

# DOES ISOTOPIC FRACTIONATION OCCUR DURING ROOT WATER UPTAKE?

REPORTING A GLOBAL DIVERGENCE IN THE ISOTOPIC COMPOSITION OF PLANT WATER AND ITS SOURCES.

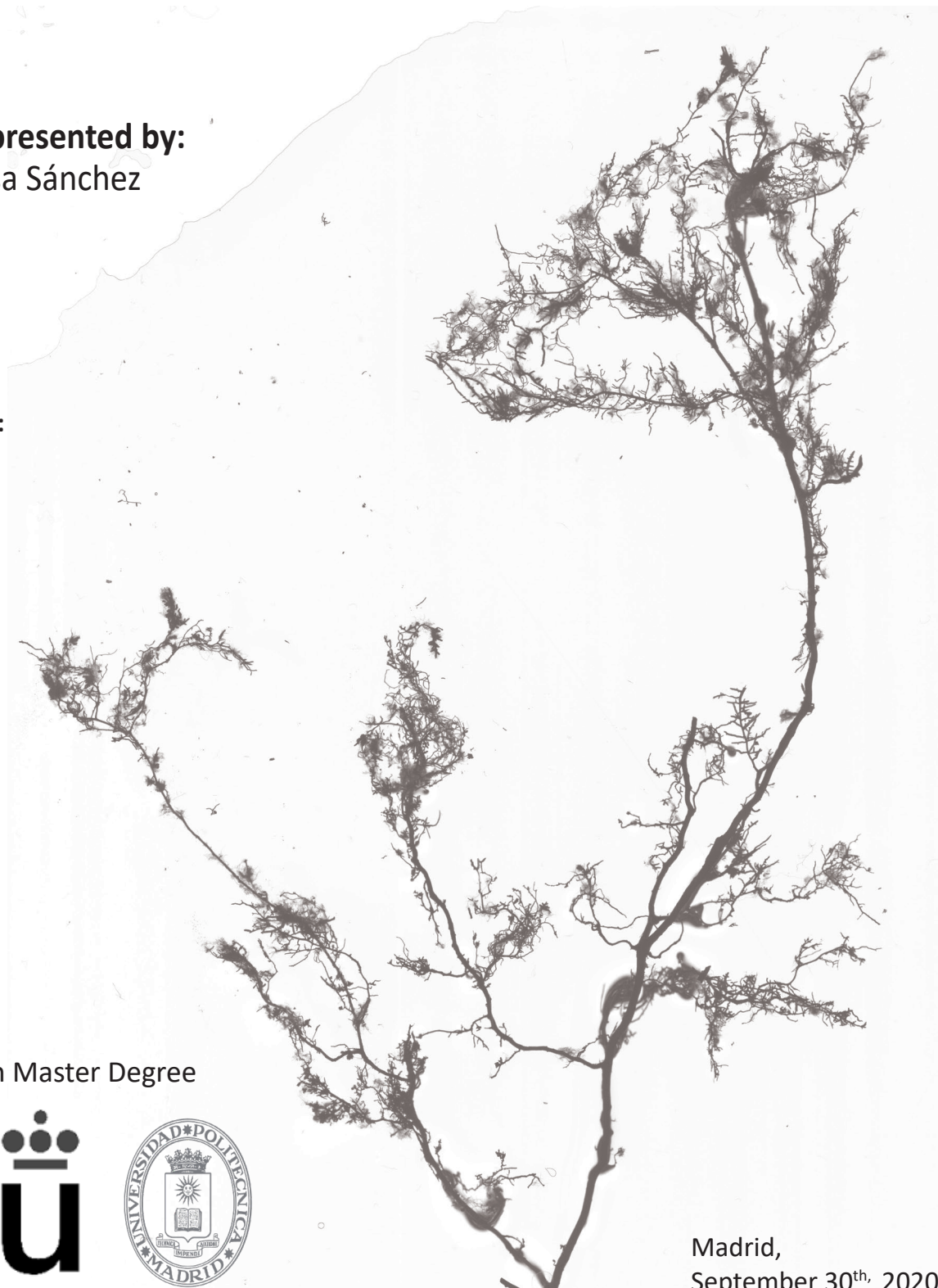
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Ecosystem Restoration Master Degree



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# Does isotopic fractionation occur during root water uptake?

Reporting a global divergence in the isotopic composition plant water and its sources

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# 1. ABSTRACT /KEYWORDS

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## ABSTRACT

The analyses of water isotopic composition serve to investigate plant water sources under the assumption that root water uptake does not entail isotopic fractionation, i.e. the isotopic composition of the plant water reflects that of the root-accessed sources. However, a growing number of studies challenge this assumption by reporting plant-source offsets in water isotopic composition, for a wide range of ecosystems. We conducted a meta-analysis to quantify the magnitude of this plant-source offset in water isotopic composition world-wide and its potential explanatory factors. We compiled 77 studies reporting dual water isotopic composition ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) and extracted plant and source (soil)  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  for 141 species. To calculate the offset, first, we fit a soil water isotopic evaporation line ( $\delta^2\text{H}$  vs.  $\delta^{18}\text{O}$ ) for each study and sampling campaign. Then, we calculated our offset with respect to this line (SW-excess) as the difference between the observed and predicted  $\delta^2\text{H}$  plant values. Effects of climate and plant functional traits on SW-excess were assessed using linear mixed models. Overall SW-excess was significantly negative: plant water was systematically more depleted in the heavier water isotopes than soil water, for  $\delta^2\text{H}$ . The sign and magnitude of the SW-excess differed among plant functional types: SW-excess was more negative in angiosperms, deciduous and broadleaved species. The SW-excess increased with mean annual precipitation. Additionally, ~90% of cases where SW-excess was negative, the estimated offset with respect to alternative water sources (precipitation and groundwater) was also negative. Thus, we conclude that this overall significant soil-plant offset in water isotopic composition cannot be attributed to alternative water sources. A consistent negative offset between plant and potential water sources could introduce biases when estimating water sources accessed by the vegetation, particularly in broadleaved forests in temperate and humid regions. So, isotopic analyses to estimate water use should be revisited

## KEYWORDS

Meta-analysis, ecohydrology, water isotopic composition, soil water line, water cycle, stable isotopes.

## RESUMEN

Los análisis de composición isotópica del agua sirven para investigar fuentes hídricas de la vegetación, suponiendo que no existe fraccionamiento isotópico durante la absorción radicular de agua, es decir, la composición isotópica de la planta refleja la de sus fuentes. Sin embargo, un creciente número de estudios contradicen este supuesto, al reportar divergencias entre la composición isotópica de la planta y sus fuentes en diferentes ecosistemas. Realizamos un meta-análisis para ver la magnitud de esta divergencia a escala global, compilando 77 estudios que recogen la composición isotópica ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) del agua extraída de plantas (141 especies) y del suelo. Primero se estimó la recta de regresión de la composición isotópica ( $\delta^2\text{H}$  vs.  $\delta^{18}\text{O}$ ) del suelo para cada campaña de muestreo y después se calculó la divergencia respecto de esta línea (SW-excess) como la diferencia entre los valores observados y predichos de  $\delta^2\text{H}$  de la planta. Los efectos del clima y de las características de las plantas fueron evaluadas mediante modelos lineales mixtos. En general, el SW-excess fue significativamente negativo: el agua de las plantas está más empobrecida en  $\delta^2\text{H}$  que el suelo. El signo y la magnitud del SW-excess varió entre tipos funcionales, siendo más negativo en especies angiospermas, caducifolias y de hoja ancha, e incrementa con la precipitación media anual. Además, ~90% de casos donde la SW-excess resultó negativa, la divergencia isotópica entre plantas y fuentes alternativas (lluvia o agua subterránea) también fue negativa. Entonces, concluimos que la divergencia generalizada entre la composición isotópica del suelo y planta no puede ser atribuida a fuentes alternativas de agua. Esta divergencia, consistentemente negativa, puede introducir sesgos importantes en la estimación de fuente hídricas de la vegetación, particularmente en bosques de hoja ancha y regiones templadas y húmedas. Es necesario entonces revisar los análisis isotópicos para estimar uso de agua.

## PALABRAS CLAVE

Meta-análisis, ecohidrología, composición isotópica del agua, recta isotópica del suelo, ciclo del agua, isótopos estables.

## 2. INTRODUCTION

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### 1. BACKGROUND

Freshwater is a key limiting resource for terrestrial ecosystems and their inhabitants. Limited access to freshwater is widespread across the globe: one third of the world largest groundwater systems are already in distress (Voss et al., 2015); over 2 billion people live in areas experiencing severe water stress (UN, 2018) and about 4 billion people, representing nearly two-thirds of the world population, experience severe water scarcity during at least one month per year (Mekonnen and Hoekstra, 2016). Current climate change scenario further challenges freshwater access. In dry areas, desertification risk is quickly escalating, reducing the resilience of dryland ecosystems and the provisioning of ecosystem services (Mirzabaev et al., 2019). Besides direct human consumption, terrestrial vegetation has a fundamental role in the water cycle, as it is responsible for 60-80% of the total evaporation fluxes in land (Schlaepfer et al., 2014; Schlesinger and Jasechko, 2014). Plants lose water via transpiration and take up water through their roots from the soil, mainly. Plants deploy a great variety of strategies and morphological adaptations to warrant a steady water supply to meet the demand of the transpiration stream under varying climatic conditions. Hence, terrestrial ecosystems vary greatly in the hydrological niches they harbour depending on their species community composition. Moreover, the terrestrial vegetation also affects the storage and flow of groundwater and surface, providing important hydrologic services, e.g. *in situ* water supply or water damage mitigation (Brauman, 2015). Hence, to handle the water scarcity problem and manage such an important and endangered resource as freshwater, future management plans of freshwater resources require a profound understanding of the role of the vegetation in the hydrological cycle and its disturbances.

The analysis of the isotopic composition of water is a widespread tool in the study of the water cycle. In water molecules, the most abundant naturally occurring stable isotopes are  $^1\text{H}$  and  $^2\text{H}$  (or D, deuterium) for hydrogen, and  $^{16}\text{O}$  and  $^{18}\text{O}$  for oxygen (Sprenger

et al., 2016 cites therein). Water isotopologues, molecules with the same chemical formula but different isotopic composition, differ slightly in the physical properties depending on their mass. The heavier isotopologues ( $^2\text{H}_2\text{O}$  and  $\text{H}_2^{18}\text{O}$ ) have a lower vibration frequency and potential energy (Dawson and Simonin, 2011). Therefore, processes that involve phase change of the molecules are subject to isotopic fractionation. This means that during the phase change (e.g. from liquid to vapor), the lighter isotopologues ( $^1\text{H}_2\text{O}$  and  $\text{H}_2^{16}\text{O}$ ) will evaporate preferentially, leading to a decompensation on the isotopic signature between the two phases.

Natural abundances of the heavier water isotopes are very low (<0.1%), therefore water isotopic composition is usually expressed in delta ( $\delta$ ) notation in relation to an international standard (McKinney et al., 1950):

Eq. 1:

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

In Eq. 1,  $E$  is the chemical species (H or O),  $x$  is its atomic mass and  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the sample and standard ratios of the heavy (rare) to the light (abundant) isotope (e.g.  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$ ). The international standard for water is the Vienna Standard Mean Ocean Water (VSMOW) (Craig, 1961)

Meteoric water, i.e. water derived from precipitation in any physical state (e.g. rain, snow, fog or hail) and surface freshwater bodies have an isotopic composition that is the result of the water body from which it originally evaporated from and then condense, resulting from evaporation and mixing processes occurring in the atmosphere. During phase changes, water pools are subject to kinetic fractionation: during evaporation, the lighter isotopologues evaporate more easily, but heavier isotopologues present in the evaporated water will condensate faster. Hence, atmospheric water vapour has a lower fraction of heavy isotopes, i.e. it is depleted in the heavy isotopes, compared to the water body from which it evaporated. Analogously, precipitation following condensation from a humid air mass has a higher ratio of the

heavy isotopes, i.e. it is enriched in the heavy isotopes compared to the water vapor that remains in the atmosphere (Barbeta et al., 2018). In addition, this kinetic fractionation is temperature dependent. Under warmer temperatures, heavier isotopes are more easily evaporated and thus atmospheric water in either phase (i.e. the source of all precipitation) becomes more enriched than during cold conditions. Importantly, this temperature-related fractionation does not affect H and O to a similar extent because O is heavier than H and hence water molecules with  $^{18}\text{O}$  are even less likely to perform a phase change than those with  $^2\text{H}$ . Still, the fractionation ratio of  $^2\text{H}$  relative to  $^{18}\text{O}$  during condensation of atmospheric water is nearly constant. This relationship is expressed as a linear equation for meteoric waters, known as global meteoric water line (GMWL, see Figure 1) that describes the relationship between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  (Dansgaard, 1964) with:

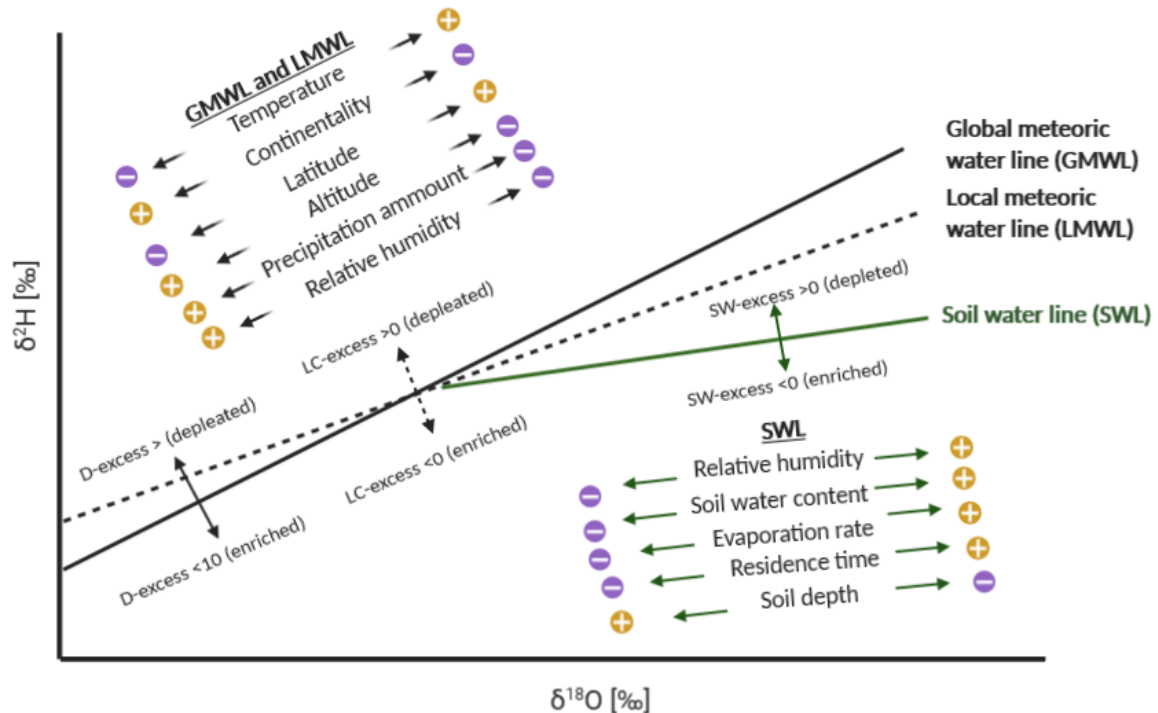
Eq. 2:

$$\delta^2H = 8 \times \delta^{18}O + 10$$

Other climatic processes affecting the relationship between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  can be assessed by calculating the deviations from the GMWL, also known as deuterium-excess or D-excess, from Eq. 2 and according Dansgaard (1964):

Eq. 3:

$$D - excess = \delta^2H - 8 \times \delta^{18}O$$



**Figure 1.** Theoretical dual isotopic plot representing the global meteoric water line (GMWL, black line), local meteoric water line (LMWL, dotted line) and soil water evaporation line (SWL, green line). Samples below or above the GMWL are enriched or depleted in heavier isotopologues compared to the GMWL, respectively. The environmental variables presented influence the isotopic composition of a sample in the direction indicated by the plus and minus symbols. Based on Sprenger (2016) and Barbeta (2018)

In addition,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of local meteoric waters for a certain region can differ from the GMWL because of the complex dynamics of water evaporation and condensation, which depend not only on air temperature, but also on air relative humidity, and hence vary with seasonality, latitude, altitude and continentality (Dansgaard, 1964; Barbeta et al., 2018). The relationship between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  at the regional scale, is described by the local meteoric water line (LMWL, Figure 1.), which is established by analysing the meteoric waters of a location periodically.

The deviation from the relationship between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of any given water sample from the LMWL can be expressed as the line conditioned excess (LC-excess) following (Landwehr and Reston, 2006)



Eq. 4:

$$LC - excess = \delta^2H - a_L \times \delta^{18}O - b_L$$

Where  $a_L$  and  $b_L$  are the slope and intercept of the LMWL

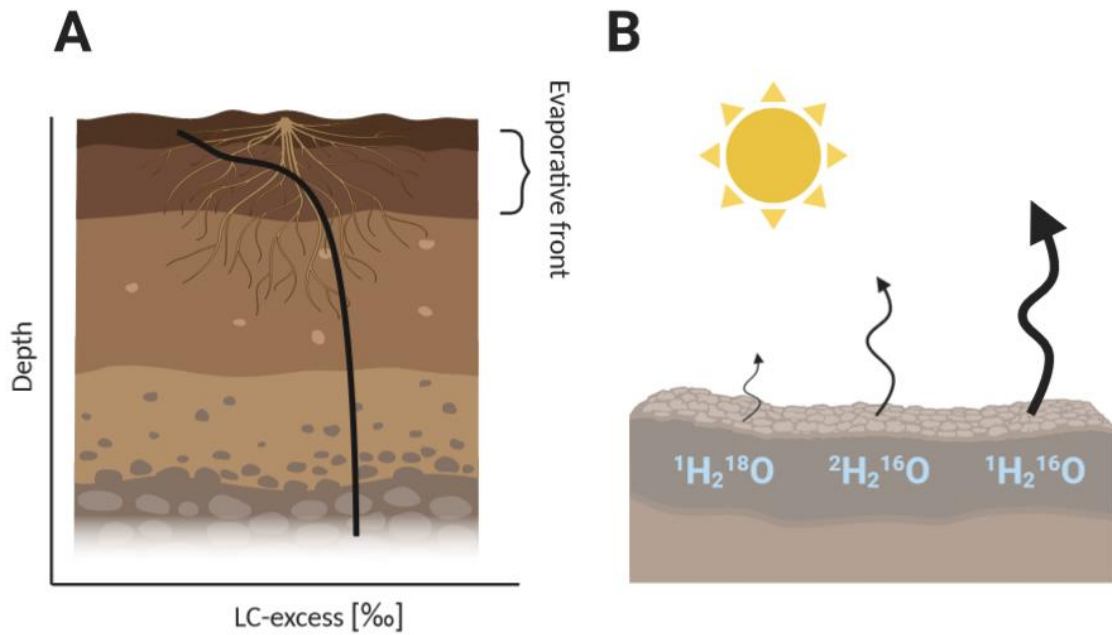
The isotopic composition of soil water depends mainly on precipitation events. Then, it is further modified by soil water evaporation that produces an isotopic enrichment, stronger for oxygen isotopes (Sprenger et al., 2016). Therefore, the isotopic composition of soil water results from a mix of the successive precipitation events in the soil plus the soil evaporative enrichment of preceding soil water (Dawson and Simonin, 2011). This evaporative enrichment occurs generally in the first 30cm of soil, but it can reach deeper horizons in dry and/or warm climates (Sprenger et al., 2016). Meanwhile, deep soil water is expected to depict an isotopic composition reflecting a mixture of the water percolating over time, which is presumed to not have been subject to evaporative enrichment. Usually, these processes result in an isotopic differentiation along the soil profile encompassing isotopically heavier water in the surface soil, compared to water in the deep horizons, as it is reflected in Figure 2

For concurrent soil water samples, the relationship between  $\delta^2H$  and  $\delta^{18}O$  is given by the soil water line (SWL, see Figure 1), also known as soil evaporation line (Barbeta et al., 2018):

Eq. 5:

$$SWL: \delta^2H = a_s \times \delta^{18}O - b_s$$

Where  $a_s$  and  $b_s$  are the slope and intercept of the SWL.



**Figure 2.** Theoretical scheme of soil evaporation enrichment. A) Common isotopic profile of a soil in depth. The black line represents the change in LC-excess (Eq. 4), is an estimate of the isotopic enrichment, along the depth profile. In the upper soil, water is usually isotopically enriched (LC-excess more negative) due to evaporation. B) Theoretical relative fractionation of the heavier ( $^1\text{H}_2^{18}\text{O}$  and  $^2\text{H}_2^{16}\text{O}$ ) and lighter ( $^1\text{H}_2^{16}\text{O}$ ) water isotopologues during soil evaporation. Longer and wider arrows represent more likelihood to perform a liquid-vapor phase change. Based on Barbata (2018) and Benettin (2018).

The slope of the SWL ( $a_s$ ) is typically less steep than that of the LMWL ( $a_l$ ), and it varies across climates depending on the evaporative fractionation and the isotopic composition of precipitation. The SWL is generally shallower in dry and warm regions because of the strong evaporation enrichment. A proper characterization of the SWL is necessary in any field study to understand water transport or biological processes (Barbata et al., 2018).

To feed the constant water demand resulting from transpiration (see Annex 1 for more basic information about this and other processes), plants take up water from the soil through their roots. Root water uptake is assumed to be a non-fractionating process (Poca et al., 2019 and cites therein) and thus, the isotopic composition of plant water should match that of the source of water. Therefore, the combined isotopic analyses of plant water together with analyses of its potential sources, mainly soil water, but also groundwater and other mobile water pools such as stream water, can inform about the

relative contribution of the different water pools to global transpiration (Barbeta and Peñuelas, 2017; Evaristo and McDonnell, 2017).

The applications of these isotopic tools is diverse and it grows as new measurement methodologies are developed, such as the capacity of in-situ sampling of soil and xylem water isotopic composition or the possibility of tracing source's isotopic signatures with high temporal resolution (Marshall et al., 2020).

The analysis of water stable isotopes is frequently applied to study the water utilization of tree plantations and agriculture (Zhang et al., 2011; Liu et al., 2019a; Muñoz-Villers et al., 2020) to determine local aquifer recharge ratio and its sources (Liu and Yamanaka, 2012), to determine the different sources of water in forests and its variability in response to drought episodes (Anderegg et al., 2013) or to predict the tolerance to varying climatic conditions. Also, stable isotopes of water are used to assess risks like saltwater intrusion on groundwater or to trace the movement of a pollutant (Schlosser et al., 1999). Another remarkable application of using stable isotopes of waters is in scientific research: from the study of our past climate (Sturm et al., 2010) and the current atmospheric disturbances (Breitenbach et al., 2010), to the pharmacology area (Schellekens et al., 2011)

In the field of ecosystem restoration, analyses of water stable isotopes can be applied to characterise the vegetation water requirements and hydrological fluxes. In addition, these techniques can be helpful for selecting the appropriate plant species in revegetation actions or other active restoration practices, enhancing plant survival and restoration success. For example, Clinton (2004) examined the relative uptake of surface water vs. groundwater of *Populus deltoides* to assess the effectiveness of using this species as a phytoremediation agent for groundwater pollution. Also this techniques give more information of the ecohydrological changes occurring with human disturbances e.g. damming (Zhao et al., 2020)

The studies and applications mentioned above rely on the assumption that the isotopic composition of plant water faithfully mirrors that of its source. Early experiments with hydroponic cultures indeed demonstrated that that there is no isotopic fractionation during root water uptake (Washburn and Smith, 1934; Zimmermann et al., 1967).

Nonetheless, this absence of fractionation may not be a valid assumption for halophytic (Lin and Sternberg, 1993; Ellsworth y Williams, 2007) and xerophytic species (Ellsworth y Williams, 2007; Poca et al., 2019). The explanation of this phenomenon was hypothesized to be related to the particular anatomy of these species adapted to salty and/or dry conditions. Xylem root cells of some species adapted to saline and/or dry environments have root adaptations (e.g. highly developed Casparian bands in their root cells) that force water to move via symplastic transport, which may cause fractionation (Lin and Strenberg, 1993; Ellsworth y Williams, 2007; Poca et al., 2019). Results from a recent study also suggest that biological interactions, specifically associations with mycorrhizal fungi, could produce discrimination against the heavier hydrogen isotopes of water during root water uptake. Poca (2019) suggested that preferential transmembrane transport mediated by aquaporins would be causing this observed fractionation but this has not been demonstrated. Still, beyond species dwelling in saline or dry environments, it appears that the mismatch between the isotopic composition of plant and source waters could be more widespread than previously believed (Brooks et al., 2010; Geris et al., 2015; Evaristo et al., 2016; Evaristo and McDonnell, 2017; Vargas et al., 2017; Wang et al., 2017; De Deurwaerder et al., 2018; Barbeta et al., 2019; Brum et al., 2019; Carrière et al., 2020)

(Barbeta, Gimeno, et al., 2020) performed a glasshouse experiment with *Fagus sylvatica* in order to reproduce this offset under controlled conditions and manipulated soil texture and water availability. They found that regardless of substrate, stem water was consistently more depleted in  $\delta^2\text{H}$  than its source water. Their results confirmed that soil-plant isotopic offset is not exclusive of halophytes and xerophytes, and cannot be solely attributed to a missing water source, since in such a glasshouse experiment the source water isotopic composition was always known. Instead, the authors suggested that the mechanism behind this offset may not be a discrimination process during root water uptake. Instead, these authors suggested that this offset would be driven by the relative depletion in  $\delta^2\text{H}$  of stored stem water, which would cause isotopic heterogeneity among pools within the plant: water in the conductive xylem tissues and stored water in the non-conductive tissues. Interestingly, this mechanism is consistent

with the findings (Zhao et al., 2016; Barbeta, Burlett, et al., 2020), that observed a more depleted  $\delta^2\text{H}$  in water in non-conductive xylem tissues, compared to xylem sap.

Hence, although scattered evidence across the literature strongly suggests that the mismatch in isotopic composition between plant and source waters would not be restricted to saline or arid environments, we still lack a systematic analysis of such mismatch in the literature. A global analysis of the magnitude of this offset and its distribution across biomes, climates and plant functional types is key for unveiling a mechanistic explanation for this phenomenon.

## 2. AIM AND HYPOTHESES OF THE STUDY

The aim of this study is to compile a database of all plant and source water isotopic compositions available in the literature, encompassing different biomes and a wide range of plant functional types, to perform a global meta-analysis of the magnitude of the soil-plant isotopic offset and also test the effect of climatic and plant related variables. We aimed to test the following hypotheses:

- 1) The slope of the SWL (Eq. 5), incorporated in the calculation of the soil-plant isotopic offset, should be steeper in more humid climates.
- 2) Under the assumption of the absence of isotopic fractionation during root water uptake and plant water transport, the isotopic composition of plant water reflects that of its sources. Hence, if soil water is the main water source, the SW-excess should not be significantly different from zero, whereas when precipitation and/or groundwater constitute the main water source, the LC-excess should not be significantly different from zero.
- 3) The absence of systematic soil-plant water isotopic offsets is consistent across different climates and plant functional types.

### 3. MATERIALS AND METHODS

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#### 1. DATA COMPILATION

To create the database, we extracted all studies cited on three previous meta-analyses (Barbeta and Peñuelas, 2017; Evaristo and McDonnell, 2017; Amin et al., 2020) and then selected only those that reported both  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ . Next, we updated our selection by performing a bibliographic search of papers published between 2016 and 2020 on Scopus, Web of Science and Google Scholar. The search was performed in April 2020 using the following terms: (water AND isotop\*) AND (dual OR (hydrogen AND oxygen)) AND (plant OR tree OR vegetat\*) AND source AND NOT nitrogen. After removing duplicated studies, title and abstract screening, we retained studies that reported: 1) plant and source (soil) water isotopic composition, 2) both  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for both source and plant waters; 3) sufficient data to calculate the soil water line, i.e. at least 3 dual isotope data extracted from the soil profile and 4) bulk source (soil) and plant water  $\delta^2\text{H}$   $\delta^{18}\text{O}$ , i.e. waters extracted following cryogenic vacuum distillation or similar procedures (e.g. Orłowski et al., 2013).

In order to collect the data from the selected papers, we created a database where we compiled the following information:

**Data related to the publication:** name of first author, year of publication, journal name, title of the study and DOI.

**Geographic data:** country; geographic area within country; latitude; longitude; elevation and ecosystem type, a factor variable with four levels: natural, agricultural, urban garden and controlled conditions (glasshouse studies).

**Climatic data:** mean annual precipitation (MAP, in mm/year), mean annual temperature (MAT, in °C), Lang aridity index ( $I_L = \text{MAP}/\text{MAT}$ ), climate class adapted from the Köppen-Geiger classification (Kottek et al., 2006), with four classes, namely: arid (BS and BW), tropical (A), warm (C) and cold (E and D); season, a categorical variable with three values: dry, wet or not-applicable, for studies where either there is no dry or wet season, or data for multiple seasons were pooled together and slope and intercept of the LMWL of the study site. For those studies where MAP and MAT were not reported, these were

extracted from the Worldclim database (Fick and Hijmans, 2017). For those studies where MAP and MAT were reported, we compared the MAP and MAP extracted from Worldclim with the reported values and we found a high significant correlation for both ( $R^2 > 87$ ), so for further analyses we used only MAP and MAT extracted from Worldclim data. For those studies where the LMWL was not reported, the slope and intercept of the LMWL were extracted from a repository of global isotopic data (Wateriso, 2020).

**Plant functional type data:** plant species, plant group (angiosperm or gymnosperm), leaf habit (deciduous, semi-deciduous or evergreen), leaf shape (broadleaf or narrow leaved) and growth form (trees, shrubs and non-woody).

**Isotopic composition of plant water:**  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  (in ‰) and tissue sampled, water extraction and analysis method.

**Isotopic composition of sources:**  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  composition of soil samples, date and season of the sampling and isotopic composition of other possible sources such as rain, stream water and groundwater. For soil samples, we also recorded the sampling depth (when provided), as well as the water extraction and analysis method.

The isotopic composition data ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) was extracted from tables or figures of the article, or obtained directly from the author's database, when provided. Data from figures was extracted using WebPlotDigitizer (Rohatgi, 2020).

In addition, for those studies where multiple samplings were performed, we also recorded the sampling date (year and month). Finally, we also recorded the sampling plot for studies where data from ecologically distinct plots (e.g. high and low elevation plots as in Berry et al., 2014) were sampled. We grouped all the data from a specific sampling date and plot, within a study and hereafter, we refer to these groups as *campaigns*.

## 2. SOIL WATER LINE AND OFFSET CALCULATION

The database was processed in R (R Core Team, 2020), using the *tidyverse* package (Wickham et al., 2019).

For each study, we established different campaigns considering 1) the study, 2) the sampling date (using year and month) and 3) the sampling plot, when they were ecologically meaningful. For each campaign, we calculated the soil water line according to Eq. 5. Significant regression lines were selected according to  $p$ -values ( $P < 0.05$ ),  $R^2$  ( $R^2 > 0.5$ ) and sample size ( $n \geq 3$ ).

Since the main water source for plants is the soil, we can calculate the isotopic offset between a plant water sample and the soil. Here, we refer to this offset as soil water [line conditioned] excess (SW-excess hereafter), according to the definition of Barbeta (2019). This SW-excess is a measure of the deviation of a given plant water sample and its soil water line. This variable is calculated for each plant dual isotope data pair ( $\delta^2H_p$  &  $\delta^{18}O_p$ ) and its corresponding SWL, according to:

Eq. 6:

$$SW - excess = \delta^2H_p - a_s \times \delta^{18}O_p - b_s$$

In Eq. 6,  $a_s$  and  $b_s$  are the slope and intercept of the corresponding SWL. Positive SW-excess values indicate enrichment in  $\delta^2H$  of plant samples with respect to their corresponding SWL and are positioned above soil water in the dual isotopic plot, while negative SW-excess values indicate depletion in  $\delta^2H$  compared to the SWL and are positioned below the SWL in the dual isotopic plot). We calculated the SW-excess value for each plant sample and then we calculated the mean SW-excess for each species and campaign.

Also, we calculated the LC-excess for each plant sample according to Eq. 4, using the slope and intercept of the corresponding LMWL, and then averaged these values per species and campaign. Plant samples with positive LC-excess values indicate enrichment in  $\delta^2H$  with respect to the LMWL and negative LC-excess values indicate depletion in  $\delta^2H$  with respect to the LMWL.



## 1. STATISTICAL ANALYSES

For the statistical analysis, we used the following R packages: *lme4* (Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), *MuMin* (Barton, 2009), and *performance* (Dominique et al., 2020). We assessed the effects of climate and plant type on the slope of the SWL and calculated SW-excess using hierarchical linear mixed models (LMMs). For our LMMs we used the following structure: our random factors were the study and sampling season, nested within study and our fixed factors were climatic variables (climate class, MAP, MAT and Lang index, which we tested individually and also combining MAP and MAT) for both SWL slope and SW-excess, and plant functional type variables (leaf shape, leaf habit, growth form and plant group, tested individually), only for SW-excess. Season was not included in the fixed part of the model because it could not be assigned to >30% of the campaigns. In addition, to assess the global value of the calculated variables (SWL slope and SW-excess), we also performed our LMMs without the fixed effects (null models). Finally, to assess differences among categories, within groups, we used post-hoc tests.

The size of the database (and hence the degrees of freedom) used in each LMMs depended on the fixed variables included: null models included all campaigns; but to assess climatic effects, we discarded those campaigns where the hydrological regime was managed, *i.e.* glasshouse studies, irrigated crops or urban gardens. Finally, to assess the effect of certain functional traits such as leaf shape, leaf habit or plant group, we only selected woody species (trees or shrubs). We did so, to avoid large differences in sample size among categories, as all non-woody species were angiosperms, and the vast majority were broad-leaved deciduous species.

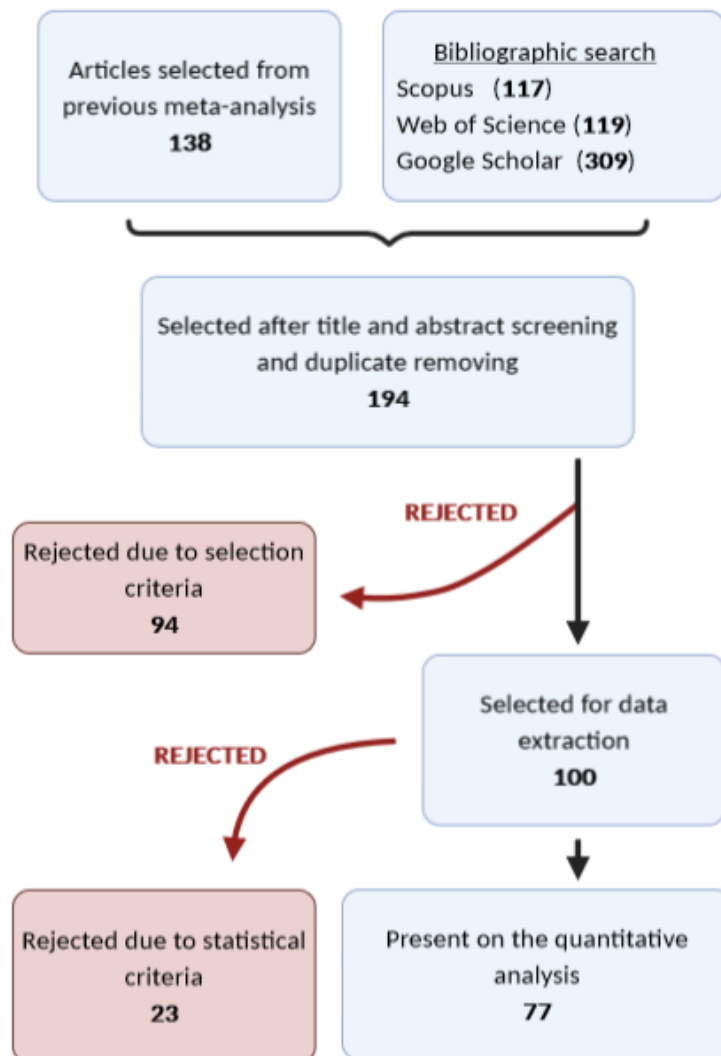
Models were generated with different and biologically meaningful combinations of the response variables, avoiding collinearity among fixed factors. We compared our models based on the Akaike information criterion (AIC, citation) and chose the ones with the smallest AIC, and when two or more models had similar AIC's ( $\pm 2$ ), we selected the one with less degrees of freedom. All models were tested for normality, heteroscedasticity, linearity and covariance.

## 4. RESULTS

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### 1. DATA EXTRACTION

The process of paper selection for data extraction is summarized in Figure 3. Our initial selection rendered 194 studies, after title and abstract screening of the outcomes of each bibliographical search and duplicates removing. Then, we rejected papers based on our defined selection criteria (see Materials and methods), retaining 100 studies. Finally, 77 of those were included in the quantitative analysis, rejecting 23 studies due to a non-significant or poor fit of the SWL.



**Figure 3.** Flow information scheme of the systematic review phases based on PRISMA Statement (Moher et al., 2009).

The studies finally included in the quantitative analysis are shown in Table 1:

**Table 1.** Authors, year of publication, journal, country of the study and the title of the 77 articles present in the quantitative analysis.

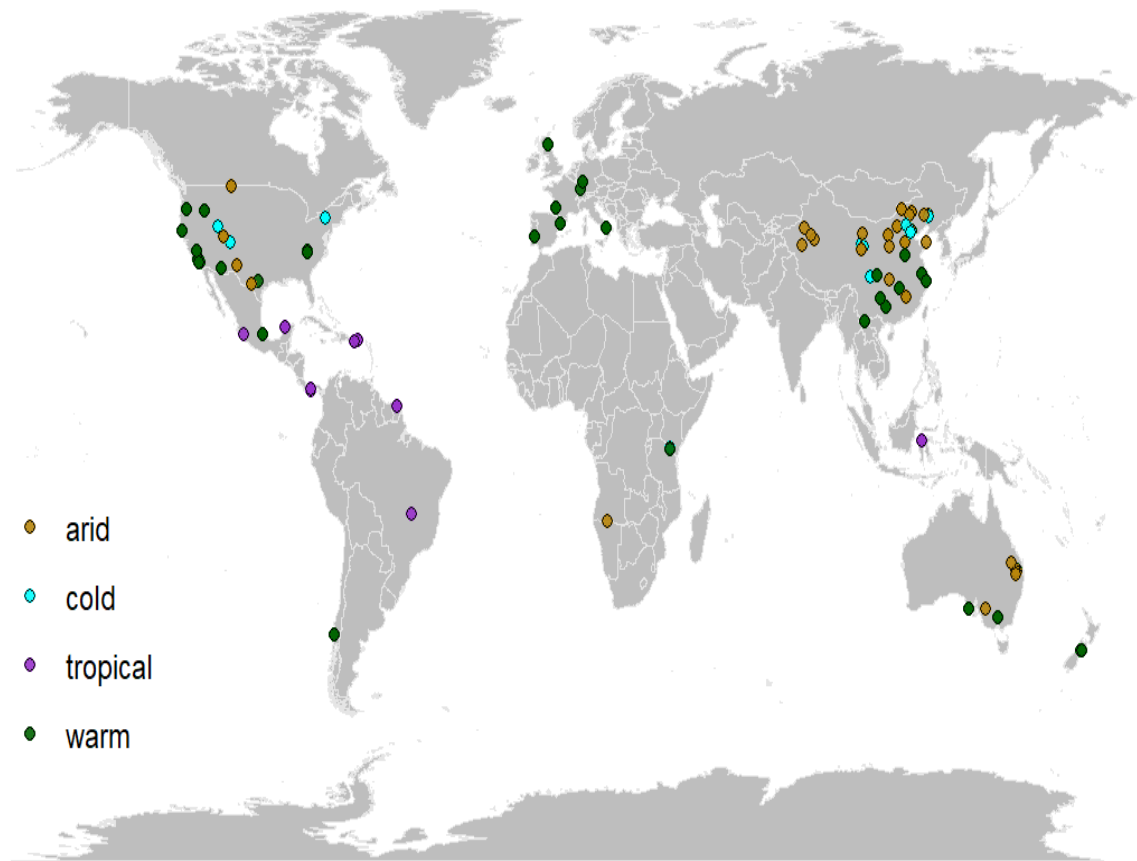
Author and year	Journal	Country	Title
Anderegg et al. (2013)	Global Change Biology	USA	Drought characteristics' role in widespread aspen forest mortality across Colorado. USA
Barbeta et al. (2015)	Global Change Biology	Spain	The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest
Barbeta et al. (2020)	New Phytologist	France	An explanation for the isotopic offset between soil and stem water in a temperate tree species
Barbeta et al. (2019)	Hydrology and Earth System Sciences	France	Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest
Berry et al. (2014)	Oecologia	USA	Cloud immersion: an important water source for spruce and fir saplings in the southern Appalachian Mountains
Beyer et al. (2016)	Journal of Hydrology	Namibia	A deuterium-based labeling technique for the investigation of rooting depths, water uptake dynamics and unsaturated zone water transport in semiarid environments
Bijoor et al. (2012)	Urban Ecosystems	USA	Water sources of urban trees in the Los Angeles metropolitan area
Bode et al. (2020)	Ecohydrology	Tanzania	Water-isotope ecohydrology of Mount Kilimanjaro
Bowling et al. (2017)	Ecohydrology	USA	Revisiting streamside trees that do not use stream water: can the two water worlds hypothesis and snowpack isotopic effects explain a missing water source?
Brandes et al. (2007)	Plant, Cell and Environment	Germany	Assessing environmental and physiological controls over water relations in a Scots pine ( <i>Pinus sylvestris</i> L.) stand through analyses of stable isotope composition of water and organic matter
Brinkmann et al. (2018)	New phytologist	Switzerland	Employing stable isotopes to determine the residence times of soil water and the temporal origin of water taken up by <i>Fagus sylvatica</i> and <i>Picea abies</i> in a temperate forest
Brooks et al. (2010)	Nature Geoscience	USA	Ecohydrologic separation of water between trees and streams in a Mediterranean climate
Cao et al. (2018)	Agricultural Water Management	China	The effects of rainfall and irrigation on cherry root water uptake under drip irrigation
Chi et al. (2019)	Ecology and evolution	China	Increased snowfall weakens complementarity of summer water use by different plant functional groups
Cramer et al. (1999)	Agricultural Water Management	Australia	Transpiration and groundwater uptake from farm forest plots of <i>Casuarina glauca</i> and <i>Eucalyptus camaldulensis</i> in saline areas of southeast Queensland, Australia
De Deurwaerder et al. (2018)	Tree Physiology	French Guiana	Liana and tree below-ground water competition-evidence for water resource partitioning during the dry season
Dong et al. (2020)	Journal of Arid Land	China	Stable oxygen-hydrogen isotopes reveal water use strategies of <i>Tamarix taklamakanensis</i> in the Taklimakan Desert, China
Dubbert et al.	New Phytologist	Portugal	A pool-weighted perspective on the two-water-worlds hypothesis

(2019)			
Dudley et al. (2018)	Ecohydrology	New Zealand	Water sources for woody shrubs on hillslopes: An investigation using isotopic and sap flow methods
Dwivedi et al. (2020)	Ecohydrology	USA	Vegetation source water identification using isotopic and hydrometric observations from a subhumid mountain catchment
Estrada-Medina et al. (2013)	Trees - Structure and Function	Mexico	Source water phenology and growth of two tropical dry forest tree species growing on shallow karst soils
Evaristo et al. (2016)	Hydrological Processes	Puerto Rico	Insights into plant water uptake from xylem-water isotope measurements in two tropical catchments with contrasting moisture conditions
Feikema et al. (2010)	Plant and soil	Australia	The water balance and water sources of a <i>Eucalyptus</i> plantation over shallow saline groundwater
Gaines et al. (2016)	Tree Physiology	USA	Reliance on shallow soil water in a mixed-hardwood forest in central Pennsylvania
Geris et al. (2015)	Hydrological Processes	United Kingdom	Ecohydrological separation in wet northern environments? A preliminary assessment using different soil water extraction techniques
Geris et al. (2017)	Science of the Total Environment	United Kingdom	Spatial and temporal patterns of soil water storage and vegetation water use in humid northern catchments
Gierke et al. (2016)	Hydrogeology Journal	USA	Soil-water dynamics and tree water uptake in the Sacramento Mountains of New Mexico (USA): a stable isotope study
Goldsmith et al. (2012)	Ecohydrology	Mexico	Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest
Gómez-Navarro et al. (2019)	Ecohydrology	USA	Spatiotemporal variability in water sources of urban soils and trees in the semiarid, irrigated Salt Lake Valley
Hartsough et al. (2008)	Arctic, Antarctic, and Alpine Research	Mexico	Stable isotope characterization of the ecohydrological cycle at a tropical treeline site
Herve et al. (2016)	Hydrological Processes	Chile	Assessing the two water world hypothesis and water sources for native and exotic evergreen species in south-central Chile
Holland et al. (2006)	Australian Journal of Botany	Australia	Tree water sources over shallow, saline groundwater in the lower River Murray, south-eastern Australia: Implications for groundwater recharge mechanisms
Jia et al. (2018)	Fresenius Environmental	China	The seasonal water use patterns of <i>Populus pseudo-simmonii</i> Kitag in the Otindag Sandy Land
Jones et al. (2020)	Hydrogeology Journal	Australia	Field investigation of potential terrestrial groundwater-dependent ecosystems within Australia Great Artesian Basin
Knighton et al. (2020)	Ecohydrology	USA	Using isotopes to incorporate tree water storage and mixing dynamics into a distributed ecohydrologic modelling framework
Kulmatiski et al. (2006)	Plant and Soil	USA	Exotic plant communities shift water-use timing in a shrub-steppe ecosystem
Leng et al. (2013)	Vegetation Science	China	Differential water uptake among plant species in humid alpine meadows
Liu et al. (2014)	Ecohydrology	China	Dry-season water utilization by trees growing on thin karst soils in a seasonal tropical rainforest of Xishuangbanna, Southwest China

Liu et al. (2019)	Forests	China	The depth of water taken up by Walnut trees during different phenological stages in an irrigated arid hilly area in the Taihang Mountains
Liu et al. (2011)	European Journal of Soil Biology	China	Analyzing relationships among water uptake patterns, rootlet biomass distribution and soil water content profile in a subalpine shrubland using water isotopes
Luo et al. (2019)	Journal of Hydrology	China	Examination of the ecohydrological separation hypothesis in a humid subtropical area: Comparison of three methods
Ma et al. (2016)	Science of the Total Environment	China	Using stable isotopes to determine seasonal variations in water uptake of summer maize under different fertilization treatments
Marttila et al. (2018)	Ecohydrology	New Zealand	Does transpiration from invasive stream side willows dominate low-flow conditions? An investigation using hydrometric and isotopic methods in a headwater catchment
McCole et al. (2007)	Journal of Hydrology	USA	Seasonal water use patterns of <i>Juniperus ashei</i> on the Edwards Plateau, Texas, based on stable isotopes in water
McCutcheon et al. (2017)	Hydrological Processes	USA	An evaluation of the ecohydrological separation hypothesis in a semiarid catchment
Moore et al. (2016)	Ecohydrology	USA	Flood water legacy as a persistent source for riparian vegetation during prolonged drought: an isotopic study of <i>Arundo donax</i> on the Rio Grande
Muñoz-Villers et al. (2020)	Hydrology and Earth System Sciences	Mexico	Coffee and shade trees show complementary use of soil water in a traditional agroforestry ecosystem
Muñoz-Villers et al. (2018)	Oecologia	Mexico	Reduced dry season transpiration is coupled with shallow soil water use in tropical montane forest trees
Nehemy et al. (2019)	Hydrology and Earth System Sciences Discussions	Switzerland	How plant water status drives tree source water partitioning
Newberry et al. (2017)	Ecohydrology	Switzerland	Cryogenic vacuum artifacts do not affect plant water-uptake studies using stable isotope analysis
Nie et al. (2011)	Plant and soil	China	Seasonal water use patterns of woody species growing on the continuous dolostone outcrops and nearby thin soils in subtropical China
Ohte et al. (2003)	Ecological Applications	China	Water utilization of natural and planted trees in the semiarid desert of Inner Mongolia, China
Poca et al. (2019)	Plant and Soil	Argentina	Isotope fractionation during root water uptake by <i>Acacia caven</i> is enhanced by arbuscular mycorrhizas
Qian et al. (2017)	Journal of Hydrology	China	Assessing the ecohydrological separation hypothesis and seasonal variations in water use by <i>Ginkgo biloba</i> L. in a subtropical riparian area
Qian et al. (2017)	Chemical speciation and bioavailability	China	Water sources of riparian plants during a rainy season in Taihu Lake Basin, China: a stable isotope study
Ripullone et al. (2020)	Tree physiology	Italy	Variation in the access to deep soil water pools explains tree-to-tree differences in drought-triggered dieback of Mediterranean oaks
Rong et al. (2011)	Hydrological Processes	China	Isotopic analysis of water sources of mountainous plant uptake in a karst plateau of southwest China

Rose et al. (2003)	Oecologia	USA	Water source utilization by <i>Pinus jeffreyi</i> and <i>Arctostaphylos patula</i> on thin soils over bedrock
Rossatto et al. (2012)	Environmental and Experimental Botany	Brasil	Depth of water uptake in woody plants relates to groundwater level and vegetation structure along a topographic gradient in a neotropical savanna
Schwendenmann (2019)	-	Indonesia	Data: H and O isotope signatures of soil and xylem samples, cacao agroforest, Indonesia.
Schwendenmann and Jost (2019)	-	Panama	Data: Water stable isotope signature of soil and xylem samples under different land use systems, Panama.
Simonin et al. (2014)	Ecohydrology	USA	Vegetation induced changes in the stable isotope composition of near surface humidity
Song et al. (2016)	Environmental and Experimental Botany	China	Water use patterns of <i>Pinus sylvestris</i> var. <i>mongolica</i> trees of different ages in a semiarid sandy lands of Northeast China
Song et al. (2014)	Trees - Structure and Function	China	Water utilization of <i>Pinus sylvestris</i> var. <i>mongolica</i> in a sparse wood grassland in the semiarid sandy region of Northeast China
Sun et al. (2019)	Hydrology Research	China	Short-term changing patterns of stem water isotopes in shallow soils underlain by fractured bedrock
Swaffer et al. (2014)	Hydrological Processes	Australia	Water use strategies of two co-occurring tree species in a semi-arid karst environment
Wang et al. (2019)	Science of the Total Environment	China	Inter-comparison of stable isotope mixing models for determining plant water source partitioning
Wei et al. (2013)	Trees - Structure and Function	China	Stable isotopic observation of water use sources of <i>Pinus sylvestris</i> var. <i>mongolica</i> in Horqin Sandy Land, China
West et al. (2007)	Oecologia	USA	Seasonal variations in moisture use in a piñon-juniper woodland
Wu et al. (2016)	Science of the Total Environment	China	Contrasting water use pattern of introduced and native plants in an alpine desert ecosystem, Tibet Plateau, China
Wu et al. (2016)	Environmental Earth Sciences	China	Differential soil moisture pulse uptake by coexisting plants in an alpine <i>Achnatherum splendens</i> grassland community
Yang et al. (2015)	Agricultural and Forest Meteorology	China	Seasonal variations in depth of water uptake for a subtropical coniferous plantation subjected to drought in an East Asian monsoon region
Zhang et al. (2011)	Field Crops Research	China	Coupling a two-tip linear mixing model with a $\delta D$ - $\delta^{18}O$ plot to determine water sources consumed by maize during different growth stages
Zhao et al. (2020)	Land Degradation and Development	China	Does damming streams alter the water use strategies of riparian trees? A case study in a subtropic climate
Zhou et al. (2019)	Journal of Hydrology	China	Variation in depth of water uptake for <i>Pinus sylvestris</i> var. <i>mongolica</i> along a precipitation gradient in sandy regions
Zhu et al. (2016)	PLoS ONE	China	Seasonal Dynamics of Water Use Strategy of Two <i>Salix</i> Shrubs in Alpine Sandy Land, Tibetan Plateau

The data of the quantitative analysis presents 94 different geographic locations, as seen in Figure 4. Most sites belonged to warm climates (43 observations) followed by arid (29 observations) and tropical and cold climates (11 observations each one)



*Figure 4. World map showing each sampling location. Colours depict climate classes.*

The resulting databases included more than 5.000 observations of dual isotopic measurements for plants and more than 7.000 for soil samples. The 77 selected studies included 196 different campaigns. We calculated one SWL for each campaign, and then we calculated a mean SW-excess and LC-excess for each species within each campaign. This rendered a database with 361 observations. More information about the different characteristics of the database are found in Table 2.

**Table 2.** Total number of observations (Obs.), studies, campaigns and species for each climatic class and plant type. Mean  $\pm$  Sd values of the slope of the SWL line (unitless) and SW-excess (in %) are provided for each group.

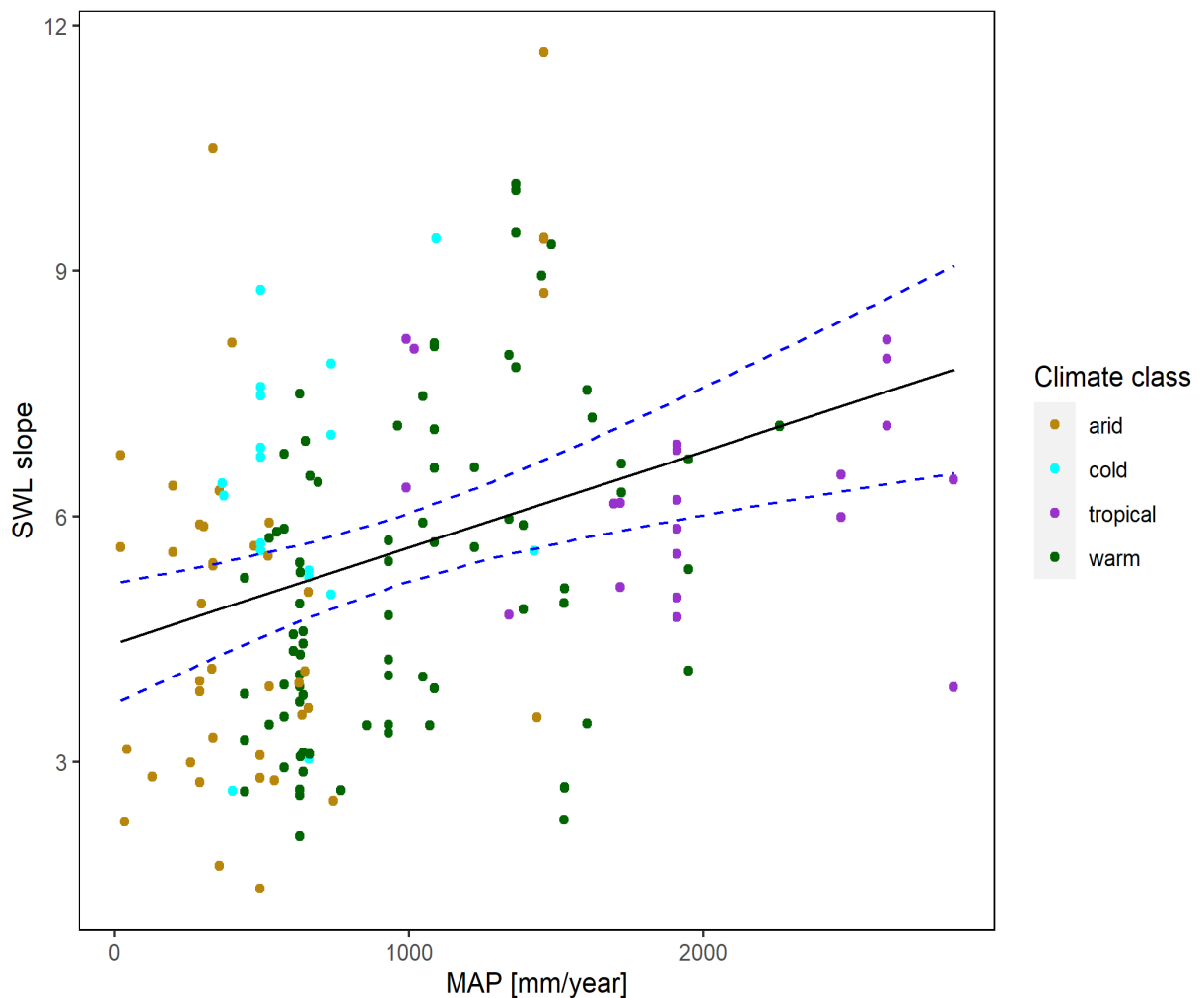
	Obs.	Studies	Campaigns	Species	SWL slope	SW-excess
<b>TOTAL*</b>	361	77	196	141	5.49 $\pm$ 2.30	-1.79 $\pm$ 7.88
<b>Climate</b>						
Tropical	49	8	21	17	6.68 $\pm$ 1.20	-3.27 $\pm$ 3.47
Warm	196	32	83	60	4.91 $\pm$ 1.88	-1.88 $\pm$ 8.15
Arid	71	21	48	28	5.39 $\pm$ 2.66	-1.01 $\pm$ 9.56
Cold	39	11	29	20	7.34 $\pm$ 2.95	-0.41 $\pm$ 7.938
<b>Plant group</b>						
Angiosperm	205	-	-	93	-	-1.63 $\pm$ 8.10
Gymnosperm	69	-	-	21	-	0.17 $\pm$ 6.20
<b>Growth form</b>						
Tree	209	-	-	75	-	-1.93 $\pm$ 8.00
Shrub	44	-	-	17	-	1.16 $\pm$ 6.46
Non-woody	41	-	-	23	-	-2.06 $\pm$ 6.45
<b>Leaf habit</b>						
Evergreen	195	-	-	66	-	-0.57 $\pm$ 7.29
Deciduous	65	-	-	32	-	-3.47 $\pm$ 9.29
Semi-deciduous	3	-	-	3	-	-9.67 $\pm$ 0.96
<b>Leaf form</b>						
Broadleaved	153	-	-	67	-	-1.44 $\pm$ 8.67
Narrow leaved	81	-	-	26	-	-0.12 $\pm$ 6.19

\*Note that the sum of observations for climatic classes differs from the overall number of observations. because glasshouse studies have been excluded for the climatic classification. Also, the sum of observations from plant groups does not match the total number of observations because species identity was not always reported. For example, some studies reported the isotopic composition of plant water from a list of species without distinguishing the specific species; in the case where this list consisted of a mix of trees from different plant groups, we could use this study for assessing the effect of growth form (tree, shrub or non-woody), but not of plant group (angiosperms or gymnosperms).



## 2. WORLDWIDE PATTERNS OF THE SOIL WATER LINE

The mean slope of the SWL across all studies was  $5.49 \pm 2.30$  (unitless), lower than that of the GMWL. According to the results of the null model, this value was significantly positive (Table 3). Following model selection based on the AIC, the best model for predicting the effects of climate on the slope of the SWL, included MAP (in mm/year) alone as fixed factor, whereas models including other climatic variables; such as climate class, MAT or the Lang aridity index; were assigned higher AIC values. MAP had a positive significant effect on the slope of the SWL (Table 3, Figure 5). The slope of the SWL increased by 1 unit per each 500 mm of MAP (Figure 5).



**Figure 5.** Prediction of the slope of the soil water evaporation line (SWL slope, unitless) with mean annual precipitation (MAP). Each point is the slope of the SWL from a study campaign (see main text for definition of study campaign). Colours depict different climate classes. The black line is the linear fit of the main effect of the linear mixed model and the blue dotted line represent the standard error (see Table 3).

**Table 3.** Results from the null and best models for the slope of the soil water line. The random factors of these linear mixed models were study and sampling season, nested within study

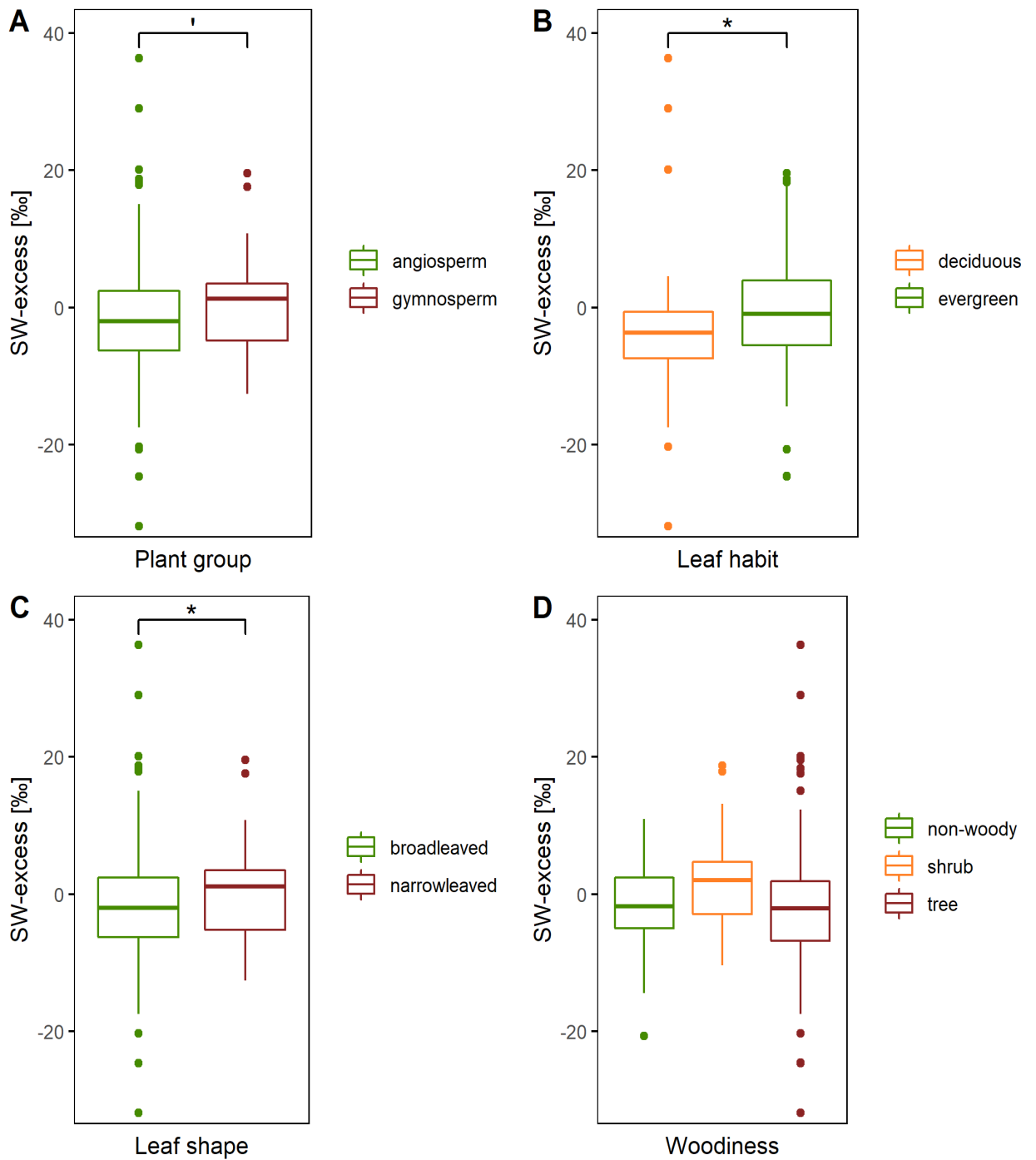
PREDICTORS	ESTIMATE	STD ERROR	DEGREE FREEDOM	T-VALUE	P-VALUE
SWL SLOPE (NULL)					
INTERCEPT	5.545	0.2270	82.48	24.22	<b>&lt;0.001</b>
SWL (CLIMATE: PRECIPITATION, MAP IN MM/YEAR)					
INTERCEPT	4.449	0.3840	80.51	11.69	<b>&lt;0.001</b>
MAP	0.002	0.0003	83.89	3.58	<b>&lt;0.001</b>

\*Significant ( $P < 0.05$ ) P-values are indicated in **bold font**.

### 3. WORLDWIDE PATTERNS OF THE PLANT-SOIL ISOTOPIC OFFSET

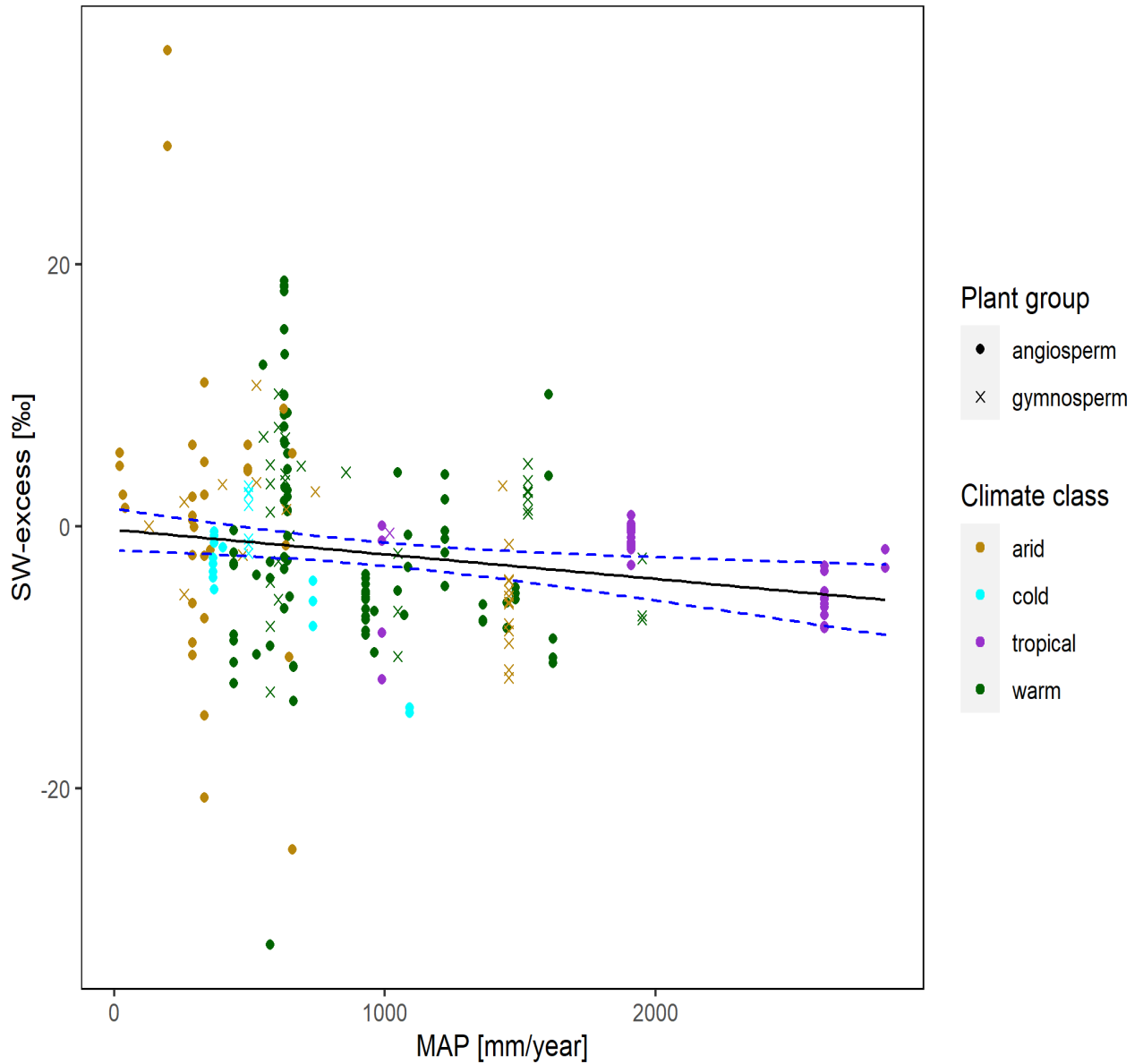
The results of the null model for the SW-excess showed that the overall SW-excess was significantly negative after considering the random variability among studies and climatic season (Table 4). The mean overall SW-excess was  $-1.508 \pm 0.705\%$ . When analysing the effects of climate, we found that the best model (lowest AIC value) included MAP, instead of other climatic variables such as climate class, MAT or the Lang aridity index. We found that MAP had a significant negative effect on the SW-excess (Table 4), meaning that the magnitude of the soil-plant isotopic offset increased (became more negative) as MAP increased (Figure 6)

Regarding the effects of plant functional traits and plant groups, we analysed these on separate models (Figure 6). Plant group (angiosperm vs. gymnosperm) only had a marginally significant effect ( $P = 0.076$ ) on SW-excess (Table 4). We found that angiosperms had a marginally (see Table 4) larger (more negative) SW-excess than gymnosperms. Regarding the effect of leaf morphology (broadleaf vs. narrowleaf), we found that species with broad leaves (mostly angiosperms) had a larger (more negative) SW-excess than those with narrow leaves (mostly gymnosperms, see Table 4, Figure 7). These results were consistent with the effects observed for leaf habit. Deciduous species (mostly angiosperms with broad leaves) had a larger (more negative) SW-excess than evergreen species (mostly gymnosperms with narrow leaves, Table 4). Deciduous species had a larger (more negative) SW-excess than evergreen species (Figure 7). Finally, we did not find significant differences among growth forms (trees, shrubs and non-woody species, Table 4, Figure 7).



**Figure 6.** Four boxplots of the SW-excess [‰] for different plant functional type and groups: A) plant group, B) leaf habit, C) leaf shape, D) growth form. Asterisks (\*) and dots (.) indicate significant ( $P < 0.05$ ) and marginally significant ( $P < 0.1$ ) differences between groups according to the results of the linear mixed models (Table 4).

When analysing together the effects of climate and plant type on the SW-excess, we found that the best model was the one that included MAP and plant group (angiosperm and gymnosperm), but not their interaction. Mean annual precipitation (MAP) had a negative significant overall effect on the SW-excess (Table 4), whereas there were no significant differences between plant groups (Figure 8).



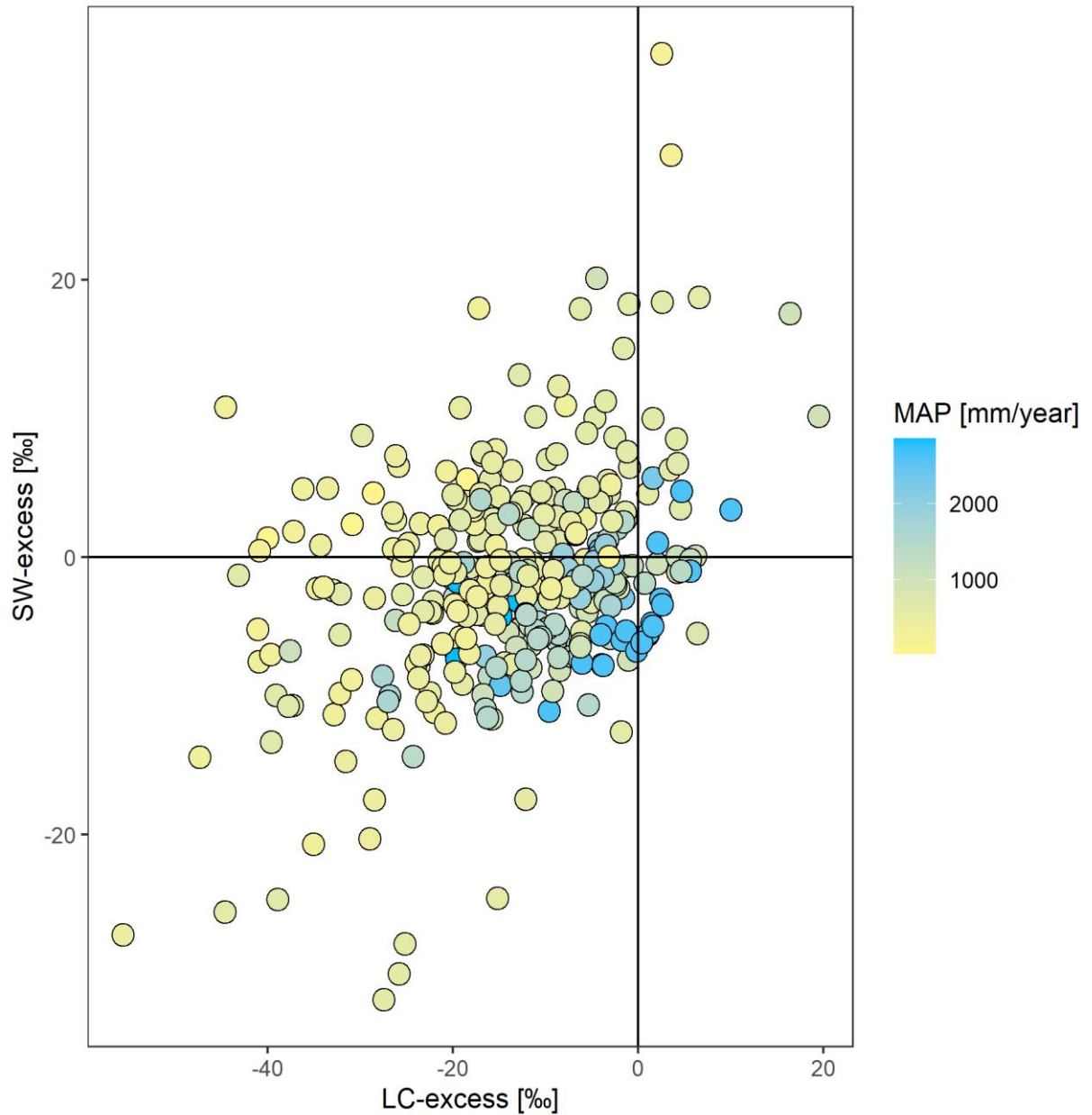
**Figure 7.** Predicted SW-excess [‰] with mean annual precipitation (MAP, [mm/year]). Each point is the mean SW-excess of a species from a study campaign (see main text for definition of study campaign). Colours and shapes depict different climate classes and plant groups, respectively. The line is a linear fit of the main effect of the linear mixed and the dotted blue line is the standard error (see general model, Table 3).

**Table 4.** Results from the null and best models for the SW-excess [‰]. Predictor variables included climatic values, plant functional traits (or groups) or both. The random factors of these linear mixed models were study and sampling season, nested within study

PREDICTORS	ESTIMATE	STD ERROR	DEGREE FREEDOM	T-VALUE	P-VALUE
SW-EXCESS (NULL MODEL)					
INTERCEPT	-1.508	0.705	64.07	-2.13	<b>0.036</b>
SW-EXCESS (CLIMATE: PRECIPITATION)					
INTERCEPT	1.011	1.350	56.08	0.75	0.451
MAP	-0.002	0.001	52.40	-2.34	<b>0.022</b>
SW-EXCESS (PLANT GROUP)					
INTERCEPT (ANGIOSPERM AS REFERENCE)	-1.545	1.094	55.60	-1.41	0.160
GYMNOSPERM	2.378	1.334	2.06	1.74	<i>0.071</i>
SW-EXCESS (LEAF SHAPE)					
INTERCEPT (BROADLEAF AS REFERENCE)	-1.859	1.128	58.04	-1.64	0.104
NARROW LEAF	2.865	1.339	195.51	2.14	<b>0.037</b>
SW-EXCESS (GROWTH FORM)					
INTERCEPT (NON-WOODY AS REFERENCE)	-0.846	1.610	134.07	-0.52	0.602
SHRUB	-0.343	1.96	203.22	-0.17	0.861
TREE	-0.823	1.69	174.76	-0.48	0.628
SW-EXCESS (GENERAL MODEL: PRECIPITATION AND PLANT GROUP)					
INTERCEPT (ANGIOSPERM AS REFERENCE)	2.260	2.010	53.30	1.11	0.260
GYMNOSPERM	1.680	1.380	160.50	1.21	0.230
MAP	-0.004	0.001	45.40	-2.50	<b>0.029</b>

\*Significant ( $P < 0.05$ ) and marginally significant ( $P < 0.1$ ) are indicated in **bold** and *italic* font, respectively.

Also, we studied the relation of the LC-excess and the SW-excess of each species and campaign, and we found that 60% of the samples had both negative SW-excess and LC-excess, being the LC-excess negative in almost 90% of the negative SW-excess samples. Also 28% of the samples with positive SW-excess have negative LC-excess values (as seen in Figure 8).



**Figure 8.** SW-excess plotted against LC-excess. Each point represents the mean of species sampled in a specific campaign. Colours indicate mean annual precipitation (MAP, in mm/year) according to the indicated colour scale.

## 5. DISCUSSION

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### 1. GLOBAL PATTERNS OF THE PLANT-SOIL ISOTOPIC OFFSET

Our global analysis of the dual isotopic composition of plant and source waters revealed that there is a significant mismatch in  $\delta^2\text{H}$  between plant water and its source. We analysed plant and soil water isotopic composition for a total of 196 sampling campaigns and 141 species, and for 60% of our observations, we found both negative values for the line conditioned excess (LC-excess), which indicates a mismatch in isotopic composition between the plant water and precipitation and groundwater, and for the soil water excess (SW-excess), which indicates a mismatch in isotopic composition between soil and plant water. This result indicates that the mismatch between plant and soil water in isotopic composition cannot be solely attributed to the fact that plants would be accessing alternative water sources, such as precipitation and/or groundwater. Our results are in line with the growing number of studies that reported relevant source-plant water isotopic offsets (Barbeta et al., 2019; Poca et al., 2019; Barbeta, Gimeno, et al., 2020; Carrière et al., 2020a). Importantly, the fact that at the global scale the isotopic composition of plant water was significantly more depleted in the dual-isotope space may challenge the general assumption that root water uptake and plant water transport are non-fractionating processes (Washburn and Smith, 1934; Zimmermann et al., 1967; Dawson and Simonin, 2011). Even though our broad-scale analysis does not allow us to unravel the mechanisms underlying these source-plant water isotopic offsets, they still provide some suggestions on the hypotheses that would be worth exploring to explain such offsets (Lin and Sternberg, 1993; Ellsworth and Williams, 2007; Zhao et al., 2016; Poca et al., 2019; Barbeta, Burlett, et al., 2020; Barbeta, Gimeno, et al., 2020). We argue so because our dataset indicates that soil-plant water isotopic offsets vary with climate and depending on the plant functional type or certain plant traits. Still, regardless of the specific underlying mechanisms, our results show that these mismatches between source and plant water isotopic composition may be more common than previously expected.

The SW-excess differed with plant traits (Figure 6). We found that the species with broad and deciduous leaves and angiosperms depicted larger SW-excess values than species with narrow and evergreen leaves and gymnosperms. These results suggest that physiological and anatomical traits are involved in the mechanisms driving source-plant water isotopic offsets. Angiosperms and gymnosperms have different wood anatomy and associated density, which for

instance, result in a different water storage capacity (Köcher et al., 2013; Matheny et al., 2015; Oliva-Carrasco et al., 2015). Our results partially agree with previous findings such as those of Zhao et al. (2016) who found significant differences in the  $\delta^2\text{H}$  of sap and the bulk stem water in poplar and hypothesised that stem water in non-conducting tissues (i.e. outside xylem vessels) was depleted in  $\delta^2\text{H}$  relative to sap water and suggested that this was a result of aquaporin-facilitated water transport in woody living cells. Similarly, water transport through aquaporins has also been hypothesized to underlay previously observed source-plant isotopic water offsets in plants forming mycorrhizal associations (Poca et al., 2019). Regardless of the mechanism, it seems that water in non-conducting xylem tissues would be isotopically-depleted compared to sap and source water (Barbeta, Burrett, et al., 2020; Barbeta, Gimeno, et al., 2020). Our finding that the SW-excess was different among plant groups; with contrasting water storage capacity, and parenchyma fraction (living cells); supports this explanation.

The sign and magnitude of the SW-excess was not only associated with biological traits, but also with the climate of the field sites included in the analysis. First, we found that mean annual precipitation (MAP) was positively correlated with the slope of the soil water evaporation line (SWL). As expected, in wetter sites, the slope of the SWL was steeper and closer to that of the global meteoric water line (GMWL) (Figure 5). In wet climates, soil evaporative losses are compensated by frequent rain events that refill soil water storage. As a consequence, the evaporative enrichment of the surface soil produced by the kinetic fractionation is frequently compensated by the water inputs of rain events that shift the soil water line closer to the local meteoric water line (LMWL) (Benettin et al., 2018). In the case of the SW-excess, MAP had a significantly negative effect on both the climate-only and the combined (climate and plant group or plant trait) models (figure 7). The SW-excess was more negative as MAP increased, which implies that plant water was more isotopically-depleted in  $\delta^2\text{H}$  compared to its source (soil water), in wetter places. One possible explanation is that in wetter climates, woody plants store larger amounts of water in their non-conducting woody tissues (Hartzell et al., 2017). This explanation is concurrent with previous observations, where sap water was found to be depleted in  $\delta^2\text{H}$  relative to the source of root water uptake. This would cause apparent isotopic fractionation as plant samples would fall below their corresponding soil water line (SW-excess significantly negative), when soil water was actually the most likely source. However, plants may access alternative water sources, which could be more depleted in heavy isotopes than the soil water. For example, plants that accessed groundwater, stream water or rainfall; with a lighter isotopic composition; may also plot below the SWL and hence depict a negative SW-excess. In such cases, we would then expect a LC-excess (relative to the LMWL, Eq. 4) close to zero, as



these mobile water pools would fall on the LMWL (Barbeta et al., 2018). Nonetheless, when combining the analyses of the SW-excess and the LC-excess we found that almost 90% of our negative SW-excess cases exhibited also a negative LC-excess. Simultaneous negative LC-excess and SW-excess cannot be explained by the use of mobile waters with an isotopic composition similar to that of the waters falling within the LMWL. Other nonexclusive processes, such as heterogeneities in within the soil matrix in water isotopic composition (Orlowski et al., 2013) or root discrimination (Lin and Sternberg, 1993; Ellsworth and Williams, 2007; Poca et al., 2019), may also be simultaneously driving these negative source-plant water isotopic offsets, but previous empirical evidence suggests that heterogeneities in isotopic composition among pools within the plant tissue is a more likely underlying cause.

## 2. LIMITATIONS OF THE STUDY

Our results challenge the general assumption that plant water isotopic composition should match that of its source. Instead, we show that plant water appears to be consistently more depleted in  $\delta^2\text{H}$  with respect to its source, whether it is soil water or meteoric waters. Nonetheless, we cannot provide a detailed mechanistic explanation for the underlying causes of these patterns. Yet, in this discussion, we provide some plausible explanations for the observed negative SW-excess, supported by the observed LC-excess. When the calculated LC-excess is close to zero, but the SW-excess is negative, this would mean that the plant is accessing another source of water (precipitation or groundwater) different to the soil and more depleted in isotopic composition. Still, when both the LC-excess and SW-excess are negative and not close to zero, then the plant is not reflecting faithfully the water source accessed. Previous studies (Zhao et al., 2016; Barbeta, Burlett, et al., 2020) explained observed source-plant isotopic offsets with the storage water hypothesis, meaning that the measured isotopic depletion on plants is due to the sampling of  $\delta^2\text{H}$  depleted storage water together with the sap water (which should resemble its source composition). Our study cannot confirm this hypothesis, but our results suggest do not discard it as our different patterns of source-plant isotopic offsets for different plant groups differing in certain traits could be explained by this hypothesis. Still, even if our results did not disagree with the hypothesis of the plant-soil isotopic offset being caused by storage waters, it these are not sufficient to fully infer that depleted stem storage water would be fully responsible for these observed offsets, as there are numerous functional traits related to anatomy that affect the water storage capacity within groups that were not included in our analyses, e.g wood density or vessel lumen area (Zanne et al., 2010). It is necessary to explore other possibilities beyond the stem water storage hypothesis. To do so, additional plant traits

should be incorporated to our analyses, as these could be simultaneously driving the observed source-plant isotopic offsets

Another limitation of our study is the unbalanced number of observations across plant groups and geographical regions in our database. First, our database was largely dominated by observations from angiosperms (205 data in contrast to 69 of gymnosperm, see Table 2 for more information about the data distribution), evergreen species, trees and broadleaved species. Second, some regions of the world are overrepresented in our database, while we lack observations in many regions of the world. Most of our studies collected data from sampling sites between 0° and 50°N latitudes. The USA and China are the countries that rendered more studies and hence the distribution of the studies is biased towards shrub and tree communities in warm and arid (and semi-arid) regions. As a result, in our database the distribution of climate classes is decompensated, with temperate ecosystems (warm) being the most represented.

### 3. IMPLICATIONS FOR RESOURCE MANAGEMENT AND ECOSYSTEM RESTORATION

Our results provide evidence that the assumption that plant water resembles isotopically its belowground source needs to be reconsidered. In many cases, this may not prevent the identification of plant water sources using the isotopic composition of water. However, it may lead to biases in the estimation of plant water sources in the not so rare cases where the SW-excess is of notable magnitude. As such, the existence of this significant soil-plant water isotopic does not deny the validity of plant water source studies using the isotopic composition of water. Rather, it is a reminder that there is a need to fully understand the isotopic processes behind the observed source-plant offsets to minimize the uncertainty associate to the use of stable isotope techniques in ecohydrology.

Our results call for caution when inferring water use from analyses of water isotopic composition, but they should not discourage researchers, resource managers and restoration practitioners to apply this methodology to assess the local water fluxes and groundwater recharge ratios, learn about the water use patterns of the vegetation or test water efficiency of crops and plantations, to develop better plans of water use towards sustainability. Water used by the vegetation, inferred from the analyses of water stable isotopic composition can provide very valuable information to enhance the survival and resilience of ecosystem restoration plantations and the effectiveness and success of revegetation actions. Nevertheless, it is important to be aware of pitfalls when assessing water use patterns by the vegetation, when source-plant isotopic offsets would be non-negligible. This would be particularly relevant in broadleaved deciduous forest, and in very humid and wet climates. We suggest that measuring

both  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  across various layers of the soil profile within a given date and occasional sampling of sap and storage water separately (e.g. as done on Magh et al., 2020) or Barbeta, Burlett, et al., 2020) could be a good option to assess the potential effect of source-plant isotopic offsets. Alternatively, a reliable modelling frame derived from our study could serve to estimate the expected magnitude of the source-plant offset from climatic water-availability and incorporate it into the calculations to infer the water sources, as sampling only sap water may be inaccessible for most projects. Nevertheless, further field research is needed to clarify whether the suggested mechanistic explanations actually hold.

## 6. CONCLUSIONS

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- ✚ The slope of the SWL is globally controlled by mean annual precipitation. The wetter the climate of a given location, the closer the soil water evaporation line is to the local meteoric water line.
- ✚ We found a significant and negative source-plant water offset in isotopic composition: plant water had a more depleted isotopic composition in  $\delta^2\text{H}$  than its sources. This result challenges the long-standing assumption that plant water isotopic composition faithfully reflects that of its sources.
- ✚ Access to alternative water sources of meteoric origin could not explain the observed soil-plant isotopic offsets
- ✚ Mean annual precipitation was the best predictor of the observed source-plant water isotopic offsets compared to mean annual temperature or their effect combined
- ✚ Soil-plant water isotopic offsets vary with plant traits, which hints to the biological role in driving apparent isotopic fractionation processes along the soil-plant-atmosphere hydraulic continuum.

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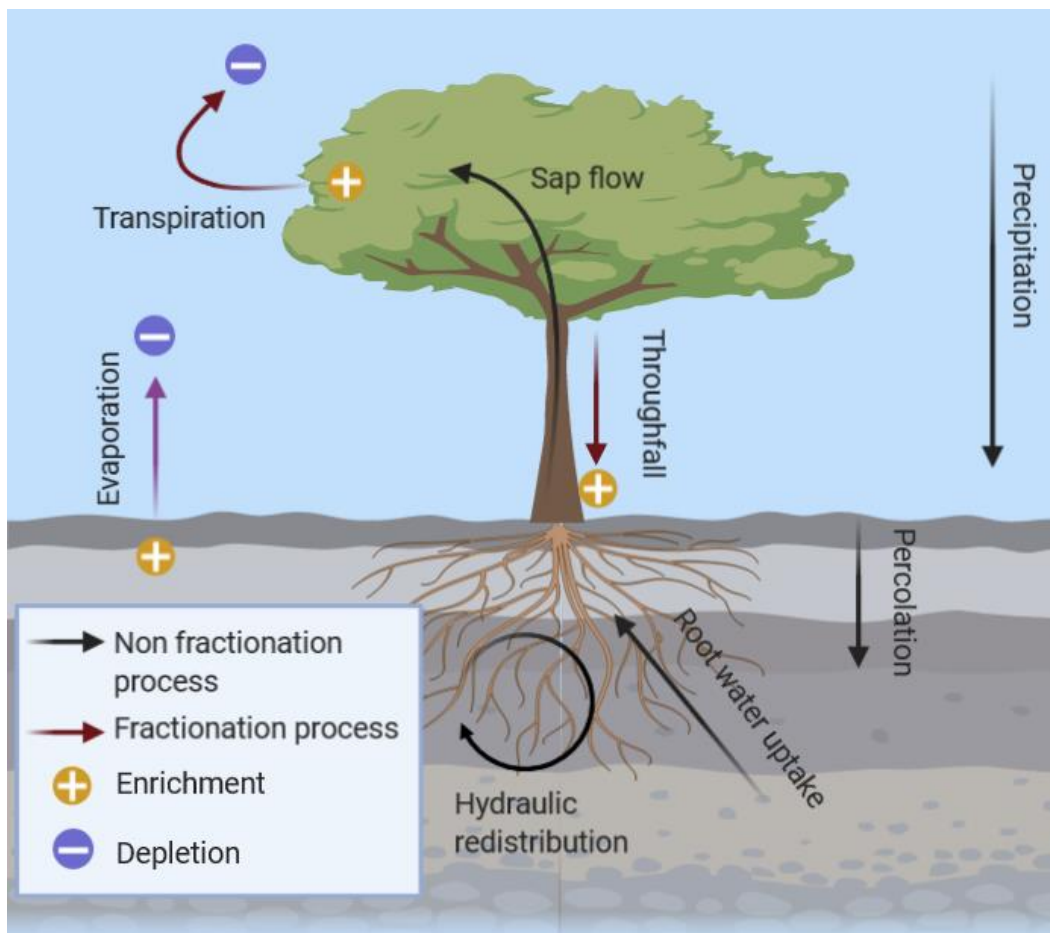
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## ANNEX 1: OTHER SOIL-PLANT-ATMOSPHERE ISOTOPIC INTERACTIONS

For this study, the plant-soil-atmosphere interaction we focus more on are the root water uptake and evaporation (and also precipitation). But we wanted to provide basic information of other interactions to help the reader to understand the whole isotopic cycle (Figure A.1).



**Figure A.1.** Scheme of the isotopic interactions between soil, plants and atmosphere on tree species. Based on Sprenger (2016)

In the plant-atmosphere interaction, water on the leaves (and photosynthetic tissues) remain enriched in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  because lighter isotopologues change phase preferentially during transpiration, while vapor water gets depleted (Dubbert and Werner, 2019). The isotopic composition of water vapor transpired from plant is the result of the transpiration process but depends on the plants source isotopic

composition (assuming that there is no fractionation during root water uptake and sap flow).

Another plant-atmosphere interaction involves vegetations aerial architecture, (canopy and trunk for trees) which participate in the isotopic fractionation process as the throughfall is an input of isotopic enriched waters, because the thin layer of water accumulated in the vegetation's surface after a precipitation event is subject to evaporation enrichment (Sprenger et al., 2016).

At the soil-plant level, the dynamic processes are not still well defined (Dubbart and Werner, 2019). The root interface interacts with soil in the root water uptake, but also participates in the hydraulic redistribution process: a passive transport of soil water by roots in a hydraulic gradient (from wet to dry). This process doesn't involve fractionation but can alter the isotopic signature of the depth profile (Sprenger et al., 2016).

All these processes has to be taken into account to understand the fluxes of isotopes through the soil-plant-atmosphere continuum.