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METHODS PAPER

Bagging: a cheaper, faster, non-destructive transpiration water sampling method for tracer studies

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Abstract
Purpose Stable isotope tracer experiments provide a powerful tool for understanding plant root distributions, resource uptake, niche partitioning and water cycling. Plant water is typically collected from pre-transpiring tissues to avoid the effects of evaporative isotope enrichment at the leaf surface, but extracting water from these plant samples is difficult and expensive. The purpose of this study was to test a simple transpiration bagging approach for measuring hydrologic tracer uptake.
Methods Sampling was performed as part of a depth-specific tracer experiment in which $^{2}H_2O$ was injected to target depths (5, 15, 30, 60, or 150 cm) in different replicated plots. One day following injections, leaves from three species were sealed in bags for 16 h and transpired water was collected. Water from pre-transpiring stem tissue was then collected in a separate set of samples and extracted using cryogenic distillation.
Results Deuterium concentrations from the two techniques were correlated ($R^2 = 0.84$) and both approaches produced similar descriptions of vertical root distributions for three dominant plant species. ^{18}O concentrations from the two techniques were not correlated.
Conclusion Bagging transpired water produced similar estimates of ^{2}H tracer uptake as the standard sampling technique. Bagging requires no destructive sampling, specialized laboratory equipment, training or consumables and is expected to halve sampling costs. While effective in this tracer experiment, bagging may be not be effective in natural abundance experiments, or tracer experiments with very small plants or small transpiration rates (i.e., early-season or arid sites).

Keywords Ecohydrology · Stable isotope · Root distribution · Tracer · Water uptake

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Introduction

Plant root distributions have long been a focus of ecologists (Grant and Dietrich 2017). For over 100 years it has been suggested that different rooting distributions may allow plant species to coexist by providing some species (e.g., deep-rooted trees) access to soil resources that are not available to other species (e.g., shallow-rooted grasses; Walker and Noy-Meir 1982; Ward, et al. 2013). Rooting distributions are also important to hydrologic cycles and plant responses to climate change (Orellana et al. 2012). Despite their importance, it remains difficult to measure water uptake by plants in the field (Holdo, 2013; Smithwick et al. 2014; Silvertown et al., 2015).

Stable isotope techniques have gained attention for their ability to describe water uptake patterns by different plants in the field (van der Heijden et al., 2015; Newberry et al. 2017; Rothfuss and Javaux, 2017; Barbeta et al. 2018; Dubbert and Werner 2019; Beyer

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et al. 2020). It is notable that isotope approaches measure water uptake and not root biomass because there is growing evidence that water uptake can be poorly correlated with root biomass (Schymanski et al., 2008; Kulmatiski et al. 2017). There are two broad classes of stable isotope approaches: natural abundance and tracer (Bishop and Dambrine 1995; da SL Sternberg et al. 2005; Rothfuss and Javaux 2017). Natural abundance approaches typically rely on naturally occurring, vertical isotope enrichment profiles in the soil that result from evaporation, though some studies also distinguish vadose from water table sources (Dawson and Ehleringer 1993; Asbjornsen et al., 2011). Evaporative enrichment produces isotopically-enriched soil water near the surface, however, these profiles typically only develop to 50 cm or less and can be diluted or confounded by different rain events (Ogle and Reynolds, 2004; Rothfuss and Javaux, 2017; Dubbert and Werner 2019). Tracer techniques introduce water enriched in heavy isotopes (typically of $^{2}H_2O$) to the soil (Mamolos et al. 1995; Sternberg et al. 2002; Beyer et al. 2016). Tracer can be flooded at the surface or injected to target depths (Bakhshandeh et al. 2016 l Volkmann et al. 2016). Injections require more labor but provide more detailed information about the vertical and horizontal distribution of root water uptake even in deep (i.e., > 50 cm) soils and in sites with non-descript evaporation enrichment profiles (Berry and Kulmatiski, 2017; Warren et al. 2015). Tracer techniques also have the advantage that they are less likely to be biased by natural variations in isotope concentrations across soil depths, across species, or due to exchange between plant tissues and xylem water (Kulmatiski et al. 2010; Barbeta et al. 2020; Chen et al. 2020).

Both natural abundance and tracer approaches rely on the fact that plant roots and stems do not distinguish $^{2}H_2O$ from H_2O, so it can be assumed that water in the stem of plants represents the average concentration of ^{2}H absorbed across the root system (Ehleringer and Dawson 1992; Newberry et al. 2017; but see Zhao et al. 2016; Barbeta et al. 2020; Chen et al. 2020). Because evaporative enrichment of 50–100 ‰ can occur at the leaf surface, enriched leaf samples may reflect either enriched soil water uptake or enrichment at the leaf surface (Dongmann et al. 1974; Cooper and DeNiro 1989; Kahmen et al. 2013). To ensure that sampled water reflects soil water uptake and not leaf enrichment, it has been important to collect stem water from plant tissues below transpiring tissues (Ehleringer and Dawson 1992; Cernusak et al. 2016). Isotope concentrations are typically low and reported in delta notation in units of parts per thousand (i.e., [{Rsa – Rstd}/Rstd] * 1000, where R is the ratio of, for example, ^{2}H to H, sa = sample, and std. = standard – typically Vienna mean standard ocean water).

In practice, there are several problems with sampling stem water (Fischer et al. 2019; Barbeta et al. 2020). First, extracting stem water is difficult (Millar et al. 2018; Beyer et al. 2020). Many water extraction procedures have been developed, but these techniques typically require some combination of costly vacuum pumps that can create pressures of 0.03 hPa, centrifuges, scientific glass-working, heating and cooling blocks and liquid nitrogen (Newberry et al. 2017; Millar et al. 2018; Fischer et al. 2019). Sample preparation in commercial labs can cost $10- $100 USD per sample. Second, isotope values in extracted stem water can be susceptible to temperature, extraction conditions, and organic contamination (West et al. 2010; Cui and Tian 2020). Third, stem water extraction requires destructive sampling. In our tracer experiments, we have found that it can be difficult to collect sufficient pre-transpiring tissues from small or uncommon plants or early in the growing season to be able to extract 1–2 mL of pre-transpiring sample water for isotope analyses.

Given the difficulties associated with stem water extraction, there has been a recent effort to develop simpler water sampling techniques (Millar et al. 2018; Fischer et al. 2019; Kübert et al. 2020). Induction modules on isotope analyzers that extract water directly from stem tissue have been an important advance, but they require that a technician attends to samples which take 5 to 10 min for analysis and results can be poorly correlated with cryogenically-extracted water (Cui and Tian 2020). Continuous sampling of water vapor in the field is also promising, but this approach requires either extensive sample collection and/or an isotope analyzer in the field (Wang et al. 2012; Volkmann et al. 2016; Kübert et al. 2020). Vapor equilibration techniques are also promising and require little specialized equipment, but they do require some preparation (grinding and centrifuging) and a technician's attention during analysis (Fischer et al. 2019). In contrast, cryogenic extraction requires specialized equipment and trained technicians, but the water samples produced can be run using an autosampler allowing 60 samples to be run with little attention from a technician.

Bagging of transpired water offers another potential option (Menchaca et al. 2007). To date, bagging has not

been used because evaporative enrichment at the leaf surface can easily obscure signals of evaporative enrichment in soil-obtained water (i.e., shallow, highly-enriched soil water vs. deep, less-enriched soil water; Cernusak et al. 2016). Leaf-surface enrichment, however, may not obscure signals in tracer experiments where sampled water may demonstrate deuterium concentrations of 1000's ‰ (Kulmatiski et al. 2010; Cernusak et al. 2016). Bagging transpired water can be expected to represent an integrated measure of transpiration water isotope concentrations during the collection period (Wang et al. 2012; Dubbert et al. 2014). If collected over many hours, there is an opportunity for sampled water to have equilibrated with leaf water (Roden and Ehleringer 1999; Menchaca et al. 2007; Dubbert et al. 2014). Further, bags are likely to create a humid, warm environment that will encourage transpiration and isotope equilibration and preclude evaporative enrichment (Menchaca et al. 2007). Bagged water is also less likely to be affected by organic contamination than stem-extracted water (Fischer et al. 2019). However, we are not aware of a study comparing results from bagged transpiration water to cryogenically-extracted pre-transpiring water (Menchaca et al. 2007; Millar et al. 2018; Fischer et al. 2019).

Methods

Here we tested a bagging approach to capture transpired water (Menchaca et al. 2007; Beyer et al. 2016). Plastic bags (18 cm by 19 cm zipper top, freezer bags) were secured to plants and left for ~16 h – enough time to collect at least 1 mL of transpired water from each plant. At the same time, non-transpiring plant tissues were collected from separate plants in the same experiment and stem water was extracted using batch cryogenic extraction (Vendramini and Sternberg 2007; Kulmatiski et al. 2010). Separate plants were sampled because stem water sampling is destructive and because we did not know whether or not bagging would change evaporative demand and water uptake patterns, so we did not want to collect stem water samples from previously bagged plants. Values from the two techniques were compared directly through correlation. Sampling was performed as part of an experiment that was designed to describe the vertical rooting profiles of dominant plants in a mesic grassland. More specifically, plant tissues were collected from plots in which $^{2}H_2O$ was injected to one of five soil depths (Kulmatiski et al. 2020). Research was conducted during peak growing season, 2016 in an ex-arable field converted to tallgrass prairie at Cedar Creek Ecosystem Science Reserve, East Bethel, MN, USA (45.396989, −93.191277). Mean annual precipitation is 796 mm and mean annual temperature is 6.7 °C (PRISM Climate Group).

Tracer injections The tracer experiment broadly followed the approaches described by Kulmatiski et al. (2017). Briefly, in 2016, 25 plots (3 m diameter) were placed 5 m apart in a grid. Each plot was randomly assigned to one of five replicates of each of five soil depths (5, 15, 30, 60 and 150 cm). Within each plot, pilot holes to the target depth were drilled in a 15 cm by 15 cm grid, using a 1 cm drill bit and a hammer-drill (Hilti TE-60, Tulsa, Oklahoma). Custom-made syringes using 16-gauge thin-walled hypodermic tubing (Vita Needle Company, Needham, Massachusetts, USA) were used to inject 1 mL of 70% $^{2}H_20$ (Cambridge Isotopes, Tewksbury, MA). This tracer injection was followed by 2 mL tap water injection to clear tracer from the syringe.

Tracer concentrations in transpired water One day after tracer injection, clear plastic sandwich bags were secured to leaves. Without compressing leaves together, as much leaf tissue as possible was placed in each bag (18 cm by 19 cm). Bags were placed on plants between 9 am and 2 pm and left in place until the following morning. Previous work suggests that leaf and surrounding water equilibrate within a few hours (Roden and Ehleringer 1999). Cooler evening temperatures encouraged condensation in the bags. Condensed water was pipetted into 2 mL vials and refrigerated until sample analysis. One to three bags were placed on each species in each sampled plot. The dominant forb *Artemisia ludoviciana* (6.13% total biomass, Nutt.), C3 grass *Poa pratensis* (36.21%, L.) and C4 grass *Schizachyrium scoparium* (3.50%, Nash) were sampled from all injection depths (i.e., 5, 15, 30, 60, 150 cm; Ownbey, 1991). These three species represent 46% of total biomass in the field (measured in Cedar Creek's 2016 Experiment 002 survey, which was performed one week after tracer injections and plant sampling).

Tracer concentrations in non-transpiring plant tissues Two days after injections in each plot (Kulmatiski and Beard 2013; Mazzacavallo and

Kulmatiski 2015; Warren et al. 2015), non-transpiring tissues from one to three individuals of each of three target species were clipped with rinsed clippers, placed in pre-made 19-mm wide, medium-walled borosilicate tubes (Pyrex, Corning, NY, USA), sealed with parafilm and placed on ice. For each species, non-green tissues from within 2 cm of the root crown were sampled. Samples were moved to a freezer within six hours. Water from plant tissues was extracted using batch cryogenic distillation (Vendramini and Sternberg 2007; Kulmatiski et al. 2010) within two weeks from sampling date. More specifically, each sample vial was placed in liquid N, evacuated to 50 milliTorr, and sealed with a torch. The top of the sample tube, which contained the plant sample, was placed in an aluminum block and heated to 95 °C for four hours. At the same time, the bottom of the tube was placed in an aluminum block that was sitting in a liquid N bath. After four hours, or when ice placed on the sample tube did not cause any condensation, the tube was cracked and sample water was transferred to a 2 mL vial.

Isotope analyses Water samples from both techniques were analyzed for hydrogen and oxygen isotopes on a wavelength scanned cavity ring-down spectrometer (Picarro L-2120i; Picarro Instruments, CA, USA). Raw delta values of the measured hydrogen and oxygen were normalized to the VSMOW-SLAP scale using three calibrated waters (δ values of 12, 242 and 745 ‰). These calibrated waters (i.e., standards) were included for roughly every ten samples. The isotopic data for plant extracts were checked and adjusted for spectral contamination using ChemCorrect® software (Picarro Inc.). Deuterium isotope values [in delta notation (δ)] were converted to deuterium excess values (δ_e) to control for natural isotope enrichment caused by evaporation as follows: $\delta_e = \delta^2H - [(8 * \delta^{18}O) + 10]$ (Craig 1961; Kulmatiski et al., 2010; Mazzacavallo and Kulmatiski, 2015).

Statistical analyses

Two general approaches were used to compare sampling techniques. First, paired T-tests were used to test for differences between sample values from the two techniques and simple correlations were made to describe the relationship between sample values from the two technique. Because samples were from different individual plants and sometimes different replicate plots and because the number of replicate samples within plots varied, sample values were averaged by species and depth treatment prior to comparisons. We also compared the variation among samples by comparing standard deviations of data from the two sampling techniques.

To provide a more biologically relevant test, we compared the rooting profiles that each sampling technique described. Differences among tracer uptake rooting profiles were tested using generalized additive mixed models (GAMMs; Wood 2004). GAMMs were used to approximate the continuous soil profiles of tracer uptake with depth using a beta likelihood with a logit link for the linear predictor (soil depth; Wood 2004; Kulmatiski et al. 2017). GAMMs had four "knots" to allow for a smooth interpolation between the five sample depths. Models were fit either with bagged and stem water samples together (all together model) or separate (all separate model) and the model with the lowest AIC was the model that best balances goodness-of-fit against parsimony. Models were fit in R (R Core Research Team 2004) using the gam function from the mgcv package (Wood 2004).

Results

A total of 86 bagged and 74 stem water samples were collected. Bagged water δ_e values ranged from 23 ‰ to 9311 ‰. Stem water δ_e values ranged from 3 ‰ to 8375 ‰. At the plot level (i.e., after species replicate samples within plots were averaged), there were 44 bagged and 63 stem water samples. When these samples were paired by plant species and plot, δ_e values were correlated ($F_{1,37} = 60.7$, $P < 0.001$, $R^2 = 0.63$). To incorporate all values (i.e., values that represented the same species and depth of injection, but not necessarily the same replicate plot), the mean value across replicate depth plots were compared. This produced a balanced, paired comparison of 15 bagged samples and 15 stem water samples (e.g., bagged water from *P. pratensis* in 30 cm plots compared to stem water *P. pratensis* in 30 cm plots). There was no difference between these bagged and stem water δ_e values ($T_{1,14} = 0.043$, $P = 0.67$) and these δ_e values were correlated (Fig. 1; $F_{1,14} = 66.3$, $P < 0.001$, $R^2 = 0.84$).

The biological interpretation of results from the two techniques was similar: there was no difference between

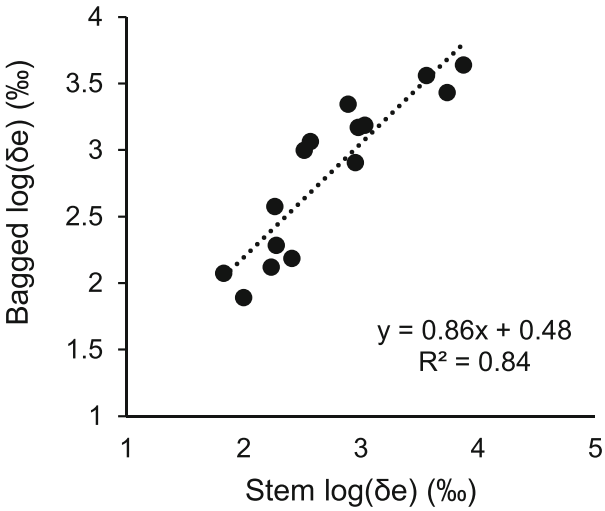
models that combined bagging and stem water data (all together models) and models that separated bagging and stem water data (all separate models; Table 1; Fig. 2). This pattern was consistent for each species individually and for all species together (Table 1: Fig. 2).

Across all bagged and all stem samples, ^{18}O concentrations were -2.2 ± 0.5 ‰ and 0.1 ± 1.3 ‰, respectively. Mean ^{18}O concentrations from plots did not differ ($T_{1,14} = 1.54$; $P = 0.14$), but there was no correlation between bagged and stem ^{18}O values at the plot level ($F_{1,29} = 0.46$, $P = 0.50$).

Discussion

There has been a growing interest in simplifying plant water sampling in hydrological isotope experiments (Newberry et al. 2017; Millar et al. 2018; Fischer et al. 2019; Beyer et al. 2020). Here we show that a simple transpiration bagging technique produced similar results to a traditional, but more cumbersome stem-water extraction technique (Menchaca et al. 2007). Tracer ^{2}H concentrations in bagged and stem-water samples were well correlated and both techniques produced similar descriptions of plant species rooting distributions. Both techniques required similar effort in the field, though the stem-water technique required destructive plant sampling and bagging did not. More importantly, stem-water collection using cryogenic distillation requires specialized equipment, training and consumable costs and bagging does not (Menchaca et al. 2007; Beyer et al. 2020). In summary, for a tracer experiment, the bagging technique was faster, cheaper, did not require destructive sampling and produced similar results to traditional stem-water collection and cryogenic distillation. It is important to note, however, that a lack of correlation between ^{18}O values from the two techniques suggested that while bagging was effective in a ^{2}H tracer experiment, that bagging may not be appropriate for natural abundance experiments.

Sampling plant water near the leaf surface has been avoided because evaporation can result in leaf tissue ^{2}H enrichment that masks differences in stem water isotope concentrations that are used to infer the depth of soil water uptake (Dongmann et al. 1974; Cooper and DeNiro 1989; Kahmen et al. 2013; Barbeta et al. 2020). However, we found no evidence of leaf-level evaporative enrichment in bagged samples. Bagged ^{18}O concentrations (-2.2 ± 0.5 ‰) were not more enriched than stem-water ^{18}O concentrations (0.1 ± 1.3 ‰). Similarly, the mean of the ten smallest bagged and stem-water ^{2}H concentrations (i.e., samples that were unlikely to be affected by tracer uptake) were 33 and 49 ‰, respectively. We suspect that bagging plants for 16 h provided a closed system that allowed equilibration between transpired water and leaf water and minimized the effects of evaporative enrichment at the leaf surface (Roden and Ehleringer 1999; Menchaca et al. 2007; Dubbert et al. 2014).



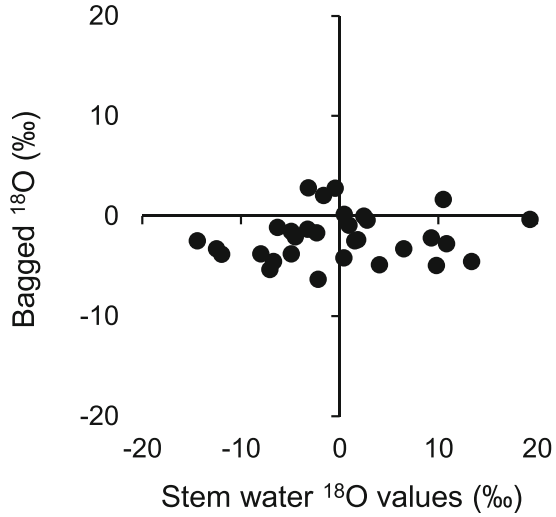


Fig. 1 Correlation between (a) deuterium excess (δ_e) and (b) ^{18}O in transpired water (bagged) and cryogenically-extracted water from pre-transpiring plant stems. Samples were collected from plants in plots that received equivalent amounts of $^{2}H_2O$ at different target depths (5–150 cm) in a tallgrass ecosystem, Minnesota, USA. Values in delta notation and parts per thousand units (‰)

Table 1 AIC table for models of tracer uptake by depth measured using either a transpiration bagging technique or a traditional stem water extraction technique. Bagging and stem water measurements were combined in the 'All together' models of tracer uptake by depth and separated in the 'All separate' models. Results shown for three plant species (*A. ludoviciana, P. pratensis and S. scopularum*) and for the three species combined

Model	logLik	AIC	ΔlogLik	ΔAIC	df
A. ludoviciana					
All together*	−470	950	0	0	5
All separate*	−467	951	3	1	8
P. pratensis					
All together*	−225	459	0	0	5
All separate	−226	465	1	6	5
S. scopularum					
All together*	−370	749	0	0	5
All separate	−372	756	2	7	6
All species					
All together*	−1082	2175	0	0	5
All separate	−1082	2181	0	6	8

Abbreviations: logLik, log likelihood; AIC, Akaike's information criterion; df, degrees of freedom

*Indicates top model based on ΔAIC <2 criteria

There was no evidence of leaf-level enrichment in this experiment, but even if some enrichment had occurred, it is likely that the large ^{2}H concentrations often measured in tracer experiments would have obscured these effects (Kulmatiski et al. 2010; Berry and Kulmatiski, 2017). Further, if it were to have occurred, leaf enrichment would likely have occurred in all species, so bias caused by enrichment would be limited to variation in enrichment among species (e.g., between C3 and C4 plants) which would have an even smaller effect on tracer concentrations. In short, bagging has not been used due to concerns of evaporative enrichment at the leaf surface, but this effect was not evident, or likely to be important in a tracer experiment such as this one (Wang et al. 2012; Volkmann et al. 2016; Kübert et al. 2020).

Bagging and stem-water sampling produced similar descriptions of vertical root distributions (Fig. 2). Both techniques revealed decreasing tracer uptake with soil depth, and that *A. ludoviciana* had the shallowest and *S. scopularum* had the deepest mean depth of tracer uptake. However, among the three sampled species, mean tracer uptake depth was between 15.6 and 18.4 cm for bagged samples and between 7.3 and 9.7 cm for stem samples. The mean depth of uptake was deeper for bagged samples because bagged samples had non-significantly smaller ^{2}H concentrations in 5 cm plots and larger ^{2}H concentrations in 15 and 30 cm plots than stem-water samples. The cause of these differences is not clear, but likely reflected the fact that bagged samples described the isotope concentrations in

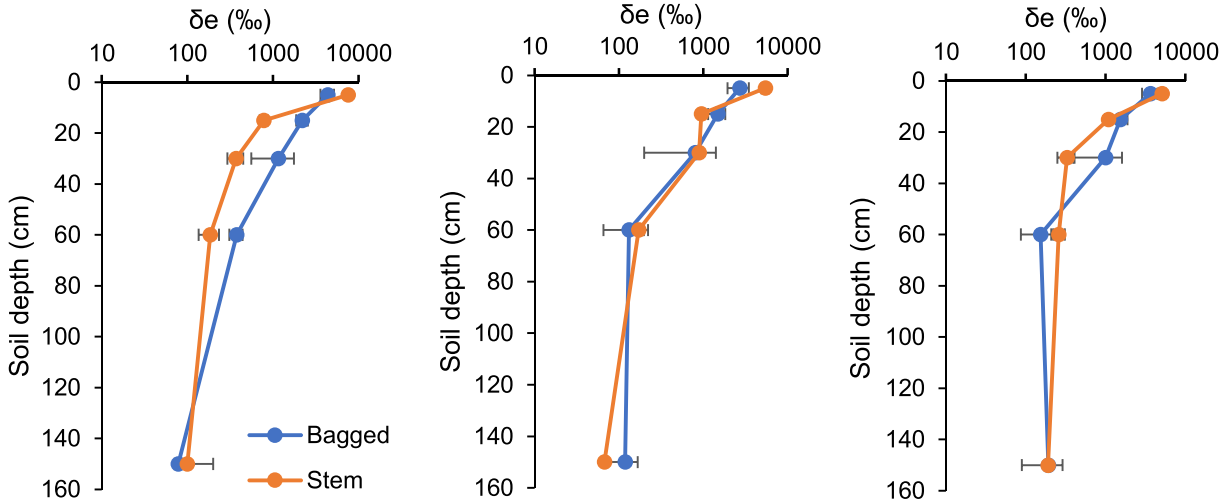


Fig. 2 Deuterium excess concentrations (δ_e; $\log_{10}$ scale) in bagged transpiration water (bagged) or cryogenically-extracted water from pre-transpiring tissues (stem) from three dominant plant species: a) *A. ludoviciana*, b) *S. scoparium,* and c) *P. pratensis* in plots that received $^{2}H_2O$ injections to 5, 15, 30, 60 or 150 cm. General additive mixed models did not support separating rooting patterns from the two techniques. Values in delta notation and parts per thousand units (‰). Error bars represent variation among replicate field plots

transpired water that was allowed to equilibrate with leaf water for 16 h, whereas stem-water samples represented the water in a few cm of stem tissue (Kühnhammer et al. 2020).

Different sampling techniques have different strengths and weaknesses. While stem water extraction is the standard method for collecting stem water samples for isotope analyses, it was not immediately obvious that stem water extraction provided more biologically relevant data than transpiration bagging in this experiment (Barbeta et al. 2020; Chen et al. 2020; Kühnhammer et al. 2020). A recent study indicated that cryogenic distillation can produce samples that are less enriched than transpiration water (Chen et al. 2020). Further, because stem water samples represent only a few cm of transpiration water they are unlikely to represent changes in water uptake that may occur within a day (Volkmann et al. 2016; Kühnhammer et al. 2020). Techniques such as bagging that integrate longer transpiration times may be more appropriate than stem water sampling (Volkmann et al. 2016). While it may be possible to develop detailed models of isotope fractionation for the soil through different plants and into a transpiration-collecting bag (Chen et al. 2020; Kühnhammer et al. 2020); perhaps a more biologically relevant approach will be to use results from these different techniques to make predictions about plant water uptake, water cycling and plant landscape abundance and validate these predictions against observed data. Soil water data was not available for this study, but this approach has been used successfully in other studies (Holdo 2013; Mazzacavallo and Kulmatiski 2015; Kühnhammer et al. 2020; Kulmatiski et al. 2020).

Costs

There are many approaches used to extract stem water, and costs are likely to vary among them Kübert et al. 2020). In commercial labs, sample extraction costs vary widely from $10 to $100 per sample. It is common for sample extraction to double analysis costs. Our laboratory uses a custom batch distillation extraction procedure (Vendramini and Sternberg 2007). This procedure was developed to allow the collection of large, relatively dry samples from semi-arid systems. The procedure requires a high vacuum pump (~$1500), custom made vacuum line (~$1500), pressure gauge (~$300), glassworking station (~$500), and custom-milled aluminum blocks (~$1500). After these initial costs, glass tubing costs about $0.75 USD per sample and must be made by hand. A minimally-trained technician can produce about 150 sample tubes a day. The same technician can evacuate, isolate (flame off) and extract roughly 50 samples a day. Propane, oxygen and liquid nitrogen add roughly another $0.25 USD per sample. Thus, sample preparation requires roughly $1 USD per sample in materials and 10 min of labor. Broken glassware tends to result in a 10% extraction failure rate. All of this equipment, training and materials are unnecessary with the bagging approach. Thus, bagging can be expected to cut costs in half or double sample sizes.

Applications and limitations

This study was performed as part of a tracer experiment in a mesic grassland in the middle of a growing season. Results demonstrate that transpiration bagging was a faster, cheaper method of sampling plant water uptake in these conditions. However, a lack of correlation between ^{18}O values from the bagged and stem-water samples suggested that bagging may not be appropriate for natural abundance experiments. Further, it may be difficult to collect 1 mL of transpired water when leaf area or transpiration rates are small; for example, early in the season or in arid systems. Larger, white bags and longer bagging periods may be necessary in these conditions. Heat stress (e.g., wilting) was not noticed in this study but could be a problem in hotter, sunnier conditions or where bags have to be left in place longer to accumulate sufficient water. With only 160 samples total from three herbaceous species in one experiment, results will need to be tested on more species (e.g., woody species) in more systems. However, results showed that, in this tracer experiment, transpiration bagging produced similar descriptions of plant rooting distributions with less effort than stem-water sampling techniques.

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Authors' contributions AK and LEF conceived of and executed the research and analyzed and wrote the manuscript.

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Compliance with ethical standards

Conflicts of interest The authors have no conflicts of interest or competing interests to declare.

Ethics approval There were no ethics approvals related to this research.

Consent to participate Not applicable.

Consent for publication Both authors consent publication of this manuscript.

Code availability No unique code is associated with this manuscript.

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