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Spatial variation in throughfall, soil, and plant water isotopes in a temperate forest

Running Head: Variation of water isotopes in a temperate forest

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Abstract

Studies of stable isotopes of water in the environment have been fundamental to advancing our understanding of how water moves through the soil-plant-atmosphere continuum; however, much of this research focuses on how water isotopes vary in time, rather than in space. We examined the spatial variation in the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of throughfall and bulk soil water, as well as branch xylem and bulk leaf water of *Picea abies* (Norway Spruce) and *Fagus sylvatica* (Beech), in a 1 ha forest plot in the northern Alps of Switzerland. Means and ranges of water isotope ratios varied considerably among throughfall, soil, and xylem samples. Soil water isotope ratios were often poorly explained by soil characteristics and often not predictable from proximal samples. Branch xylem water isotope values varied less than either soil water or bulk leaf water. The isotopic range observed within an individual tree crown was often similar to that observed among different crowns. As a result of the heterogeneity in isotope ratios, inferences about the depth of plant root water uptake drawn from a two end-member mixing model were highly sensitive to the soil sampling location. Our results clearly demonstrate that studies using water isotopes to infer root water uptake must explicitly consider how to characterize soil water, incorporating measures of both vertical and lateral variation. By accounting for this spatial variation and the processes that shape it, we can improve the application of water isotopes to studies of plant ecophysiology, ecohydrology, soil hydrology, and paleoclimatology.

Key words

ecohydrologic separation, hydrogen isotopes, leaf water enrichment, mixing models, oxygen isotopes, root water uptake, spatial autocorrelation

Introduction

Tracing stable isotopes of water ($^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/\text{H}$) through the soil-plant-atmosphere continuum has been essential for addressing many ecohydrological questions. Recent applications include studies of the transit times and flow paths of water in soils (Sprenger, Seeger, & Blume, 2016b), the depth of plant root water uptake (Goldsmith et al., 2012), leaf physiological response to climate (Bögelein, Thomas, & Kahmen, 2017), the relative roles of evaporation versus transpiration in returning water to the atmosphere (Jasechko et al., 2013), and the reconstruction of past climate conditions (Saurer, Spahni, Frank, & Joos, 2014).

Addressing such questions relies on understanding the isotopic fractionation and mixing processes that act on water as it moves through an ecosystem (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002). Precipitation is subject to evaporative fractionation, isotopic exchange, and mixing as it passes through the canopy (Allen, Keim, & McDonnell, 2015). This throughfall is further altered as it infiltrates into the soil and variably mixes with existing pools of soil water or becomes isotopically enriched by evaporation from the soil surface (Sprenger, Leistert, & Gimbel, 2016a, Benettin et al., 2018). Water may then be taken up by plant roots at different locations in the soil, a process that generally occurs without fractionation (but see Ellsworth & Williams, 2007; Zhao, Wang, Cernusak, & Liu, 2016). Water that reaches the leaves from the plant xylem is subject to evaporative enrichment, while some of the remaining leaf water is incorporated into photosynthetic assimilates.

Much of our understanding of how water isotopes move through ecosystems is based on temporal sampling – studying how water isotopes vary at a given location as a function of time. On average, the studies used in a recent synthesis on plant root water uptake from soil and groundwater measured the water isotopes at 8 different time points, with 4 replicate individuals of 3 different plant species and 3 replicate soil profiles ($n = 76$ studies from 2010-2016; Evaristo & McDonnell, 2017). While many of these studies contrasted locations (e.g. ridge top, slope and valley bottom; Gaines, Stanley, Meinzer, & McCulloh, 2016), none of them explicitly measured the spatial variation in soil or plant water isotopes within a given location.

Explicitly measuring the spatial variation in water isotopes is critical to understanding the scales at which patterns occur, as well as for revealing the processes that drive those patterns

(e.g., Vachaud, de Silans, Balabanis, & Vauclin, 1985). Studies of spatial variation are often based on the premise that two observations made close to one another in space are more likely to be similar than two observations made farther apart. The presence and scale of these spatial autocorrelations have important implications for both study design and interpretation of results (Fortin, Drapeau, & Legendre, 1989; Hurlbert, 1984; Legendre, 1993). For instance, how do we know whether changes in the depth of plant root water uptake inferred from stable water isotopes at a given site are representative of that site? Moreover, how do we know whether the depth of plant water uptake at one site is representative of other neighboring sites? Or perhaps most importantly, how can we use spatial variation to better understand the processes that lead to differences in the depth of plant water uptake?

There has been considerable research studying large-scale spatial patterns in the isotopes of precipitation (Bowen, 2003), surface water (Brooks, Gibson, Birks, & Weber, 2014), and groundwater (West, February, & Bowen, 2014), as well as predicting spatial patterns of leaf water isotopes based on precipitation isotopes (West, Sobek, & Ehleringer, 2008). Mapped water isotope patterns, often referred to as ‘isoscapes,’ have led to novel insights into hydrological processes and served as important tools for visualization (Bowen, 2010). However, our understanding of fine-scale spatial patterns of water isotopes as they move through an ecosystem, beyond their variation as a function of soil depth, remains limited.

We studied the spatial variation in stable isotopes of throughfall, bulk soil, branch xylem and bulk leaf water in a 1 ha forest plot in the northern Alps of Switzerland. Understanding the spatial variation in water isotopes along the soil-plant-atmosphere continuum can inform many different applications of water isotopes, particularly with respect to uncovering processes that cannot be inferred through temporal studies alone. Our objectives were to describe 1) how water isotope ratios vary among these different pools, 2) the extent to which the variation within and among pools is correlated in space, and 3) the underlying processes that structure that variation by studying relationships between soil characteristics and soil water isotope ratios.

Methods

Site Description

The study was carried out in a 1 ha plot established near Leissigen, Switzerland (SW corner: 46.651°, 7.754°; 722 m asl). The site is a northwest-facing (ca. 290°) forest slope ($25 \pm 6^\circ$). It is dominated by *Fagus sylvatica* L. (Beech) and *Picea abies* (L.) H. Karst. (Norway Spruce). Leaf area index was determined at the time of sampling from 41 random locations within the site under diffuse light conditions with alternating open sky and closed canopy readings facing due north at 1.5 m height with a 45° view cap (LAI-2000; LI-COR, Lincoln Nebraska). LAI was estimated at $4.2 \text{ m}^2 \text{ m}^{-2}$, although this is uncorrected for clumping and leaf shape and may be an underestimate (Cutini, Matteucci, & Mugnozza, 1998). The site is actively managed for commercial timber; the last harvests were carried out between 2006 and 2010 and removed ca. 10% of the standing biomass (Braun; *pers. comm.*). The soils are classified as vertic cambisols. Persistent seeps are found in several places. Mean annual precipitation at the site is 1268 ± 138 mm and is relatively well-distributed throughout the year; mean monthly temperatures range from -0.6 to 17.6 °C (Meteotest, Bern, Switzerland).

Sampling

To assess the spatial variation in throughfall and soil water isotopes, we established 150 fixed sampling points at random locations within the plot (**Figure 1**). We placed a throughfall collector at each location on 1 July 2015. Collectors consisted of a 15 cm diameter funnel sealed to a 50 ml collection vial nested in the soil. Evaporation was prevented by using a layer of mineral oil at least 1 cm thick. Two open precipitation collectors were simultaneously established in a field about 400 m from the site.

We collected precipitation, stream, throughfall, and soil water samples on 14 July 2015. Event precipitation and throughfall water originated from small events on 5 (0.9 mm) and 7 (2.5 mm) July. Prior to that, the most recent precipitation was a 6-day event (77 mm) that began on 18 June. Long-term (1970-2015) precipitation patterns were determined from monthly data collected ca. 30 km from Leissigen in Belp. A single water sample was collected from a stream located ca. 50 m from the plot. Soil samples integrating 0-10 cm depth below the soil surface were collected ($n = 150$), as were additional samples from 40-50 cm depth wherever soils were not too rocky or shallow ($n = 8$). All samples were immediately sealed in glass vials and placed in coolers for transport. On the same day, we sampled paired branch and leaf water samples randomly from 23 canopy emergent

individuals of *P. abies* and 35 individuals of *F. sylvatica*. The diameters at breast height (1.3 m) of individual *P. abies* (56 ± 11 cm) and *F. sylvatica* (47 ± 7 cm) that were sampled were similar. For three individuals of each of these species, we also assessed intra-crown variability by sampling five separate branches. Fully sunlit branches were collected with pole pruners using a technician suspended below a helicopter between 14:00 and 16:00. Branches 1-2 m in length were dropped to the ground. Bark and cambium were stripped from fully suberized branches, then xylem samples and leaf tissue from the same branches were immediately sealed in glass vials.

Stable Isotope Analysis

Soil, branch, and leaf water samples were extracted using cryogenic vacuum distillation following the methods of West, Patrickson, & Ehleringer (2006) in an 80 °C water bath with a liquid nitrogen cold trap at a pressure < 50 Pa. Soil water samples were extracted for 4 hours, while branch and leaf water were extracted for 2 hours. Following extraction, samples were analyzed for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ isotope ratios by means of isotope ratio mass spectrometry using a high temperature conversion/elemental analyzer (TC/EA) linked to a Delta Plus XP MS via a ConFlo III interface (Thermo Fisher Scientific, Bremen, Germany). Isotope ratios are expressed in per mil (‰) as:

$$\delta^N E = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000$$

where N represents the heavy isotope of the element E , and R is the ratio of the heavy to the light isotope ($^{18}\text{O}/^{16}\text{O}$ or $^2\text{H}/\text{H}$). Two calibration standards were used to adjust the ratios relative to V-SMOW. Long-term precision of the instrument is 0.4‰ for $\delta^{18}\text{O}$ and 1.7 ‰ for $\delta^2\text{H}$. All sample extractions and analyses were carried out at the Paul Scherrer Institute.

Soil Analysis

To explore sources of variation in soil water isotopes, additional soil samples ($n = 30$) were collected at 10 cm depth from a random subset of the soil-water isotope sampling locations for determination of soil moisture, texture and chemical properties. Litter depth was

determined at each location with a ruler. Percent clay, silt and sand in each sample were determined using a sedimentation method (Swiss Agricultural Research Institute Reference Method: KOM), while percent organic matter was determined using chromic acid wet oxidation (Swiss Agricultural Research Institute Reference Method: Corg). The total cation exchange capacity (CEC), as well as percent K, Ca, Mg, Na and H, were determined using methods appropriate for the soil pH (Swiss Agricultural Research Institute Reference Method: KUKI-KUKII). Soil analyses were carried out at Sol Conseil (Gland, Switzerland) and additional details on methods are found in Flish et al. (2017).

Statistical Analysis

Statistical analysis was carried out using R 3.3.2 (R Core Team, 2016) and MATLAB 2015a (Mathworks, Massachusetts, USA). For the purposes of spatial analyses, trees where intra-tree variation was quantified were summarized with a single mean value. To test for the presence of spatial autocorrelation in the throughfall and precipitation samples, we calculated a global Moran's I using the *moran.test* function in the R package "spdep." To do so, we first constructed a matrix of points that are the nearest neighbors to one another in space via using the *knearest* (*k* nearest neighbors) function. The results were robust for changes in *k* (the number of neighbors to be returned). We then attached spatial weights to the matrix using the *nb2listw* function and performed the Moran's I test. To test how the difference between samples changes as a function of the distance between them, we then constructed variograms for each source. To do so, we used the *variogram* function in the R package "gstat" using 10 evenly spaced bins, with widths of 4.6 m, up to a cutoff lag distance fixed at 1/3 of the maximum point-to-point distance (46 m). Bin counts ranged from 61 to 719 and the effects of different bin sizes are described in **Supplementary Table 1**. Distances were defined with respect to the hillslope plane (i.e., not projected to a horizontal plane). A spherical function was then fit to the variograms using *fit.variogram* to determine the nugget, range and sill. Practically speaking, the range indicates the maximum distance at which sample values are autocorrelated, the nugget serves as an indication of small-scale variation that is not explained by proximity, and the partial sill is maximum variance that is explainable by proximity (equal to the total variance minus the nugget value). If two samples were collected from locations that are infinitely close to each other and they had the same value, the associated nugget would be zero; if their values were only as similar to each other as they were to values from more distant locations, the nugget effect would be maximal and there

would be no spatial autocorrelation (partial sill of zero). Variogram models were used to interpolate by ordinary kriging and map spatial heterogeneity of $\delta^{18}\text{O}$ in throughfall and shallow soil water. Further information on these methods can be found in Bivand, Pebesma, & Gómez-Rubio (2013).

To study the depth at which plants take up water, we carried out a series of exercises where we solved for the proportional contributions of shallow and deep soil water to tree xylem water using a standard two end-member mixing model. We then studied how spatial heterogeneity in the end members would affect the interpretation of these results. In the first exercise, a distribution of solutions was obtained by solving the mixing model for each tree paired with the nearest deep and shallow soil water sample. In the second exercise, a distribution of solutions was calculated for each tree by solving the mixing model for every possible combination of shallow and deep soil water from all samples. Here, the results are presented as a function of tree diameter at breast height to explore the effects of tree size on the proportional contributions of shallow and deep soil water. In the third exercise, a distribution of solutions was obtained by assuming a hypothetical representative sampling approach for a study of plant water uptake at a hypothetical site. To do so, we first calculated the average number of xylem water samples and soil profiles used in the 2010-2016 studies reviewed within (Evaristo & McDonnell, 2017). The average study sampled four trees and used three independent soil sampling locations. We applied this to our sampling and calculated the proportion of shallow soil-water uptake for the means of four xylem samples of each of our two species with three shallow and three deep soil samples, all randomly selected from observed data via Monte-Carlo iteration (10,000 runs). The spread of the resultant distributions were interpreted as a measure of the sensitivity of source-water attribution (i.e. shallow vs. deep) to the spatial heterogeneity of the soil water samples used as end members. To study how sample size affects the uncertainty in soil, xylem, and bulk leaf water isotope samples, we performed Monte-Carlo iterations (1,000 runs) to subsample our observed data at different sample sizes until we arrived at $n - 1$ samples.

To study the factors that may shape variation in soil water isotopes, we studied how the difference of the mean of deep soil water isotope samples from individual shallow soil water isotope samples ($\Delta_{\text{shallow-deep soil water}}$) varied as a function of soil characteristics. As soil characteristics may be correlated (i.e. collinearity), we first assessed the pairwise correlation

of potential predictors. Based on these results, we used percent clay, soil moisture, organic matter and their interactions as predictors in a multiple linear regression model, as well as in a generalized least squares model accounting for spatial autocorrelation, then compared these models using the Akaike Information Criterion (AIC).

All results will be made available through Dryad Digital Data Repository upon acceptance of the manuscript.

Results

The average monthly $\delta^2\text{H}$ of precipitation varied from -97.9‰ in February to -42.2‰ in July, while $\delta^{18}\text{O}$ varied from -12.8‰ to -6.4‰ at a nearby long-term monitoring station (**Supplementary Table 2**; [FOEN, 2016]). Relative to the global meteoric water line (GMWL: $\delta^2\text{H} = 8 * \delta^{18}\text{O} + 10$), the local meteoric water line (LMWL) had a similar slope and intercept ($\delta^2\text{H} = 8.0 * \delta^{18}\text{O} + 9.3$).

The relationships between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water isotopes in precipitation, throughfall, soil and plants are presented in **Figure 2** and **Table 1**. The average $\delta^{18}\text{O}$ ($-3.3 \pm 0.8\text{‰}$) and $\delta^2\text{H}$ ($-18.9 \pm 2.7\text{‰}$) of throughfall collected prior to the soil and plant sampling were generally enriched compared to the $\delta^{18}\text{O}$ (-4.1‰) and $\delta^2\text{H}$ (-22.6‰) of precipitation collected at an open location about 400 m from the site, presumably due to canopy interception and evaporation. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of shallow (0-10 cm) soil waters were depleted compared to throughfall, presumably because they contain precipitation from earlier months, and there was no significant relationship between throughfall and soil water isotope ratios (OLS regression; $p > 0.1$). Deeper (40-50 cm) soil water isotopes were isotopically similar to that of stream water. The mean $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water in *P. abies* branches were significantly enriched compared to those in *F. sylvatica* branches (t-test; $p < 0.01$), suggesting a deeper root water uptake for *F. sylvatica*. Moreover, *F. sylvatica* demonstrated significantly higher midday leaf water enrichment ($\Delta^{18}\text{O}_{\text{leaf-branch}}$ and $\Delta^2\text{H}_{\text{leaf-branch}}$) compared to *P. abies* (t-test; $p < 0.001$). The unique canopy structure of each species may lead to differences in biophysical conditions (e.g., the ratio of ambient air vapor pressure to leaf intracellular vapor pressure) that would explain the differences in leaf water enrichment (Bögelein, Thomas, & Kahmen, 2017).

The variability between and within pools of water is presented for $\delta^{18}\text{O}$ in **Figure 3** and for both isotopes in **Table 1**. The range of water isotopes at 0-10 cm soil depth (10.7 ‰ for $\delta^{18}\text{O}$) far exceeded that of the throughfall event (4.2 ‰ for $\delta^{18}\text{O}$), 40-50 cm soil water (3.3 ‰ for $\delta^{18}\text{O}$), or branch xylem water (4.2 ‰ for $\delta^{18}\text{O}$). *P. abies* demonstrated an overlapping and larger range than *F. sylvatica* in δ^{18} . Notably, the range of branch xylem water within an individual tree crown of *P. abies* and *F. sylvatica* was nearly as large as the range among all the trees sampled in the plot. For instance, the range in branch water isotopes of one of the *P. abies* trees was 3.2 ‰ for $\delta^{18}\text{O}$ and 11.0 ‰ for $\delta^2\text{H}$, comparable to 4.2 ‰ for $\delta^{18}\text{O}$ and 21.3 ‰ for $\delta^2\text{H}$ among all other sampled individuals of *P. abies*. For both species, the range of bulk leaf water isotopes was higher than the respective range of their branch xylem water isotopes. Similar to branch water isotopes, the range of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of leaf water within an individual was often similar to that among all other sampled individuals.

Variograms and statistical measures of spatial variation, used to better understand the scales at which similarity in isotope ratios are (or are not) a function of proximity, are presented in **Figure 4** and **Table 2**. The $\delta^{18}\text{O}$ of throughfall demonstrated significant spatial autocorrelation (Moran's I; $p < 0.001$) up to a distance of 13.9 m. The $\delta^{18}\text{O}$ of soil water (10 cm depth) also demonstrated significant spatial autocorrelation (Moran's I; $p < 0.001$) up to a distance of 6.2 m. However, there is very limited visual evidence for convergence towards zero semivariance, suggesting that there is substantial variation unaccounted for in the sampling. This is despite fitted variogram models indicating that soil water nugget effects were minor (Table 2); the observation of these nugget effects may depend on variogram assumptions (see **Supplementary Table S1**). Ultimately, these results imply that individual soil or throughfall samples may not be representative of proximal locations. Variograms and measures of spatial variation are qualitatively similar for $\delta^2\text{H}$, although the ranges are longer.

The differences between the spatial patterns of throughfall and shallow soil water, as well as how they compare to xylem water can be observed from interpolated $\delta^{18}\text{O}$ maps (**Figure 5**; also see **Figure S1**). Throughfall $\delta^{18}\text{O}$ does not correlate with soil water $\delta^{18}\text{O}$ ($p > 0.1$). Similarly, there is no visual evidence for a relationship between soil and xylem water $\delta^{18}\text{O}$.

Soil water isotope ratios, here reported as the deviation of each shallow soil water isotope ratio from the mean deep soil water isotope ratio ($\Delta_{\text{shallow-deep soil water}}$), varied significantly as a

function of soil moisture and soil texture (**Figure S2**). For both $\Delta^{18}\text{O}_{\text{shallow-deep soil water}}$ and $\Delta^2\text{H}_{\text{shallow-deep soil water}}$, the most parsimonious model included soil moisture and percent clay, but not their interaction ($F_{2,26} > 5$, $p < 0.03$). The incorporation of a spatial covariance structure did not improve model fit. Deuterium excess (calculated as $d = \delta^2\text{H} - 8 * \delta^{18}\text{O}$) was uncorrelated with factors we would expect to relate to evaporation rates (soil moisture and leaf litter depth), indicating that soil surface evaporation was not likely to account for the observed patterns ($p > 0.05$). Finally, there were no significant relationships between $\Delta_{\text{deep-shallow soil water}}$ and either total cation exchange capacity or individual cations for either isotope (**Figure S3** and **Figure S4**).

Discussion

By tracing water from precipitation to the leaf, our results demonstrate how the distribution of water isotope ratios varies through the soil-plant atmosphere continuum. Changes in the magnitude of variability from one pool of water to the next indicates both the effects of fractionating processes, as well as the integration and mixing of water sources from different times and locations. Beyond enabling inferences regarding how water moves through this forest, this unprecedented level of detail regarding the spatial heterogeneity of soil and plant water isotopes informs what can be inferred from past studies and what sampling considerations should be made moving forward.

Effects of canopy interception on throughfall water isotopes

During a precipitation event, processes associated with canopy interception introduce spatial variability in water isotopes. Assuming homogenous precipitation inputs during the precipitation event that occurred prior to sampling, canopy interception resulted in a 4.2 ‰ range in the $\delta^{18}\text{O}$ of throughfall, with a mean $\Delta^{18}\text{O}_{\text{throughfall-precipitation}}$ of 0.8 ‰. This is likely owing to the time-dependent differences in isotopic composition of precipitation during the event reaching the canopy and the spatially distinct mixing, exchange, and evaporative fractionation processes that subsequently occur within the canopy (Allen, Keim, Barnard, McDonnell, & Brooks, 2017). For example, drip from one location that occurs for a short period during high intensity precipitation may only reflect a small fraction of the total storm duration. This can be contrasted with another location where precipitation passes through the

canopy throughout the storm event without much interception and thus has isotopic ratios similar to open precipitation.

The isotopic composition of throughfall for this event demonstrated significant spatial autocorrelation over short distances. Fine-scale spatial autocorrelation has previously been observed in throughfall amount (Staelens, De Schrijver, Verheyen, & Verhoest, 2006); however, evidence for autocorrelation in the isotopic composition of throughfall is more limited. Given that throughfall isotopic differences from precipitation are related to canopy characteristics, the apparent spatial variation in species and canopy gaps likely structured the throughfall heterogeneity. However, the presence of a nugget in our variogram model means that there is still significant variability that we are unable to account for in our sampling. For instance, this may mean that there is some combination of sampling error or drivers of heterogeneity at scales smaller than that at which we sampled.

More generally, the effects of canopy interception are often not accounted for when considering the isotope ratios of water in soil and plants. Rather, it is assumed that precipitation isotope ratios are an accurate representation of these isotope ratios. However, there is growing recognition that using throughfall isotope ratios in place of precipitation can improve the estimation of hydrological processes (Allen et al., 2017). Even for the one throughfall event observed here, both the mean and the individual spatially explicit values of throughfall were likely altered relative to precipitation. As such, assuming precipitation as a model input to soil or plants would affect the results and interpretation. If the spatial autocorrelation pattern is consistent over time, then we would expect systematic spatial biases in inputs to soil water. While this cannot be assessed here, it is important to consider sampling designs that appropriately and adequately characterize the water entering the soil surface.

Effects of soil infiltration and retention on soil water isotopes

Shallow soil water was generally depleted relative to mean annual precipitation at the time of sampling, but demonstrated a range of 10.7 ‰ in $\delta^{18}\text{O}$ across the 1 ha area we sampled. The single throughfall event that occurred prior to sampling was likely too small to account for significant water infiltrating into the soil. As therefore may be expected, the weak spatial autocorrelation observed in soil water isotopes does not likely reflect the infiltration of this

throughfall event and there is no correlation between their values, which can be visualized using the interpolated maps of the two sources (**Figure 5**). Shallow soil water locations that were enriched in $\delta^{18}\text{O}$ relative to the throughfall event we sampled may reflect either evaporation or the persistence of isotopically heavier prior precipitation events (Sprenger et al., 2016a). Shallow soil water locations that were depleted in $\delta^{18}\text{O}$ may reflect the result of a combination of 1) the persistence of isotopically lighter prior precipitation events (e.g., from winter), or 2) a differentially rising water table across the hill slope that is flushing the shallow soil with groundwater. Four sampling locations that appeared to be seeps where exfiltration was occurring had water (mean = -9.7‰ $\delta^{18}\text{O}$) more similar to mean annual precipitation than much of the shallow soil water. Similarly, the few locations where 40-50 cm deep soil water could be collected demonstrated isotope ratios that suggested a temporal lag or a bias towards winter precipitation.

Inferring plant root water uptake using xylem water isotopes

The smaller range of branch xylem water isotope ratios, as compared to soil water isotope ratios, suggests that roots extend both laterally and vertically through soil and integrate waters with distinct isotope ratios (**Figure 3**). In the case of both species, xylem water was more similar to deeper soil water. Moreover, both the plot of xylem water isotope ratios and the mixing models indicated deeper root water uptake by *F. sylvatica* than *P. abies*, which is consistent with previous research comparing the fine root biomass as a function of soil depth in mixed stands of the two species (Bolte & Villanueva, 2006; Schmid & Kazda, 2001). However, as discussed below, the range of deep soil water overlapped substantially with shallow soil water and inferences regarding depth of water uptake are not consistent on a tree-by-tree basis depending on the specific soil water sample locations that are considered.

Effects of evaporative enrichment on leaf water isotopes

The variability of water isotope ratios in bulk leaf water was greater than that of the xylem water that supplies the leaves. This variability likely arises from the effects of different rates of leaf water evaporation superimposed upon differences in branch source water. Branch source water may differ within a crown when different roots take up isotopically distinct sources of water that travel through different flow paths within the xylem and into different branches in the crown (referred to as sectorality; Schulte & Brooks, 2003; Zimmermann,

1983). The high intra-crown variability of branch xylem water in *P. abies* compared with *F. sylvatica* may serve as an indication that distinct sources accessed by *P. abies* may supply different branches and remain distinguishable even upon reaching the crown. Differences in leaf position that lead to variation in microclimate, as well as differences in leaf age, morphology, or biochemistry may also result in variable rates of leaf gas exchange and H₂¹⁸O bulk leaf water enrichment (Cernusak et al., 2016). The range of both the δ¹⁸O of branch water (-11.9 to -8.6 ‰) and leaf water enrichment relative to branch water (Δ¹⁸O_{leaf-branch}: -24.5 to -18.8 ‰) within a single crown of *P. abies* demonstrates that while both source water differences and evaporative enrichment contribute to the observed variation in bulk leaf water isotope ratios, leaf water evaporation still plays a larger role. These results have important implications for the interpretation of hydrogen and oxygen isotopes from leaf water that are incorporated into plant assimilates (e.g., sugars and cellulose).

Drivers of Variability

The high spatial variation observed, particularly in soil water isotopes, raises questions about the processes that may contribute to the variability observed at this scale. In particular, there is renewed interest in soil water isotope fractionation driven by observed differences in soil and plant water isotopes relative to precipitation and stream water isotopes (Evaristo, Jasechko, & McDonnell, 2015; Goldsmith et al., 2012; McDonnell, 2014; Brooks et al., 2010). There is increasing evidence that the magnitude of soil water isotope fractionation may be related to soil texture (e.g., surface area; (Golvan, Michelot, & Boisson, 1997) and chemical properties (e.g., cation exchange capacity; (Oerter et al., 2014). In addition to resulting in different liquid-vapor isotopic fractionation factors among soils (Lin & Horita, 2016), differences in soil properties may contribute to observed differences in soil water isotope recovery depending on the laboratory method used for water extraction (Gaj, Kaufhold, & McDonnell, 2017a; Gaj, Kaufhold, Koeniger, & Beyer, 2017b; Orłowski, Pratt, & McDonnell, 2016b), or even variation among labs using the same method (Orłowski et al., 2018). While we cannot exclude any effects based on the cryogenic vacuum distillation method used here, all samples were treated equally (but see Orłowski, Breur, & McDonnell, 2016a). Rather, we focus on the processes that may contribute to soil water variation, particularly with respect to the effect of soil texture on water retention and mixing, as well as the possibility of an isotopic fractionation associated with increasing soil cation exchange capacity (Oerter et al., 2014). Increasing soil moisture and decreasing soil particle size (e.g.

higher percentage of silt) were significantly related to the isotope ratio of shallow soil water (**Figure S2**). However, this could result from differences in transit properties associated with texture. Furthermore, we found no evidence here for a relationship between soil water isotopes and increasing cation exchange capacity. Moving forward, both *in situ* studies of soil pore water vapor (Oerter & Bowen, 2017) and laboratory bulk soil water studies (Gaj et al., 2017b) of soil water isotopes should consider both spatial (lateral) and vertical differences in soil characteristics that may result in isotopic heterogeneity.

Variability in Geographic Space

Ultimately, the high variation that we observe in water isotopes at this scale indicates that the choice of experimental design will have clear effects on the results and their interpretation. In particular, the overlapping distributions of shallow and deep soil water have consequences for inferring relative sources of plant water uptake, as demonstrated by mixing model solutions in **Figure 6**. The exercises demonstrate that heterogeneity in soil and xylem water samples yield wide distributions of possible source water mixtures in the xylem. This is the case irrespective of whether source contributions are calculated for each tree based on the nearest soil waters (**Figure 6A**), for each tree for all potential source waters (**Figure 6B**), or from means of subsampled sets as would be typical of a study of plant water uptake (**Figure 6C**). This final scenario, where we subsample our dataset using sample sizes that are typical of previous studies (Evaristo & McDonnell, 2017), provides a means of assessing the reliability of results of plant root water uptake studies to date. Although *F. sylvatica* seems to use less shallow water than *P. abies*, only 26% of the subsampling iterations using four trees of each species and three soil cores yielded statistically significant support for that inference (2-sample t test, $\alpha = 0.05$). Thus, using simple mixing models to identify source contributions likely leads to frequent misinterpretations, especially when sample sizes are small, because of the tremendous variability among individual trees and soil samples.

It is also of note that the mixing model results only account for differences in soil water isotopes as a function of vertical soil depth. However, lateral differences in soil water isotopes were similar in magnitude to vertical differences. For all the locations with paired 10 and 40 cm depth soil water observations ($n = 8$), the absolute value of differences between the two depths ($2.2 \pm 1.4\text{‰ } \delta^{18}\text{O}$ and $15.8 \pm 9.1\text{‰ } \delta^2\text{H}$) was not statistically different from the absolute value of differences between the observations ($1.5 \pm 1.6\text{‰ } \delta^{18}\text{O}$ and $12.4 \pm 9.1\text{‰}$

$\delta^2\text{H}$) of the nearest neighboring 10 cm depths (3.1 m average lateral distance; 2-sample t-test; $p > 0.1$). As such, it is possible that plants that appear to be taking up water from 10 to 40 cm soil depth are simply using water from 10 cm depth in different locations within the lateral spread of their roots.

Many studies interpret plant root water uptake based on a limited number of soil profiles established at locations that are not specified relative to the locations of the plant sampling. Taken together, our results indicate the need to inform sampling design with a better understanding of the variability of water isotopes within the given scale of the study. For instance, our data resampling experiment suggests that approximately 50 soil water samples, 15 branch xylem water samples, and 20 bulk leaf water samples would be necessary to obtain reliable estimates of the standard deviations in water isotopes we observed in our plot (Figure 7), although this depends on the nature of the study. These results are site and species-specific; similar approaches should be pursued at other locations and scales in order to improve our ability to confidently interpret environmental processes using stable isotopes of water.

Conclusions

By studying the spatial variation in throughfall, soil and plant water isotopes, we demonstrate how the water isotope signal propagates as it moves through the soil-plant atmosphere continuum, as well as how it varies in space. Sites with different characteristics (e.g., topography or soils) may have different patterns. While we observed some evidence for spatial autocorrelation of this signal within different pools (e.g., throughfall), there was considerable variation in soil water isotope ratios that raise important questions of how best to characterize and relate soil and plant water isotopes in space. While plant root water uptake across space (and time) may integrate much of this variation, fractionation associated with evaporative enrichment of leaf water re-introduces considerable intra- and inter-canopy variation. Accounting for these variations should lead to more accurate interpretations of oxygen and hydrogen isotopes in plant tissue (Gessler, Ferrio, Hommel, & Treydte, 2014).

Moreover, it is unclear how the small-scale variations explored here influence the interpretation of large-scale patterns, where other processes presumably dominate (Allen, Kirchner, & Goldsmith 2018; Esper et al. 2018; Treydte et al. 2007). As such, there is a clear need to describe patterns of stable isotopes of water in the soil-plant-atmosphere across scales

and to better understand the processes that structure those patterns. While the study of stable isotopes of water has historically been limited by the resource-intensive nature of making observations, continuous improvements in existing methods (e.g. simultaneous measurement of both hydrogen and oxygen) and the emergence of new methods (continuous and real time *in situ* measurements; Oerter, Perelet, & Pardyjak, 2017; Volkmann & Kühnhammer, 2016; Volkmann & Weiler, 2014) hold great promise for improving our understanding of stable isotopes of water. Our results demonstrate the critical need to leverage these new advances to study pattern and process in both time and in space.

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Accepted Article

Tables

Table 1. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water from different sources collected on one day in a 1 ha forest plot established near Leissigen, Switzerland. Data represent means ± 1 SD with range in parentheses.

Source	$\delta^{18}\text{O}$ (‰)	$\delta^2\text{H}$ (‰)
Event precipitation	-4.1	-22.6
Stream	-11.5	-80.8
Event throughfall	-3.3 ± 0.8 (-5.5 to -1.3)	-18.9 ± 2.7 (-25.4 to -11.6)
Soil (0-10 cm)	-7.2 ± 1.7 (-11.0 to -0.3)	-53.4 ± 10.6 (-83.4 to -33.2)
Soil (40-50 cm)	-11.2 ± 1 (-12.5 to -9.1)	-81.5 ± 7.1 (-88.6 to -67.3)
Branch (<i>P. abies</i>)	-9.1 ± 1 (-11.9 to -7.7)	-69.8 ± 5.7 (-82.0 to -60.6)
Leaf (<i>P. abies</i>)	11.7 ± 1.3 (8.9 to 13.6)	-21.1 ± 3.9 (-29.1 to -14.9)
Branch (<i>F. sylvatica</i>)	-10 ± 0.8 (-11.8 to -8.3)	-83.5 ± 6.3 (-96.6 to -71.4)
Leaf (<i>F. sylvatica</i>)	14.8 ± 1.7 (10.4 to 17.9)	-23.9 ± 5.6 (-34.9 to -11.9)

Accepted

Table 2. Characteristics of spatial autocorrelation observed in the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water sampled from throughfall and soil in a 1 ha forest plot established near Leissigen, Switzerland. Significance for Moran's I is indicated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Source	$\delta^{18}\text{O}$			$\delta^2\text{H}$				
	Moran's I	Nugget	Range (m)	Partial sill	Moran's I	Nugget	Range (m)	Partial sill
Throughfall	0.16***	0.3	13.9	0.3	0.05*	5.2	25.0	1.7
Soil	0.14***	0	6.2	2.6	0.07***	0	6.8	105.9

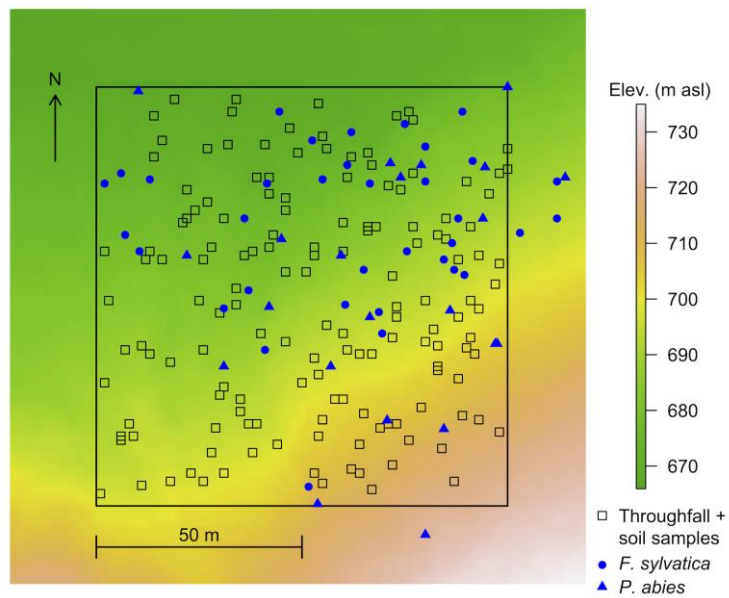


Figure 1. Locations for the sampling of throughfall, bulk soil, plant xylem, and leaf water isotopes in a 1 ha forest plot established near Leissigen, Switzerland. Locations are presented on a 2 m digital elevation model (swissAlti^{3D}; Swiss Federal Office of Topography 2016).

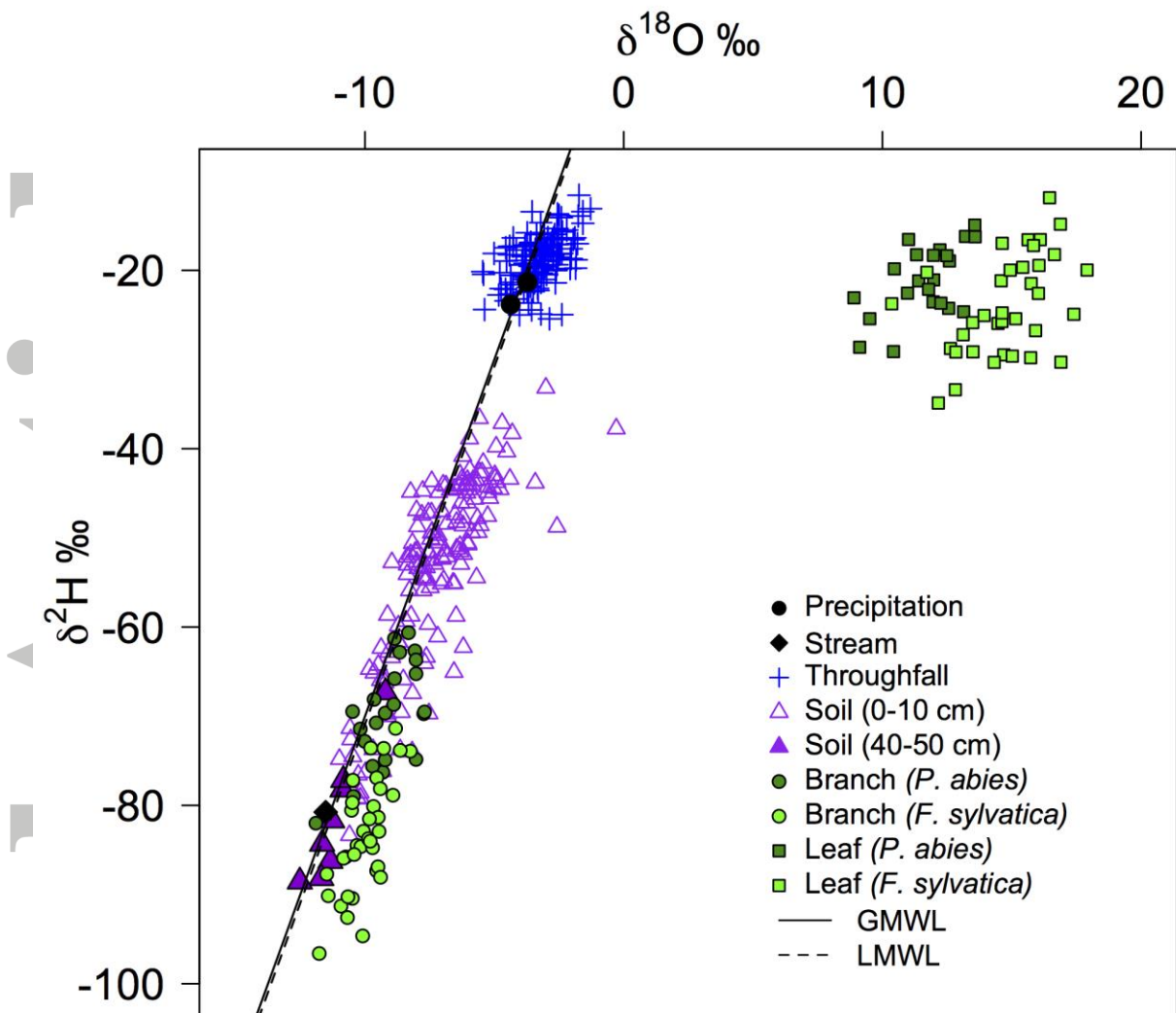


Figure 2. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water from precipitation, a stream, throughfall, bulk soil, branch xylem, and leaves observed in a 1 ha forest plot. The solid line represents the global meteoric water line (GMWL) and the dashed line represents the local meteoric water line (LMWL).

Accepted

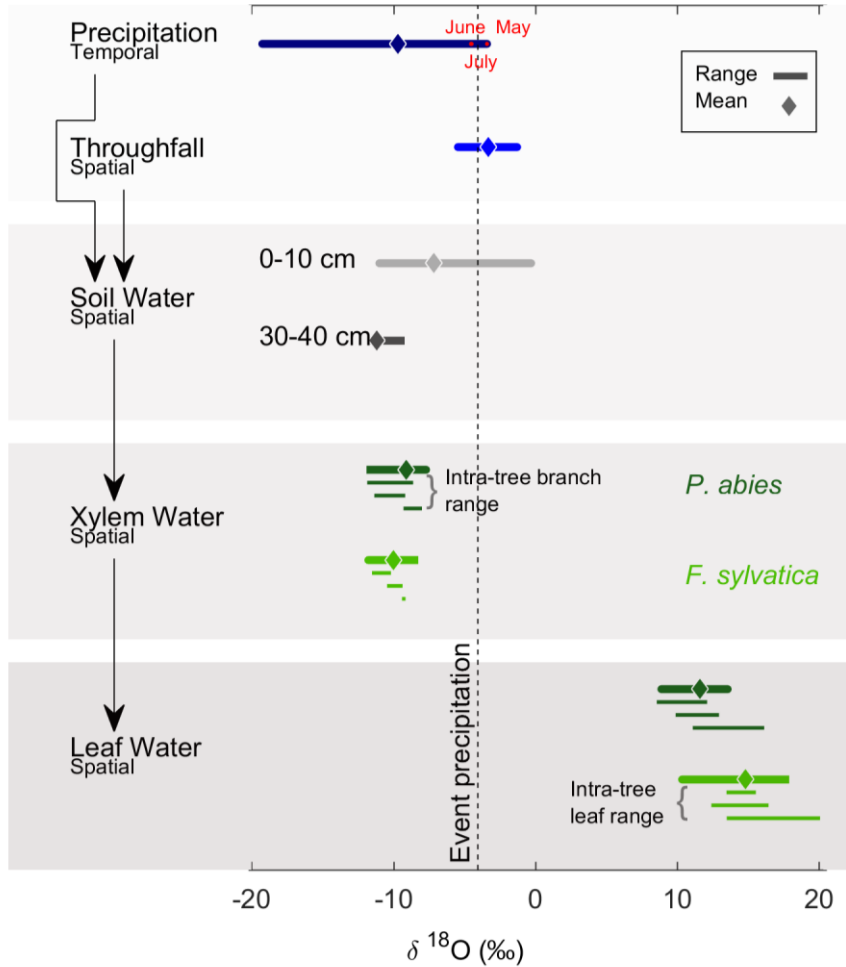


Figure 3. Changes in the mean and range of the $\delta^{18}\text{O}$ of water from precipitation, throughfall, bulk shallow and deep soil, branch xylem, and leaf tissue observed in a 1 ha forest plot. Precipitation represents mean and range of monthly values from samples collected over time (1970-2015) from a nearby monitoring station, while throughfall, soil, branch xylem and leaf water samples were collected one time from many locations within the plot (see text for sample sizes).

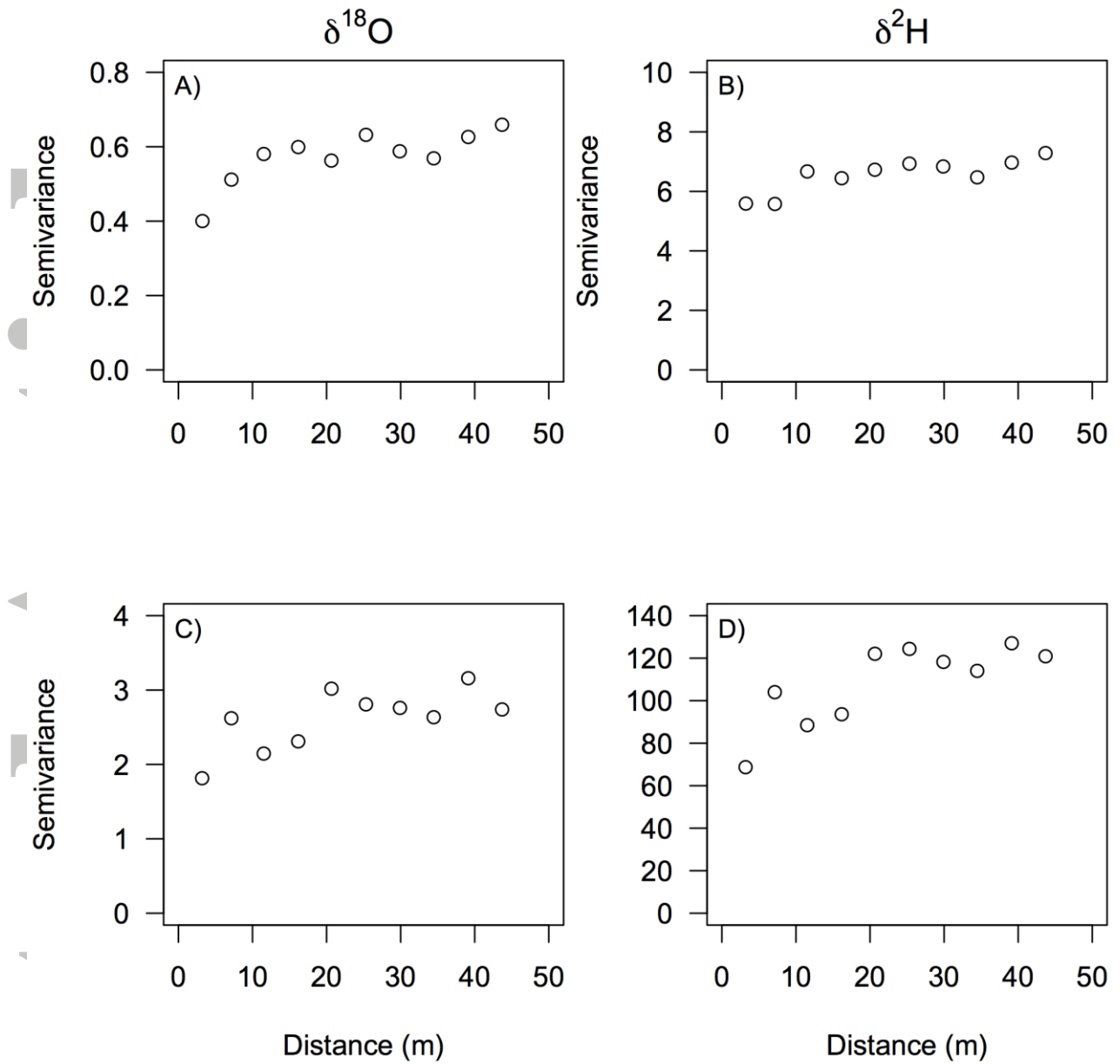


Figure 4. Empirical variograms for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water in throughfall (A and B) and soil (C and D) observed in a 1 ha forest plot.

Accepted

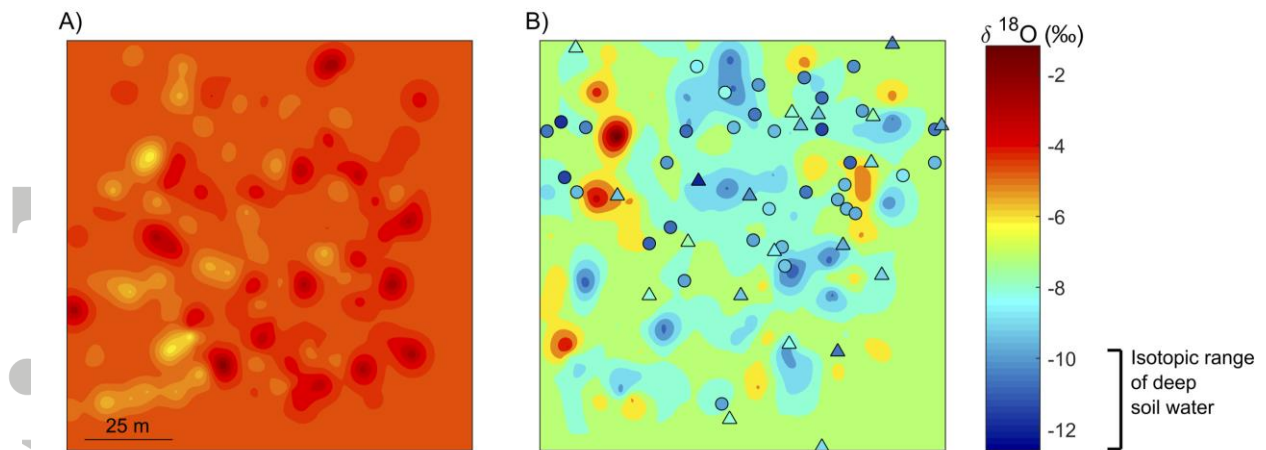


Figure 5. Kriged layers of the $\delta^{18}\text{O}$ of A) 150 throughfall and B) 150 soil water (0-10 cm depth) with *P. abies* (triangles) and *F. sylvatica* (circles) xylem water observed in a 1 ha forest plot. Correlations between a subset of these soil-water isotope values and soil characteristics are shown in Figures S1, S2 and S3).

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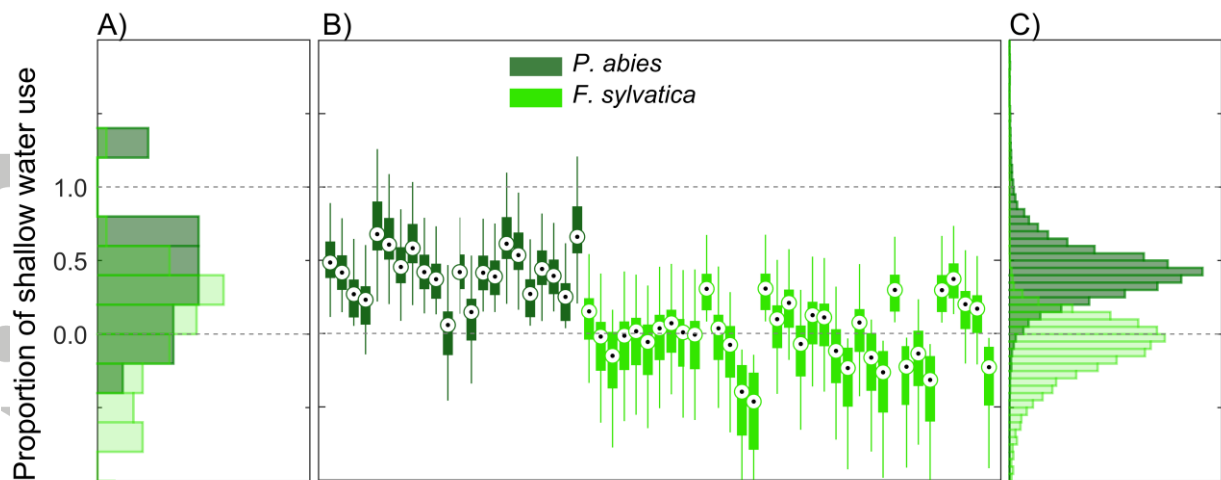


Figure 6. Proportion of water taken up by plants from shallow and deep soil inferred from a two end-member mixing model of the observed $\delta^{18}\text{O}$ of shallow (0-10 cm) and deep (40-50 cm) soil and xylem water. Source water contributions were solved for A) each tree paired with the nearest shallow and deep soil water samples, B) each tree with every combination of shallow and deep soil water (boxplots for each tree with quartiles and whiskers extending to 95% CI [1.57 inter-quartile range]), and C) a hypothetical representative sampling approach for a study of plant water uptake at a site using the means of four randomly selected xylem samples of each species, three shallow and three deep soil samples, then represented as probability density functions from a Monte-Carlo iteration (see Methods). Individual trees of each species in (B) are ordered from small to large diameter at breast height (1.3 m) from left to right.

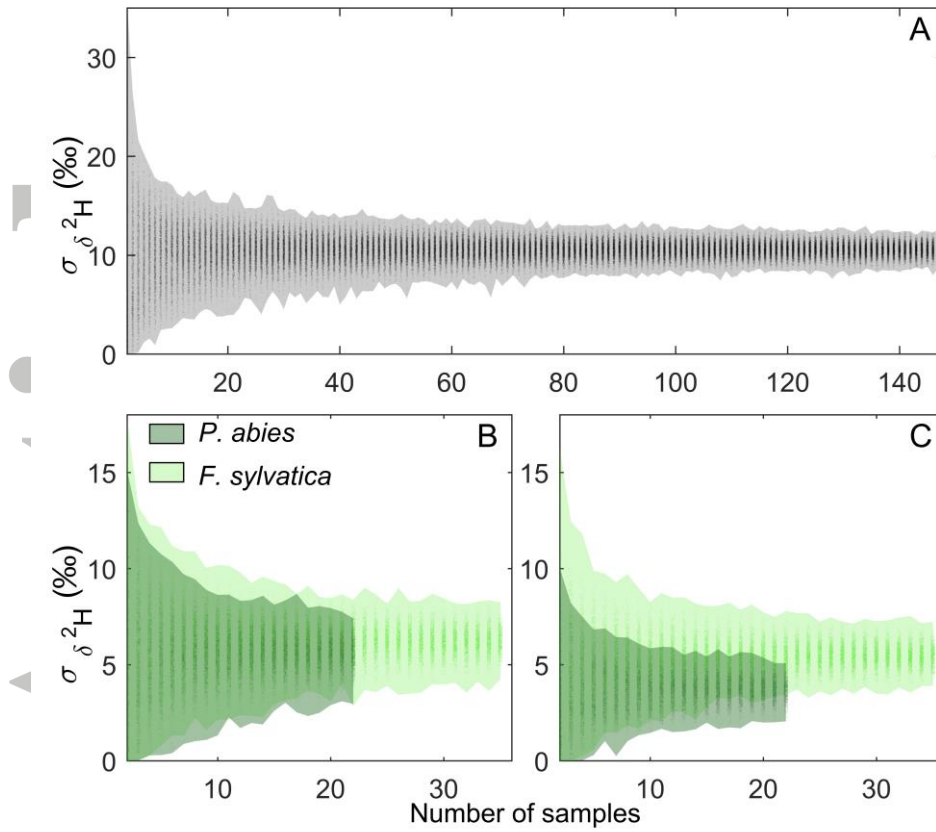


Figure 7. The standard deviation of $\delta^{18}\text{O}$ in A) soil, B) plant xylem and C) bulk leaf water as a function of the number of samples, as generated from a Monte-Carlo iteration (1,000 draws) using the observed isotope ratios.

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