman, 2002). The flora within these ecosystems have slowly evolved over time, adapting to the local climate and hydrological cycle. However, given the dramatic changes occurring to the climate and hydrology both globally and locally, there are numerous implications for the ecology of these ecosystems.

It has been observed throughout many places in the world that the temperature is rising, snowpack is declining, snow melts are occurring earlier, and the snow line is receding up in elevation (Barnett, 2005; Hennessy, 2008; Pepin et al., 2015; Räisänen, 2008; U.S. Global Change Research Program, 2017). Winter warming has caused plants to budburst earlier potentially exposing their soft tissues to late winter freezes (Weijers, Beckers, & Löffler, 2018). Future increases to winter warming has the potential to effect the endodormancy period of plants which can make them incapable of budburst and therefore they would not be able to repair damage to their existing tissues or grow new tissues (Asse et al., 2018). Reduced snowpack results in reduced soil moisture available to plants during the winter and spring time periods when budburst and transpiration starts up with tissue production (Barnett, 2005). Likewise, earlier snowmelt results in reduced soil moisture later in the spring time which can result in increased drought stress for the plants (Barnett, 2005). A receding snowline means that the temperature is warming at higher elevations which means that plants that could not exist in colder climates can encroach into areas that they previously could not inhabit (Hennessy, 2008).

Despite these findings, to the best of the authors knowledge, there are no studies that investigate how ecosystems and their ecohydrological processes such as transpiration are likely to be impacted by the spatial and temporal changes in water availability resulting from a shift from seasonal to ephemeral snowmelt. This study looks at the ephemeral snowpack/melt system in the Santa Catalina Mountains to give an example of the conditions that can be expected if and when seasonal systems become ephemeral. The main objectives of this study were to identify 1) the source of
water that trees are taking up water from and 2) whether that source of water changes with season. In answering these two questions it will be determined what plant water use strategies are used to survive in an ephemeral snowpack/melt environment, and by extension, what will happen when a system shifts from a seasonal to an ephemeral snowpack environment.

1.2 The Soil-Plant-Atmosphere Continuum (SPAC)

To elaborate on the implications of a shift from a seasonal to an ephemeral snowpack/melt environment, it is first necessary to have an understanding of how water moves through the soil-plant-atmosphere continuum (SPAC). The SPAC is the movement of water as it precipitates from the atmosphere, infiltrates into the soil, and transpires from plants back into the atmosphere.

In the Southwestern United States, precipitation largely falls during two seasons: 1) the North American Monsoon from July to September and 2) winter from December to March (Ajami, Troch, Maddock, Meixner, & Eastoe, 2011; Brown-Mitic et al., 2007; Eastoe & Dettman, 2016; Wahi, Hogan, Ekwarzel, Baillie, & Eastoe, 2008). Monsoon storms originate in the Gulfs of Mexico and California (Eastoe & Dettman, 2016; Wahi et al., 2008). These storms are largely localized to areas of a few km and are generally characterized as high intensity and low duration (Ajami et al., 2011; Eastoe & Dettman, 2016; Wahi et al., 2008). The winter storms originate in the Pacific Ocean and are largely regional in area and are generally characterized as low intensity and high duration (Ajami et al., 2011; Eastoe & Dettman, 2016; Wahi et al., 2008). In the Southwest, most of water that ends up in the aquifer originates in the mountains, making them critical areas to study (Ajami et al., 2011). Precipitation has an isotopic signature that has been formed from latitude, continental, altitude, and seasonal effects (Clark & Fritz, 1997). Each precipitation event delivers water that has unique isotopic values from other precipitation events, with marked differences between summer and winter storms (Anderson, von Blanckenburg, & White, 2007). Precipitation that falls during winter months has relatively low isotopic values, while precipitation that falls during the summer months has relatively high isotopic values (Eastoe & Dettman, 2016; Eastoe, Gu, & Long, 2004).

After water has precipitated out of the atmosphere in the form of rain and snow, it begins moving from the land surface into the subsurface through the processes of infiltration. The water then
moves through the unsaturated zone (redistribution) and eventually recharges the saturated zone (Dingman, 2002). Intensity and duration of a rain storm, the antecedent soil moisture, and the hydraulic conductivity of the soil, all affect the rate of infiltration (Ajami et al., 2011; Earman et al., 2006; Gierke, Newton, & Phillips, 2016; A. A. Harpold et al., 2015). Before mixing with any shallow soil water the water that infiltrates into the ground initially has the isotopic signature of the precipitation. After the water infiltrates into the subsurface it acts as either mobile or immobile water. Mobile water, bypass flow, is water that moves rapidly through the unsaturated zone by means of preferential pathways created by macropores, resulting from roots, animal burrows, or cracking (Dingman, 2002; Mathieu & Bariac, 1996). Immobile water is water that moves slowly through the unsaturated zone by means of micropores and is often electrostatically attracted to soil particles (Dingman, 2002; Mathieu & Bariac, 1996). Water can evaporate from the land surface and from the soil. In the evaporative process the light isotopes of water preferentially evaporate off leaving the source water increasingly enriched in heavy isotopes (Clark & Fritz, 1997; Dansgaard, 1964). Thus, immobile waters that are subject to continued evaporative losses at shallow soil depths will become increasingly enriched compared to the original precipitation event (Barnes & Allison, 1988; Zimmermann, 1967). These immobile waters will generally be trapped in the unsaturated zone, until a sufficiently strong precipitation event (high intensity and long duration) produces a water front that pushes antecedent water moisture down into the soil profile towards the saturated zone (Gazis & Feng, 2004). Though these isolated pockets of immobile water can have highly enriched isotopic signatures, they are so small by volume that if they mix with mobile water, the ground water Table, or are taken up by plants, their relatively extreme isotopic signatures can be averaged out when mixed with other waters. As mobile water moves through the unsaturated zone it can either mix or not mix with the antecedent water in the soil matrix. If the mobile water mixes with the antecedent water as it moves towards the saturated zone/groundwater, the isotopic value of the antecedent water will make negligible effect on the larger volume of mobile water. It is possible for mobile water, that is more isotopically depleted/enriched than pockets of antecedent water to travel deeper into the soil column and then get deposited as immobile water (Bertrand et al., 2014; Beven & Germann, 2013; Mathieu & Bariac, 1996). Regardless of the method by which water percolates through the unsaturated zone, once it mixes with the groundwater, the isotopic value becomes approximately equal to the annual

Many plants that grow in seasonal or ephemeral snow environments have evolved to have periods of active growth and dormancy. In these environment’s plants tend to go dormant beginning in fall through the winter and are active beginning in spring through the summer. Plants, including the Douglas Fir, have developed root systems that tap into water from the shallow soil (0 – 20 cm), deep soil (30 – 100 cm), and groundwater (variable depth dependent on site) (Andrews, Flanagan, Sharp, & Cai, 2012; Brooks, Meinzer, Coulombe, & Gregg, 2002; Warren, Meinzer, Brooks, & Domec, 2005). Having access to multiple sources of water allows plants to survive periods when one source of water is not available. Additionally, the Douglas Fir has been shown to hydraulically redistribute water both laterally and horizontally within the root zone to keep the soil profile wetted (Brooks et al., 2002). The hydraulic redistribution process is likely increased in effectiveness due to root grafting amongst the trees in a Douglas Fir stand (Lavender & Hermann, 2014). The water that is taken up by a plant, into its sap wood, is an integration of the various sources of water that the roots are tapping into (Ehleringer & Dawson, 1992). Thus, the sap water has an isotopic value that is an integration of the isotopic values of the various waters being taken up by the plant (Ehleringer & Dawson, 1992). Once water has entered a plants root system fractionation of the water seizes to alter the isotopic signature of the water until it reaches the leaves (Ehleringer & Dawson, 1992). However, it has been shown that new plant growth, such as fresh green stems, can experience isotopic fractionation like leaves (Dawson & Ehleringer, 1993). It is uncertain to what extent plants can control which source of water they are tapping into and to what extent they can switch from one source to another.

1.3 Stable Isotopes of Water as Tracers
To study the movement of water through the environment it is common to use the stable isotopes of water (δD and δ18O) as natural tracers. The use of these stable isotopes is predicated on the notion that each source (precipitation, soil depth, groundwater, and stems) has a different isotopic signature from one another (J. P. Brunel, Walker, & Kennettsmith, 1995; J. P. W. Brunel, G. R.; Walker, C. D.; Dighton, J. C.; Dennett-Smith, A., 1993). Some studies have used stable isotopes to determine the source water of plants (Busch, Ingraham, & Smith, 1992; Ellsworth & Williams,
Some studies have looked at the temporal variability of source water (Andrews et al., 2012; Bertrand et al., 2014; Gierke et al., 2016). Other studies have used stable isotopes to look at various aspects of snowpack and snowmelt (Earman et al., 2006; Gustafson, Brooks, Molotch, & Veatch, 2010; Lee, 2010; Unnikrishna, 2002). Thus, it appears that the technique of using stable isotopes to study plant water use is a robust established method.

When using stable isotopes of water to determine the source water of a plant it is common to make a number of assumptions, as summarized by Brunel et al. (1995): 1) There is no significant fractionation of stable isotopes of water during uptake of soil water by the plant, 2) there are no significant errors associated with the sampling of isotopes nor in the extraction and analysis of water from plants and soils, 3) there is no significant variability of isotopic composition in the sap water within the tree except in the vicinity of the leaf water, 4) the isotopic composition of the soil water is laterally homogeneous within the rooting area of the tree, and 5) the time of sampling was such that time delays associated with transport of isotopes up the tree were not important.

Some studies show that caution must be exercised when using these assumptions under various circumstances. Ellsworth and Williams (2007) show that some plants roots use a symplastic pathway which causes fractionation of root water as compared with the surrounding soil water, violating assumption 1. Dawson and Ehleringer (1993) show that “green” stems (which are new growth stems) can experience enrichment, violating assumption 3. DeNiro and Cooper (1990) show that enrichment occurs in some woody stomata bearing stems, violating assumption 3. White et al. (1984) shows that in some plants there is mixing of sapwood water (stem samples) with that of heartwood water which has distinctly different isotopic values than that of sapwood water in some plants, violating assumption 3. Most of these violations of the general assumptions either will not apply or can be avoided by careful planning. This study is using the Douglas Fir which has been used in many studies and if sampled properly does not violate any of the assumptions.

1.4 Stable Isotope Analysis

Isotope Ratio Infrared Spectrometers (IRIS) have become a primary means for earth scientists to analyze stable isotopes $\delta^{18}$O and $\delta$D (Wassenaar et al., 2012). These instruments use an auto-
sampler to inject water into a vaporizer, which in turn delivers water vapor to the IRIS for measurement. However, since these instruments can only analyze water, solid samples such as soil and tree stems need to be processed with cryogenic vacuum distillation (CVD) to remove water from the samples, before being analyzed for isotopes on an IRIS system. Picarro Inc. has developed an Induction Module (IM), capable of extracting matrix-bound water by vaporizing the water at a high temperature and delivering the water vapor to a cavity ring-down spectrometer (CRDS) in a single in-line system that extracts, treats, and measures the isotopic composition of a sample.

When using IRIS instrumentation, four factors need to be considered: 1) memory effect, 2) organic contaminants, 3) instrument drift, and 4) post processing corrections. Memory effect is the carryover of sample from one analysis to the next, caused by water vapor condensing on conveyance surfaces before entering the spectrometer (Lis, Wassenaar, & Hendry, 2008; D. Penna et al., 2012). Memory effects can be avoided by analyzing a sample multiple times to ensure that water from a previous sample has been flushed from the system, and averaging the last three analyses (Brand, Geilmann, Crosson, & Rella, 2009; Lis et al., 2008; D. Penna et al., 2012). Organic contamination is the presence of ethanol, methanol, and other organic acids within the liquid sample solution (Brand et al., 2009; Schultz, Griffis, Lee, & Baker, 2011; Adam G. West, Goldsmith, Brooks, & Dawson, 2010; A. G. West, Goldsmith, Matimati, & Dawson, 2011). Since these organic contaminants contain O-H bonds, they have isotopologues that overlap the H2O isotopologues being measured by IRIS instruments thereby affecting the measured isotope ratios (Brand et al., 2009). Organic contamination within a study can be marginalized by, 1) not using plant leaves which have been shown to contain elevated levels of organic contamination (Schultz et al., 2011; Adam G. West et al., 2010; Zhao et al., 2011), 2) treating samples with activated charcoal (Martin-Gomez et al., 2015; Adam G. West et al., 2010), and 3) chemical conversion of organic material into water by means of oxidation prior to analysis (Brand, 2010; Lazarus, Germino, & Vander Veen, 2016). IRIS systems display a random slow temporal instrumental drift (Lis et al., 2008). Instrument drift can be corrected using internal standards being spaced between samples (Lis et al., 2008). Post processing corrections are intended to deal with errors largely related to organic contamination. Many studies have identified the need to post process results.
based on spectral analysis of organic contamination (Brand et al., 2009; Johnson et al., 2017; Lazarus et al., 2016; Martin-Gomez et al., 2015; Schultz et al., 2011).

This study analyzed samples on three different IRIS systems: 1) Picarro L2130-I cavity ring-down spectrometer equipped with an Induction Module (IM-CRDS), housed in the Center for Environmental Physics and Mineralogy (CEPM) lab at the University of Arizona, 2) Picarro L2120-I cavity ring-down spectrometer (CRDS) equipped with a V1102-I high-precision vaporizer and auto sampler, housed at the tree ring lab of the University of Arizona, and 3) IRIS equipped with vaporizer and auto sampler, housed at the University of Arizona Biosphere 2.

All spectrometers required standards to correct for instrument drift. Procedures for analyzing samples varied between the IRIS systems and sample type. The CRDS and IRIS both used an auto sampler which retrieved 1.5 mL of liquid sample and injected it into the spectrometer for analysis. The IM-CRDS had three procedures for preparing liquid, soil, and stem samples for insertion into a capped vial with septa which was then inserted into the IM-CRDS for analysis. Liquids were injected onto glass dots, soils were placed into a metallic cylinder, and stems were sliced and placed into a metallic clip. Each sample was analyzed several times until the memory effect of the last sample was eliminated and the last three analyses had an acceptable standard deviation. At this point a linear offset of the standard is used to find the true isotopic value of the sample.

1.5 Objectives
This thesis presents isotopic data collected in the Santa Catalina Mountains, from 05/2014 to 07/2015 and from 03/2016 to 10/2016, which was used to answer two main questions. Regarding the isotopic data, it is hypothesized that: 1) the isotopic signature of precipitation will vary with the season within the study area, 2) the isotopic signature of soil water will vary with depth and season, 3) the isotopic signature of groundwater will be equal to the annual weighted average isotopic value of precipitation, 4) the isotopic signature of Douglas Fir will vary with the season.

The main objectives of this study were to identify 1) the source of water that trees are taking water from and 2) whether that source of water changes with season. In answering these two questions it will be determined what plant water use strategies are used to survive in an ephemeral
snowpack/melt environment, and by extension, what will happen when a system shifts from a seasonal to an ephemeral snowpack environment.

1.6 Study Site
The study site is comprised of 4 locations near the Santa Catalina Mountain Critical Zone Observatory (SCM-CZO). The SCM-CZO is situated on Mt. Bigelow, at an elevation of 2573 m above sea level, within the Santa Catalina Mountains part of the National Coronado Forest, Northeast of the city of Tucson, AZ (Figure 1). The ecosystem at the study site is a second-growth subalpine mixed conifer forest (Brown-Mitic et al., 2007). The forest is dominated by *Pseudotsuga menziesii* (Douglas Fir), *Abies concolor* (White Fir), and *Pinus ponderosa* (Ponderosa Pine) (Brown-Mitic et al., 2007; Nelson et al., 2014). Additionally, underbrush such as ferns grow when there is enough water to support them and die off during the intermittent dry periods.
The soil at the study site is comprised of four distinct layers: 1) a layer of litter both fresh and at various stages of decomposition has a thickness of approximately 0.5 cm, 2) a layer of rich organic soil that is a few centimeters thick, 3) a layer of sandy loam with variable thickness, and 4) a layer of clay loam with variable thickness (Brown-Mitic et al., 2007; Nelson et al., 2014). Throughout
these layers is dispersed a multitude of cobbles and stones of varying sizes and shapes (Restaino, Peterson, & Littell, 2016). The thickness of these layers is highly variable from one location to the next, throughout the study site, resulting in a range of soil depths approximately between 0.25 m to 1.5 m (Ajami et al., 2011; Brown-Mitic et al., 2007).

The climate at the site is semi-arid with air temperatures ranging from a low of -5° C during the winter to a high of 32° C during the summer (Brown-Mitic et al., 2007). The area experiences an average annual total precipitation ranging from 690 mm to 940 mm, that largely falls during two wet seasons (Brown-Mitic et al., 2007). Of this precipitation, approximately 54% falls during the summer and 46% falls during the winter (Wahi et al., 2008). During the summer, the North American Monsoon (July – September) brings moisture from the Gulf of California and the Gulf of Mexico (Eastoe & Dettman, 2016). The monsoon storms are highly localized and are characterized as having high-intensity and short-duration (Ajami et al., 2011; Eastoe & Dettman, 2016). During the winter (November – March), Pacific cold fronts bring precipitation from the west and southwest (Eastoe & Dettman, 2016). The frontal winter storms are regionally distributed (falling on a large area) and are characterized as being low-intensity and long-duration (Ajami et al., 2011; Eastoe & Dettman, 2016).

1.7 Structure of the Following Chapters

The following chapter is an abstract of my M.S. research, followed by descriptions of the remaining appendices (technical information about field sampling, lab procedures, and supplemental data), summary of results, and future research directions. My M.S. research is presented as an individual research paper in Appendix A of this document. The manuscript, Temporal dynamics of tree source water in sky island ecosystems with ephemeral snow pack: A case study using Pseudotsuga meziesii (Douglas Fir), is in preparation for submission to the journal Hydrological Processes. Tables and figures associated with Appendix A appear at the end of Appendix A, with supplemental table data appearing in Appendix E. Appendices B through D include: a paper published in Rapid Communications in Mass Spectrometry that I second authored titled, Performance of induction module cavity ring-down spectroscopy (IM-CRDS) for measuring delta O-18 and delta H-2 values of soil, stem, and leaf waters; field protocols; and lab protocols.
2. **PRESENT STUDY**
The methods, results, and conclusions of this research are included in Appendix A. The following abstract summarizes our research. Sections following the abstract are descriptions of Appendices B through E.

2.1 **Abstract of Appendix A: Temporal dynamics of tree source water in sky island ecosystems with ephemeral snow pack: A case study using Pseudotsuga Meziesii (Douglas Fir)**
Throughout the world, water is the limiting resource for both natural and human development. A primary source of water throughout the world comes from seasonal snowpack and snowmelt which largely occurs in the mountains. Our results show that the precipitation that falls in the Santa Catalina Mountains has seasonal isotopic variations, with summer monsoon being more enriched than that of winter precipitation. The isotopic signature of soil moisture has a seasonal trend determined by a combination of seasonal precipitation and dynamic soil moisture flow, this also results in a slight seasonal variation of the isotopic signature of groundwater. The Douglas Fir (*Pseudotsuga meziesii*) utilizes water from the shallow soils (0 – 20 cm), deep soils (30 – 100 cm), and groundwater. The main source of water for the Douglas Fir throughout most of the year is the shallow soils, but during the dry pre-winter season the source of water switches to groundwater due to a drying soil profile. Hydraulic redistribution during the dry pre-winter season likely causes wetting in the shallow soils. If climate change predictions are correct a shift in precipitation could alter the source water of the Douglas Fir such that they may become increasingly water stressed. If Douglas Fir is unable to survive under this increased water stress, it will have important implications for water resources in the region.

2.2 **Abstract of Appendix B: Performance of induction module cavity ring-down spectroscopy (IM-CRDS) for measuring delta O-18 and delta H-2 values of soil, stem, and leaf waters**
Rationale: Induction module isotope ratio infrared spectroscopy (IM-IRIS) has been proposed as a rapid and cost-effective alternative to cryogenic vacuum distillation (CVD) and isotope ratio mass spectrometry (IRMS) for analysis of δ18O and δ2H in matrix-bound waters. Although an IM-IRIS system is commercially available, it has not yet been thoroughly assessed for many of the
sample types of interest. In the current study, we characterized the performance of IM-IRIS relative to CVD and IRMS and analyzed the mechanisms responsible for differences between the two methods.

Methods: To characterize the performance of IM-IRIS, we collected a set of $n = 75$ soil, stem, and leaf water samples that were physically and chemically diverse and expected to vary in isotopic composition. We measured $\delta^{18}O$ and $\delta^2H$ of each sample with four techniques: CVD and IRMS, CVD and IRIS, CVD and IM-IRIS, and IM-IRIS alone. To understand the basis of differences between the techniques, we calculated the isotopic errors for each of the three IRIS methods relative to CVD and IRMS, and then analyzed the relationships among these errors and suites of diagnostic spectral parameters that are indicative of organic contamination. We attributed IRIS errors that were correlated to the diagnostic spectral parameters to organic contamination, and those that were uncorrelated to the spectral parameters to other mechanisms.

Results: The IM-IRIS technique accurately assessed $\delta^{18}O$ and $\delta^2H$ of pure waters, but exhibited progressively increasing errors for soil waters, stem waters, and leaf waters. The mechanisms underlying the $\delta^{18}O$ and $\delta^2H$ errors differed by sample type: for soils, errors were attributable to subsampling of isotopically heterogeneous source material, whereas for stems and leaves, errors were attributable to spectral interference. Several lines of evidence indicate that methanol was the primary source of spectral interference. However, one or more unidentified compounds also made secondary contributions.

Conclusions: There are currently many types of water samples with organic solutes for which water isotope measurements with IM-IRIS include significant errors from spectral interference. The performance of IM-IRIS systems could be improved through: (i) identification of the compounds that cause spectral interference when solid samples are extracted, and either (ii) modification of the combustion step to completely oxidize these compounds to CO$_2$, and/or (iii) incorporation of corrections for these compounds into the spectral fitting models used by the IRIS analyzers.
2.3 Appendix C: Field Protocols
This appendix describes the protocol that was used in collecting soil, stem and precipitation samples at the study site. The study site consists of three locations on the periphery of the Santa Catalina Mountain Critical Zone Observatory (SCM-CZO) located on Mt. Bigelow. Samples were collected at a two-week interval. Precipitation samples were collected in a standard rain gage with few centimeters of mineral oil to retard evaporation. Soil samples were collected with a soil corer down to 0.40 meter and from a trench down to 1 meter. Stem samples were collected from trees within 25 meters of the point where soil samples were collected. All samples were collected into 20 milliliter glass vials, which were wrapped in parafilm, placed in plastic zip-lock bags, and then placed in a cooler with ice packs. In the lab all samples were placed into a refrigerator, until they could be analyzed.

2.4 Appendix D: Lab Protocols
This appendix describes the protocol that was used in analyzing soil, stem, and liquid sample on the Picarro L2120-I Cavity Ring-Down Spectrometer equipped with an induction module (IM-CRDS). All samples were stored in a refrigerator in the lab until they could be analyzed. First, the system was allowed to reach the operating condition of 250 ppm of H2O present in the spectrometer. Second, based on the type of sample that was being analyzed (soil, stem, liquid), blanks were run at a different gas pressure. Third, based on the type of sample that was being analyzed (soil, stem, liquid), standards were run to correct for instrument drift. Fourth, samples were analyzed with guidance provided by the lab protocols regarding when a sample analysis was complete. No more than five samples were analyzed for an analyzed standard. Lastly, the power down instructions were important for proper storage of the spectrometer while it was not in use.

2.5 Appendix E: Supplemental Data
This appendix presents tables of results related to the manuscript presented in Appendix A, but not appropriate for inclusion in the Hydrological Processes publication. Tables 1 and 2 were also presented in Appendix A, but reappear here for ease of access to the reader. Table 3 gives the isotopic value for all precipitation samples that were used in this study, including samples collected by the Papuga lab and the Gregg Barron-Gafford Research Group. Table 4 shows all the soil and stem samples that were used in this study as well as any precipitation sample that was collected by
the Papuga lab. Table 5 shows the seasonal average of each sample type collected. Table 6 shows the isotopic value for all groundwater samplers that were collected and analyzed by staff from the SCM-CZO and used in this study. Table 7 shows each of the sampling days and compares the isotopic value of the stem sample with the most similar source waters isotopic value. Table 8 shows the source water that was dominantly used dependent on the season.

2.6 Summary of Results

It has been shown that there is a seasonal precipitation isotopic trend with more enriched monsoon precipitation and more depleted winter precipitation. It has been shown that a combination of the seasonal precipitation and a dynamic soil moisture flow regime combine to produce seasonal soil moisture trends and likewise produce a slight seasonal groundwater trend. It has been shown that the Douglas Fir has minor variation in its stem water throughout the year. These findings have culminated in an understanding that the Douglas Fir trees within the Santa Catalina Mountains are using water from the shallow soils (0 – 20 cm), the deep soils (30 – 100 cm), and from the groundwater. Furthermore, the findings suggest that there is a seasonal transition from the predominantly used shallow soil water to the groundwater in the pre-winter season, likely due to the water within the soil profile becoming increasingly difficult to retrieve. Lastly, results have been presented that indicate the process of hydraulic redistribution being employed by the Douglas Fir during the dry pre-winter period.

2.7 Future Research Opportunities

There are research opportunities for future study related to the work presented here. Our results show that the Douglas Fir utilizes water from the shallow soil, deep soil, and groundwater. To verify this more samples need to be collected and analyzed, preferably with a shorter sampling interval. Our results suggest that there may be a seasonal transition from shallow soil water to the groundwater during the “prewinter” time period. Again, further sampling at a higher temporal resolution would help verify this and may find additional examples of seasonal water use shifts occurring. Future studies should also look at the density of vegetation at elevations and measure its change with time in reference to local temperature changes and snowpack dynamics. In particular, it is important for these studies to be conducted in various global regions with seasonal snowpack that are predicted to become ephemeral in the future.
Another area for further study is with regards to the process of hydraulic redistribution and how this process is utilized by the Douglas Fir in the Santa Catalina Mountains. It is likely that the Douglas Fir uses its deep tap roots to access groundwater during periods of water stress within the shallow and deep soils. However, it is still unclear as to why the Douglas Fir would switch from one source to the other if the groundwater is available year-round. If the groundwater was available year-round it would be easiest to continually tap this source of water as the main source of water. An exception would be if the Douglas Fir “saves” the groundwater for periods of water scarcity within the soil profile and competes with other plants for the available soil water for periods of adequate water supply. Further study is required to couple the soil matric potential throughout the soil profile with isotope data that informs on the source of water being used by the Douglas Fir. In this way it could be seen that as water becomes increasingly difficult to retrieve from the shallow and deep soils the Douglas Fir begins to tap groundwater, if available. Furthermore, it would be possible to see that as water became increasingly accessible in the shallow and deep soils the Douglas Fir would cease tapping the groundwater and resume accessing water from the shallow and deep soils.
3. REFERENCES


Weijers, S., Beckers, N., & Löffler, J. (2018). Recent spring warming limits near-treeline deciduous and evergreen alpine dwarf shrub growth. Ecosphere, 9(6), n/a-n/a. doi:10.1002/ecs2.2328


APPENDIX A: TEMPORAL DYNAMICS OF TREE SOURCE WATER IN SKY ISLAND ECOSYSTEMS WITH EPHEMERAL SNOW PACK: A CASE STUDY USING PSEUDOTSUGA MEZIESII (DOUGLAS FIR)

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Temporal dynamics of tree source water in sky island ecosystems with ephemeral snow pack: A case study using Pseudotsuga meziesii (Douglas Fir)

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ABSTRACT
Throughout the world, water is the limiting resource for both natural and human development. A primary source of water throughout the world comes from seasonal snowpack and snowmelt which largely occurs in the mountains. Our results show that the precipitation that falls in the Santa Catalina Mountains has seasonal isotopic variations, with summer monsoon being more enriched than that of winter precipitation. The isotopic signature of soil moisture has a seasonal trend determined by a combination of seasonal precipitation and dynamic soil moisture flow, this also results in a slight seasonal variation of the isotopic signature of groundwater. The Douglas Fir (Pseudotsuga meziesii) utilizes water from the shallow soils (0 – 20 cm), deep soils (30 – 100 cm), and groundwater. The main source of water for the Douglas Fir throughout most of the year is the shallow soils, but during the dry pre-winter season the source of water switches to groundwater due to a drying soil profile. Hydraulic redistribution during the dry pre-winter season likely causes wetting in the shallow soils. If climate change predictions are correct a shift in precipitation could alter the source water of the Douglas Fir such that they may become increasingly water stressed. If Douglas Fir is unable to survive under this increased water stress, it will have important implications for water resources in the region.

1. INTRODUCTION
Water is a major limiting resource to development in arid and semi-arid regions throughout the world. One primary source of usable water comes from seasonal snowpack and snowmelt which largely occurs in the mountains (Brown-Mitic et al., 2007; Earman, Campbell, Phillips, & Newman, 2006; A. Harpold et al., 2012; A. A. Harpold et al., 2015; Luca, 2018; Nelson, Kurc,
Seasonal snowpack has been defined as: snowpack that has a predictable melt timing and volume of melt water, whereas ephemeral snowpack is snowpack that lasts for less than 60 days and produces less/earlier snowmelt than seasonal snowpack (Petersky & Harpold, 2018). Global climate change models predict that many regions around the world, including the southwestern United States, will become drier and hotter, have reduced winter snowpack, and have an earlier onset of spring melt (Barnett, Adam, & Lettenmaier, 2005; Beniston, 2003; Grundstein & Mote, 2010; Luca, 2018; López-Moreno, Goyette, & Beniston, 2009; Räisänen, 2008; Schmucki, Marty, Fierz, Weingartner, & Lehning, 2017; U.S. Global Change Research Program, 2017; Vicuña, Garreaud, & McPhee, 2011; You et al., 2017). These changes have already had an adverse effect on regional water resources and are predicted to continue to do so (Grundstein & Mote, 2010; Hennessy, 2008; Rocca, Brown, Macdonald, & Carrico, 2014; Schmucki et al., 2017; U.S. Global Change Research Program, 2017). As the global average temperature continues to increase, many areas that have relied on seasonal snowpack may shift to ephemeral snowpack in the future (Barnett et al., 2005; Luca, 2018; López-Moreno et al., 2009; Petersky & Harpold, 2018; Schmucki et al., 2017; Vicuña et al., 2011), which are likely to have major consequences for water resources.

In many watersheds multiple ecosystems, such as pinon-juniper woodlands, lower/upper montane forests, subalpine forests, and riparian corridors, rely on the seasonal snowpack and snowmelt occurring in the mountains (Archer & Predick, 2008; Brown-Mitic et al., 2007; A. A. Harpold et al., 2015; Luca, 2018; Nelson et al., 2014; Rocca et al., 2014). These ecosystems are home to numerous plant species that rely on snowmelt to support increased transpiration at the start of their growing season (Brown-Mitic et al., 2007; Gergel, Nijssen, Abatzoglou, Lettenmaier, & Stumbaugh, 2017; Grundstein & Mote, 2010; A. Harpold et al., 2012; A. A. Harpold et al., 2015; Luca, 2018; Nelson et al., 2014; Pederson et al., 2011). In the process of transpiration, water is absorbed by the root system of plants, translocated through the vascular system to the stomata on the stems/leaves, and ultimately evaporating to the atmosphere (Dingman, 2002). Each plant has a different root system that collects water from different sources, such as the shallow soil, deep soil, or groundwater to use toward transpiration. Plants that exist in seasonal snowpack systems have adapted their growth cycle to coincide with the predictable timing and volume of snowmelt associated with seasonal snowpack systems. If the snowpack becomes ephemeral, this could result
in a reduced volume of snowmelt being available to plants at the beginning of their growing season which could result in less productivity (Petersky & Harpold, 2018; Schmucki et al., 2017). Additionally, the increased temperature, which causes earlier snowmelt, can cause plants to bud earlier; if this happens, soft plant tissues become vulnerable to damage from freezes, which can inhibit leaf production (Asse et al., 2018; Ma, Huang, Hänninen, & Berninger, 2018; Weijers, Beckers, & Löffler, 2018; Wipf, 2009). Reduced snowmelt may also reduce the water stores that are used by plants through spring into summer, potentially resulting in die off. Thus, any change to the snowpack is important to these snowmelt dependent ecosystems.

Global climate change models predict many regions of the world, including the southwestern United States, will become hotter and drier as well as have reduced snowpack and earlier snowmelt (Barnett et al., 2005; Grundstein & Mote, 2010; Luca, 2018; López-Moreno et al., 2009; Petersky & Harpold, 2018; Schmucki et al., 2017; U.S. Global Change Research Program, 2017; Vicuña et al., 2011; You et al., 2017). The spring snowmelt produces increased soil moisture that lasts into the summer and provides water to plants during the beginning of their productivity (Brown-Mitic et al., 2007; Gergel et al., 2017; Grundstein & Mote, 2010; A. Harpold et al., 2012; A. A. Harpold et al., 2015; Luca, 2018; Nelson et al., 2014; Pederson et al., 2011). As the climate becomes increasingly hot and dry the amount of water that is returned to the atmosphere by the processes of evaporation and transpiration will increase and diminish the soil moisture (Archer & Predick, 2008; U.S. Global Change Research Program, 2017). With less soil moisture available, possible implications could be increased plant water stress, reduced production, increased die off, and increased forest fires (Archer & Predick, 2008; Gergel et al., 2017; Grundstein & Mote, 2010; Rocca et al., 2014). Thus, less and earlier snowmelt have the potential to significantly impact regional hydrology and ecology.

Despite these perceived problems, to the best of the author’s knowledge, few studies investigate how ecosystems and their ecohydrological processes such as transpiration are likely to be impacted by the spatial and temporal changes in water availability resulting from a shift from seasonal to ephemeral snowmelt. Many plants that grow in seasonal or ephemeral snow environments have evolved to have periods of active growth and dormancy. In these environments, plants tend to go dormant in fall, remain dormant through the winter and are become active beginning in spring
through the summer. The plants have differing root systems capable of accessing water from various depths of the soil profile down to the groundwater as water availability changes. Reduced snowpack or earlier snowmelt, will change the timing of availability of water for plants during the beginning of growing season having important consequences for their phenological activity (Archer & Predick, 2008; Nelson et al., 2014).

To study the movement of water through the environment it is common to use the stable isotopes of water (δD and δ¹⁸O) as natural tracers. The use of these stable isotopes is predicated on the notion that each source (precipitation, soil depth, groundwater, and stems) has a different isotopic signature from one another (J. P. Brunel, Walker, & Kennettsmith, 1995; J. P. W. Brunel, G. R.; Walker, C. D.; Dighton, J. C.; Dennett-Smith, A., 1993). Some studies have used stable isotopes to determine the source water of plants (Busch, Ingraham, & Smith, 1992; Ellsworth & Williams, 2007; Penna et al., 2013; Todd & James, 1991; White, Cook, Lawrence, & Broecker, 1985) and the temporal variability of that source water (Andrews, Flanagan, Sharp, & Cai, 2012; Bertrand et al., 2014; Gierke, Newton, & Phillips, 2016). Other studies have used stable isotopes to look at various aspects of snowpack and snowmelt (Earman et al., 2006; Gustafson, Brooks, Molotch, & Veatch, 2010; Lee, 2010; Unnikrishna, 2002). Thus, the technique of using stable isotopes to study plant water use in snow-dependent ecosystems is a robust established method.

Here we use the stable isotope method to understand the temporal variability of plant source water use in ecosystems with ephemeral snowpack. We focus on Douglas Fir because it is largely distributed throughout North America and other parts of the world with seasonal and ephemeral snowpack (Lavender & Hermann, 2014; Restaino, Peterson, & Littell, 2016). Douglas Fir has developed a root system capable of accessing water throughout the soil profile as well as the groundwater (Andrews et al., 2012; J. R. Brooks, Meinzer, Coulombe, & Gregg, 2002; Warren, Meinzer, Brooks, & Domec, 2005). Having access to multiple sources of water allows plants to survive periods when one source of water is not available. Additionally, Douglas Fir has been shown to hydraulically redistribute water both laterally and horizontally within the root zone to keep the soil profile moist (J. R. Brooks et al., 2002). The hydraulic redistribution process is likely increased in effectiveness due to root grafting amongst the trees in a Douglas Fir stand (Lavender & Hermann, 2014). While these various survival mechanisms have been associated with Douglas
Fir, how they are implemented to access different water sources, how they vary temporally throughout the year, and how a changing snowpack and snowmelt might affect them remain largely unknown.

This study looks at the ephemeral system in the Santa Catalina Mountains as an example of the conditions that can be expected if and when seasonal systems become ephemeral. It is hypothesized that: 1) the isotopic signature of precipitation will vary with the season within the study area, 2) the isotopic signature of soil water will vary with depth and season, 3) the isotopic signature of groundwater will be equal to the annual weighted average isotopic value of precipitation, 4) the isotopic signature of Douglas Fir stem water will vary with the season. The main objectives of this study were to identify 1) the source of water within the subsurface that trees are taking up water from and 2) whether that source of water changes with season.

2. METHODS

2.1 Study Site
The study site is comprised of four locations near the Santa Catalina Mountain Critical Zone Observatory (SCM-CZO). The SCM-CZO is situated on Mt. Bigelow, at an elevation of 2573 m above sea level, within the Santa Catalina Mountains portion of the National Coronado Forest, Northeast of the city of Tucson, AZ (Figure 1). The ecosystem at the study site is a second-growth subalpine mixed conifer forest (Brown-Mitic et al., 2007). The forest is dominated by Pseudotsuga menziesii (Douglas Fir), Abies concolor (White Fir), and Pinus ponderosa (Ponderosa Pine) (Brown-Mitic et al., 2007; Nelson et al., 2014).

The soil at the study site is comprised of four distinct layers. The thickness of these layers is highly variable from one location to the next resulting in a range of soil depths approximately between 0.25 m to 1.5 m (Ajami, Troch, Maddock, Meixner, & Eastoe, 2011; Brown-Mitic et al., 2007). Despite the varying thicknesses of the layers, the soil profile is composed of: 1) a layer of litter less than a centimeter thick, 2) a layer of rich organic soil that is a few centimeters thick, 3) a layer of sandy loam with variable thickness, and 4) a layer of clay loam with variable thickness (Brown-
Mitic et al., 2007; Nelson et al., 2014). Throughout these layers is dispersed a multitude of cobbles and stones of varying sizes and shapes (Restaino et al., 2016).

The climate at the site is semi-arid with air temperatures ranging from a low of -5° C during the winter to a high of 32° C during the summer (Brown-Mitic et al., 2007). The area experiences an average annual total precipitation ranging from 690 mm to 940 mm, that largely falls during two wet seasons (Brown-Mitic et al., 2007). Of this precipitation, approximately 54% falls during the summer and 46% falls during the winter (Wahi, Hogan, Ekwurzel, Baillie, & Eastoe, 2008). During the summer, the North American Monsoon (July – September) brings moisture from the Gulf of California and the Gulf of Mexico (C. J. Eastoe & Dettman, 2016). The monsoon storms are highly localized and are characterized as having high-intensity and short-duration (Ajami et al., 2011; C. J. Eastoe & Dettman, 2016). During the winter (November – March), Pacific cold fronts bring precipitation from the west and southwest (C. J. Eastoe & Dettman, 2016). The frontal winter storms are regionally distributed (falling on a large area) and are characterized as being low-intensity and long-duration (Ajami et al., 2011; C. J. Eastoe & Dettman, 2016).

2.2 Sampling

Sampling was conducted during two periods, 1) 06/23/2014 through 05/06/2015, and 2) 03/05/2016 through 10/01/2016. Samples were collected at two-week intervals to capture large temporal variability in the isotopic values associated with the precipitation, soil and stem samples. Cumulative precipitation was collected in standard rain samplers that were partially filled with mineral oil to hinder evaporation until samples could be retrieved. The samplers were either attached to a tree near to the soil/stem sample collection, or they were in the open near to the SCM-CZO meteorological tower. The cumulative precipitation samples collected by the CZO were collected at a variable interval. Additional precipitation data (e.g. depth) was collected by staff from the SCM-CZO and is available through their website (http://criticalzone.org/catalina-jemez/). Soil samples from 06/19/2014 to 07/09/2016 were collected with a split core soil sampler, and samples from 07/23/2016 to 10/01/2016 were collected from a trench. Soil moisture data were collected by staff associated with the Greg Barron-Gafford Research Group. Groundwater seepage was collected from an ephemeral spring, located at coordinates 32.426293 -110.761113 at Marshall Gulch (Figure 1) by staff from the SCM-CZO. The CZO identifies the spring as “SEEP”
and collects grab samples from a custom-made system that piped the groundwater into a tipping bucket for volume measurements (additional info is available at the CZO website). While these groundwater samples do not come from the exact location where the other samples were collected, the precipitation was likely the same isotopic value between the two locations. With the same dynamics governing infiltration and recharge between the locations it is reasonable to assume that the differences in groundwater between both locations is negligible. Because there were no monitoring wells at the Mt. Bigelow location to collect groundwater samples, these samples give the best representation possible for the groundwater of the area. Stems were cut from three random Douglas Fir trees within proximity (< 25 m) to the point where soil samples were collected.

At the time of collection, all field samples were placed into 20 milliliter (ml) glass vials with a polycone cap, labeled (site id, date, depth (for soils), numbered (for stems)), sealed with parafilm, and then stored in a cooler with an ice pack. Upon arriving at the lab, the soil and stem samples were immediately placed in a refrigerated storage unit; the precipitation samples were filtered with cellulose filters (to remove debris and oil), stored in new 20 ml glass vials with a polycone cap, labeled (site id and date), sealed with parafilm, and then stored in a refrigerated unit.

2.3 Stable Isotope Analysis
This study analyzed samples on three different IRIS systems: 1) Picarro L2130-I cavity ring-down spectrometer equipped with an Induction Module (IM-CRDS), housed in the Center for Environmental Physics and Mineralogy lab at the University of Arizona, 2) Picarro L2120-I cavity ring-down spectrometer (CRDS) equipped with a V1102-I high-precision vaporizer and auto sampler, housed at the tree ring lab of the University of Arizona, and 3) IRIS equipped with vaporizer and auto sampler, housed at the University of Arizona Biosphere 2. All isotopic values are written in delta notation in relation to VSMOW (Clark & Fritz, 1997).

All spectrometers required standards to correct for instrument drift. Procedures for analyzing samples varied between the IRIS systems and sample type. The CRDS and IRIS both used an auto sampler which retrieved 1.5 mL of liquid sample and injected it into the spectrometer for analysis. The IM-CRDS had three procedures for preparing liquid, soil, and stem samples for insertion into a caped vial with septa which was then inserted into the IM-CRDS for analysis. Liquids were
injected onto glass dots, soils were placed into a metallic cylinder, and stems were sliced and placed into a metallic clip. Each sample was analyzed several times until the memory effect of the last sample was eliminated and the last three analyses had an acceptable standard deviation. At this point a linear offset of the standard was used to find the true isotopic value of the sample.

2.4 Data Analysis
The isotope comparison method is an established method to determine the source water of a plant (J. Renee Brooks, Barnard, Coulombe, & McDonnell, 2010; J. P. W. Brunel, G. R.; Walker, C. D.; Dighton, J. C.; Dennett-Smith, A., 1993; Ehleringer & Dawson, 1992; Flanagan & Ehleringer, 1991; Gierke et al., 2016). The method is predicated on various source waters having distinct isotopic values from one another to determine which source or sources are contributing to the stem water of a plant (J. Renee Brooks et al., 2010; J. P. W. Brunel, G. R.; Walker, C. D.; Dighton, J. C.; Dennett-Smith, A., 1993; Ehleringer & Dawson, 1992; Flanagan & Ehleringer, 1991; Gierke et al., 2016). The isotope comparison method makes a direct comparison of the isotopic value of stem samples with that of the source waters. The source water nearest in value is assumed to represent the source of the water used by the plant (J. Renee Brooks et al., 2010; J. P. W. Brunel, G. R.; Walker, C. D.; Dighton, J. C.; Dennett-Smith, A., 1993; Ehleringer & Dawson, 1992; Flanagan & Ehleringer, 1991; Gierke et al., 2016).

3. RESULTS

3.1 Precipitation

3.1.1 Depth
Long term observation of the Southwest regional climate shows that precipitation is highly variable and can fall at any time of the year, but predominantly falls during the winter and monsoon wet seasons (Brown-Mitic et al., 2007; C. J. Eastoe & Dettman, 2016). This study spanned three water years (WY), 2014 – 2016, during which annual precipitation was 430, 850, and 652 mm, respectively (Table 1). Water years 2014 and 2016 were below the average annual precipitation while WY 2015 was in the mid-range of the average annual precipitation. During the study period the total precipitation was 1742 mm of which 1031 mm (59%) fell during monsoon, 324 mm (19%)
fell during winter, 233 mm (13%) fell during the pre-winter period, and 154 mm (9%) fell during the pre-monsoon period (Table 2).

3.1.2 Isotopic Value
The $\delta^{18}$O value of precipitation ranged from -19.20 ‰ to -1.63 ‰ throughout the study period. The weighted seasonal average $\delta^{18}$O value for all precipitation samples in the study was -7.97 ‰, for winter samples was -9.16 ‰, for pre-monsoon samples was -8.15 ‰, for monsoon samples was -7.41 ‰, and for pre-winter samples was -10.45 ‰ (Table 2). The most isotopically depleted precipitation samples were obtained after larger precipitation events (Figure 3A). The Global Meteoric Water Line (GMWL) used in this study is from Harmon Craig, $\delta D = 8.0 \delta^{18}$O + 10 ‰ (Figure 2A). The Local Meteoric Water Line (LMWL), as defined by our precipitation samples, is $\delta D = 7.49 \delta^{18}$O + 8.59 ‰ (Figure 2A). The proximity of the LMWL to the GMWL is likely due to a relatively small data set to derive the LMWL from.

3.2 Soil

3.2.1 Soil Moisture Content
This study used data collected from three soil moisture sensors at three different depths within the soil profile 10, 30, and 60 cm (Figure 3B). The soil moisture data was limited to the period from 12/03/2014 to 04/12/2016. At all depths, the soil moisture content SMC showed the same general response to precipitation events, i.e. wetting during a precipitation event and drying between precipitation events (Figure 3B). However, the soil at these depths never appeared to have a SMC lower than 15% which means the soil is partially wetted year-round (Figure 3B). Generally, the 10 cm depth appeared to have a higher SMC then the deeper depths, except following large precipitation events when the deeper depths appeared to become more wetted than the shallow depth (Figure 3B). Further, the SMC was greater at all depths during the winter wet season than during the monsoonal season (Figure 3B). This trend is likely due to the relatively longer duration of precipitation events and the slow release of snow melt during the winter wet season (Brown-Mitic et al., 2007; A. A. Harpold et al., 2015) compared with the short, intense precipitation events which largely evaporated during the monsoon wet season (A. A. Harpold et al., 2015).
3.2.2 Isotopic Value of Soil with Regards to Depth and Season

The $\delta^{18}O$ value of soil water ranged from -19.58 ‰ to 3.18 ‰ throughout the study period. During dates where a precipitation sample was collected in conjunction with the soil and stem samples, it was generally observed that the 10 cm soil depth was more isotopically enriched in $\delta^{18}O$ and $\delta^{D}$ than the precipitation suggesting some amount of evaporation within the top soils. Below 10 cm, soils became more isotopically depleted with greater depth.

The seasonal isotopic trend of soil water showed increased isotopic depletion in $\delta^{18}O$ and $\delta^{D}$ with increasing depth from 10 – 100 cm (Figure 5). The most highly enriched samples were collected during the pre-monsoon season when the region tends to be at hottest and driest. The most highly depleted samples were collected during the pre-winter season.

3.2.2.1 Shallow Soil

The most highly enriched samples occurred during the pre-monsoon season with $\delta^{18}O$ values of 0.43, 1.51, and 3.18 ‰ likely due to this being the driest and hottest time of year, when evaporative losses would be at their greatest. The single most highly depleted sample occurred during the winter season with a $\delta^{18}O$ value of -19.58 ‰, likely the result of winter snowmelt seeping into the 20 cm depth. The collection of shallow soil samples created a trend line (similar to the GMWL and LMWL) of $\delta^{D} = 4.88 \delta^{18}O - 28.48$ ‰ (Figure 2B).

3.2.2.2 Deep Soil

The deep soil samples were less isotopically enriched in $\delta^{18}O$ and $\delta^{D}$ than the shallow soil samples (Figure 4). The most highly enriched samples occurred during the monsoon season with $\delta^{18}O$ values of -6.46 and -4.99 ‰ likely resulting from heavy monsoon rains penetrating the deep soil. The most highly depleted sample occurred during the monsoon season with a $\delta^{18}O$ value of -17.04 ‰. The collection of deep soil samples creates a trend line (similar to the GMWL and LMWL) of $\delta^{D} = 6.04 \delta^{18}O - 7.30$ ‰ (Figure 2C).
3.3 Groundwater

3.3.1 Isotopic Value of Groundwater
The δ¹⁸O value of groundwater ranged from -9.66 ‰ to -7.09 ‰ throughout the study period. The isotopic value of groundwater had some variance, but much less than was seen in precipitation, soil, and stem samples. The average δ¹⁸O value for all of the groundwater samples was -8.74 ‰. The groundwater had a distinct isotopic signature when compared to the deepest soil samples collected. The groundwater was most highly enriched during the monsoon season and most highly depleted during the pre-monsoon season, possibly due to late winter melt.

3.4 Stem

3.4.1 Isotopic Value of Stems with Regards to Season
The δ¹⁸O value of stem samples ranged from -10.91 ‰ to 1.01 ‰ throughout the study period. The average δ¹⁸O value for all of the stem samples was -5.99 ‰. The stem samples were most highly enriched in δ¹⁸O and δD during the winter season and most highly depleted during the pre-winter season. The collection of stem samples created a trend line (similar to the GMWL and LMWL) of δD = 3.13 δ¹⁸O – 53.65 ‰ (Figure 2D).

3.4.2 Isotopic Comparison Method
A direct comparison of the δ¹⁸O value for stems with all soil depths and the seasonal average groundwater values showed that the shallow soil depth samples were most similar to the stem samples. Likewise, two-isotope plots of all samples showed that stem samples largely plot amongst the shallow soil samples (Figures 6 and 7). A comparison of the trend lines derived from the stem, shallow soil, and deep soil samples revealed that the stem trend line is more closely associated with the shallow soil trend line (Figure 2D). Statistically, 56% of the stem samples had an δ¹⁸O value similar to shallow soil samples, 22% were similar to deep soil samples and 22% were similar to groundwater. Seasonally a similar trend is revealed as the average soil depth δ¹⁸O value that matches the δ¹⁸O value of stems is derived from the shallow soil samples overall and for three of the four seasons.
4. DISCUSSION

4.1 Variability in Isotopic Composition of Precipitation, Soil Water, and Ground Water

Our results support that summer monsoonal precipitation is enriched in $\delta^{18}O$ and $\delta^D$ as compared to winter precipitation which is depleted in $\delta^{18}O$ and $\delta^D$ (Clark & Fritz, 1997; Dansgaard, 1964; C. J. Eastoe & Dettman, 2016). The seasonal average $\delta^{18}O$ value for winter is -9.16‰ and for the summer monsoon is -7.41‰ (Table 2). The LMWL created by the precipitation samples intersects the GMWL at the upper right end (Figure 2A) indicating that local precipitation is largely derived from warm region/equatorial sources (Clark & Fritz, 1997). This is supported by the finding that 59.16% of this study's precipitation fell during the monsoon seasons (Table 2). Eastoe and Dettman (2016), using 30 years of isotopic data for precipitation, found that summer monsoon precipitation has been dominant in the Tucson basin since the late 1990's. These findings indicate that the isotopic signature of precipitation varies with the season within the study area. We note the possibility that our precipitation data included samples collected under the tree canopy which could have resulted in an isotopic value that erred on the side of enrichment in $\delta^{18}O$ and $\delta^D$. However, no noticeable difference between temporally similar precipitation samples collected under the tree canopy and samples collected in the open were observed in our study.

Consistent with previous studies, near the surface, the soil water becomes increasingly enriched in $\delta^{18}O$ and $\delta^D$ due to the water vapor from evaporated soil water below being depleted in isotopes and mixing with the soil water above (Barnes & Allison, 1988; Clark & Fritz, 1997; Mathieu & Bariac, 1996; Zimmermann, 1967). (Barnes & Allison, 1988; Zimmermann, 1967). Below the depth of atmospheric demand, the soil water becomes increasingly depleted in $\delta^{18}O$ and $\delta^D$ until it resembles the mean annual precipitation (Barnes & Allison, 1988; Clark & Fritz, 1997; Mathieu & Bariac, 1996; Zimmermann, 1967). The approximate termination depth of evaporation is the deepest depth within the soil profile at which evaporation occurs and is where the most enriched soil water is likely to occur (Barnes & Allison, 1988). Our samples suggest that the approximate termination depth of evaporation was at the 10 cm depth, as revealed by 75% of the sample days having the most enriched soil water at the 10 cm depth. Overall, 86% of the sample days had the most enriched soil water come from the shallow soil and 14% of the sample days had the most enriched soil water come from the deep soil. The sample days where the most enriched sample
was below the 10 cm depth may be explained by one of three scenarios: 1) new precipitation pushes the isotopically enriched antecedent soil moisture downwards into deeper soil layers, 2) isotopically enriched new precipitation bypasses zones of isotopically depleted antecedent immobile water and goes into storage (Bertrand et al., 2014; Beven & Germann, 2013; Mathieu & Bariac, 1996), and 3) plants may retrieve isotopically enriched water during daytime transpiration and hydraulically redistribute the water into lower soil layers during night (J. R. Brooks, Meinzer, Warren, Domec, & Coulombe, 2006). However, the lack of co-location in our sampling design limits our ability to ascertain by which means this is occurring because of possible differences in soil water flow dynamics and evapotranspiration rates. On average, shallow samples were more enriched in $\delta^{18}O$ and $\delta D$ than the deeper samples. Furthermore, each soil depth had a unique isotopic value from all other soil depths, indicating that it would be possible to compare the isotopic signature with that of the stems to identify the trees source water (J. P. Brunel et al., 1995; Busch et al., 1992).

Seasonally, the soil profile has a trend like the daily soil profile with the termination depth of evaporation occurring at the 10 cm depth (Figure 5). From the surface downwards to 10 cm soil water becomes increasingly enriched, representative of the continuously high evaporative demand found in the Southwest. Below the 10 cm depth the soil water is more isotopically depleted in $\delta^{18}O$ and $\delta D$ due to large pre-winter and winter precipitation events that infiltrate deeper into the soil and stay in storage for a prolonged period as was found by Brooks et al. (2009) and Gierke et al. (2016). However, whereas Brooks et al. (2009) found that the deep soil was recharged by Autumn (pre-winter) precipitation and Gierke et al. (2016) found that it was recharged by winter precipitation, it appears that the deep soil at the Santa Catalina Mountains is being recharged by both winter and monsoon precipitation. This is particularly noticeable when comparing site visits with relatively enriched precipitation and soil samples with a proceeding site visit with relatively depleted precipitation and soil samples which completely displaces the enriched soil moisture that preceded it.

The isotopic value of groundwater is often observed to be equal to the mean weighted annual isotopic value of precipitation (Clark & Fritz, 1997; Ehleringer & Dawson, 1992; Flanagan & Ehleringer, 1991). During our study, the mean weighted annual $\delta^{18}O$ value of precipitation was -
7.97 ‰ and the average δ¹⁸O value of groundwater was -8.74 ‰. It is possible that the set of precipitation samples that were collected for this study are inadequate to accurately depict the mean weighted annual isotopic value of precipitation. However, it is more likely that this difference is due to seasonal recharge trends (Clark & Fritz, 1997; C. Eastoe & Towne, 2018). As discussed above, the Santa Catalina Mountains have a dynamic soil moisture regime described by 1) seasonal precipitation and 2) dynamic soil moisture flow. Groundwater is largely recharged through macropores during large precipitation events during the winter (depleted isotopes) and monsoon (enriched isotopes) seasons as well as snow melt during the late winter and early spring period. Melting and sublimation can both lead to ice becoming increasingly enriched in δ¹⁸O and δD as the melt water and gas are depleted in δ¹⁸O and δD (Earman et al., 2006). The result of this process can manifest in enriched melt water being released from the snowpack later in the winter season or during the spring melt which can contrast with the generally more isotopically depleted precipitation that falls during the winter season. This dynamic form of recharging the groundwater is a possible cause for the slight variations in seasonal δ¹⁸O values that is seen in the Santa Catalina Mountains. Consistent with this theory, in the Santa Catalina Mountains, groundwater has been shown to be recharged primarily in the wettest 30% of months, mostly associated with the winter season (C. Eastoe & Towne, 2018).

In summary, the precipitation, soil water, and ground water samples all have isotopic values that vary with the season. The soil water also varies with depth on any given sampling day. Because each sample has a unique isotopic value it is possible to use the isotopic comparison method to determine the source water of the Douglas Fir stem samples. Additionally, because there is seasonal variability in these sources it is possible to determine if the Douglas Fir changes between source waters from one season to the next.

4.2 Source Water for Trees in Sky Island Ecosystems
Using knowledge of temporal variability in source waters for Douglas Firs allows for determination of the conditions which produced the source water and what conditions can limit its continuation. Likewise, conditions that are conducive to the survival of Douglas Fir are likely beneficial to the rest of the plants within the Santa Catalina Mountains ecosystem and other ephemeral snowpack/melt systems around the globe.
A single isotope comparison of the δ^{18}O value of samples shows that in the Santa Catalina Mountains the Douglas Fir appears to utilize source water from all three subsurface compartments, shallow soils, deep soils, and groundwater. Comparison of each stem sample collected with the available source waters shows that 56% most closely resemble the shallow soils, 22% resemble the deeper soils, and 22% resemble the groundwater. The use of multiple source waters by the Douglas Fir is supported by other studies that have found comparable results (Andrews et al., 2012; Warren et al., 2005). However, these studies were conducted in the Pacific Northwest and Southwestern Alberta, both representing significantly different ecosystems than that of the semiarid Santa Catalina Mountains.

A two-isotope comparison using the δ^{18}O and δD isotopes allows for a quick visual comparison of the stem samples and the various source water samples (Figure 7). The stem samples of this study largely plot amongst both the shallow and deep soil samples, but also in proximity to some of the groundwater samples (Figure 7). This suggests that the Douglas Fir trees within the Santa Catalina Mountains utilize water from all three of these sources, which is consistent with the findings of the single isotope comparison. With most of the shallow soil samples plotting in the same area as all the stem samples, it is likely that the two-isotope comparison is identifying the shallow soil samples as the primary source of water for the Douglas Fir trees (Figure 7). Similarly, a fraction of the deep soil samples plot amongst the stem samples, which likely identifies it as a source of water for the Douglas Fir trees, but to a lesser extent (Figure 7). Lastly, though the groundwater samples do not plot around the stem samples, some to plot near the stem samples suggesting that there could be occasions when groundwater becomes a resource for these trees (Figure 7) possibly via hydraulic redistribution.

Hydraulic redistribution has been shown to be a common technique used by Douglas Fir to move stores of water from one area of the soil profile to another (J. R. Brooks et al., 2002). It has been shown that during dry periods the Douglas Fir will access water from the groundwater during the day and redistribute it into the shallower soil profile during the night (Andrews et al., 2012; J. R. Brooks et al., 2002). In the Santa Catalina Mountains, with its relatively shallow soil above a fractured bedrock, it is possible that the Douglas Fir is hydraulically redistributing water from the
groundwater and relocating it into the shallower soil profile during periods of water stress. This may be evidenced by the dramatic enrichment in $\delta^{18}$O and $\delta$D of soil water throughout the soil profile with no recorded precipitation during the pre-winter period. The soil samples collected on 10/23/2014 show a soil profile depleted in $\delta^{18}$O and $\delta$D throughout, remnant of the soil profile proceeding this date, followed by soil samples collected on 11/6/2014 which show a large enrichment in $\delta^{18}$O and $\delta$D in the soil profiles isotopic values, but no precipitation was observed at this time or collected at the site. The soil profile continued to be isotopically enriched in $\delta^{18}$O and $\delta$D through the samples collected on 11/20/2014 to the samples collected on 12/9/2014 when a highly depleted winter precipitation event started to infiltrate into the soil profile, but only reached a depth of approximately 20 cm. One possibility for this could be enriched snow melt infiltrating through the soil or alternatively this could be further effects of hydraulic redistribution. An additional possibility is that the enriched soil profile could have been produced by enriched water in the upper shallow soil infiltrating and mixing with deeper soil moisture. Unfortunately, this is outside the scope of our study, but with additional measurements and monitoring of soil moisture or other variables this process could be isolated.

Both isotopic comparisons show that the primary source water for the Douglas Fir is from the shallow soils and to a lesser extent it uses water from the deep soils and the groundwater. That the shallow soil is the primary source water is beneficial because it can be wetted up by relatively small precipitation events throughout the year. However, global climate change is predicted to result in reduced snowpack/melt (Barnett et al., 2005; Beniston, 2003; Grundstein & Mote, 2010; Luca, 2018; López-Moreno et al., 2009; Räisänen, 2008; Schmucki et al., 2017; U.S. Global Change Research Program, 2017; Vicuña et al., 2011; You et al., 2017) which would result in reduced shallow soil moisture during spring (Archer & Predick, 2008; Barnett, 2005; A. Harpold et al., 2012; U.S. Global Change Research Program, 2017) when plant transpiration and production are at their fullest (Petersky & Harpold, 2018; Schmucki et al., 2017). This is likely to lead to increased plant water stress in spring and summer. The deep soil and groundwater are wetted up by relatively large precipitation events during the monsoon and winter seasons as well as snowmelt in late winter, early spring. However, global climate change is predicted to produce more monsoon storms of greater intensity and less duration (U.S. Global Change Research Program, 2017), which is expected to result in greater runoff, with less deep soil moisture and groundwater recharge.
Additionally, reduced winter snowpack will likely result in reduced deep soil moisture and groundwater recharge (Earman et al., 2006). Similar scenarios are likely to become increasingly common in various seasonal snow dominant regions around the globe as they transition into ephemeral snow systems.

4.3 Seasonal Variability in Source Water

While our results show that Douglas Fir utilizes water from the shallow soil, deep soil, and groundwater, predominantly utilizing water from the shallow 10 cm depth, an evaluation of source water for each stem sample and averaging the number of times each source occurs for each season shows a slightly different picture. The shallow soil water is still the dominant source of stem water throughout most of the year, but during the pre-winter period the groundwater becomes the dominant source of stem water. This suggests a seasonal shift in source waters. It is likely that after the monsoon season when the shallow and deep soils become drier due to limited precipitation the Douglas Fir switches to the more abundant source of groundwater (Andrews et al., 2012; Bertrand et al., 2014; J. R. Brooks et al., 2002).

A two-isotope comparison of the seasons reveals that during each season the stem samples plot amongst both the shallow and deep soils (Figure 6). This finding is consistent with the single isotope comparison and is important because it shows that two tracers (δ¹⁸O and δD) both agree with the findings of the other. However, the two-isotope comparison does not clearly indicate a shift in seasonal source water use by the Douglas Fir.

For most of the year the primary seasonal source of water is from the shallow soils. As discussed above there are both benefits and disadvantages for the Douglas Fir relying so heavily on shallow soil as its primary source water. To better determine if there is a seasonal shift in the source water of Douglas Fir or any other representative plant within an ecosystem further study is required.

5. CONCLUSION

It has been observed throughout many places in the world that the temperature is rising, snowpack is declining, snow melts are occurring earlier, and the snow line is receding up in elevation (Barnett, 2005; Hennessy, 2008; Pepin et al., 2015; Räisänen, 2008; U.S. Global Change Research
Winter warming has caused plants to budburst earlier potentially exposing their soft tissues to late winter freezes (Weijers et al., 2018). Future increases to winter warming has the potential to effect the endodormancy period of plants which would make them incapable of budburst and therefore they could not repair damage to their existing tissues or grow new tissues (Asse et al., 2018). Reduced snowpack results in reduced soil moisture available to plants during the winter and spring time periods when budburst and transpiration starts up with tissue production (Barnett, 2005). Likewise, earlier snowmelt results in reduced soil moisture later in the spring time which can result in increased drought stress for the plants (Barnett, 2005). A receding snowline means that the temperature is warming at higher elevations which means that plants that could not exist in colder climates can encroach into areas that they previously could not inhabit (Hennessy, 2008). Hence, it is important to understand the source water of plants, such as the Douglas Fir, and whether that source water changes seasonally to determine the conditions which produced the source water and what conditions can limit its continuation and thereby risk the plant and its surrounding environments survival.

This study has made several important contributions to the understanding of the ecohydrological implications of changing snow melt patterns in the Santa Catalina Mountains of the southwestern United States and how they apply to other ecosystems throughout the globe. First, we show that there is a seasonal precipitation isotopic trend with summer monsoon precipitation more enriched in $\delta^{18}O$ and $\delta$D and winter precipitation more depleted in more depleted. Second, we show that a combination of the seasonal precipitation and a dynamic soil moisture flow regime combine to produce seasonal soil moisture trends and likewise produce a slight seasonal groundwater trend. The seasonal precipitation component results in the wetting up of the soil profile during large precipitation events during the winter and monsoon periods which persist, at deeper depths, into the proceeding dry seasons. Third, we showed that Douglas Fir has minor variation in its stem water isotopic composition throughout the year. Overall, our findings suggest that the Douglas Fir within the Santa Catalina Mountains use water from the shallow soils (0 – 20 cm), the deep soils (30 – 100 cm), and from the groundwater. However, there is a seasonal transition from the predominantly used shallow soil water to the groundwater in the pre-winter season, likely due to the water within the soil profile becoming increasingly difficult to retrieve. Lastly, results have been presented that indicate the process of hydraulic redistribution potentially being employed by
the Douglas Fir during the dry pre-winter period. If there is less snowpack or snowmelt occurs earlier, then there will be less water available to Douglas Fir and other plants during the beginning of the growing season. This could result in increased water stress in plants which could cause reduced new growth and increased die off, especially in plants that do not have an ability to access multiple sources of water. These findings contribute to the relatively few studies pertaining to the Santa Catalina Mountains and the sky islands of the desert Southwestern United States in general. This study also functions to inform the global community about the conditions that can be expected when transitioning from a seasonal snow dominated environment to an ephemeral snowmelt environment.
REFERENCES


Table 1: The total and seasonal precipitation amount and percent for the 3 water years that span the period of study.

<table>
<thead>
<tr>
<th>Season</th>
<th>Precip [mm]</th>
<th>Precip [%]</th>
<th>Precip [mm]</th>
<th>Precip [%]</th>
<th>Precip [mm]</th>
<th>Precip [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>430.36</td>
<td>100.00</td>
<td>850.43</td>
<td>100.00</td>
<td>652.46</td>
<td>100.00</td>
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<td>Winter</td>
<td>66.66</td>
<td>15.49</td>
<td>264.68</td>
<td>31.12</td>
<td>59.45</td>
<td>9.11</td>
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<td>Pre-Monsoon</td>
<td>12.61</td>
<td>2.93</td>
<td>81.09</td>
<td>9.53</td>
<td>72.94</td>
<td>11.18</td>
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<td>Monsoon</td>
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<td>61.26</td>
<td>418.99</td>
<td>49.27</td>
<td>372.36</td>
<td>57.07</td>
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<tr>
<td>Pre-winter</td>
<td>87.47</td>
<td>20.32</td>
<td>85.68</td>
<td>10.07</td>
<td>147.72</td>
<td>22.64</td>
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</table>

Table 2: The total and seasonal average isotopic values of precipitation.

<table>
<thead>
<tr>
<th>Season</th>
<th>Precip [mm]</th>
<th>Precip [%]</th>
<th>δ18O [‰]</th>
<th>δ2H [‰]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>1742.10</td>
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<td>-7.97</td>
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<td>Winter</td>
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<tr>
<td>Pre-winter</td>
<td>233.40</td>
<td>13.40</td>
<td>-10.45</td>
<td>-67.85</td>
</tr>
</tbody>
</table>

**Figure Captions**

Figure 1: Site map

Figure 2: Global meteoric water line & local meteoric water line (2A, top left), shallow soil water line (2B, top right), deep soil water line (2C, bottom left), stem water line (2D, bottom right)

Figure 3. Daily precipitation (3A, top), daily soil moisture at depths of 10, 30, and 60 cm (3B, second from top), avg isotopic value of precipitation (3C, third from top), avg isotopic value of soil at depths of 10 cm, 40 cm, and ground water seepage (3D, fourth from top), avg isotopic value of stems (3E, bottom).

Figure 4. Isotopic values for each daily sample collected (precipitation, soils at 10, 40, 80 cms, groundwater, and stems), as well as the average for those daily samples, separated seasonally.

Figure 5: Average and standard error of the seasonal δ18O for soil water at each depth.

Figure 6: All seasonal samples (precipitation, shallow soil, deep soil, ground water, and stem) plotted atop of the GMWL and LMWL; winter samples (6A, top left), pre-monsoon samples (6B, top right), monsoon samples (6C, bottom left), pre-winter samples (6D, bottom right)
Figure 7: All samples (precipitation, shallow soil, deep soil, ground water, and stem) plotted together atop the GMWL and LMWL.
Figure 2
Figure 3
Figure 4
Figure 5
Figure 7
APPENDIX B: PERFORMANCE OF INDUCTION MODULE CAVITY RING-DOWN
SPECTROSCOPY (IM-CRDS) FOR MEASURING DELTA O-18 AND DELTA H-2 VALUES
OF SOIL, STEM, AND LEAF WATERS

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Performance of induction module-cavity ring-down spectroscopy (IM-CRDS) for analysis of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in soil, stem, and leaf waters

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ABSTRACT

Rational: Induction module cavity ring-down spectroscopy (IM-CRDS) has been proposed as a rapid and cost-effective alternative to cryogenic vacuum distillation (CVD) and isotope ratio mass spectrometry (IRMS) for analysis of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in matrix-bound waters. In the current study, we characterized the performance of IM-CRDS relative to CVD and IRMS and analyzed the mechanisms responsible for differences between the two methods.

Methods: We collected a set of $n = 75$ soil, stem, and leaf water samples, and measured $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of each sample with four techniques: CVD and IRMS, CVD and CRDS, CVD and IM-CRDS, and IM-CRDS alone. We then calculated the isotopic errors for each of the three CRDS methods relative to CVD and IRMS, and analyzed the relationships among these errors and suites of diagnostic spectral parameters that are indicative of organic contamination.

Results: The IM-CRDS technique accurately assessed $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of pure waters, but exhibited progressively increasing errors for soil waters, stem waters, and leaf waters. For soils, errors were attributable to subsampling of isotopically heterogeneous source material, whereas for stems and leaves, errors were attributable to spectral interference. Unexpectedly, the magnitude of spectral interference was higher for the solid samples analyzed directly via IM-CRDS as compared to those originally extracted via CVD and then analyzed via IM-CRDS.

Conclusions: There are many types of matrix-bound water samples for which IM-CRDS measurements include significant errors from spectral interference. As a result, spectral analysis...
and validation should be incorporated into post-processing procedures for IM-CRDS systems. In the future, IM-CRDS performance could be improved through: (i) identification of the compounds that cause spectral interference, and either (ii) modification of the combustion step to completely oxidize these compounds to CO₂, and/or (iii) incorporation of corrections for these compounds into the spectral fitting models used by the CRDS analyzers.

1. INTRODUCTION

In the environmental sciences, measurements of the stable isotope composition of oxygen (δ¹⁸O) and hydrogen (δ²H) are routinely performed on liquid water samples extracted from solid matrices. Traditionally, the measurement approach has involved the extraction of waters from solid matrices via cryogenic vacuum distillation (CVD) and subsequent analysis of the distillates via isotope ratio mass spectrometry (IRMS). While the IRMS measurements originally represented the bottleneck in this analysis pathway, improvement in continuous flow techniques eventually turned the table (Gehre, Geilmann, Richter, Werner, & Brand, 2004), leaving CVD as the rate-limiting step (Adam G. West, Patrickson, & Ehleringer, 2006). More recently, the emergence of isotope ratio infrared spectroscopy (IRIS) as a higher-throughput alternative to IRMS has accentuated the long-standing throughput limitations associated with CVD, and highlighted a new set of challenges associated with the organic contaminants that co-extract with water in this technique (Brand, Geilmann, Crosson, & Rella, 2009; Schmidt et al., 2012; Schultz, Griffis, Lee, & Baker, 2011; Adam G. West, Goldsmith, Brooks, & Dawson, 2010; A. G. West, Goldsmith, Matimati, & Dawson, 2011). In combination, these factors have intensified interest in the development of new methods that integrate the extraction step with isotope analysis via IRIS.

To date, two methods have been developed as alternatives to CVD: an induction module and a microwave extraction chamber. The original version of the induction module was designed to extract liquid water into a dry nitrogen stream by induction heating, to carry the resulting vapor through a ceramic micropyrolysis column heated to 1200°C to pyrolyze organic contaminants, and then to pass the cleaned vapor directly into an IRIS analyzer (Berkelhammer et al., 2013). A revised version of the induction module was then designed to combust, rather than pyrolyze, the organic contaminants by replacing the ceramic micropyrolysis column with a metal catalyst heated to only 400°C (Chang, Wolf, Gerlein-Safdi, & Caylor, 2016; Lazarus, Germino, & Vander Veen,
In a parallel effort, a microwave extraction chamber was designed to extract liquid water into a dry air stream by microwave heating within a sealed vessel, to cool the resulting water vapor in a condensation chamber, and then to carry the cooled vapor directly into an IRIS analyzer (Munksgaard, Cheesman, Wurster, Cernusak, & Bird, 2014). While the initial tests of the induction module and microwave extraction chamber have indicated that both techniques have promise, neither has yet been thoroughly characterized relative to traditional CVD and IRMS, and only the induction module is currently commercially available.

Here, we report a study of the performance of an induction module coupled to an IRIS analyzer for isotopic analysis of soil, stem, and leaf waters. The specific system under evaluation included an induction module coupled to a cavity ring-down spectrometer (IM-CRDS). The specific study objectives were: (i) to quantify the accuracy of IM-CRDS relative to CVD and IRMS, (ii) to diagnose the mechanisms responsible for errors in IM-CRDS relative to CVD and IRMS, and (iii) to determine the remedies needed to correct the errors. To pursue these objectives, we collected a physically and chemically diverse set of soil, stem, and leaf water samples expected to vary in isotopic composition. We measured δ¹⁸O and δ²H of each sample with four techniques (Figure 1). Building on previous work (Schmidt et al., 2012), we analyzed the relationships between the errors observed for each CRDS method and the suites of spectral parameters that are indicative of organic contamination (Table 1). We then used these relationships to diagnose the types of mechanisms most likely responsible for the observed errors (Table 2). The key assumption underlying this approach is that CRDS errors that are correlated to the diagnostic spectral parameters are likely to be the result of organic contamination, whereas CRDS errors that are uncorrelated to the spectral parameters are likely to be the result of other mechanisms.

2. METHODS

2.1 Sample Collection

We collected 25 different samples with 3 replicates each, for a total of n = 75 samples. The samples included pure waters (n = 4 x 3), leaf waters (n = 8 x 3), stem waters (n = 9 x 3), and soil waters (n = 4 x 3). The pure waters were of known isotopic composition and were included as internal standards, to check for fractionations during sample handling and analysis. The soil, stem, and leaf
waters were selected to represent a diverse range of soil types and perennial plant species that (i) are of key interest for ecological studies and (ii) exhibit variability in physical and chemical properties that might affect water extraction. The soil samples included a sandy loam and a clay loam with low organic content from the Desert Laboratory on Tumamoc Hill (ca. 2 km west of downtown Tucson), as well as a silty loam from Mt. Bigelow (ca. 30 km northeast of downtown Tucson in the Santa Catalina Mts.) and a commercially available potting mix that both had high organic content. The plant samples included stems and leaves from *Larrea tridentata*, *Prosopis velutina*, *Acacia constricta*, *Parkinsonia microphylla*, *Fouquieria splendens*, and *Ambrosia deltoidea*, all collected from Tumamoc Hill. Basal and apical succulent stem segments were also included from the cactus species *Opuntia engelmannii* and *Cylindropuntia versicolor* at Tumamoc Hill; for analysis, these samples are grouped with the ‘stem’ and ‘leaf’ samples, respectively. Additionally, stem samples were included from *Pseudotsuga menziesii*, collected from Mt. Bigelow. The soil and plant collections were made at midday during several parts of the annual cycle, including the summer of 2014 (Jul.-Aug.), fall of 2014 (Oct.-Nov.), winter of 2015 (Mar.-Apr.), and summer of 2015 (Aug.-Sept.). At the field sites, the soils, stems, and leaves were collected into 20 mL air-tight glass vials with Polyseal cone-lined screw caps, sealed with Parafilm, and kept on ice. After transfer to the laboratory, samples were frozen at -4°C until analysis. For analysis, each frozen soil, stem, and leaf sample was divided into two fractions, one for CVD (groups I-III), and the other for IM-CRDS (group IV).

### 2.2 Cryogenic Vacuum Distillation

A CVD system was constructed at the Laboratory of Tree-Ring Research, University of Arizona (Tucson, AZ, USA) for this analysis. The extraction system consisted of six distillation units, each comprised of a glass sample holder and collection tube, connected to a main vacuum line. For extractions, the main vacuum line was pumped down to a pressure of 10 millitorr with a direct-drive vacuum pump (Edwards, E2M2). The vials containing the frozen samples were weighed, uncapped, placed in the sample holders, and attached to the distillation units. The attached sample holders were submerged in liquid nitrogen for 15 minutes; the distillation units were opened to the main vacuum line for 15 minutes; and then the distillation units were sealed under vacuum. The pressure was monitored in each distillation unit with vacuum gauges (Granville-Phillips, Convectron Vacuum Measurement System Series 275) to ensure that there were no detectable
leaks. The sample holders were then submerged in deionized water and heated to 100°C by immersion heaters to evaporate the liquid water, while the collection tubes were submerged in liquid nitrogen to condense the resulting water vapor. Each extraction continued until the pressure in the distillation unit returned to the original level, which ranged from a minimum of ~ 60 minutes (i.e., for liquid waters) to a maximum of ~ 3 hours (i.e., for soils). Distillates were then collected and transferred to a refrigerator until the completeness of the extractions was assessed. Extraction completeness was assessed by weighing the residual solid samples immediately after extraction, transferring them to a drying oven, holding at 55°C for 72 hours, and then re-weighing. Of the \( n = 75 \) samples, \( n = 67 \) were initially extracted with an efficiency > 97%. The \( n = 8 \) samples with lower initial extraction efficiencies were discarded and the extractions were repeated on new subsets of material from the original collections. Once the extractions were complete, the distilled samples were divided into three fractions, one for analysis via IRMS, a second for analysis via regular CRDS, and a third for analysis via IM-CRDS (groups I-III; Figure 1).

2.3 Activated Carbon Treatment
We applied an activated carbon treatment to the distillates that were analyzed via IRMS and regular CRDS (groups I and II). A coarse activated carbon (4-12 mesh) was homogenized to a fine powder with a mortar and pestle to achieve maximum surface area. The homogenized activated carbon was added to each distillate vial in order to achieve a 20% w/w slurry. The slurries were incubated for 24 hours at 4°C, with periodic vortexing to achieve thorough mixing. After the incubation period, each mixture was filtered through a 0.2 μm syringe-tip filter (Sigma Aldrich, 54145-U), transferred to a fresh vial, and sealed with Parafilm. To test for any fractionation associated with the activated carbon treatment, subsets from one set of distilled standards were set aside as controls that did not receive the activated carbon treatment, but were otherwise handled identically. Since analysis via IM-CRDS involves on-line treatment with activated carbon, the distillate fraction for IM-CRDS did not receive the manual treatment with activated carbon.

2.4 Isotope Ratio Mass Spectrometry (IRMS)
Samples in group I were analyzed with a Finnigan Delta S Isotope Ratio Mass Spectrometer in the Environmental Isotope Laboratory, Department of Geosciences, University of Arizona (Tucson, AZ, USA). For oxygen, samples were equilibrated with CO₂ gas at approximately 15°C in an
automated equilibration device coupled to the mass spectrometer. For hydrogen, samples were reacted at 750°C with Cr metal using a Finnigan H/Device coupled to the mass spectrometer. Standardization was based on internal distilled water standards referenced to VSMOW2 and SLAP2. The analytical precision for these methods was ± 0.08‰ for $\delta^{18}O$ and ± 0.9‰ for $\delta^2H$ on the basis of repeated internal standards.

2.5 Cavity Ring-Down Spectroscopy (CRDS)

Samples in group II were analyzed via regular CRDS. These analyses were performed with a L2120-i cavity ring-down spectrometer equipped with a V1102-i high-precision vaporizer (Picarro, Inc., Santa Clara, CA, USA) and autosampler (HTC PAL, Leap Technologies, Carrboro, NC, USA). The L2120-i measures three of the major isotopologues of water based on absorption at three near-infrared absorption lines close to 7184 cm$^{-1}$ (1392 nm). The specific lines that are utilized are 7183.685 cm$^{-1}$ (1392.043 nm) for $^1$H$^1$H$^{16}$O, 7183.585 cm$^{-1}$ (1392.063 nm) for $^1$H$^1$H$^{18}$O, and 7183.972 cm$^{-1}$ (1391.988 nm) for $^1$H$^2$H$^{16}$O ("IUPAC critical evaluation of the rotational-vibrational spectra of water vapor, Part III: Energy levels and transition wavenumbers for H.sub.2.sup.16O.(Report)," 2013; Tennyson et al., 2009; Tennyson et al., 2010; Tennyson et al., 2013). All of the measurements were performed in the air carrier mode, with air provided from a cylinder of ultra-high-purity compressed air (<1 ppm H$_2$O, <0.01 ppm THC, <0.01 ppm CO, <0.001 ppm NOx, <0.001 ppm SO$_2$; Ultrapure Air, Scott-Marrin, Inc., Riverside, CA, USA). For analyses, 1.5 mL aliquots of each sample were pipetted into 1.8 mL glass vials (VWR, 66020-950), with polypropylene screw caps and bonded PTFE-silicone septa (VWR, 46610-700). The autosampler sampled the vials and injected the samples into the vaporizer on a 9 minute cycle, using a 10 uL syringe (SGE 10R-C/T-5/0.47C, Trajan Scientific Americas, Inc., Austin, TX, USA) which was rinsed twice in N-methyl-2-pyrrolidone (99.5%, Acros Organics, Fisher Scientific, Pittsburgh, PA, USA) before each injection. Each sample was measured 10 times; the vaporizer septum was replaced every 250 injections; and the vaporizer was run at 110°C. The analytical precision for this method was ± 0.20‰ for $\delta^{18}O$ and ± 0.7‰ for $\delta^2H$ on the basis of repeated internal standards.

2.6 Induction Module – Cavity Ring-Down Spectroscopy (IM-CRDS)
Samples in groups III and IV were analyzed via IM-CRDS. These analyses were performed with a L2130-i cavity ring-down spectrometer equipped with an A0213 induction module (Picarro, Inc., Santa Clara, CA, USA). The L2130-i measures three of the major isotopologues of water based on absorption at three near-infrared absorption lines close to 7200 cm\(^{-1}\) (1389 nm). The specific lines that are utilized are 7200.133 cm\(^{-1}\) (1388.863 nm) for \(^1\)H\(^2\)H\(^{16}\)O, 7199.961 cm\(^{-1}\) (1388.896 nm) for \(^1\)H\(^1\)H\(^{18}\)O, and 7200.302 cm\(^{-1}\) (1388.831 nm) for \(^1\)H\(^2\)H\(^{16}\)O\([13-15]\). All of the measurements were performed in the air carrier mode, with air provided from a cylinder of ultra-zero compressed air (<3 ppm H\(_2\)O, <0.1 ppm THC, <1 ppm CO, <1 ppm CO\(_2\); ALPHAGAZ1, Air Liquide, Houston, TX, USA). Samples were prepared for analysis in three different ways: (i) liquid samples were injected onto a glass-fiber filter paper disc and placed into a metal clip; (ii) leaf and stem samples were placed directly into a metal clip; (iii) soil samples were placed into a metallic cylinder and sealed off with metal wool. After each sample was prepared, it was placed into a glass vial, sealed with a septum, and loaded into the IM for extraction. To optimize the extractions for the different types of samples, slightly different values of the manufacturer’s parameters ‘heatTime,’ ‘polyA,’ ‘polyB,’ and ‘polyC’ were used. In the group III sample set, all of the samples were extracted with heatTime = 180 seconds with a heating profile defined by polyA = 0.00021, polyB = 0.00001, and polyC = 13. In the group IV sample set, one replicate of each of the pure water internal standards and all of the solid leaf samples were extracted with heatTime = 180 seconds with a heating profile defined by polyA = 0.00021, polyB = 0.00001, and polyC = 13. The second replicate of each of the pure water internal standards and the solid stem samples were also extracted with heatTime = 180 seconds, but with a heating profile defined by polyA = 0.00003, polyB = 0.03, and polyC = 15. The third replicate of each of the pure water internal standard and the solid soil samples were extracted with heatTime = 480 seconds and a heating profile defined by polyA = 0.00003, polyB = 0.4, and polyC = 25. On the basis of repeated internal standards, the analytical precision for these three methods was ± 0.15, 0.18, and 0.20\(\%\) for \(\delta^{18}\)O and ± 1.2, 1.4, and 1.0\(\%\) for \(\delta^2\)H, respectively.

### 2.7 Data Analysis

#### 2.7.1 Notation

For all of the measurements, isotope ratios were expressed relative to the international standard VSMOW (Vienna Standard Mean Ocean Water):
\[
\text{Eqn. 1} \quad \delta^{18}\text{O or } \delta^2\text{H (‰)} = (R_{\text{sample}}/R_{\text{VSMOW}} - 1) \times 1000
\]

where \(R_{\text{sample}}\) and \(R_{\text{VSMOW}}\) represent the ratios of the abundance of the heavy and light isotopologues in the samples and international standard, respectively (i.e., \(^1\text{H}^1\text{H}^{18}\text{O}/^1\text{H}^1\text{H}^{16}\text{O}\) and \(^1\text{H}^2\text{H}^{16}\text{O}/^1\text{H}^1\text{H}^{16}\text{O}\)). The raw \(\delta^{18}\text{O}\) and \(\delta^2\text{H}\) values were calibrated based on working standards that were analyzed concurrently with the samples. Different working standards were used for each type of analysis, but each set bracketed the isotope ratios of the unknowns and were used to generate first-order linear models relating the raw isotope ratios to the true values.

### 2.7.2 Effects of Cryogenic Vacuum Distillation and Manual Activated Carbon Treatment

We checked whether the combination of CVD and manual activated carbon treatment had introduced isotope artifacts by comparing the true isotopic compositions of the standards (i.e., obtained by IRMS before the distillations) to the isotopic compositions that were obtained by IRMS after the distillations and activated carbon treatments (i.e., \(n = 12\) samples). To determine the basis of any differences, we also compared a subset of the standards that did and did not receive the activated carbon treatment (i.e., \(n = 4\) samples).

### 2.7.3 Similarities and Differences Between CRDS Versus IRMS

We used linear mixed-effects models to characterize differences between CRDS and IRMS values while accounting for the effects of sample type. All analyses were performed in R (Team, 2016). For each combination of predictor and response variables, we fit a mixed-effects model using the ‘lmer(’ function from the package ‘lme4’ (Douglas, Martin, Ben, & Steve, 2015). Each model included (i) the IRMS measurement as the response variable, (ii) one of the CRDS measurements as the fixed effect predictor (with intercept and slope), and (iii) the sample type as the random effect predictor (with intercept only). We assessed the significance of each predictor using the package ‘lmerTest’ (Kuznetsova, Brockhoff, & Christensen, 2017). Models where the random effect predictor was not significant were re-fit with the fixed effect only using the function ‘lm(’ in base R. The overall model fit was then summarized with: (i) P-values for individual fixed and random effects; (ii) the root mean square error (RMSE), and (iii) the marginal (i.e., fixed effect only) or conditional (i.e., fixed and random effect) \(R^2\) (Nakagawa & Schielzeth, 2013).
2.7.4 Mechanisms Underlying CRDS Versus IRMS Differences

2.7.4.1 Overall Approach
First, we calculated the difference between δ\(^{18}\)O and δ\(^{2}\)H determined by IRMS versus each of the CRDS methods: \(\Delta \delta^{18}O_{CRDS-IRMS} = \delta^{18}O_{CRDS} - \delta^{18}O_{IRMS}\) and \(\Delta \delta^{2}H_{CRDS-IRMS} = \delta^{2}H_{CRDS} - \delta^{2}H_{IRMS}\), where all terms are in units of ‰. For convenience, we will refer to \(\Delta \delta^{18}O_{CRDS-IRMS}\) and \(\Delta \delta^{2}H_{CRDS-IRMS}\) as ‘isotopic error terms.’ Next, we used mixed-effects models to identify the spectral parameters that were the best predictors of the isotopic error terms. The model analysis procedure was analogous to that described in the previous section, with the following exception: each model included (i) one of the isotope error terms as the response variable, (ii) a spectral parameter as the fixed effect predictor (with intercept and slope), and (iii) the sample type as the random effect predictor (with intercept only). Finally, we performed pairwise comparisons of the spectral parameters that were the best predictors of the isotopic error terms in each individual CRDS method to determine whether the modes of spectral interference were similar for the three CRDS methods. Details about the spectral parameters associated with each CRDS method are provided in the following sections.

2.7.4.2. Distillates Analyzed with CRDS Alone
The parameters that can be used to diagnose spectral interference in the L2120-i are the residuals of the fit between the observed absorption spectrum and the spectrum of pure water (‘organic_res’), the change in the intercept or slope of the spectral baseline (‘organic_shift’ and ‘organic_slope’), the absorption associated with methanol (‘organic_MeOHamp1’), and the absorption associated with methane (‘organic_ch4conc’) (Table 1). We retrieved the raw values of these parameters from the analyzer’s ‘Datalog_Private’ directory and calculated mean values over the duration of each injection. Although these parameters are inherently sensitive to the water concentration during measurement, the introduction of samples to the L2120-i via the autosampler and vaporizer produced square pulses of water with little variation in the maximum water concentration, so the spectral parameters from the L2120-i were not detrended for the effects of water concentration.
2.7.4.3. Distillates and Solids Analyzed with IM-CRDS

The parameters that can be used to diagnose spectral interference in the L2130-i are the residuals of the fit between the observed absorption spectrum and the spectrum of pure water (‘residuals’), the change in the intercept, slope or curvature of the spectral baseline (‘baseline_shift’, ‘slope_shift’, and ‘baseline_curvature’), and absorption associated with methane (‘CH4’) (Table 1). We retrieved the raw values of these parameters from the analyzer’s ‘Datalog_Private’ directory and calculated mean values over the duration of each injection. However, since the introduction of samples to the L2130-i via the induction module produced shark-fin type pulses of water with a moderate amount of variation in the maximum water concentration, we also detrended the spectral parameters from the L2130-i for the effects of water concentration, following the method recommended by a recent study (Lazarus et al., 2016).

2.7.5 Approaches for Resolving CRDS Versus IRMS Differences

2.7.5.1 Distillates Analyzed with CRDS Alone

We calculated organic-corrected $\delta^{18}$O$_{\text{CRDS}}$ and $\delta^2$H$_{\text{CRDS}}$ values as described by the manufacturer and as recommended by a previous study (Martín-Gómez et al., 2015). Briefly, we retrieved the organic-filtered amplitudes of the $^1$H$^1$H$^{18}$O, $^1$H$^1$H$^{16}$O and $^1$H$^2$H$^{16}$O peaks from the analyzer’s ‘Datalog_Private’ directory (organic_77, organic_splinemax, organic_82, respectively) and the instrument-specific intercept and slope from the analyzer’s ‘InstrCal_Air.ini’ script. We then calculated organic-corrected $\delta^{18}$O$_{\text{CRDS}}$ and $\delta^2$H$_{\text{CRDS}}$ values as:

\[ Y = X\beta + \varepsilon \]

where $Y$ represents a vector of corrected (but uncalibrated) $\delta^{18}$O$_{\text{CRDS}}$ or $\delta^2$H$_{\text{CRDS}}$ values, $X$ represents a vector of the fixed effect predictors (ratio of organic_77/organic_splinemax or organic_82/organic_splinemax), $\beta$ represents a vector of regression coefficients (the instrument-specific intercept and slope), and $\varepsilon$ represents a vector of random errors. The organic-corrected values were then calibrated to the isotope standards with the same approach that was originally applied to the organic-uncorrected values.
2.7.5.2. Distillates and Solids Analyzed with IM-CRDS

In the L2130-i, organic-filtered amplitudes of the $^1\text{H}^1\text{H}^{18}\text{O}$, $^1\text{H}^1\text{H}^{16}\text{O}$ and $^1\text{H}^2\text{H}^{16}\text{O}$ peaks are not reported as they are in the L2120-i. As a result, organic-corrected $\delta^{18}\text{O}_{\text{CRDS}}$ and $\delta^2\text{H}_{\text{CRDS}}$ values cannot be calculated for the L2130-i using the mechanistic model that applies to the L2120-i. Instead, we calculated isotopic corrections based on the empirical models we developed relating the spectral parameter ‘residuals’ to the $\Delta\delta^{18}\text{O}_{\text{CRDS-IRMS}}$ and $\Delta\delta^2\text{H}_{\text{CRDS-IRMS}}$ values:

Eqn. 3 $Y \text{correction} = X\beta + Z\cdot u + \varepsilon$

where $Y \text{correction}$ represents a vector of isotopic corrections for $\delta^{18}\text{O}$ or $\delta^2\text{H}$, $X$ represents a vector of fixed effect predictors (the ‘residuals’ parameter), $Z$ represents a vector of random effect predictors (sample type), $\beta$ represents a vector of fixed effect coefficients, $u$ represents a vector of random effect coefficients, and $\varepsilon$ represents a vector of random errors. We then applied the corrections as:

Eqn. 4 $Y = Y_{\text{raw}} - Y \text{correction}$

where $Y_{\text{raw}}$ represents the raw $\delta^{18}\text{O}_{\text{CRDS}}$ or $\delta^2\text{H}_{\text{CRDS}}$ values and $Y$ represents the organic-corrected $\delta^{18}\text{O}_{\text{CRDS}}$ or $\delta^2\text{H}_{\text{CRDS}}$ values. Since these models utilize regression coefficients determined from the sample set, we use them only to illustrate the degree to which the accuracy and precision of the IM-CRDS measurements could improve if the observed spectral interference were corrected.

3. Results

3.1 Effects of Cryogenic Vacuum Distillation and Manual Activated Carbon Treatment

In the standard waters, there was a slight trend towards enrichment of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ after CVD and manual activated carbon treatment as compared to the original true values (Figure 2). This effect was statistically significant for $\delta^{18}\text{O}$ ($P_{\text{fixed}} = 0.026$), but not for $\delta^2\text{H}$ ($P_{\text{fixed}} > 0.050$). The differences in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ between the standards before versus after activated carbon treatment were not statistically significant for either isotope ($P_{\text{fixed}} > 0.050$ for both). Since all of these errors fell either within or very close to the precision of the IRMS, no statistical corrections were applied to the samples for the effects of CVD or manual activated carbon treatment.
3.2 Similarities and Differences Between CRDS Versus IRMS

Across the three CRDS methods, there was significant agreement between the CRDS and IRMS values (Figure 3; \( P_{\text{fixed}} < 0.001 \) for each comparison; N.B., all of the isotope data are available in the online supplement). In general, agreement between the various techniques was highest for pure waters, and decreased progressively for soil waters, stem waters, and leaf waters. For all three sample types, the L2120-\( i \) analyses of distillates tended to underestimate the true values of \( \delta^{18}O \) and \( \delta^2H \) (Figure 3, A, D), whereas the L2130-\( i \) analyses of both distillates and solids tended to overestimate the true values of \( \delta^{18}O \) and \( \delta^2H \) (Figure 3, B-C, E-F). The relationships between the CRDS and IRMS values differed significantly between sample types for group II (\( P_{\text{random}} < 0.001 \) for \( \delta^{18}O \) and \( \delta^2H \)), but did not differ between sample types for group IV (\( P_{\text{random}} > 0.050 \) for \( \delta^{18}O \) and \( \delta^2H \)). For group III, the sample type effect was not significant for \( \delta^{18}O \) (\( P_{\text{random}} > 0.050 \)), and was only marginally significant for \( \delta^2H \) (\( P_{\text{random}} = 0.040 \)). For \( \delta^{18}O \), the relationships between the CRDS and IRMS values were strongest for group II (Figure 3, A; RMSE = 2.28‰, conditional \( R^2 = 0.942 \)) and group III (Figure 3, B; RMSE = 2.48‰, marginal \( R^2 = 0.951 \)), followed by group IV (Figure 3, C; RMSE = 3.99‰, marginal \( R^2 = 0.872 \)). For \( \delta^2H \), the relationships between the CRDS and IRMS values were strongest for group III (Figure 3, E; RMSE = 4.3‰, conditional \( R^2 = 0.994 \)), followed by group IV (Figure 3, F; RMSE = 13.0‰, marginal \( R^2 = 0.943 \)), and group II (Figure 3, D; RMSE = 15.7‰, marginal \( R^2 = 0.890 \)).

3.3 Mechanisms Underlying CRDS Versus IRMS Differences

For the measurements of the liquid distillates with the L2120-\( i \), the isotopic error terms (\( \Delta \delta^{18}O_{\text{CRDS-IRM}} \), \( \Delta \delta^2H_{\text{CRDS-IRM}} \)) were completely unrelated to ‘organic_res’, ‘organic_shift’ and ‘organic_slope’, for all of the sample types (Figure 4, A-C and F-H; \( P_{\text{fixed}} > 0.050 \) for all). In contrast, both isotopic error terms had significant positive linear relationships with ‘organic_MeOHampl’ (Figure 4, D, I; \( P_{\text{fixed}} < 0.001 \) for both). For \( \Delta \delta^{18}O \), these relationships differed slightly between sample types (\( P_{\text{random}} = 0.005 \)), but for \( \Delta \delta^2H \), there were no differences between sample types (\( P_{\text{random}} > 0.100 \)). In both cases, the models based on ‘organic_MeOHampl’ could account for the vast majority of the variation in the isotopic error terms (\( \Delta \delta^{18}O \): RMSE = 0.26‰, conditional \( R^2 = 0.992 \); \( \Delta \delta^2H \): RMSE = 1.6‰, marginal \( R^2 = 0.994 \)). Additionally, both isotopic error terms had significant negative linear relationships with ‘ch4_ppm’ (Figure 4, E, J;
For the measurements of the liquid distillates with the L2130-i with induction module, both isotopic error terms had significant linear relationships with ‘residuals’, ‘slope_shift’, ‘baseline curvature’, and ‘ch4_ppm’ (Figure 5, A, C-F, H-J; \(P_{\text{fixed}} < 0.001\) for all). A similar trend was evident with ‘baseline_shift,’ but several outliers weakened the overall relationship (Figure 5, B, G; \(P_{\text{fixed}} = 0.093\) and 0.045, respectively). Qualitatively, samples with large isotopic error terms tended to be characterized by positive values of ‘residuals’, ‘baseline_shift’, and ‘baseline_curvature,’ but negative values of ‘slope_shift’ and ‘ch4_ppm’ (Figure 5, A-J). Quantitatively, the stem samples from *Pseudotsuga menziesii* departed from the general pattern to a large degree in ‘baseline_shift’, and to a lesser degree also in ‘slope_shift’, and ‘baseline curvature’ (Figure 5, B-D, G-I). As a result, the isotopic error terms had the strongest overall relationships with ‘residuals’ and ‘ch4_ppm’ (Figure 5, A, E-F, J). For both \(\Delta \delta^{18}O\) and \(\Delta \delta^2H\), the relationships with ‘residuals’ differed significantly between sample types (\(P_{\text{random}} < 0.001\) and \(P = 0.008\), respectively), but the relationships with ‘ch4_ppm’ did not (\(P_{\text{random}} > 0.050\)). Overall, the models based on ‘residuals’ and ‘ch4_ppm’ accounted for similarly large fractions of the variation in the isotopic error terms, with ‘residuals’ being a slightly more powerful predictor than ‘ch4_ppm’ (i.e., for ‘residuals’, \(\Delta \delta^{18}O\): RMSE = 0.42‰, conditional \(R^2 = 0.985\); \(\Delta \delta^2H\): RMSE = 1.4‰, conditional \(R^2 = 0.920\); for ‘ch4_ppm’, \(\Delta \delta^{18}O\): RMSE = 0.67‰, marginal \(R^2 = 0.968\); \(\Delta \delta^2H\): RMSE = 1.6‰, marginal \(R^2 = 0.910\)).

For the measurements of the solid samples with the L2130-i with induction module, the overall relationships between the isotopic error terms and the diagnostic spectral parameters were similar to those observed with the distillates analyzed via IM-CRDS, but with substantially more noise and stronger differences between sample types (Figure 6). Both isotopic error terms had significant linear relationships with all of the spectral parameters (Figure 6, A-J; for \(\Delta \delta^{18}O\) and \(\Delta \delta^2H\), \(P_{\text{fixed}} < 0.001\) for ‘residuals’, ‘slope_shift’, ‘ch4_ppm’; \(P_{\text{fixed}} < 0.01\) for ‘baseline_curvature’; \(P_{\text{fixed}} < 0.05\).
In general, the relationships between spectral parameters were similar to those observed for group III: samples with large positive isotopic error terms tended to be characterized by positive values of ‘residuals’, ‘baseline_shift’, and ‘baseline_curvature,’ but negative values of ‘slop_shift’ and ‘ch4_ppm’ (Figure 6, A-J). However, the differences between sample types were more pronounced here: sample type was a significant random effect in nine out of the ten models (for Δδ¹⁸O and Δδ²H, P随机 < 0.001 for ‘residuals’, ‘slop_shift’, ‘ch4_ppm’; P随机 < 0.01 for ‘baseline_curvature’; whereas for ‘baseline_shift’, P固定 < 0.001 for Δδ¹⁸O and P > 0.050 for Δδ²H). In these models, including sample type as a predictor led to systematic variation in the random intercept, with pure water typically having the lowest intercepts, and soil water, stem water, and leaf water having progressively higher intercepts. The leaf and stem samples that had the largest isotopic error terms and/or largest deviations from the pure water spectral parameters were derived from *Pseudotsuga menziesii*, *Ambrosia deltoidea*, and *Opuntia engelmannii*. Overall, the models based on ‘residuals’ and ‘ch4_ppm’ accounted for the largest fractions of the variation in the isotopic error terms, with ‘residuals’ again being a somewhat more powerful predictor than ‘ch4_ppm’ (i.e., for ‘residuals’, Δδ¹⁸O: RMSE = 3.41‰, conditional R² = 0.833; Δδ²H: RMSE = 11.3‰, conditional R² = 0.516; for ‘ch4_ppm’, Δδ¹⁸O: RMSE = 3.75‰, marginal R² = 0.802; Δδ²H: RMSE = 11.4‰, marginal R² = 0.505).

### 3.4 Mechanisms Underlying Differences Among the Three CRDS Methods

The ‘organic_MeOHampil’ parameter values from the L2120-i analyses of distillates were strongly and positively linearly correlated to the ‘residuals’ parameter values from the L2130-i analyses of distillates (Figure 7, A; P固定 < 0.001). The relationship between the two spectral parameters varied slightly between sample types, with intercepts highest for pure water and decreasing progressively for soil water, stem water, and leaf water (P随机 = 0.008). Taking these effects into account, variation in the ‘organic_MeOHampil’ parameter values from group II explained nearly all of the variation in the ‘residuals’ parameter values from group III (conditional R² = 0.982). In comparison, the ‘residuals’ parameter values from the L2130-i analyses of distillates and solids were only weakly correlated with one another (Figure 7, B; P固定 < 0.001). The relationship between the two spectral parameters varied strongly between sample types, with intercepts lowest for soil water and increasing progressively for pure water, stem water, and leaf water (P随机 < 0.001). Taking these effects into account, variation in the ‘residuals’ parameter values from group
III explained approximately half of the variation in the ‘residuals’ parameter values from group IV (conditional $R^2 = 0.585$).

### 3.5 Approaches for Resolving CRDS Versus IRMS Differences

Within the L2120-$i$ measurements of the distillates, the post-processing correction based on the organic-filtered amplitudes of the $^1H^1H^{18}O$, $^1H^1H^{16}O$ and $^1H^2H^{16}O$ peaks brought the corrected CRDS values into good agreement with the corresponding IRMS values (Figure 8, A, D; $P_{\text{fixed}} < 0.001$). There were no effects of sample type on these relationships ($P_{\text{random}} > 0.050$). For $\delta^{18}O$, a type I regression between IRMS and corrected CRDS values had intercept of $-0.42 \pm 0.05\%o$, slope of $0.959 \pm 0.005$, marginal $R^2$ of 0.998 and RMSE of $0.47\%o$ (Figure 8, A). For $\delta^2H$, a type I regression between IRMS and corrected CRDS values had intercept of $-3.5 \pm 0.2\%o$, slope of $0.978 \pm 0.003$, marginal $R^2$ of 0.999 and RMSE of 1.6$\%o$ (Figure 8, D).

Within the L2130-$i$ measurements of the distillates, the post-processing correction based on ‘residuals’ also brought the corrected CRDS values into good agreement with the corresponding IRMS values (Figure 8, B, E; $P_{\text{fixed}} < 0.001$). There were no effects of sample type on these relationships ($P_{\text{random}} > 0.050$). For $\delta^{18}O$, a regression between IRMS and corrected CRDS values had intercept of $-0.01 \pm 0.05\%o$, slope of $0.993 \pm 0.004$, marginal $R^2$ of 0.999 and RMSE of $0.41\%o$ (Figure 8, B). For $\delta^2H$, a regression between IRMS and corrected CRDS values had intercept of $-0.1 \pm 0.2\%o$, slope of $0.998 \pm 0.003$, marginal $R^2$ of 0.999 and RMSE of 1.4$\%o$ (Figure 8, E).

Within the L2130-$i$ measurements of the solids, the post-processing correction based on ‘residuals’ brought the corrected CRDS values into closer agreement with the corresponding IRMS values (Figure 8, C, F; $P_{\text{fixed}} < 0.001$). Sample type still had a marginal effect on the relationships for $\delta^{18}O$ ($P_{\text{random}} = 0.070$), but no effect for $\delta^2H$ ($P_{\text{random}} > 0.050$). For $\delta^{18}O$, a regression between IRMS and corrected CRDS values had intercept of $-0.45 \pm 1.18\%o$, slope of $0.788 \pm 0.051$, conditional $R^2$ of 0.906 and RMSE of $2.98\%o$ (Figure 8, C). For $\delta^2H$, a regression between IRMS and corrected CRDS values had intercept of $-1.3 \pm 1.6\%o$, slope of $0.962 \pm 0.023$, marginal $R^2$ of 0.958 and RMSE of 11.2$\%o$ (Figure 8, F).
4. Discussion

4.1 Accuracy of CRDS Methods Relative to Cryogenic Vacuum Distillation and IRMS

All three CRDS methods performed satisfactorily for the pure water internal standards, as expected based on previous measurements with the L2120-i with vaporizer, L2120-i with micro-combustion module (MCM) (Martín-Gómez et al., 2015), L2120-i with IM (Lazarus et al., 2016), and L2130-i with MCM (Chang et al., 2016). For the soil distillates, the L2120-i with vaporizer and the L2130-i with IM produced consistent results with relatively minor errors that were within the lower end of the range of errors previously reported for soil distillates (Martín-Gómez et al., 2015; Schmidt et al., 2012; Adam G. West et al., 2010; A. G. West et al., 2011; Zhao et al., 2011). For the soil samples, the errors from the L2130-i with IM were distributed around a mean of approximately zero, but extended across a substantially wider range than was observed for the soil distillates. There were no previous measurements of solid soils analyzed with the IM available for comparison. For the stem and leaf distillates, the mean errors associated with the L2120-i with vaporizer and the L2130-i with IM were much greater than those for the soil distillates, and the largest errors were within the upper end of the range of previous reports (Martín-Gómez et al., 2015; Schmidt et al., 2012; Adam G. West et al., 2010; A. G. West et al., 2011; Zhao et al., 2011). For the solid stem and leaf samples, the magnitude of the errors from the L2130-i with IM varied substantially between species, and the largest errors were approximately four times greater than those previously reported for Artemisia tridentata stems analyzed with a L2120-i with IM (Lazarus et al., 2016).

4.2 Mechanisms Responsible for Errors in CRDS Methods

The vast majority of the errors in all three CRDS methods appear to be attributable to mechanisms that can be broadly classified as spectral interference (i.e., within classes 4-6 in Table 2). The primary lines of evidence supporting this attribution are: (i) the mathematical signs of the errors observed for each of the three CRDS methods are characteristic of spectral interference in those methods (Figure 3); and (ii) there are strong correlations between the isotopic errors within each of the three CRDS methods and spectral parameters that are characteristic of spectral interference (Figures 4, 5, 6). However, we also find evidence supporting the attribution of a small component of the errors in the IM-CRDS method to mechanisms other than spectral interference (i.e., within
classes 1-3 in Table 2). The primary lines of evidence supporting this attribution are: (i) there are deviations from the mathematical sign of the errors characteristic of spectral inference for certain samples and (ii) the errors for these samples are uncorrelated to the spectral parameters that are characteristic of spectral interference (Figure 5). Below, we discuss the evidence for attribution of the isotopic errors to the spectral and non-spectral mechanisms.

4.2.1 Spectral Mechanisms
One of the primary lines of evidence that spectral interference is responsible for the major component of the CRDS errors is the variation in mathematical sign of the isotopic errors: negative in group II, but positive in groups III and IV (Figure 3). The tendency for errors in the L2120-i measurements to be negative for both δ¹⁸O and δ²H has previously been reported for soil and plant extracts analyzed with both the L1102-i and the L2120-i instruments (Lazarus et al., 2016; Martín-Gómez et al., 2015; Schmidt et al., 2012; Adam G. West et al., 2010; A. G. West et al., 2011; Zhao et al., 2011). The tendency for errors in the L2130-i measurements to be positive for both δ¹⁸O and δ²H has also previously been reported for soil and plant extracts analyzed with the L2130-i (Munksgaard et al., 2014). These patterns result from the interaction of three factors: (i) the different spectral features that these instruments use to measure the water isotopes (i.e., around 7184 versus 7200 cm⁻¹, respectively), (ii) the differential effects of certain contaminants on those spectral features, and (iii) the abundance of those contaminants in the samples.

A number of compounds are known to produce spectral interference with the water vapor features in the target spectral regions, particularly alcohols and methane (Hendry, Richman, & Wassenaar, 2011). While methane interference can be important for atmospheric measurements, methanol and ethanol tend to be more important for soil and plant measurements. Methanol and ethanol are ubiquitous metabolic intermediates in living plants and microorganisms, and are also produced within dead organic matter through abiotic as well as biotic mechanisms (Gray, Monson, & Fierer, 2010; Warneke et al., 1999). In the water vapor feature around 7184 cm⁻¹, absorption features associated with methanol and ethanol differentially affect the amplitudes of the target ¹H¹H¹⁶O, ¹H¹H¹⁸O, and ¹H²H¹⁶O absorption lines, with the result that water samples appear to be depleted in δ¹⁸O and δ²H when either alcohol is present (Brand et al., 2009; Martin-Gomez et al., 2015). In the water vapor feature around 7200 cm⁻¹, the absorption features associated with methanol and
ethanol also differentially affect the amplitudes of the target $^1$H$^1$H$^{16}$O, $^1$H$^1$H$^{18}$O, and $^1$H$^2$H$^{16}$O absorption lines, but here with the result that water samples appear to be enriched in $\delta^{18}$O and $\delta^2$H when methanol is present and depleted in $\delta^{18}$O and $\delta^2$H when ethanol is present (Chang et al., 2016; Schultz et al., 2011). In combination, these considerations lead us to conclude that methanol was the primary spectral contaminant in the sample set used in this study.

The strong correlations between the CRDS errors and spectral parameters provide a second line of evidence that spectral interference is responsible for the major component of the CRDS errors (Figures 4-6). In the L2120-i measurements, the fact that the ‘organic_MeOHampl’ parameter was so strongly associated with the isotopic errors supports the interpretation that methanol was the primary spectral contaminant in the distillates (Figure 4). Given that methanol is not incorporated into the spectral model used in the L2130-i, and that the ‘residuals’ parameter from the L2130-i measurements of distillates was linearly associated with the ‘organic_MeOHampl’ parameter from the L2120-i measurements, it appears that either residual methanol or a product of incomplete oxidation of methanol is responsible for the majority of the errors in the L2130-i measurements of distillates (Figures 5 & Figure 7, A). Previous studies of the MCM have indicated that the effectiveness of the MCM declines continuously as methanol contamination increases, and that the mode of failure appears to be partial conversion of MeOH to CO$_2$, rather than formation of alternative oxidation products (Chang et al., 2016; Martin-Gomez et al., 2015). Therefore, the majority of the errors in both the L2120-i and the L2130-i measurements of distillates in this study seem very likely to be attributable to spectral interference from methanol. The small number of outliers from the methanol error modes are the Pseudotsuga menziesii stem samples (Figure 5, B-D, G-I). The biased baseline parameters for these samples are likely attributable to spectral interference from compounds that have weaker, broader absorbance features than methanol, such as ethanol (Schultz et al., 2011; A. G. West et al., 2011).

In the L2130-i measurements of solids, the basis of the errors appears to be relatively more complex. Compared to the L2130-i measurements of the stem and leaf distillates, the corresponding measurements of solids were associated with approximately two-fold higher values for the isotopic errors, two-fold wider ranges for the spectral parameters, and substantially increased noise in the relationship between the isotopic errors and the spectral parameters (Figure
6). In combination, these patterns indicate that spectral interference was both higher and more variable for stem and leaf samples extracted via the induction module as compared to those extracted via CVD. There are three key differences between the cryogenic extractions and the induction extractions that could potentially be responsible for this result: (i) the cryogenic extractions occurred at approximately 10 millitorr, whereas the induction extractions occurred at atmospheric pressure; (ii) the cryogenic extractions occurred at a temperature of approximately 100°C, whereas the induction extractions occurred at temperatures of 180-200°C; and (iii) the distillates from the cryogenic extractions were stored in the liquid phase for several weeks prior to analysis, whereas the vapors from the induction extractions were analyzed in the gas phase within less than a minute of extraction.

Since carrying out extractions under reduced pressure could have the effect of increasing the volatilization of compounds that otherwise would not extract under atmospheric pressure, the pressure difference between the two techniques seems likely to favor a decreased, rather than increased, contaminant load. However, if the higher temperatures in the induction extractions resulted in the volatilization of a larger fraction of the same contaminants and/or additional contaminants than volatilized in the cryogenic extractions, then the temperature difference between the two techniques might explain different contaminant loads. Similarly, if heating the solid samples to any given extraction temperature resulted in the volatilization of reactive organic compounds with spectral features close to the water vapor features of interest, then the difference in time-to-analysis between the two techniques could also make a contribution to the observed patterns. This line of reasoning suggests that either and/or both of the last two mechanisms may be involved.

In an earlier study of the effect of temperature on CVD of plant waters, higher extraction temperatures were found to have species-specific effects, causing increases in spectral contamination in some species, and decreases in others (Schmidt et al., 2012). Such effects could potentially explain the increased slope and the scatter in the regressions of the ‘residuals’ values from the distillates versus solids in the L2130-1 (Figure 7, B). However, if the different extraction temperatures only altered the amount of methanol that was volatilized, then the relationships between the isotopic errors and the spectral parameters would be expected to be invariant for the
distillates versus the solids. This is not the case: although the relationships between the isotopic errors and the spectral parameters are qualitatively similar for the two sample types, there is a marked increase in the total noise associated with the solid samples, and the increase in noise was greatest in the samples with the lowest quality spectral fits (Figure 5 versus Figure 6). These patterns suggest that, in addition to methanol, one or more additional compounds are contributing to the overall spectral interference observed in the analyses of solids.

4.2.2 Non-Spectral Mechanisms

In several recent studies, it has been proposed that significant components of the isotopic errors in IM-CRDS may be attributable to processes other than spectral interference (i.e., corresponding to mechanisms in classes 1-3 in Table 2). For example, one study has pointed out that the induction module is likely to yield apparent errors when small subsamples are taken from a material where the isotopic composition of water is spatially heterogeneous (Munksgaard et al., 2014). A second has pointed out that using the micro-combustion module on samples with high alcohol content is likely to lead to systematic errors related to the addition of combustion-derived water vapor carrying the very enriched oxygen isotopic composition of air (Martin-Gomez et al., 2015). A third has suggested that even though induction heating appears to retrieve the vast majority of the liquid water from solid samples, this extraction mechanism may nonetheless produce some sort of substantial error that is not due to spectral interference (Lazarus et al., 2016). In the current study, the only clear evidence we find for non-spectral errors in any of the CRDS analyses comes from the solid soil samples (Figure 6). These samples had substantial isotopic errors that had a mean close to zero and were not correlated with any of the spectral parameters, a pattern that is most consistent with variable subsampling of an isotopically heterogeneous matrix (i.e., class 1 in Table 2). In contrast, we find no evidence of systematic errors related to incomplete extraction of matrix-bound water (i.e., class 2 in Table 2), or to the addition of combustion-derived water vapor carrying the very enriched oxygen isotopic composition of air (i.e., class 3 in Table 2), or to any other undefined mechanisms.

The absence of errors attributable to incomplete extraction of matrix-bound water is likely due to the fact that we optimized the IM extraction recipes in advance of the experiment in order to achieve complete extractions for each sample type. Analogously, the absence of errors attributable
to combustion-derived water vapor is likely due to the fact that the manual activated carbon treatment and the in-line activated carbon cartridge were both effective at removing ethanol before samples reached the heated catalyst. Based on the spectral parameters in the L2120-i, a total of \( n = 32 \) samples had methanol concentrations that were significantly higher than the pure waters, whereas only \( n = 2 \) samples had ethanol concentrations that were significantly higher than the pure waters. Calibration of the L2120-i parameters to mixtures of ultrapure methanol and ethanol indicates that the most MeOH-contaminated samples had approximately 0.2\% methanol (v/v), and the only two EtOH-contaminated samples had approximately 0.3 and 3.0\% ethanol (v/v). For the complete oxidation of methanol (i.e., \( 2\text{CH}_3\text{OH} + 3\text{O}_2 \rightarrow 2\text{CO}_2 + 4\text{H}_2\text{O} \)), all of the hydrogen in the water vapor is derived from the methanol, whereas a maximum of 50\% of the oxygen in the water vapor is derived from the methanol; for the complete oxidation of ethanol (i.e., \( \text{C}_2\text{H}_5\text{OH} + 3\text{O}_2 \rightarrow 2\text{CO}_2 + 3\text{H}_2\text{O} \)), all of the hydrogen in the water vapor is derived from the ethanol, whereas a maximum of 30\% of the oxygen in the water vapor is derived from the ethanol. In both cases, the additional oxygen in the water vapor produced by combustion is derived from the \( \text{O}_2 \) in the carrier gas (Martin-Gomez et al., 2015). Since compressed air was used as the carrier gas for the L2130-i, we expect that its \( \delta^{18}\text{O} \) was similar to that of the natural atmosphere (23.8 \( \pm \) 0.3\‰) (Coplen & Shrestha, 2016), i.e., approximately 35\‰ higher than the most depleted organic-derived samples in this study. As a result, the maximum \( \delta^{18}\text{O} \) error potentially attributable to water vapor produced from the combustion of methanol in the IM is on the order of 0.04\‰. For the two ethanol-contaminated samples, the maximum \( \delta^{18}\text{O} \) errors attributable to this mechanism are on the order of 0.07 and 0.74\‰, respectively. The fact that two of these three values are less than the precision of the analyzer explains why the vast majority of the observed \( \delta^{18}\text{O} \) errors in this study are not attributable to artifacts from combustion-derived water vapor.

4.3 Approaches for Correcting Errors in CRDS Methods

Given (i) that the isotope errors in the CRDS results are primarily the result of spectral interference, and (ii) that spectral interference is a deterministic function of the identity and abundance of the interfering compound(s), there are potentially several alternative ways to approach post-processing corrections for the isotope results. In principle, if several samples that differed in water isotopic composition were each contaminated by exactly the same amount(s) of the same interfering compound(s), then the absolute value for each sample would be inaccurate, but the relative
differences between samples would still be accurate because the errors would be consistent. In this situation, a simple offset correction would be sufficient to correct for the effects of spectral interference. In practice, however, the samples in this study exhibited substantial variation in the amounts as well as some variation in the identities of the interfering compounds, such that both the absolute values of each sample and the relative differences between samples were inaccurate. In this situation, approaches that take into account both the identities and the amounts of the interfering compounds are needed to correct for the effects of spectral interference.

For the L2120-\textit{i} measurements of distillates, the post-processing correction based on the organic-filtered amplitudes of the $^{1}\text{H}^{2}\text{H}^{18}\text{O}$, $^{1}\text{H}^{1}\text{H}^{16}\text{O}$ and $^{1}\text{H}^{2}\text{H}^{16}\text{O}$ peaks was highly effective at correcting spectral interference (Figure 8, A, D). However, it is notable that the intercepts and slopes that we calculated for regressions between the corrected CRDS values and the IRMS values are nearly identical to those reported for an independent set of samples that were measured on a different L2120-\textit{i} analyzer but corrected analogously (Martin-Gomez et al., 2015). The slight positive bias of the corrected CRDS measurements in the two studies could be the result of the additive effect of laboratory uncertainties and potential sample alteration during transport and storage, as proposed previously (Martin-Gomez et al., 2015), or of a small but systematic bias in the spectral algorithm used to compute the organic-filtered peak amplitudes. Therefore, we concur with the earlier assessment (Martin-Gomez et al., 2015) that the post-processing correction based on the organic-filtered peak amplitudes is indeed promising, and that further tests of its accuracy are also warranted.

For the L2130-\textit{i} measurements of distillates and solids, the post-processing corrections based on the ‘residuals’ parameter were highly and moderately effective, respectively (Figure 8, B-C, E-F). Since these corrections utilized regression coefficients determined from our specific sample set, it is unclear how successfully they might perform on independent sets of measurements. However, if our inference is correct that the dominant error mode in the L2130-\textit{i} measurements is attributable to spectral interference from methanol, then the path forward is clear. The first step is revising the L2130-\textit{i} spectral fitting model to include calculation of the component of absorbance due to methanol, as well as the organic-filtered peak amplitudes, as is already done for the L2120-\textit{i}. The second step is identifying the additional species that generate spectral interference when solid
samples are extracted with the induction module, and then developing an appropriate hardware and/or software remedy. In principle, the additional interfering species could be compound(s) that are preferentially extracted under the higher-temperature, higher-pressure conditions during induction extraction, and/or oxidation products of such compounds. It is also possible that they are highly reactive compound(s)—and/or oxidation products of such compounds—that are extracted under both the cryogenic and induction conditions, but preferentially degrade in the liquid distillates during the period before isotopic analysis.

To review candidate interfering species, we compiled a list of compounds that: (i) are routinely observed in the gas phase in the atmosphere (Rothman et al., 2013), (ii) have O-H bonds that might have absorption features similar to those of water (Brand et al., 2009) and (iii) are not halogens. Aside from methanol, three compounds satisfied these criteria: formic acid (CH$_2$O$_2$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH). Since all three compounds occur naturally within plant metabolism and are also intermediates and/or products of the low-temperature oxidation of alcohols (Chang et al., 2016; Dennis, Rees-Owen, Brooks, Carter, & Dawson, 2014; Sarathy, Oßwald, Hansen, & Kohse-Höinghaus, 2014), it is possible that any or all may be carried into CRDS analyzers after IM extractions. While it is unclear whether hydroxyl radical could persist long enough to cause optical effects, the HITRAN compilation indicates that hydroxyl radical does have absorption lines that directly interfere with the water vapor lines around 7200 cm$^{-1}$. Conversely, while hydrogen peroxide and formic acid are likely persistent enough to cause optical effects, HITRAN coverage of the 7200 cm$^{-1}$ region is lacking for both compounds. Hydrogen peroxide is a particularly interesting candidate because (i) it has a boiling point of 150.2°C at atmospheric pressure that would favor co-extraction with water in both the CVDs and the induction extractions, and (ii) has a level of reactivity potentially consistent with persistence through IM-CRDS analysis as well as degradation within stored liquid distillates. Formic acid is also a plausible candidate, but evidence to date suggests that it may not be abundant enough in plant extracts to cause significant spectral interference (Dennis et al., 2014). CRDS measurements are needed to provide direct tests of whether any of these three compounds are abundant enough after IM extractions to generate the absorption features that interfere with the water vapor and methanol features around 7200 cm$^{-1}$.
4.4 Additional Considerations: Capital Costs, Operating Costs, and Speed

The techniques compared in this study differ not only in accuracy, but also in capital costs, operating costs, and speed. In terms of capital costs, CRDS systems with either the vaporizer and autosampler or induction module cost on the order of $100k, whereas IRMS systems with peripherals for water isotope analysis approach 2.5 times that amount. Conceptually, the capital costs should be prorated over the lifetime of the instruments. However, because the CRDS systems are relatively new, it is not yet clear how their lifetimes compare to those of IRMS systems. In terms of speed and operating costs, the maximum throughput rate for CVD and manual activated carbon treatment was 80 samples per week. Taking into account the costs of labor and consumables, CVD averaged $7 per sample. When CVD was used to generate liquid extracts for IRMS, the throughput remained limited by CVD and the total costs for analysis of $\delta^{18}O$ and $\delta^2H$ averaged $43 per sample (i.e., group I). When CVD was used with CRDS alone, throughput also remained limited by the speed of CVD, but the total cost of analysis averaged $8 per sample (i.e., group II). When CVD was used with IM-CRDS, throughput decreased to 40 samples per week, and costs averaged $28 per sample (i.e., group III). When solid samples were directly analyzed via IM-CRDS, throughput was also 40 samples per week, and costs averaged $21 per sample (i.e., group IV). The reduced throughput of the IM-CRDS analyses was partially due to the need to manually load samples into the IM, and partially due to the number of analytical replicates needed to obtain consistent results for samples with high levels of organic contamination (i.e., on average, $n = 7$ analytical replicates per sample).

5. Conclusions

The IM-CRDS technique has the potential to provide accurate analysis of the isotopic composition of water in soil, stem, and leaf samples under the following conditions: (i) the isotopic composition of the water in the solid samples is spatially homogeneous relative to the scale of sampling, (ii) the heating parameters in the induction module recipe are optimized to achieve complete extraction of liquid water from the solid sample matrix, (iii) the activated carbon filter reduces the total amount of co-extracted volatile organic compounds to a level that does not overwhelm the oxidation capacity of the heated catalyst; (iv) the heated catalyst produces a complete oxidation of the remaining organic compounds to CO$_2$ and H$_2$O; and (v) the total amount of completely oxidized organic compounds is low enough that the nascent water from oxidation does not detectably
contaminate the $\delta^{18}$O of the sample. If these conditions are satisfied, IM-CRDS can provide isotopic analysis of small samples with comparable accuracy, higher speed, and lower cost than CVD and IRMS. However, there are currently many types of water samples that do not satisfy these conditions, and for which IM-CRDS analysis consequently yields variably inaccurate results. The variable accuracy leads to a need for high analytical replication, which both reduces the overall speed and increases the overall cost of IM-CRDS.

Given (i) that the magnitude of the IM-CRDS errors observed in this study is similar to the magnitude of the ecological signals that are of interest in many natural abundance studies and (ii) that spectral interference is the dominant error mode, our interpretation is that measurements from existing IM-CRDS systems can only be considered to be robust if they are subject to spectral analysis and validation. We recommend that users of existing IM-CRDS systems take the following precautions to detect spectral interference: (i) perform calibration experiments to identify threshold values of the diagnostic spectral parameters that delineate acceptable versus unacceptable isotopic errors for particular applications and sample types; (ii) integrate screening of the diagnostic spectral parameters into routine laboratory QA/QC procedures; (iii) discard any analyses where the level of spectral interference results in isotopic errors that exceed the bias thresholds; (iv) cross-check a subset of the analyses that appear to be within the maximum acceptable bias thresholds against CVD and IRMS. Overall, these recommendations are very similar to those previously advocated for other forms of CRDS analysis (Martin-Gomez et al., 2015; Schmidt et al., 2012; Schultz et al., 2011; Adam G. West et al., 2010; A. G. West et al., 2011). However, implementing spectral screening procedures for IM-CRDS does require the additional step to detrend the spectral parameters for the effects of water concentration (Lazarus et al., 2016).

In terms of future development efforts, the current combustion-based version of the IM has already fully overcome the problems related to isotope fractionation that were associated with the original pyrolysis-based version of the device (Saad, Hsiao, Chapellet-Volpini, & Vu, 2013). The primary remaining challenge is resolution of the problems resulting from spectral interference. Towards this end, there are two or three steps with the potential to substantially improve the accuracy of IM-CRDS: (i) identification of all of the compounds that cause spectral interference when solid
samples are extracted, and either (ii) modification of the combustion step to completely oxidize these compounds to CO$_2$, and/or (iii) incorporation of corrections for the absorbance associated with these compounds into the spectral fitting models used by the CRDS analyzers to calculate the absorbance of the water isotopologue peaks. Identification of the interfering species is likely to be challenging because there is not yet a comprehensive spectral library that has high resolution reference spectra in the wavelength regions targeted by the laser-based analyzers. However, if spectral interference can be successfully eliminated, IM-CRDS will have the potential to offer equal or greater accuracy than CVD and IRMS. Improved accuracy would likely reduce the need for high levels of analytical replication, translating into increases in the speed and cost-effectiveness of IM-CRDS. Development of an automated sample loader could also lead to further improvements in speed and cost-effectiveness. With these types of improvements, IM-CRDS could offer unique advantages for the analysis of the isotopic composition of small samples of matrix-bound water.

Data availability
The data generated in this study are available as Supplementary Information.

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References


List of Tables

Table 1. Parameters used to diagnose spectral interference

Table 2. Classes of mechanisms potentially responsible for IM-CRDS errors

List of Figures

Figure 1. Flow chart of experimental design. In this study, we evaluated the performance of IM-CRDS for analysis of δ¹⁸O and δ²H of pure water, soil water, stem water, and leaf water through comparison to several alternative techniques. Abbreviations: IM, induction module; CRDS, cavity ring-down spectroscopy; IRMS, isotope ratio mass spectrometry. N.B., the heated catalyst component of the IM is also referred to as a ‘micro-combustion module’ (MCM).

Figure 2. Effects of cryogenic vacuum distillation and manual activated carbon (AC) treatment. Points indicate means +/- standard deviation for pure water standards without AC (n = 4) and with AC (n = 12); shaded regions indicate IRMS precision for δ¹⁸O (1σ = 0.13‰) and δ²H (1σ = 0.9‰). Abbreviations: Δδ¹⁸O and Δδ²H, differences calculated as values after treatments – true values.

Figure 3. Overall differences in δ¹⁸O and δ²H between CRDS versus IRMS. Points indicate means +/- standard deviation for pure waters (blue; n = 12), leaf waters (green; n = 24), stem waters (yellow; n = 27), and soil waters (brown; n = 12) in each of the three treatment groups (II, III, IV; as defined in Table I).

Figure 4. Group II – Mechanisms driving differences in δ¹⁸O and δ²H between CRDS versus IRMS. Isotopic errors are plotted as a function of the raw (non-normalized) spectral indices. Points indicate means +/- standard deviation for individual samples of pure waters (blue; n = 12), leaf waters (green; n = 24), stem waters (yellow; n = 27), and soil waters (brown; n = 12) in treatment group II (i.e., liquid distillates analyzed with L2120-i without induction module; Table 1). Dashed lines indicate the zero positions on each axis.
Figure 5. Group III – Mechanisms driving differences in δ¹⁸O and δ²H between CRDS versus IRMS. Isotopic errors are plotted as a function of normalized spectral indices. Points indicate means +/- standard deviation for individual samples of pure waters (blue; n = 12), leaf waters (green; n = 24), stem waters (yellow; n = 27), and soil waters (brown; n = 12) in treatment group III (i.e., liquid distillates analyzed with L2130-i with induction module; Table 1). Dashed lines indicate the zero positions on each axis.

Figure 6. Group IV – Mechanisms driving differences in δ¹⁸O and δ²H between CRDS versus IRMS. Isotopic errors are plotted as a function of normalized spectral indices. Points indicate means +/- standard deviation for individual samples of pure waters (blue; n = 12), leaf waters (green; n = 24), stem waters (yellow; n = 27), and soil waters (brown; n = 12) in treatment group IV (i.e., solid samples analyzed with L2130-i with induction module; Table 1). Dashed lines indicate the zero positions on each axis.

Figure 7. Mechanisms driving differences in δ¹⁸O and δ²H among the three CRDS methods. For each sample, plots compare the values of the spectral parameters that best predicted isotopic errors in each type of CRDS analysis. Points indicate means +/- standard deviation for individual samples of pure waters (n = 12), leaf waters (n = 24), stem waters (n = 27), and soil waters (n = 12).

Figure 8. Approaches for correcting differences in δ¹⁸O and δ²H between CRDS versus IRMS. Points indicate means +/- standard deviation for individual samples of pure waters (n = 12), leaf waters (n = 24), stem waters (n = 27), and soil waters (n = 12) in each of the three treatment groups (II, III, IV; as defined in Table 1). Solid lines indicate the 1:1 relationship between corrected CRDS values versus IRMS measurements.
<table>
<thead>
<tr>
<th>Instrument</th>
<th>Parameter name</th>
<th>Parameter definition</th>
<th>Diagnostic meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2120-i</td>
<td>organic_res</td>
<td>RMS residuals of the least-squares fit (organics)</td>
<td>Indicates how well the spectral model fits the measured absorption spectrum; poor fit may indicate organic interference</td>
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<td>Indicates whether the slope of the baseline underlying the absorption spectrum has been distorted relative to original factory calibration</td>
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<td>L2120-i</td>
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<td>Absorption of MeOH peak</td>
<td>Indicates whether methanol (MeOH) is present in the sample, and if present then in what concentration</td>
</tr>
<tr>
<td>L2120-i</td>
<td>organic_ch4conc</td>
<td>CH₄ mole fraction with no calibration</td>
<td>Indicates whether methane (CH₄) is present in the sample, and if present then in what concentration</td>
</tr>
<tr>
<td>L2130-i</td>
<td>residuals</td>
<td>RMS residuals of the least-squares fit</td>
<td>Indicates how well the spectral model fits the measured absorption spectrum; poor fit may indicate organic interference</td>
</tr>
<tr>
<td>L2130-i</td>
<td>baseline_shift</td>
<td>Change in constant term of fitted baseline</td>
<td>Indicates whether the y-intercept of the baseline underlying the absorption spectrum has been distorted relative to original factory calibration</td>
</tr>
<tr>
<td>L2130-i</td>
<td>slope_shift</td>
<td>Change in linear term of fitted baseline</td>
<td>Indicates whether the slope of the baseline underlying the absorption spectrum has been distorted relative to original factory calibration</td>
</tr>
<tr>
<td>L2130-i</td>
<td>baseline_curvature</td>
<td>Quadratic term in fitted baseline</td>
<td>Indicates whether any curvature has been introduced into the baseline underlying the absorption spectrum</td>
</tr>
<tr>
<td>L2130-i</td>
<td>CH4</td>
<td>Final methane fraction after bottle calibration</td>
<td>Indicates whether methane (CH₄) is present in the sample, and if present then in what concentration</td>
</tr>
</tbody>
</table>
Table 2. Classes of mechanisms potentially responsible for IM-CRDS errors

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism</th>
<th>Evidence for mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Differences in physical subsampling of solid material</td>
<td>In any CRDS analysis, isotopic errors are uncorrelated to spectral indices, and are distributed around a mean value of zero</td>
</tr>
<tr>
<td>2</td>
<td>Incomplete extraction of liquid water from solid matrix</td>
<td>In any CRDS analysis, isotopic errors are uncorrelated to spectral indices, and are preferentially distributed around a negative mean value</td>
</tr>
<tr>
<td>3</td>
<td>Contributions from water vapor formed during organic oxidation</td>
<td>In CRDS alone, isotopic errors correlated to spectral indices; in IM-CRDS solid or liquid analysis, isotopic errors uncorrelated to spectral indices, and larger for $\delta^{18}$O than $\delta^{2}$H</td>
</tr>
<tr>
<td>4</td>
<td>Interference from compounds that are co-extracted during cryogenic distillation, but can be oxidized</td>
<td>In CRDS alone, isotopic errors are large and correlated to spectral indices; in IM-CRDS solid and liquid analysis, errors are reduced or absent</td>
</tr>
<tr>
<td>5</td>
<td>Interference from compounds that are the products of oxidation reactions in the micro-combustion module</td>
<td>In IM-CRDS solid or liquid analysis, isotopic errors are correlated to spectral indices; in CRDS alone, isotopic errors are uncorrelated to spectral indices</td>
</tr>
<tr>
<td>6</td>
<td>Interference from compounds that are not co-extracted during cryogenic distillation, but are during induction extraction</td>
<td>In IM-CRDS solid analysis, isotopic errors are correlated to spectral indices; in IM-CRDS liquid analysis, isotopic errors are uncorrelated to spectral indices</td>
</tr>
</tbody>
</table>

Figure 1.
Figure 2.

Figure 3.
APPENDIX C: FIELD PROTOCOLS

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1. SUMMARY
This appendix describes the protocol that was used in collecting soil, stem and precipitation samples at the study site. The study site consists of three locations on the periphery of the Santa Catalina Mountain Critical Zone Observatory (SCM-CZO) located on Mt. Bigelow. Samples were collected at a two-week interval. Precipitation samples were collected in a standard rain gage with few centimeters of mineral oil to retard evaporation. Soil samples were collected with a soil corer down to 0.40 meter and from a trench down to 1 meter. Stem samples were collected from trees within 25 meters of the point where soil samples were collected. All samples were collected into 20 milliliter glass vials, which were wrapped in parafilm, placed in plastic zip-lock bags, and then placed in a cooler with ice packs. In the lab all samples were placed into a refrigerator, until they could be analyzed.

2. SITE DESCRIPTION
The study site is located on the periphery of the Santa Catalina Mountain Critical Zone Observatory (SCM-CZO). The SCM-CZO is situated on Mt. Bigelow, at an elevation of 2573 m above sea level, within the Santa Catalina Mountains part of the National Coronado Forest, Northeast of the city of Tucson, AZ. Dr Shirley Papuga runs a phenocam study at three locations in proximity to the SCM-CZO. Samples will be collected around these three phenocam locations.

The ecosystem that defines the study area is a coniferous forest, which is defined by its cone-bearing trees, such as pine, fir, and spruce. The Douglas Fir (Pseudotsuga menziesii) was selected as the representative taxa of the study site, as it is the dominant species in the area.

3. SAMPLE COLLECTION
Every two weeks at each of the three sites within the study area, three types of samples will be collected. The three types of samples that will collected are: plant xylem tissue from the Douglas Fir; a soil core down to a depth of 0.45 meter or a trench down to 1 meter; and any precipitation that has fallen since last collection. To distinguish the distinct initial isotopic signature of the water before it infiltrates into the ground, precipitation samples will be collected as they occur.
3.1. Plant Xylem Tissue Samples

3.1.1. Locate one of the three phenocams

3.1.2. Using the phenocam as the origin, determine a circular area around the phenocam with a radius of 25 meters.

3.1.3. Identify a mature Douglas fir within the 25 meter radius.
   3.1.3.1. Refer to section 3 for help identifying a Douglas fir.
   3.1.3.2. A good Douglas fir specimen for the study will have:
      3.1.3.2.1. A diameter at breast height (d.b.h.) between 0.25 meters.
      3.1.3.2.2. Branches that are near enough to the ground to be reached with a hedge trimmer
      3.1.3.2.3. Healthy greenness to its leaves.
      3.1.3.2.4. Will not appear to be physically damaged by fire, lightning strike, cracking, or gun shot.
   3.1.3.3. If there is no Douglas fir within the 25 meter radius, expand the radius until a healthy specimen is identified.

3.1.4. Record general characteristics of the specimens state of health (use your best judgment, but record what you see).

3.1.5. Taking a xylem sample.
   3.1.5.1. Identify a healthy branch.
   3.1.5.2. Identify twigs with a diameter from 0.15 cm to 0.5 cm.
   3.1.5.3. Use hedge trimmer to cut the identified twigs from the branch.
   3.1.5.4. Cut twigs into pieces from 2 cm to 4 cm in length.
   3.1.5.5. Collect enough twig pieces to fill a 20 ml glass vial.
   3.1.5.6. Label the vial with:
      3.1.5.6.1. Collection date.
      3.1.5.6.2. Collection site (1, 2, 3).
   3.1.5.7. Para-film the vial.
   3.1.5.8. Place the vial into a plastic zip-lock bag.
   3.1.5.9. Place the vial in cooler with ice pack.

3.1.6. Repeat this process for all three of the sites within the study area.

3.1.7. Storing the sample in the lab:
3.1.7.1. Immediately upon returning from the field place the samples into the refrigerator

3.2. Soil Samples – Soil Corer

3.2.1. Using the Douglas fir that was identified for sampling move out a radius of 3 - 5 meters from the tree.

3.2.2. Identify a point 3 – 5 meters away from the tree that is relatively clear:
  3.2.2.1. No large visible rocks.
  3.2.2.2. No large roots.

3.2.3. Record general characteristics of the soil area that has been identified for sampling.
  3.2.3.1. Distance from Douglas fir specimen (3 – 5 meters)
  3.2.3.2. Distance from the phenocam

3.2.4. Taking a soil sample
  3.2.4.1. Identify the sample area
  3.2.4.2. Hammer the split core soil sampler into the ground to a depth of 0.45 meters
  3.2.4.3. Use a shovel to dig around the corer exposing it all the way to the 0.45 meter depth
  3.2.4.4. Making sure not to let any soil fall out of the corer, remove the corer from the ground
  3.2.4.5. Split the core in two and let the core sections sit in one half of the split
  3.2.4.6. Use a knife to separate the sections one by one
  3.2.4.7. Place the soil from each 5 cm section into a 20 mL vial
  3.2.4.8. Label the vial.
    3.2.4.8.1. Collection date.
    3.2.4.8.2. Collection site (1,2,3).
    3.2.4.8.3. Collection depth.
  3.2.4.9. Para-film the vial.
  3.2.4.10. Place the vial into a large zip-lock bag and label
    3.2.4.10.1. Collection date
    3.2.4.10.2. Collection site (1,2,3)
  3.2.4.11. Place the large zip-lock bag into a cooler with ice
3.2.4.12. Fill in the hole and landscape to even out the sampling area with the surrounding area

3.2.5. Repeat this process for all three of the sites within the study area.

3.2.6. Upon returning to the lab after field trip store all samples in the lab refrigeration unit

3.3. Soil Samples – Trench

3.3.1. Using the Douglas fir that was identified for sampling move out a radius of 3 - 5 meters from the tree.

3.3.2. Identify a point 3 – 5 meters away from the tree that is relatively clear:
   3.3.2.1. No large visible rocks.
   3.3.2.2. No large roots.

3.3.3. Record general characteristics of the soil area that has been identified for sampling.
   3.3.3.1. Distance from Douglas fir specimen (3 – 5 meters)
   3.3.3.2. Distance from the phenocam

3.3.4. Taking a soil sample
   3.3.4.1. Identify the sample area
   3.3.4.2. Use a shovel to dig to a depth of at least 1 meter
   3.3.4.3. Place a 1 meter ruler in hole in order to identify each 5 cm increment of soil
   3.3.4.4. Scrap a few centimeters away from the side of the hole
   3.3.4.5. Use a 20 mL vial to scoop soil at each 5 cm layer
   3.3.4.6. Label the vial.
      3.3.4.6.1. Collection date.
      3.3.4.6.2. Collection site (1,2,3).
      3.3.4.6.3. Collection depth.
   3.3.4.7. Para-film the vial.
   3.3.4.8. Place the vial into a large zip-lock bag and label
      3.3.4.8.1. Collection date
      3.3.4.8.2. Collection site (1,2,3)
   3.3.4.9. Place the large zip-lock bag into a cooler with ice
3.3.4.10. Fill in the hole and landscape to even out the sampling area with the surrounding area.

3.3.5. Repeat this process for all three of the sites within the study area.

3.3.6. Upon returning to the lab after field trip store all samples in the lab refrigeration unit.

3.4. **Precipitation Samples**

3.4.1. Locate one of the three phenocams.

3.4.2. Identify a tree within proximity to the phenocam with the following characteristics:

   3.4.2.1. There is mostly open sky above the tree
   3.4.2.1.1. This may require the use of a dead tree
   3.4.2.2. The tree should be in a condition that will allow for a rain bucket to be attached
   3.4.2.2.1. The tree should not be rotted out
   3.4.2.3. The tree should not be farther than 25 meters from the phenocam

3.4.3. Attach a standard rain gauge to the tree

   3.4.3.1. Using a battery powered drill, drill out a guiding hole for the screws (with a drill bit smaller than the screws that will be used)
   3.4.3.2. Use a screw driver to insert a screw, through the rain gauge retention hole, into the tree
   3.4.3.3. Verify that the rain gauge is securely attached to the tree

3.4.4. Attach a larger funnel to the standard rain gauge

   3.4.4.1. Place the funnel into the rain gauge
   3.4.4.2. Use screws to attach the funnel to the tree
   3.4.4.2.1. This will be destructive to the funnel (this is ok)

3.4.5. Add 5 mm of mineral oil to the base of the rain gauge (this will retard evaporation)

3.4.6. Mark the standard rain gauge location.

   3.4.6.1. If allowed by the National Forest use a yellow spray paint (non-toxic) to tag the tree with the initials PL (Papuga Lab).
   3.4.6.2. If paint is not allowed by the park use yellow marking tape to identify the tree.
3.4.6.2.1. Wrap the marking tape around the tree and tie off.

3.4.7. Taking a sample

3.4.7.1. Extract the water and mineral oil from the rain gauge into a 200 ml plastic bottle
3.4.7.2. Para-film the bottle
3.4.7.3. Label the bottle
   3.4.7.3.1. Collection date
   3.4.7.3.2. Collection location (1,2,3)
3.4.7.4. Place the bottle into a zip-lock bag
3.4.7.5. Place the bottle into a cooler with ice
3.4.7.6. Place 5 mm of mineral oil into the rain gauge

3.4.8. Repeat this process for all three of the sites within the study area

3.4.9. Storing the sample in the lab:

3.4.9.1. Set up peristaltic pump
   3.4.9.1.1. Run a hose through the pump
   3.4.9.1.2. Attach a filter onto one side of the hose (will filter the air)
   3.4.9.1.3. Attach a container with a filter holder at the base
   3.4.9.1.4. Add a new filter to the filter holder
   3.4.9.1.5. Attach the hose to the top of the container
3.4.9.2. Remove the precipitation sample from the cooler
3.4.9.3. Place the sample with mineral oil into the container
3.4.9.4. Place a 20 ml vial underneath the release from the filter holder at the base of the container
3.4.9.5. Run the peristaltic pump
   3.4.9.5.1. Ensure the pump is run in the proper direction
      3.4.9.5.1.1. You want to bring air into the hose that will push the sample through the filter, while holding the mineral oil back
3.4.9.6. Para-film the vial
3.4.9.7. Label the vial
   3.4.9.7.1. Collection date
   3.4.9.7.2. Collection site
3.4.9.8. Place the vial into the refrigerator
3.4.9.9. Repeat this process for all precipitation samples that were collected.

4. **IDENTIFYING THE DOUGLAS FIR \textit{(PSEUDOTSUGA MENZIESII)}**

4.1. The tree has dense and compact foliage, holds its branches well to the ground. It grows 40 to 60 feet high

4.2. The needles are soft, flattened, slightly pointed, 1 to 1-1/2 inches long and grow around the branch to give it a full, rounded appearance. They are grooved on the upper surface, and have a white band on each side of a prominent midrib beneath.

4.3. The twigs are fine and the buds are long, pointed, dark orange-red in color and shiny. They are sometimes termed "cigar-shaped."

4.4. The cones of the Douglas fir are distinct, 1-1/2 to 4 inches long. Protruding from beneath the thin rounded scales is a conspicuous, three-pointed bract.

4.5. On older trees the reddish brown bark is broken into oblong, longitudinal plates and may be 10 to 12 inches thick. On young and smaller trees, the bark is thin, ashy gray and may have resin blisters

4.6. Photographs to identify the Douglas fir

4.6.1. Douglas fir needles

![Douglas fir needles](image1)

4.6.2. Douglas fir fruit

![Douglas fir fruit](image2)
4.6.3. Douglas fir fruit

4.6.4. Douglas fir twig

4.6.5. Douglas fir
4.6.6. Douglas fir
APPENDIX D: LAB PROTOCOL

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1. SUMMARY
This appendix describes the protocol that was used in analyzing soil, stem, and liquid sample on the Picarro L2120-I Cavity Ring-Down Spectrometer equipped with an induction module (IM-CRDS). All samples were stored in a refrigerator in the lab until they could be analyzed. First, the system was allowed to reach the operating condition of 250 ppm of H2O present in the spectrometer. Second, based on the type of sample that was being analyzed (soil, stem, liquid), blanks were run at a different gas pressure. Third, based on the type of sample that was being analyzed (soil, stem, liquid), standards were run to correct for instrument drift. Fourth, samples were analyzed with guidance provided by the lab protocols regarding when a sample analysis was complete. No more than five samples were analyzed for an analyzed standard. Lastly, the power down instructions were important for proper storage of the spectrometer while it was not in use.

2. PICARRO EQUIPMENT
   2.1. L2130-I isotopic H2O Cavity Ringdown Spectrometer Analyzer
   2.2. Induction Module (IM)
   2.3. Both the analyzer and IM are connected to an ultra-zero compressed air tank via metal tubing

3. SET UP
   3.1. Initially the analyzer should be powered on (this is visible as a green light on the lower left side of the analyzer).
   3.2. Make sure the external vacuum pump for the analyzer is also turned on when the analyzer is on (the pump should never be turned off)
   3.3. Turn on the gas (fully by turning counterclockwise)
       3.3.1. The gas flow will vary with the recipe
   3.4. Turn on the computer monitor
   3.5. Turn on the IM (there is a switch on the bottom right of the rear of the IM)
       3.5.1. The IM is the small grey box sitting on top of the analyzer.
   3.6. Turn on the coordinator launcher from the desktop by clicking on the coordinator launcher icon and selecting the coordinator for the IM
       3.6.1. Hit accept from the CRDS card screen
3.6.2. Choose the sample recipe from the dropdown menu
   3.6.2.1. ‘blank’: used for the blanks (first three runs)
   3.6.2.2. ‘sample’: used for the standards and the unknowns
3.6.3. A new pop up will ask you to purge and prepare your sample. Accept after operating conditions are met.
3.7. At this point the analyzer needs to reach operating conditions
   3.7.1. Reaching operating conditions means that the analyzer needs to dry down below H2O ppm of 250
      3.7.1.1. Use an empty vial to assist in the dry down process
      3.7.1.2. Depending on recipe the dry down may take between 30 - 60 minutes.
      3.7.1.3. To speed up the dry down set the outlet pressure on the regulator to 2.5 psi until the H2O ppm is 250 ppm. At this point return the gas flow to the operating gas flow parameter as specified for sample recipes
   3.7.2. The analyzer is running properly when the following conditions are met: (these conditions are normally met after the analyzer is turned on for 30-60 minutes)
      3.7.2.1. Cavity temperature: 80 degrees Celsius
      3.7.2.2. Cavity pressure: 50 torr
      3.7.2.3. If they are drastically different values then something is wrong, and the analyzer may need a reboot. If there is a problem, you will see a system alarm (red), at this point call Lejon.
3.8. While waiting for the analyzer to get ready for running blanks and samples prepare the following items
   3.8.1. Place two vials on the external gas flow
   3.8.2. Prepare vial caps
      3.8.2.1. Combine a plastic cap and a septum (glossed side upward, pointing out of vial)
   3.8.3. Prepare a clip with paper dots
      3.8.3.1. Take 1 clip and place 2 dots together. Insert dots into clip and clamp down.
      3.8.3.2. Bend clip with needle nose pliers 90 deg. on one end.
      3.8.3.3. Make sure the sample holder lays flat inside the vial.
   3.8.4. Remove the syringe from the DI water beaker
3.8.4.1. Dry off the syringe with kimwipes
3.8.4.2. Rinse the syringe out 3 times with standard/unknown
3.8.5. Prepare the lab notebook
3.8.6. Prepare the notepad
  3.8.6.1. Save as a .csv file
  3.8.6.2. Load into the coordinator

4. **RUNNING BLANKS**

4.1. Ensure that the gas is set to the correct pressure:
   4.1.1. Soils: 1.5 psi
   4.1.2. Woody stems: 3.0 psi
   4.1.3. Leaves: 2.5 psi
   4.1.4. Liquids: 2.5 psi
4.2. Accept to purge the system from the pop-up window
4.3. When the analyzer is ready for a sample you will be prompted by a pop-up window
4.4. Insert an empty vial into the IM and run
4.5. Discard the first run.
4.6. Include the second and third blank runs unless the following occurs
   4.6.1. Maximum H2O peaks greater than 2,000 ppm
   4.6.2. H2O volume greater than 1.0 microliters
4.7. After running the blanks, select ‘sample’ from dropdown menu to run standards and unknowns.

5. **RUNNING STANDARDS**

5.1. One standard is run per five unknowns
5.2. The recipe is determined by which unknowns are going to be run
5.3. Determining which standard to use

<table>
<thead>
<tr>
<th>sample</th>
<th>standard</th>
<th>d18O ‰</th>
<th>dD ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>liquid</td>
<td>shantz</td>
<td>-9.01</td>
<td>-68.72</td>
</tr>
<tr>
<td>soil</td>
<td>shantz</td>
<td>-9.01</td>
<td>-68.72</td>
</tr>
<tr>
<td>stem</td>
<td>destiny</td>
<td>0.24</td>
<td>-1.23</td>
</tr>
</tbody>
</table>
5.4. Standards are run as liquid unknowns (see section 5 parts 1-13 and part 14.1)
5.5. As soon as you are done with the standard re-parafilm the vial and return to the refrigerator

6. **RUNNING UNKNOWNS**

   Universal procedures (to be applied to all unknown sample procedures)

6.1. Remove an unknown from the refrigerator
6.2. Ensure to annotate sample description (unknown name and date) into lab notebook
6.3. Select the proper recipe from drop-down menu and accept
6.4. Select ‘sample’ from drop-down window and accept.
6.5. Accept and purge system.
6.6. While the analyzer is purging (approximately 30 sec) prepare the unknown
   6.6.1. Avoid evaporation by keeping the unknown vial caped until extracting a portion for analysis, after which immediately recap the vial
6.7. When the analyzer is ready, you will be prompted by a pop-up window
6.8. Place the unknown into the IM and run
6.9. Use one vial per unknown
6.10. Replace caps after 5 runs
6.11. When done analyzing the unknown re-parafilm the vial and return to the refrigerator
6.12. Run the unknown a minimum of four times. The first run is ignored when determining convergence. Run unknown a minimum of three more times to reach convergence and standard deviation parameters. It may take more than four runs to meet the acceptance criteria.
   6.12.1. Across three runs, the standard deviations must be
   6.12.1.1. D18O = 0.75 ‰
   6.12.1.2. D2H = 2.5 ‰
   6.12.2. There must not be a linear trend in the values of d18O and d2H, i.e., the errors should be fluctuating around an asymptote, rather than gradually increasing or decreasing
6.13. Review the signature of the curve for each run of a standard/unknown. Keep all the values for analysis unless:

6.13.1. The curves deviate in appearance, meaning that the IM did not properly heat up and extract all the water from our unknown for analysis.

6.13.2. If there is a curve that deviates from the other curves in appearance (which can be seen in the shape, or a significant change in the height of the peak) ignore this value when determining convergence and standard deviation.

6.13.2.1. This means that if you have two values that are developing towards convergence and then the third deviates, you should ignore this third set of isotopic values and continue with your runs until you get convergence and standard deviation.

6.13.3. A curve is not deviating from the normal curve if it is caused by a H2O volume (μl) greater or less than our parameter values of 3-10 μl

6.14. Record est. H2O volume (μl) and H2O maximum(ppm) in lab notebook

6.15. Sample types

6.15.1. Liquid

[Calibration - Higher Temperature]

polyA = 0.00021
polyB = 0.00001
polyC = 13
h2oLowThreshold = 250
preheatTime = 0
heatTime = 180
h2oEndHeatThreshold = 200

6.15.1.1. Using recipe ‘High temperature Calibration’ @ 2.5 psi gas flow

6.15.1.2. With the syringe, take 3 μL of unknown out of the vial

6.15.1.2.1. Immediately recap vial when not in use

6.15.1.3. Insert the syringe into the clip dots and inject unknown into them

6.15.1.4. Put the metal clip into a Picarro glass vial, cap and place in IM.
6.15.1.5. Run the unknown
6.15.1.6. Repeat the procedure for each successive unknown run
6.15.1.7. Reuse the clip and dots until the unknown is complete

6.15.2. Soil
[Sandy Test 25.4 480]
polyA = 0.00003
polyB = 0.4
polyC = 25
h2oLowThreshold = 250
preheatTime = 0
heatTime = 480

6.15.2.1. Using recipe ‘Sandy Test 25.4 480’ @ 1.5 psi gas flow
6.15.2.2. Prepare steel wool.
   6.15.2.2.1. Roll loose balls to a diameter just large enough to fit and remain in cylinder.
   6.15.2.2.2. Roll a long piece to use for cleaning the cylinder of soil remnants after unknowns are run
6.15.2.3. Take a clean cylinder, put steel wool ball in one end of cylinder
6.15.2.4. Pour soil into cylinder and cap with second wool ball
   6.15.2.4.1. Initially fill the cylinder halfway with soil, and see what your H2O ppm value is
   6.15.2.4.1.1. Keep the H2O ppm value between 3 and 10, with best results at 4 - 5
   6.15.2.4.1.2. Make mental note of the depth the cylinder was filled to and the H2O ppm and replicate with each successive cylinder
6.15.2.5. Put the cylinder into a Picarro glass vial, cap and place in IM
6.15.2.6. Run the unknown
6.15.2.7. Repeat the procedure for each successive unknown run
6.15.3. Stem

[Woody Stems 180]

polyA = 0.00003
polyB = 0.03
polyC = 15
h2oLowThreshold = 250
preheatTime = 0
heatTime = 180
h2oEndHeatThreshold = 200

6.15.3.1. Using recipe ‘Woody Stem 180’ @ 3.0 psi gas flow
6.15.3.2. Prepare a metal clip with a 90-degree bend on one end
6.15.3.3. Remove a stem from unknown vial
6.15.3.4. With a cutting surface underneath, slice off a small piece of the stem and discard, then cut a slice for analysis: approx. 1-2mm disc
   6.15.3.4.1. When not in use keep the stem stored in 2 ml plastic vial with cap
6.15.3.5. Place stem disc in metal clip (just like the paper dots)
6.15.3.6. Put the metal clip into a Picarro glass vial, cap and place in IM
6.15.3.7. Run unknown
6.15.3.8. Repeat the procedure for each successive unknown run
6.15.3.9. Reuse clip until folding clamps snap

7. CLOSING DOWN

7.1. After running the last unknown, in the recipe pop-up window dropdown menu, select ‘exit’
7.2. Record end gas in lab notebook
7.3. Shut off the gas!
7.4. Shut off the IM
7.5. Close the CRDS coordinator window
7.6. Do not close the CRDS Data Viewer window
7.7. Turn off the monitor
7.8. NEVER TURN OFF THE PUMP

7.9. Clean the work area up before leaving.
APPENDIX E: SUPPLEMENTAL DATA

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USA

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1. SUMMARY
This appendix presents tables of results related to the manuscript presented in Appendix A, but not appropriate for inclusion in the *Hydrological Processes* publication. Tables 1 and 2 were also presented in Appendix A, but reappear here for ease of access to the reader. Table 3 gives the isotopic value for all precipitation samples that were used in this study, including samples collected by the Papuga lab and the Gregg Barron-Gafford Research Group. Table 4 shows all the soil and stem samples that were used in this study as well as any precipitation sample that was collected by the Papuga lab. Table 5 shows the seasonal average of each sample type collected. Table 6 shows the isotopic value for all groundwater samplers that were collected and analyzed by staff from the SCM-CZO and used in this study. Table 7 shows each of the sampling days and compares the isotopic value of the stem sample with the most similar source waters isotopic value. Table 8 shows the source water that was dominantly used dependent on the season.

2. TABLE CAPTIONS
Table 1. The total and seasonal precipitation amount and percent for the 3 water years that span the period of study.
Table 2. The seasonal average isotopic value of precipitation.
Table 3. Precipitation isotopic values from the samples collected during the study period.
Table 4. Precipitation (only samples collected on days that stem and soil was collected), stem, and soil water isotopic values of samples collected during the study period. The values that are highlighted in yellow indicate a sample that was not within the target standard deviation during analysis on the Picarro.
Table 5. Seasonal isotopic values for all samples collected (precipitation, soil depth, groundwater, stem)
Table 6. Ground water seepage isotopic values for each sample in the study.
Table 7. Stem samples and the depth of soil that the isotopic comparison is associated with. The values that are highlighted in yellow indicate a sample that was not within the target standard deviation during analysis on the Picarro.
Table 8. Stem samples and the predominant depth of soil that the isotopic comparison is associated with seasonally
3. TABLES

Table 1.

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