# Forest Water Uptake Dynamics in the Long-Term Drought Experimental Site, Pfynwald – Intercomparison of Water Extraction and Isotope Analysis Methods

GEO 511 Master's Thesis

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*View on the Pfynwald forest from Leuk.*

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# Abstract

Studying the flow of water through the soil-biosphere-atmosphere continuum is crucial for understanding the dynamics of the terrestrial water cycle. In recent years, thanks to technological advancements, research in this area based on the analysis of stable water isotopes progressed rapidly. However, uncertainties accompanied these technological advancements as not all water extraction methods nor all isotope analysis technologies yielded the same results. In order to further advance our understanding of the water flow through the soil-biosphere-atmosphere continuum, these differences need to be analysed. This study compares the isotopic composition of three xylem water extraction methods (equilibrium vapor, Scholander pressure bomb and cryogenic vacuum distillation extraction) and two isotope measurement methods (laser- and mass-spectrometer) for the long-term drought experimental site of Pfynwald, Switzerland. Furthermore, the plant water status was taken into account to see if the offset between extraction methods is dependent on the plant water availability and atmospheric demand. In accordance with previous research, my analysis showed that the xylem water extraction methods yielded different water stable isotopic compositions. The results for the Scholander pressure bomb extracted and vapor equilibrium extracted in-situ largely agreed but the cryogenic vacuum distillation method for the xylem water yielded a significantly different isotopic composition. Previous studies assumed that this isotopic difference of tree xylem water originates from a fractionation process when water moves into the cell storage and some of the xylem water extraction methods sample this cell water. However, based on the results from this thesis, I propose that the pattern of cell water storage refilling and depletion might influence this offset additionally. Furthermore, my analysis reveals that the IRLS and IRMS extraction method yielded statistically significant different water stable isotopic values for the xylem but not for the soil. However, these differences were very small in comparison to the general offset between xylem water extraction methods. The plant water status was not found to have an impact on the isotopic ratios or offsets. Future research should analyse the relative contribution of the diurnal cell water storage refilling pattern and the fractionation process when water moves into the cell to the offset in the water stable isotopic composition between xylem water extraction methods. The contribution to the offset from the proposed diurnal cell water refilling pattern further highlight the need for tree xylem isotope analysis with a high temporal resolution.

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# Abbreviations

| | |
|---|---|
| CESM | Community Earth System Model |
| CLM | Community Land Model |
| $d_{excess}$ | deuterium excess |
| ESM | Earth System Model |
| GLS | Generalised Least Square |
| GMWL | Global Meteoric Water Line |
| GNIP | Global Network of Isotopes in Precipitation |
| IRLS | Isotope Ratio Laser Spectrometer |
| IRMS | Isotope Ratio Mass Spectrometer |
| LME | Linear Mixed Effect |
| LMWL | Local Meteoric Water Line |
| LWP | Leaf Water Potential |
| rmANOVA | repeated measurement Analysis of Variance |
| SD | Standard deviation |
| VSMOW2 | Vienna Standard Mean Oceanic Water 2 (Gröning et al. 2007) |
| VOC | Volatile Organic Compounds |
| VPD | Vapor Pressure Deficit |
| WSL | Swiss Federal Institute for Forest, Snow and Landscape Research |
| Xylem-CVD | Cryogenic vacuum distillation extracted xylem samples. |
| Xylem-SPB | Scholander pressure bomb extracted xylem samples. |
| Xylem-EV | Equilibrium Vapor extracted xylem samples. |

# 1. Introduction

## 1.1 Stable Water Isotopes in Tree Water Uptake Studies – The Research Gap

Water transfer through the soil-biosphere-atmosphere continuum is studied to understand the water cycle, and changes therein. Key topics for investigations in the soil-biosphere-atmosphere continuum are drought (e.g., Gessler et al. 2022; Phillips et al., 2009; Wang et al. 2018) , the role of plants in general (e.g., Jasechko et al., 2013; Joetzier et al. 2014; Wang et al. 2018), effects from land-use change (Sterling et al. 2012; Wang et al. 2018) and how to implement the soil-biosphere-atmosphere continuum in Earth-System-Models (e.g., Brady et al. 2019; Joetzjer et al. 2014). Thereby, the biosphere is in many places dominated by trees, which play a key role concerning the transport of water from the soil to the atmosphere (Jasechko et al. 2013). Major Earth-System-Models (ESM) like the Community Earth System Model (CESM) by the National Centre for Atmospheric Research and its sub-model the Community Land Model (CLM) or the Global Land Evaporation Amsterdam Model (GLEAM) implement the water transport from the soil to the atmosphere through the biosphere heavily simplified, leading to high model uncertainties (Cui et al. 2023; Denager et al. 2023; Jasechko et al. 2013). Most models implement plant physiological properties from a look-up table, which does not address local soil-plant interactions sufficiently (Denager et al. 2023). This modelling strategy furthermore excludes possible short- and long-term adaptation strategies by trees, like taking up more water from deeper soil layer, developing a larger rooting system or tree internal physiological adaptation strategies like increasing stem water conductivity (Bachofen et al. 2024; Deslauriers et al. 2017; Jiménez-Rodríguez et al. 2022; Kiorapostolou et al. 2020), leading to large model uncertainties (Denager et al. 2023; Sulis et al. 2019). However, such adaptation strategies are different between species (Eilmann & Rigling 2012; Monneveux & Belhassen 1996). It is therefore essential to conduct species-specific water uptake studies to understand and predict changes in the water flux through the soil-biosphere-atmosphere continuum under climate change. Such research is further fundamental for forest management, as it is key to find drought resistant trees to maintain ecosystem services like natural hazard protection or wood production under altering environmental conditions (Gessler 2021; Mina et al. 2017).

In recent years, thanks to new technologies, studies on tree-water-uptake and the flow of water through the soil-biosphere-atmosphere continuum advanced rapidly based on the study of stable water isotope (Sprenger & Allen 2020). Examples of such research include the comparison of the water stable isotopic composition of xylem to soil to understand water uptake patterns (e.g., Gessler et al. 2022), drought effects on plants (e.g., Lehmann et al. 2023, in review), water exchange through rivers, soil, vegetation and atmosphere continuum (e.g., Brooks et al. 2010; Goldsmith et al. 2018), and the paleoclimatic environment (e.g., Freund et al. 2023). For these applications of the xylem water isotopic composition, it is crucial to understand how and why the isotopic composition changes between the soil and plant water and how the results are affected by methodological artefacts. Several studies have shown that there is a significant difference in the isotopic composition between the water source and the stem xylem water in trees (Barbeta et al. 2020 & 2022; Brooks et al. 2010; Chen et al. 2020; Duvert et al. 2023; Fabiani et al.

2022; Orlowski et al. 2019; Radolinski et al. 2021; Vargas et al. 2017). The source of the difference in isotopic values obtained using different extraction methods is subject to scientific debates. In the literature, the following four hypotheses can be found describing the difference in the isotopic composition between the source water and xylem water:

1. Two-water-world hypothesis (Brooks et al. 2010)
2. Fractionation during root-water uptake (Vargas et al. 2017)
3. Bias due to cryogenic water distillation (Chen et al. 2020; Barbeta et al. 2022)
4. Fractionation during movement of water between different tree tissues (Barbeta et al. 2022; Zhao et al. 2016)

The two-water-world hypothesis suggests that the soil contains two water pools, one of accessible water for plants and another one that recharges streamflow and groundwater and is not used by vegetation (McDonnell 2014). Similarly, based on Brooks et al. (2010), the soil may consist of multiple water pools, of which some are used for vegetation water uptake and others are not. Brooks et al. (2010) argue that the two water pools have a different isotopic composition, leading to a different isotopic composition between the soil water and the plant xylem water. This hypothesis is opposed by others.

For example, Vargas et al. (2017) suggested that the different isotopic compositions could come from fractionation processes (discrimination against one isotopologue) during root water uptake, as they found a difference between xylem and soil water isotopic compositions, but the soil water was found to mix largely with the bound water. However, Chen et al. (2020) found that the difference between source water and xylem water isotopic composition still occurs for a rehydration experiment, contradicting the second hypothesis. In their experiment, they extracted the water from xylem samples through cryogenic vacuum distillation (CVD), rehydrated the xylem with water of a known isotopic composition and extracted the water again from the xylem with the CVD methodology. The water stable isotopic signature was different between the water used for rehydration and the water extracted from the xylem after rehydration. This suggests that neither different soil water pools nor fractionation processes during root water uptake are the reason for the distinct isotopic differences between the soil and the tree. Chen et al. (2020) used nine different tree species from different habitats but no pine trees. Based on their findings, they suggested that there is a bias in the methodology when the water is extracted via the cryogenic vapor distillation (CVD) process. According to Chen et al. (2020) this could be due to organic contaminants in the sample like alcohols.

The findings from Barbeta et al. (2022) also contradict the second hypothesis. They found that pressure extracted xylem water has a similar isotopic composition as the unbound soil water but differs from the CVD-extracted xylem water isotopic composition. Barbeta et al. (2022) used three tree species, among them Pinus pinaster (maritime pine), which is adapted to a higher variety of habitats compared to pinus sylvestris (Scots pine) (Bogino & Bravo 2014). Due to comparable water transport mechanisms in scots pine and maritime pine (Hacke et al. 2015), it can be assumed that the results from Barbeta et al. (2022) should also apply to the Scots pine stand in Pfynwald, the study site of this work. The fourth hypothesis is based on the difference in the isotopic composition of bulk stem xylem water compared to sapwood xylem water of Barbeta et al. (2022) and suggests that there is a fractionation process when water moves through different tissues in the tree. However, the study from Barbeta et al. (2022) uses two different methods to extract bulk stem water and sapwood water. But their findings are supported by Fabiani et al. (2022), who found a compartmentalization between sapwood water and heartwood water for needleleaf trees (difference between sapwood and heartwood is explained in section 1.4.1). In their study, the sapwood and heartwood xylem water samples were extracted through cryogenic vapor distillation. This strengthens the hypothesis that there is fractionation process when water moves between the wood tissues. They further showed that the variation of the water isotopic composition over time is linked between heart and sap wood. However, evidence is missing that there is no cryogenic vacuum distillation bias additionally to the tree internal differences in isotopic compositions between heartwood and sapwood. It is furthermore unclear whether the difference between sapwood and heartwood stable water isotopic values arises from a difference in the level of organic contamination.

Therefore, there is no research yet to support the third or fourth hypothesis exclusively. What is missing is the comparison of different water extraction techniques for the bulk tree xylem water for different tree species. It is furthermore possible that the difference between sapwood and heartwood are due to differences in the CVD-offset or that the alternative methods also face some sort of bias. More research is therefore needed to verify Barbeta et al. (2022), Chen et al. (2020) and Fabiani et al. (2022) concerning their conclusions on the third and fourth hypothesis.

A further dispute concerning the difference in the isotopic composition is related to drought. Barbeta et al. (2020) found a relationship between the water availability for trees and the isotopic composition of xylem water for naturally grown trees in the Mediterranean, but Vargas et al. (2017) for potted trees and Duvert et al. (2023) for tropical tress did not find such a relationship. Thus, investigations of the relationship of the isotopic composition of soil and tree water under different water availability conditions is needed.

Another issue which came forth in very recent research are the differences in the offset between tree species. Fabiani et al. (2022) found a distinct offset between soil water and tree water stable isotopes for broadleaf and needleleaf trees, indicating that tree physiology has an impact on the tree water isotopic values and their differences between sapwood and heartwood. This is supported by Duvert et al. (2023) for different tropical tree species, where they not only found a difference between the different classes of trees but also between individual coniferous and deciduous tree species. However, this thesis only focuses on the methodologies and drought for one tree species (Scots pine, Pinus sylvestris) and does not look into differences between tree species due to a constraint in the available resources.

Due to the contamination of measurements using the isotope-ratio laser spectroscopy (IRLS) compared to the isotope-ratio mass spectroscopy (IRMS), IRLS measurements need to be corrected for the bias caused by organic contaminants or/and compared to IRMS measurements (West et al. 2010; Martín-Gómez et al. 2015). The IRMS measurements are assumed to be contamination free based on previous studies (Barbeta et al. 2022; Martín-Gómez et al. 2015). According to Herzog et al. (2019), who studied the effect of drought on litter decomposition in the Pfynwald under different water availability conditions, less organic matter is to be expected in the dry soils. Therefore, it can be hypothesized that the dry soils suffer less from organic contaminants and show a better agreement between the IRLS and IRMS results.

Additionally, in regard to the tree samples, the production of volatile organic compounds (VOC), which includes alcohols, differs between trees subject to different water availabilities at the Pfynwald research site (Rissanen et al. 2022). Based on Martín-Gómez et al. (2015), alcohols are the main contaminant influencing the IRIS measurement. However, according to Rissanen et al. (2022) the release of VOC decreases under drought stress because stored VOC is not released due to closure of the stomata and the limited production of new VOC due to reduced tree productivity. Therefore, it is unclear whether the storage-effect (less release from storage) or production-effect (less production) is more important concerning the contamination of tree xylem samples.

## 1.2 Research Questions

Based on the research gaps introduced in the previous chapter, I derived the following research questions and corresponding hypotheses:

1. Is there a difference in the isotopic composition of water extracted with the equilibrium vapor method (in-situ, bulk stem water), Scholander pressure bomb (sap flow water) and the cryogenic vacuum distillation method (bulk stem water)?

The null-hypothesis (H0-1) is, that the three different water extraction methods provide the same isotopic signature, i.e., there is no significant difference in the water isotopic composition.

2. How are the observed isotopic differences between xylem and soil water related to plant water status?

The null-hypothesis is (H0-2): the plant water status does not statistically significant influence the observed isotopic difference between soil water and tree xylem.

3. Is there a difference in the water isotopic composition measured by the mass spectrometer and the laser spectrometer, and if so is it the same for the irrigation, irrigation stop, or control treatment?

The null hypothesis is (H0-3): there is no significant difference between the isotope measurements from the laser spectrometer and mass spectrometer for any of the treatments.

## 1.3 Stable Water Isotopes –Physical & Chemical Fundamentals

Water ($H_2O$) consists of combinations of hydrogen and oxygen molecules. There are in total five different stable isotopes ($^1H$, $^2H$, $^{16}O$, $^{17}O$ and $^{18}O$) of different abundance. They contain a different number of neutrons (indicated by the number) and have a distinct weight. The water molecule can contain each possible combination of the hydrogen or oxygen isotopes. This is often referred to as the water isotope. The weight difference of the different combinations of hydrogen and oxygen isotopes in the water molecule leads to fractionation when water changes its state (e.g., from liquid to gas), whereby the heavier isotope combination of $H_2O$ is enriched in the source (water body) and the lighter isotope has a relatively higher concentration in the vapor. This is called Rayleigh fractionation (Kendall & Caldwell 1998). Fractionation can also occur during biological process or chemical processes (Goldsmith et al. 2018; Oerter et al. 2014).

Fractionation changes the relative amount of heavy and light isotopes in water and is expressed relative to a standard as displayed in *Eq. 1* and *Eq. 2 (*Gat 1996), which is in this work (and most other research) the Vienna Mean Standard Ocean Water 2 (VSMOW2, Gröning et al. 2007). The comparison to a standard makes it easier to deal with the numbers, as in nature the lighter isotopes are much more abundant than the heavier ones and the ratios are thus very small. In nature, around 99.9885% of Hydrogen atoms are $^1$H and 0.0115% are $^2$H and for Oxygen it is 99.757% of $^{16}$O vs. 0.205% of $^{18}$O vs. 0.038 of $^{17}$O (Mahaffy et al. 2023; Werner & Cormier 2022). The deviation from the standard is calculated as follows:

$$\delta^{18}O\ [‰] = \left( \frac{\frac{^{18}O}{^{16}O}_{Sample} - \frac{^{18}O}{^{16}O}_{Standard}}{\frac{^{18}O}{^{16}O}_{Standard}} \right) * 1000 \qquad Eq. 1$$

$$\delta^{2}H\ [‰] = \left( \frac{\frac{^{2}H}{^{1}H}_{Sample} - \frac{^{2}H}{^{1}H}_{Standard}}{\frac{^{2}H}{^{1}H}_{Standard}} \right) * 1000 \qquad Eq. 2$$

From *Eq. 1* and *Eq. 2* one can derive that $\delta^{18}$O and $\delta^2$H increase, when the relative number of light isotopes decreases. As an example, if we assume that the standard has a ratio of 0.0111 and the ratio of the sample increases from 0.0100 (1*Heavy/100*Light) to 0.0066 (1*Heavy/98*Light), the δ-value increases from -100 ‰ to -81.6‰. By using the delta notation, it is clear that already small changes in the isotope concentration lead to a notable change in the isotopic ratio between heavy and light isotopes. In precipitation, the stable water isotope ratios for hydrogen and oxygen are usually related, following a linear relationship called the global meteoric water line (GMWL, Craig 1961):

$$\delta^2H\ [‰] = 8 * \ \delta^{18}O\ [‰] + 10\ [‰] \qquad Eq. 3$$

Fractionation during evaporation (non-equilibrium evaporation processes) leads to deviations from the GMWL. One way to express this is the deuterium excess ($d_{excess}$) value which is calculated after Dansgaard (1964):

$$d_{excess} = \delta^2H\ [‰] - \ 8 * \ \delta^{18}O\ [‰] \qquad Eq. 4$$

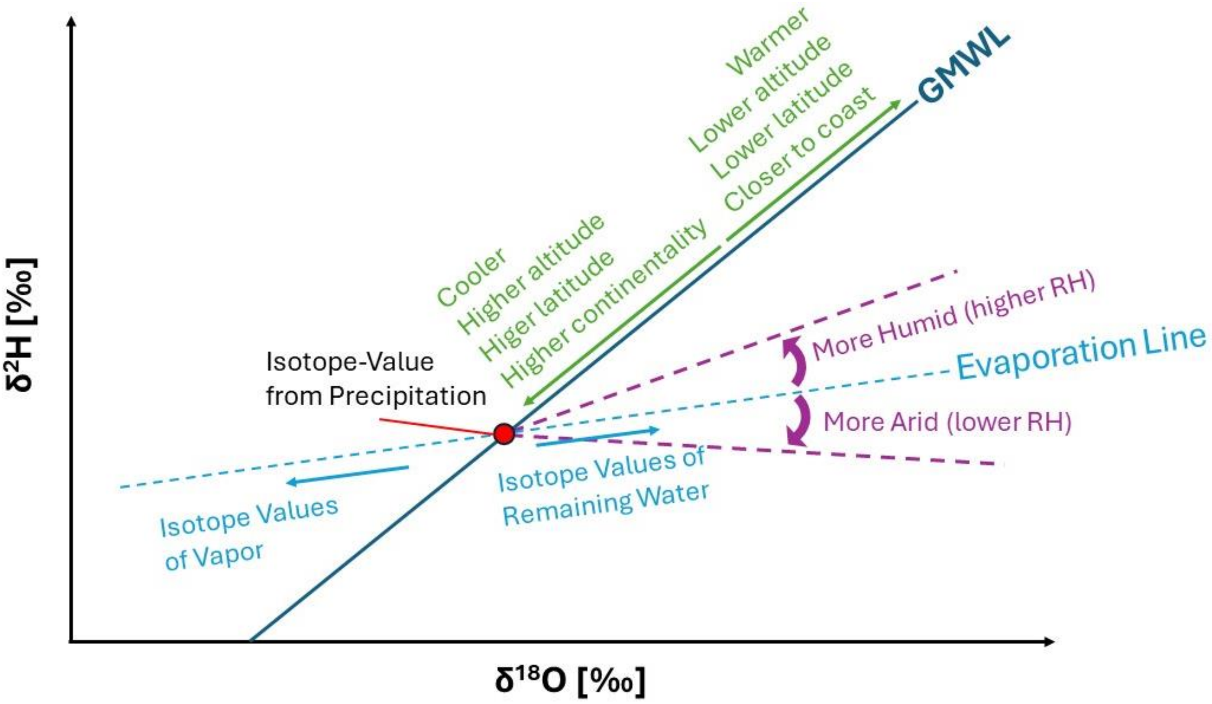


*Figure 1: Indications of how different processes lead to a deviation of the relationship between $\delta^{18}O$ and $\delta^{2}H$ from the GMWL or LMWL. Graphic made after Allen et al. (2022) & Gat (1996).*

Based on *Eq. 3* and *Eq. 4*, a *d-excess* value of around 10‰ is expected for precipitation. Where there is a negative deviation (<10‰), evaporative fractionation processes along the evaporation line occurred (Figure 1, Dansgaard 1964 & Gat 1996). However, the starting value from precipitation, which is assumed to be 10‰, varies based on weather phenomena which can lead to local differences in the expected values (Froehlich et al. 2008). Based on Bershaw (2018), on the lee side of mountains (in the wind-shadow of the mountain) the $d_{excess}$ values are expected to be <10‰ even for water that has not undergone any evaporative fractionation while the opposite is true for the luv side.

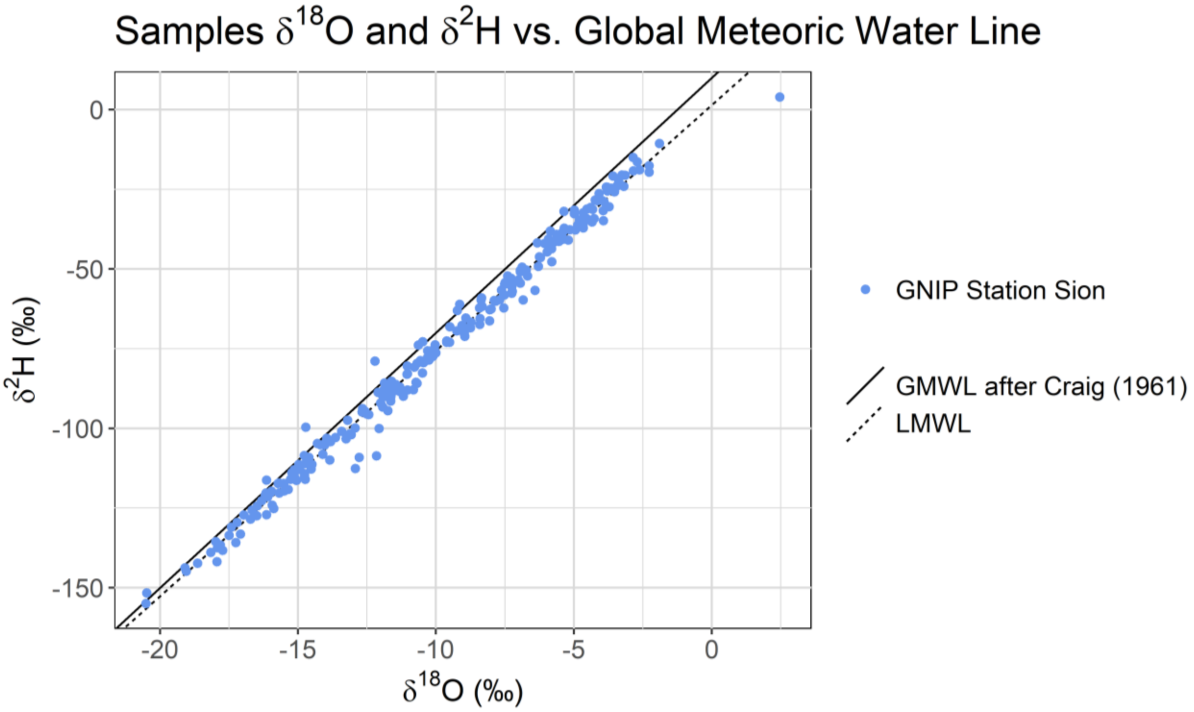


*Figure 2: Local vs. Global Meteoric Water Line (LMWL vs. GMWL). The LMWL was calculated based on the Global Network for Isotopes in Precipitation (GNIP) Data from the Sion meteorological station (Craig 1961; Meteoswiss and FOEN 2017).*

This is also the reason why in local precipitation the relationship between $\delta^{18}O$ and $\delta^{2}H$ can deviate from the GMWL. A Local Meteoric Water Line (LMWL) needs to be computed and the sample deviation in the $\delta^{18}O/\delta^{2}H$ relationship needs to be analysed in comparison to the LMWL (Figure 2). The deviation of the LMWL from the GMWL is additionally influenced by the humidity of the vapor source. The LMWL lies below the GMWL in case the vapor forming the precipitation comes from a humid source, for an arid vapor source it is the other way around (Figure 1).

The replacement of water by new precipitation and loss of water through evaporation leads to major temporal changes in the isotopic composition of the soil (Brinkmann et al. 2018; von Freyberg et al. 2020). Water in deeper soil horizons usually has a more consistent water stable isotopic composition which is lower than measured near the soil surface. The smaller magnitude of change comes from a longer water residence time in deeper soil horizons compared to shallow soil and thus mixing of water from different events (Dubbert & Werner 2019; von Freyberg et al. 2020). Furthermore, in Switzerland and areas with a similar climate, winter precipitation with a lower (more negative) isotopic composition mainly recharges the water in deeper soil layers (Allen et al. 2019; Goldsmith et al. 2018). Additionally, evaporation leads to an enrichment in heavy isotopes near the soil surface (as shown in Figure 2). This leads to a vertical gradient in the soil stable water isotopic signal, whereas lower soil horizons are expected to have a lower (more negative) isotopic value and shallow soil has higher values (Allen et al. 2019; Dubbert & Werner 2019; von Freyberg et al. 2020). The change in stable water isotopic compositions with depth can be used to assess from which depths a tree took up water, for example with a Bayesian mixing model (e.g., Gessler et al. 2022).

Additional to the vertical gradient in isotopic signal, previous research has found a spatial heterogeneity in the isotopic signals near the soil surface (Goldsmith et al. 2018). This is mainly due to spatial differences in throughfall amount (rainfall passing through the canopy), infiltration and evaporation from the soil surface. Differences particle size, moisture content or chemical properties, also lead to spatial heterogeneity in the shallow soil water stable isotopic composition (Goldsmith et al. 2018; Oerter et al. 2014).

## 1.4 Tree Physiology – Biological Fundamentals

### 1.4.1 Tree Structure

This paragraph only covers the biological fundamentals necessary to understand this thesis without a biological background and does not aim to cover the vast complexity of the anatomical structure of trees. The soil water conducting tissue in the tree is called xylem (Rathgeber et al. 2022), whereas the phloem is responsible for the transport of photosynthetic assimilates (e.g., sugar) through the tree (Deslauriers et al. 2017). The cambium, which is located between the xylem and phloem (Figure 3) is the tissue responsible for growth, producing cells for the xylem and phloem alike (Deslauriers et al. 2017; Rathgeber et al. 2022).

Therefore, when analyzing tree water uptake, only xylem water is of interest whereas phloem water should be avoided, as it can be subject to evaporative enrichment and is therefore not representative of the soil water stable isotopic composition (Barbeta et al. 2018). However, the xylem is also important in the trees structure for mechanical support. It furthermore transports and stores organic compounds, which are not used for tree growth, but, as an example, for the trees' defense against pests (Rathgeber et al. 2022). Coniferous (needleleaf) trees have a homogenous xylem structure whereas the deciduous (broadleaf) trees have a more complex and heterogenous xylem structure (Rathgeber et al. 2022), which is important to be aware of when comparing tree water uptake studies.

The xylem is subdivided into heartwood and sapwood (Figure 3). The heartwood is composed of dead xylem cells, which do not sustain the water transport function anymore, but are due to their increased thickness and stability important for the tree's structural resistance. The sapwood on the other hand is mainly responsible for the transport of water and nutrients from the soil as well as organic compounds for defense (Rathgeber et al. 2022). The transition from sapwood to heartwood is a consistent process described in further detail in Rathgeber et al. (2022) p. 67-72. An additional division visible in Figure 3 is between the early and late wood. The early wood is usually built between Mai and June and the late wood afterwards until the end of the growing season. Thereby, the main factor for the distinct formation of early and latewood cells is assumed to be the water availability and through that growth speed (Rathgeber et al. 2022).

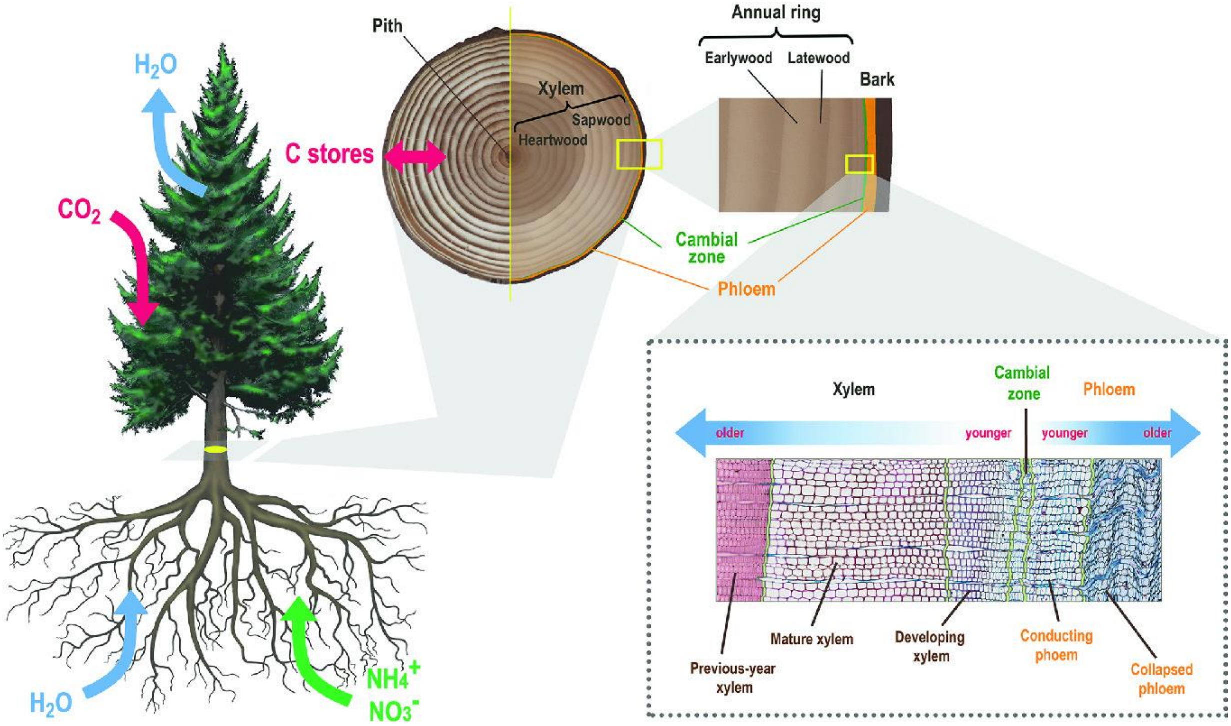


*Figure 3: Overview on the tree anatomy with a microscopic cross-section of the tree wood structure from Rathgeber et al. (2022).*

### 1.4.2 Leaf-Water-Potential as a Water Stress Indicator

Plant water status is often assessed through the leaf water potential (e.g., Shakel et al. 1997; Vesala et al. 2017), which is also the case for studies relating the tree stable water isotopic values to plant water stress (e.g., Douvert et al. 2023). Usually, the leaf water potential is measured pre-dawn and midday. The pre-dawn leaf water potential measurement is used to determine the soil water content and potential in the rooting area of the plant (Améglio et al. 1999; Ratzmann et al. 2019; Schönbeck et al. 2022). The midday leaf water potential on the other hand is an indicator for plant water stress, where high leaf water potentials indicate that the plant struggles to keep up delivering sufficient water to the leaves, either due to low soil moisture or high transpirational water demand (Ratzmann et al. 2019; Schönbeck et al. 2022; Shakel et al. 1997).

The leaf water potential is most often measured with a Scholander pressure bomb (SPB, Figure 4), also called a Scholander pressure chamber (Rodriguez-Dominguez et al. 2022). The values measured with the SPB are usually reported in negative numbers, even if the pressure applied and read on the SPB is positive. This is because the water potential is always reported in reference to the water potential of pure water under atmospheric pressure (Hillel 2003, p. 441; Nobel 2005, p. 69-70). The leaf water potential refers to the deviation from this reference, which is negative the pressure added with the SPB to equalize the leaf water potential, i.e. bring the leaf water potential to atmospheric reference pressure. The moment the leaf water potential plus the added pressure is equal to the atmospheric pressure, water starts flowing out the stem (Figure 4). This moment of equilibrium, the moment before water starts flowing out, is measured and reported as the leaf water potential (Shakel et al. 1997; Vesala et al. 2017).

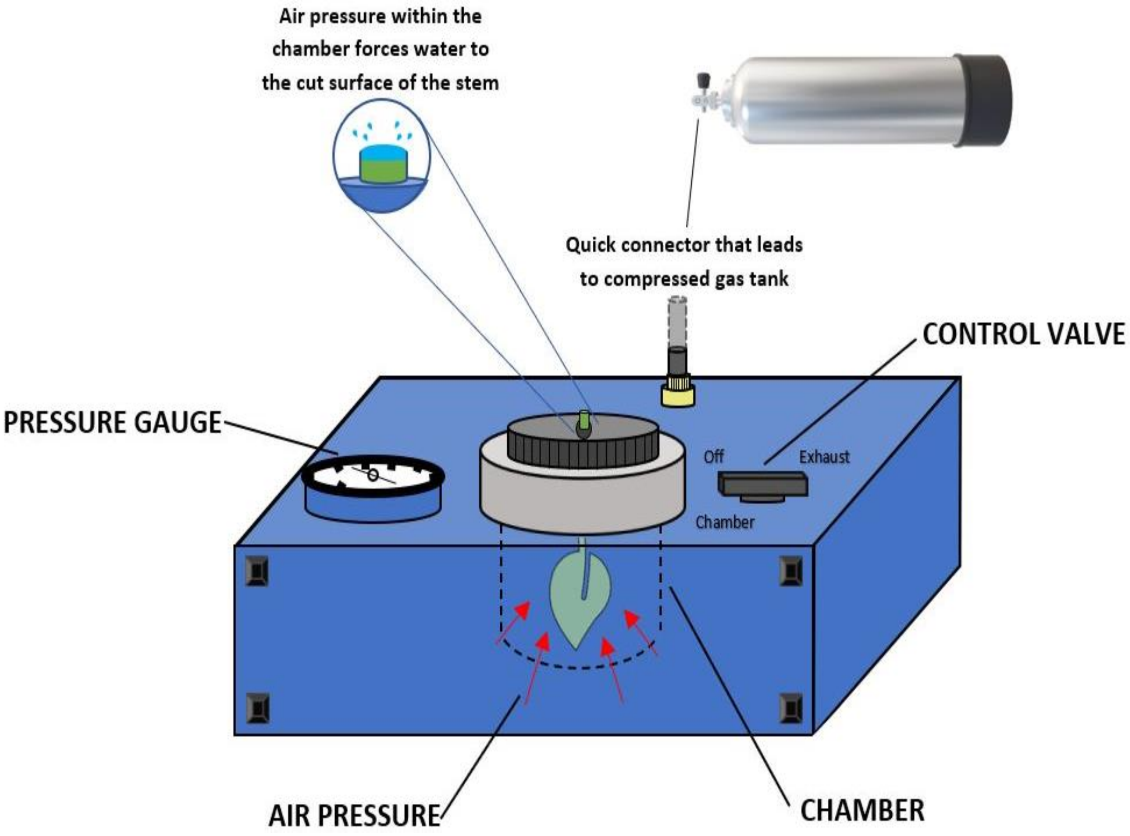


*Figure 4: Schematic overview of the main components and functioning of a Scholander pressure bomb from Bartell et al. (2021).*

The water potential is dependent on the capillary size (matrix potential), pressure (e.g., from the atmosphere), solutes (osmotic potential) and gravitational potential. Thereby, along the path of water from the soil through the plant to the atmosphere occur different water potential, whereby the water flows from the higher to the lower water potential (Figure 5, Hillel 2003, chapter 2, 6 & 19). It can be imagined the way, that the difference in water potential is the pressure exhibited on the water to flow from the higher to the lower water potential.

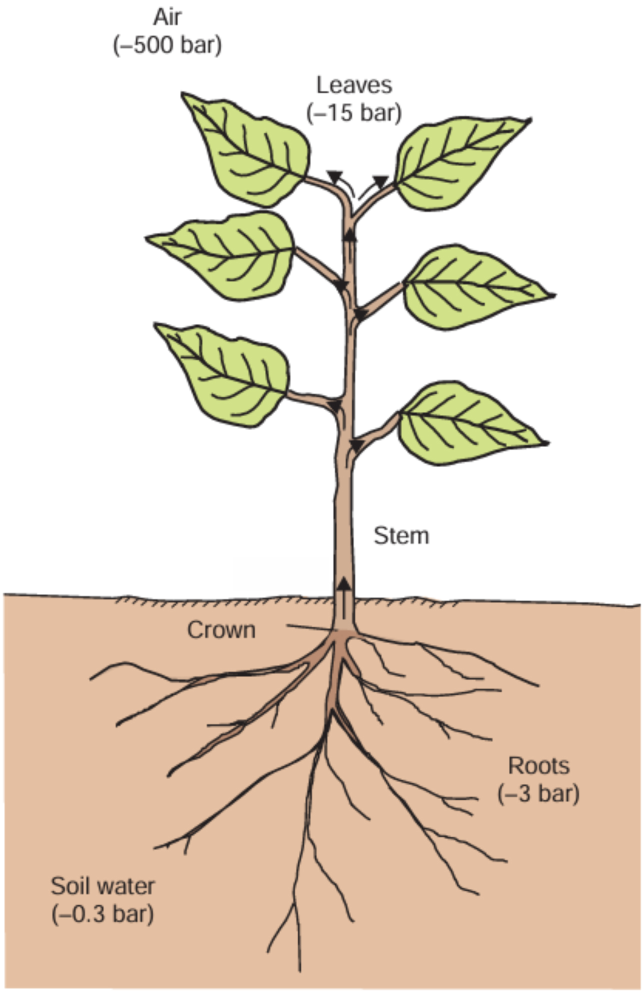


*Figure 5: Typical values of water potential along the water movement path through the soil-biosphere-atmosphere continuum (Hillel 2003, p. 375).*

In the soil, the water potential is determined mainly by the matric potential, gravimetric (gravitational) potential and the osmotic water potential. As the water potential is dependent on the shape (curvature) and size of the water surface, the water potential is lower in small pores compared to larger soil pores (Hillel 2003, chapter 2 & 6; Vesala et al. 2017). For plants it is therefore easier to access water in larger soil pores. If the soil water potential is reduced below the water potential of roots (i.e. only small soil pores have water left) the water stops flowing towards the plant roots and the plant cannot take up water anymore. The plant already faces water stress even when the root water potential is higher than the soil water potential if the water flow from the soil to the roots is not fast enough to compensate for the transpirational water loss (Hillel 2003, chapter 19). However, the root water potential is not static but can be altered by the plant to a limited extent in order to maintain water uptake in a soil of low soil water potential (Mair et al. 2023).

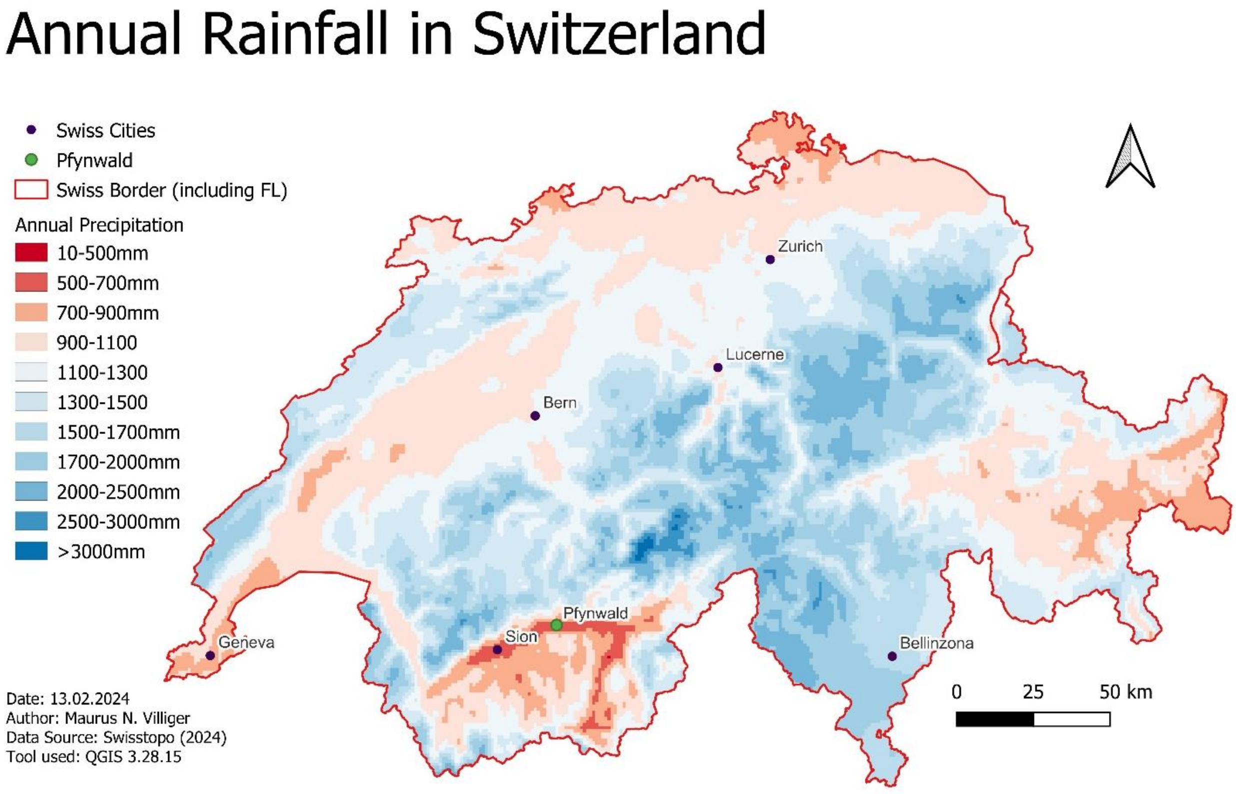
The leaf and stem water potential is mainly driven by the evaporation from the leaves and its influence on the water surface in the sub-stomatal cavities, whereas the osmotic potential is negligible in comparison (Vesala et al. 2017). Regarding Figure 5, it might be confusing why the atmospheric water potential is not zero, as the leaf water potential is given in relation to atmospheric pressure. However, the atmosphere of course does not contain pure water. The water potential in air relates to different constants, the temperature and relative humidity (Vesala et al. 2017; Nobel 2005 p. 352-377). Through the relative humidity it furthermore relates to the vapor pressure deficit in the air (Grossiord et al. 2020; Schönbeck et al. 2022; Vesala et al. 2017). However, the water loss from the plant to the atmosphere is not only controlled by the atmospheric water demand but also by the leaf water conductance, which is mainly controlled by the opening and closing of the stomata, which are small pores in the leaves to exchange gas with the atmosphere (Grossiord et al. 2020; Schönbeck et al. 2022; Vesala et al. 2017).

# 2. Materials and Methods

## 2.1 Fieldwork

### 2.1.1 Field site

The fieldwork was conducted in the Pfynwald forest in the canton of Valais (Figures 6 & 7) on five campaigns between August and October 2023. The research site forest is subject to a long-standing drought experiment by the Federal Institute for Forest, Snow and Landscape research (WSL) and contains three experimental treatments: irrigation, irrigation-stop, and control (Bose et al. 2022; WSL 2024). There were eight plots (four control and irrigation) of 1000 m$^2$, whereas later irrigation was stopped for half of the area of each irrigation plot (Bose et al. 2022; Figure 6). Each research plot is equipped with scaffolds to reach the canopy (WSL 2024). The Pfynwald research site is located at 615 m.a.s.l. in the dry valley of the canton of Valais with an average annual precipitation of 575 mm (Figure 6). At the irrigation site around 600 mm (5mm/night if there is no natural rain) of water from the nearby glacial meltwater channel is added during the growing season (WSL 2024). The channel is made of concrete so that no water leaches into the surrounding ground. The research site is equipped with an atmospheric observation station, which takes temperature, humidity and wind measurements every 10 minutes. The annual average temperature is 10.6 °C, with an average summer temperature of 19.6 °C. The xeric scots pine forest is about 100 years old and is established on an alluvial fan with deep sedimentary layers (>5m) and shallow topsoil (Bose et al. 2022; Grossiord et al. 2018).



Date: 13.02.2024
Author: Maurus N. Villiger
Data Source: Swisstopo (2024)
Tool used: QGIS 3.28.15

*Figure 6: Map of Switzerland with few major cities and the Pfynwald research site indicated. The colouring indicates annual precipitation on average between 1990-2020, which makes it visible that the Pfynwald lies in an unusually dry area of Switzerland. The data is from swisstopo (2021, 2024). FL stands for "Fürstentum Liechtenstein".*

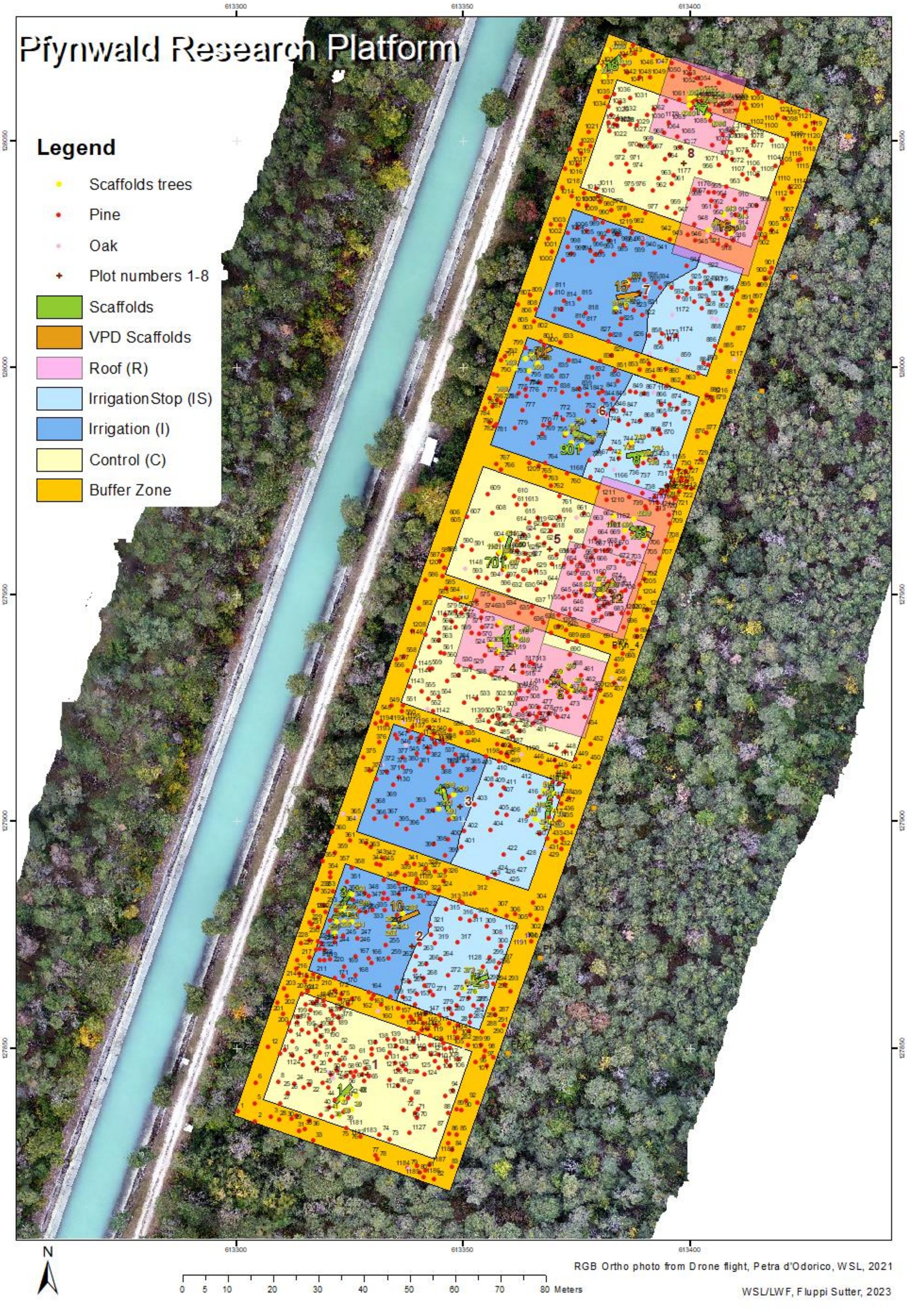


*Figure 7: Pfynwald research site by WSL. The VPD and Roofed areas are newly introduced over the course of 2023 and 2024 (WSL 2024).*

### 2.1.2 Acquisition Strategy Destructive Samples for Laboratory Extraction

To gather destructive samples, five field campaigns were conducted between August and October 2023. On each campaign at each treatment site (irrigation, irrigation stop and control), three soil cores were drilled with a soil-corer from different positions within the treatment plot to account for possible spatial heterogeneities in the near soil surface stable water isotopic signals as described in Goldsmith et al. (2018). The locations where soil cores were extracted were chosen to cover the surface heterogeneity on the forest floor between the tree stands with in-situ measurements and scaffolds that give access to the tree canopy for destructive sampling of tree twigs. From each core from the depths of 0-10cm, 10-30cm and 30-50cm samples were collected. For the xylem samples for laboratory extraction (xylem-CVD), twig samples were collected from the scaffolds 701 (control), 8 (irrigation stop) and 901 (irrigation) (green rectangles, Figure 7). Five samples were prepared for each treatment. At the control site the trees with the numbers 597, 600 and 601, at irrigation-stop site the trees with the numbers 734, 735, 736, 742 and 743 and at irrigation site the trees with the numbers 754, 755, 756 and 757 were sampled (red points, Figure 7). Not the same number of trees per treatment were sampled due to the limited access to trees from the scaffolds. Where there weren't five trees accessible, some trees were sampled twice to get five samples. On site, the bark was removed from the twigs. The soil and xylem-CVD samples were filled into 12ml exetainer, closed, sealed with parafilm, and stored in a cooling bag until arrival at the laboratory. There, the samples were stored in a freezer until water extraction. In total, 75 xylem-CVD samples and 135 soil samples were collected during the five fieldwork campaigns.

### 2.1.3 Leaf Water Potential Measurement and Xylem Samples for Field Extraction

To investigate the level of drought the trees experience at each treatment site, pre-dawn and midday leaf water potential measurements were performed on site. The twigs to measure the leaf-water-potential were collected from the same trees as were used for the xylem samples for laboratory extraction. After cutting the twigs, they were stored in humidified plastic bags and measured as soon as possible. The measurements were done on-site using a Scholander Pressure Bomb (Model 3115 Pressure Extractor from the Soilmoisture Equipment Corporation) borrowed from the institute for soil physics and terrestrial ecosystems at ETH. The leaf water potential measurement from the first campaign was performed with a different Scholander pressure bomb which yielded unreasonable leaf water potential results (see chapter 3.3). Therefore, this leaf water potential data is excluded.

The xylem samples for the on-site extraction (xylem -SPB) were collected from the trees 600 and 604 at the control site, from tree 735 and 743 at the irrigation stop site and from tree 756 and 755 from the irrigation site. Less trees were sampled compared to the xylem-CVD samples due to the high gas and work demand in the field. Water was extracted with the SPB from 3-5 twigs per tree, depending on how many were needed to fill the vial. The water was pressed out of each twig consecutively by slowly increasing the pressure until no more water outflow was detected, which was usually around 20 to 30 bars. The emerging water droplets were collected with a syringe and transferred into a small inlet vial

(1.5ml). The vial was covered after every transfer to prevent evaporation. After successful extraction, the vials were additionally sealed with parafilm and stored in a cooling container. After transferring the samples to the laboratory, the water was filtered through a 25µm mesh to get rid of larger organic contaminants which could clog the measurement system.

### 2.1.4 In-Situ Isotope Measurement

Each treatment site (irrigation, irrigation-stop, and control) contains several automated in-situ measurement systems to analyze the water stable isotopic composition. Each treatment contains eight soil probes in different depths (20cm-2m, every 20cm a probe), five tree probes in different trees at breast height inside boreholes and atmospheric measurements above and below the canopy. The irrigation and control sites additionally contain an automated rain sampler to analyze the rainfall isotopic composition on site. The water is sampled in-situ with the equilibrium vapor extraction method and transported via small tubes to a picaro laser ring-down spectrometer installed in the field for the $\delta^{18}O$ and $\delta^{2}H$ isotope ratio measurement. More on the in-situ measurements for the soil, trees and atmosphere can be found in Volkmann et al. (2014 & 2016) and Gessler et al. (2022). The automated rain samplers are a development from Dr. Elham Freund. The in-situ system is operated as a cooperation between the WSL, University of Freiburg, and University of Zurich and the measurements are maintained and post-processed by Dr. Elham Freund (UZH), Dr. Marco Lehmann (WSL), Dr. Katrin Di Bella-Meusburger (WSL) and Dr. Zhaoyong Hu (WSL). They kindly provided the post-processed data to compare it to the destructive samples retrieved in the field.

## 2.2 Laboratory Analysis

### 2.2.1 Cryogenic Vacuum Distillation for Water Extraction

For the water extraction from the xylem and soil samples the Cryogenic Vacuum Distillation (CVD) set up at WSL was used, which is similar to the one used by Orlowski et al. (2013) in Figure 8. The samples retrieved in the field can be inserted into the extraction line in the 12ml exetainers (Nr. 13 as indicated in Figure 8). The exetainers were weighed before and after extraction and equipped with a filter to prevent soil particles from entering the extraction line. The water was extracted through a vacuum (Nr. 2 Figure 8) set at < 0.05 mbar. The samples are inserted in a hot water bath at 80°C (Nr. 5 Figure 8) to facilitate and accelerate the extraction process (Orlowski et al. 2013). The extracted water is trapped in U-tubes (Nr. 14, Figure 8) which are then inserted into a liquid nitrogen bath (Nr. 7, Figure 8). The samples are left in the extraction line for at least two hours for a complete water extraction. After complete extraction, the system is filled with nitrogen gas at atmospheric pressure. The U-tubes are then removed from the extraction line and sealed with corks. As soon as the extracted water has melted in the U-tubes, the water was retrieved with a syringe and if necessary, filtered through a 25µm mesh before being inserted into 2ml vials. The vials were stored in a freezer until the isotope measurement.

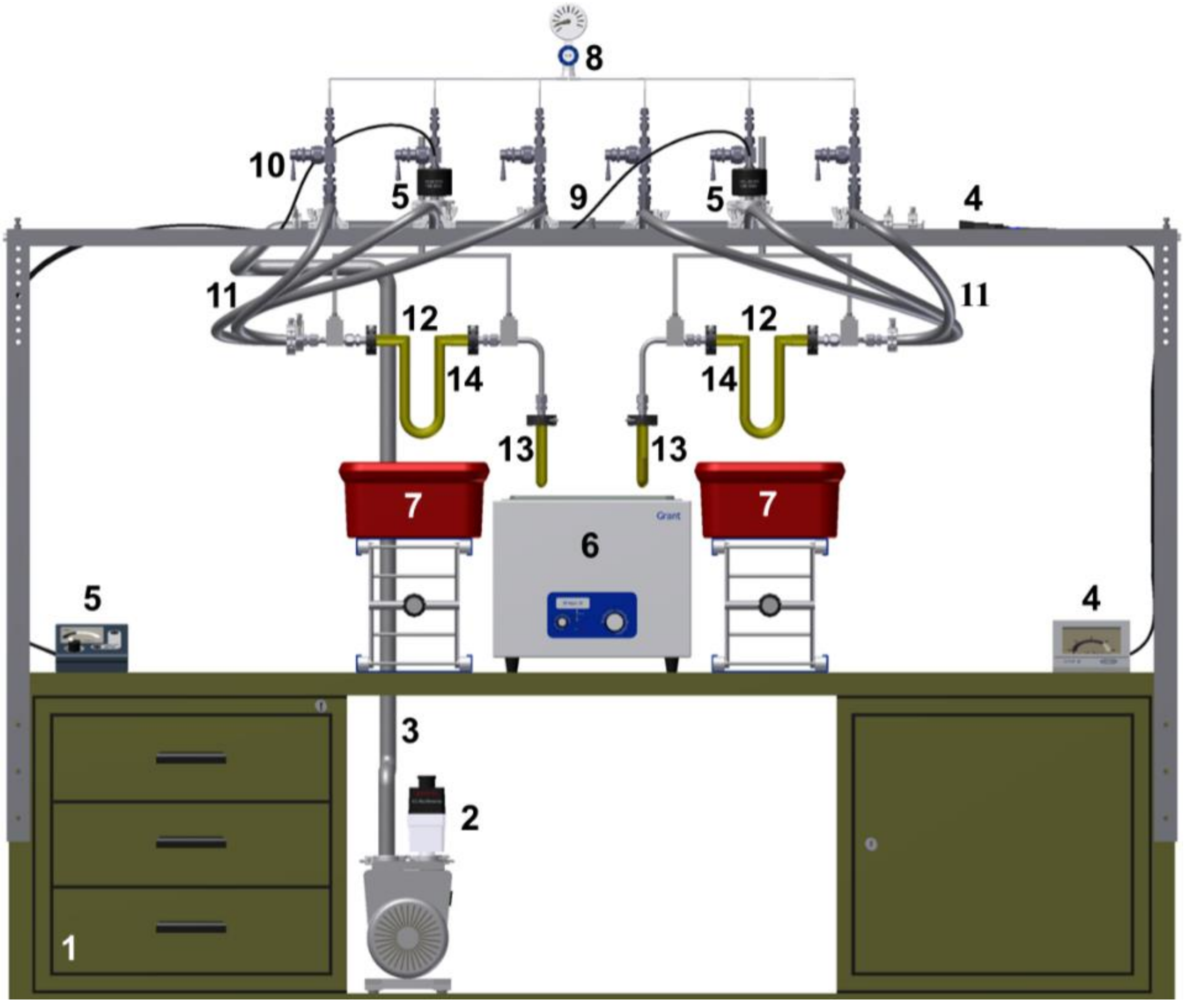


*Figure 8: CVD setup as described by Orlowski et al. (2013). For the work presented here, a setup installed by Dr. Marco Lehmann at WSL is used.*

### 2.2.2 Isotope Ratio Mass and Laser Spectrometer Measurements

The water samples were measured at the WSL with an isotope ratio laser spectrometer (IRLS) and an isotope ratio mass spectrometer (IRMS) by Dr. Marco Lehmann. Each sample is measured with both systems to compare and find out what effect the used measurement device has on the measured isotope ratio. This is important because IRLS is known to be sensitive to organic contaminants in the water samples extracted from soil or plants, as explained in the introduction (Brand et al. 2009; Chang et al. 2016; Cui et al. 2021; Martín-Gómez et al. 2015; West et al. 2010). All xylem and soil samples are measured with the IRLS and the IRMS, but only a few river samples were measured twice as no organic contamination is expected for glacial meltwater. The comparison is also important regarding the reliability of the in-situ measurements, as there only the IRLS is used to measure the stable isotopic composition. The standard used to calculate the samples $\delta^2$H and $\delta^{18}$O is the VMSOW 2 (Gröning et al. 2007). To assess the accuracy, precision and to get rid of memory effects, each sample was measured eight times by the IRLS from which the average of the last 5 measurements was reported. Additionally, the measurement of standard samples alongside the soil and xylem water samples were compared to their known isotopic value.

## 2.3 Data Analysis

The data was sorted, visualized, and analyzed in R (version 4.2.1, R Core Team 2022). More detail on the visualization methods used (e.g., packages information) can be found in the R-Project handed in alongside the thesis. The statistical analysis was started with a mixed-effect repeated measurement analysis of variance (rmANOVA) to analyze if there are significant differences in the stable water isotope values between the three treatments and between the extraction methods, following Nord (2022). The between subject predictors are treatment and sample type. The within subject predictors are the sample date and the subject ID. The within subject predictors are the repeated measurement structures (Nord 2022), which is necessary since the same subject (e.g., tree with nr. 600) is measured repeatedly over time (over different fieldwork campaigns). For the soil samples, the dataset was more challenging as not on every fieldwork campaign the same soil core could be taken out and measured again. A new soil core had to be drilled to take samples from the three different depths. Therefore, a spatial repeated measurement with depth is present. Despite each depth being supposedly different from each other, a spatial correlation cannot be excluded. Therefore, the sample ID for soil samples consists of the core number (repetition within the soil core for the three depths) and the soil depth (repeated measurement for each layer/depth in the same plot over three different soil cores). However, this multi-dimensional repeated structure as well as the two between variable predictors made it impossible to test for the prerequisites for the rmANOVA and made it difficult to run the necessary post-analysis tests.

Therefore, a more advanced statistical analysis was performed following the book "*Mixed Effects Models and Extensions in Ecology with R*" by Zuur et al. (2009) to verify the results yielded from the rmANOVA. Linear mixed effect (LME) models as well as generalized least squares (GLS) models were created and compared, which allow for a post-analysis and model verification by, for example, analyzing the residuals (Zuur et al. 2009). For both modeling approaches the parameters could be adapted to implement interactions between the variables and different repeated measurement structures. Especially the GLS modelling function from the nlme-package (Pinheiro & Bates 2023) contains different correlation structures for spatial and temporal repetition, where different data distributions of the spatial repeated structures are possible. Thereby, it was also checked if a sample ID for soil composed of either only the soil core or soil depth performed better for the LME and GLS modelling approach (see the data analysis files handed in alongside the thesis). From the literature, the distinction between two depths in stable water isotopic values for the same soil core should be stronger than the differences in stable water isotopic values between soil cores for a specific depth (see section 1.1 for explanation).

In a further step, the data was grouped once by treatment and once by sample type in order to analyze if within each treatment and each sample type the stable water isotope values are significantly different. The difference between the IRLS and IRMS measurement have been assessed additionally to the mentioned rmANOVA, LME and GLS by a one-sample test (t-test for parametric distribution, Wilcoxon test for non-parametric distributions). Thereby, the hypothetical mean is zero, as the 0-hypothesis assumes that there is no difference between the IRLS and IRMS measurement. Furthermore, a correlation analysis was performed to see whether the same samples had the same level of bias between the IRLS and IRMS measurement for $\delta^2H$ and $\delta^{18}O$. To analyze the drought effect on the measured isotopic composition depending on the sample type, an rmANOVA was conducted for the whole dataset and each treatment individually.

# 3. Results

## 3.1 Intercomparison of Xylem Water Extraction Methods

### 3.1.1 Destructive Samples Comparison – Qualitative Analysis

The intercomparison of water extraction methods is mainly performed on the IRMS measurements, as it is assumed to be the true value (Martín-Gómez et al. 2015; West et al. 2010). The IRMS dataset is composed of 263 measured samples, from which 134 samples are from the soil, 74 from xylem-CVD and 31 from xylem-SPB. Figure 9 shows the $\delta^2H$ and $\delta^{18}O$ measurements for each fieldwork campaign grouped by treatment and by sample type. Sample type is used here to refer to the different acquisition strategies and origins of water samples, this being the soil-CVD, xylem-CVD, xylem-SPB and river (irrigation water). It is visible that the soil-CVD and xylem-SPB values are usually closer together for $\delta^2H$ and $\delta^{18}O$ compared to xylem-CVD (Figure 9). This is most pronounced for the sampling dates of the 14.08 and 22.09 (Figure 9), where no rain occurred for a long period (cf. Figure 10, 11 & 16). Furthermore, a difference between treatments is visible in Figure 10 and 11. The soil-CVD samples have a lower isotopic value (are isotopically lighter) in the irrigation plot compared to the irrigation stop and control plots, with the exception of the 31.08, where two out of the three soil cores from irrigation stop yielded lower isotopic values compared to irrigation. The isotopic values of the third core at the irrigation stop plot on the 31.08 lie within expectation above the stable water isotopic values of the irrigation site cores. Regarding the xylem-CVD data, the irrigation-stop and irrigation samples plot below the control xylem-CVD stable water isotopic values (Figure 10 & 11). It is notable that irrigation and irrigation stop xylem-CVD samples remain rather stable over the whole measurement period while xylem-CVD at the control site follows roughly the GNIP Sion station values, e.g. the stable water isotopic composition of rainfall.

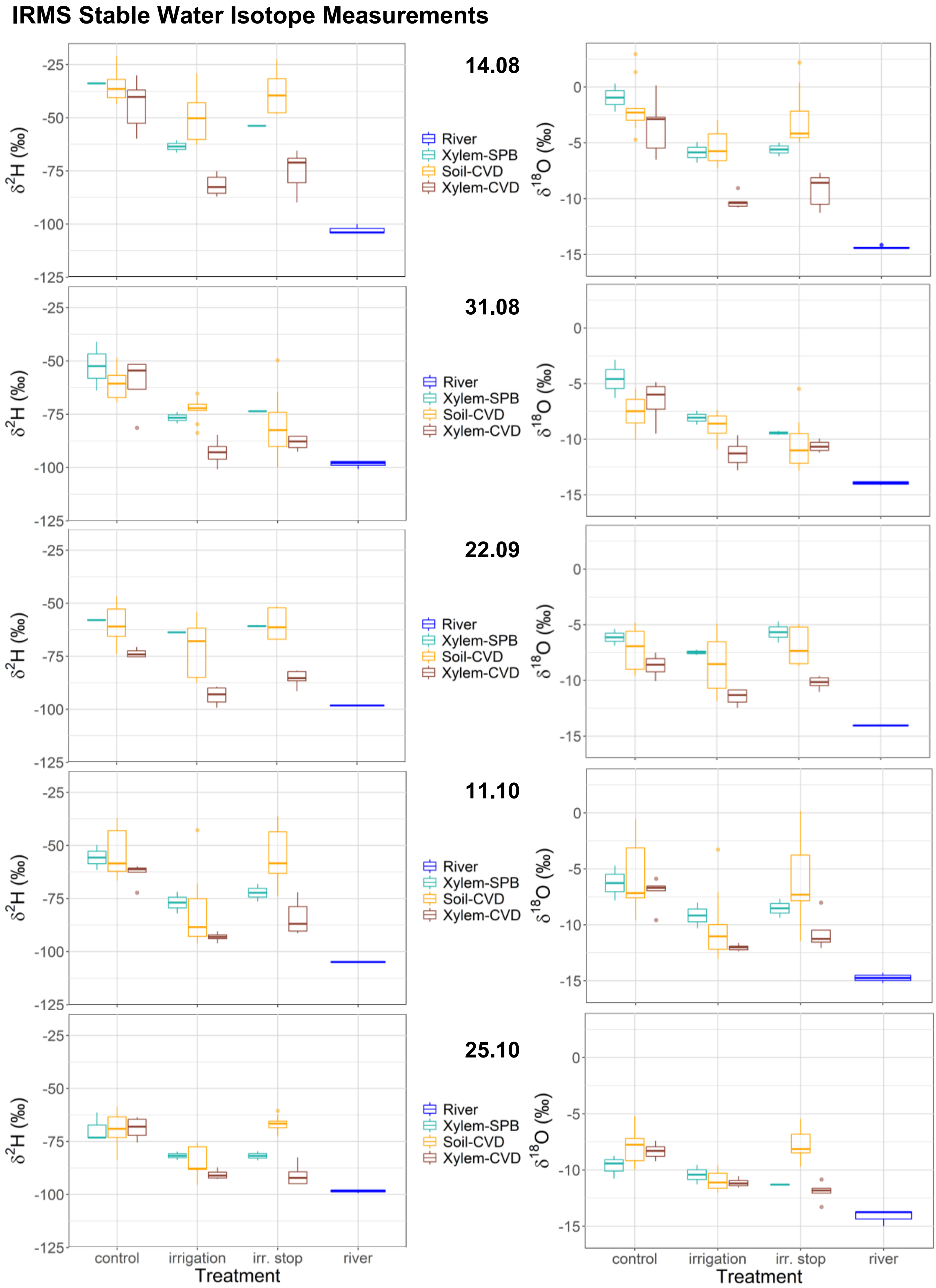


*Figure 9: The stable water isotope compositions over the different field campaigns measured by the IRMS and grouped by treatment as well as sample type. The river stable water isotope data for the 14.08, 31.08 and 22.09 were taken from the IRLS measurement. Appendix 1 shows the same plotting layout and grouping for the IRLS measurements.*

### 3.1.2 Destructive Samples Comparison – Quantitative Analysis

An overall significant influence on the stable water isotopic composition from the treatment and sample type is supported by all statistical methods applied (rmANOVA, LME and GLS p-value < 0.05). Based on the residual analysis, the LME approach performed best and is also the preferable approach based on literature (Zuur et al. 2009). Over the whole dataset, the LME model yielded a p-value below 0.0001 indicating that the treatment and sample type is a highly significant predictor for measured $\delta^2$H and $\delta^{18}$O (i.e. the stable water isotopic composition is different between and dependent on treatments and sample types). For $\delta^2$H the LME-model also indicates an interaction between the treatment and sample type, which is not supported by the rmANOVA. For $\delta^{18}$O, no interaction between sample type and treatment was found.

The xylem-CVD, xylem-SPB and soil-CVD stable water isotopes were in a next step analyzed individually to only compare the differences between treatments to better distinguish the treatment effect from the sampling method effect. The distinct $\delta^{18}$O and $\delta^2$H signals were confirmed, indicating that the treatment is responsible for differences in the stable water isotopic composition within every sample type ($p < 0.0001$ for LME). However, the residuals from the LME for soil water samples stable isotopes do not seem to follow a normal distribution either for $\delta^{18}$O or $\delta^2$H, indicating possible other neglected influences in the model.

The treatments were furthermore analyzed individually to better assess whether the sample type leads to significant differences in the measured stable water isotopic composition. For $\delta^{18}$O, the LME yields a significant difference between sample types for all treatments ($p < 0.05$). However, only for irrigation and irrigation stop the difference was highly significant ($p < 0.0001$). For control, the p-value was only of low significance with a $p \approx 0.02$.

*Table 1: Absolute differences of the IRMS stable water isotope measurement between the xylem-SPB and xylem-CVD extracted water.*

| **$\delta^{18}$O IRMS** | Mean diff. | SD | Date of max. diff. | Max. diff. | Date of min. diff. | Min. diff. |
|---|---|---|---|---|---|---|
| Control | 1.9‰ | 0.8‰ | 22.09.2023 | 2.6‰ | 11.10.2023 | 0.9‰ |
| Irrigation-stop | 3‰ | 1.4‰ | 14.08.2023 | 4.4‰ | 25.10.2023 | 0.6‰ |
| Irrigation | 2.4‰ | 1.6‰ | 22.09.2023 | 4.5‰ | 25.10.2023 | 0.7‰ |
| **$\delta^2$H IRMS** | Mean diff. | SD | Date of max. diff. | Max. diff. | Date of min. diff. | Min. diff |
| Control | 10‰ | 7‰ | 14.08.2023 | 17‰ | 25.10.2023 | 0.5‰ |
| Irrigation-stop | 18‰ | 8‰ | 22.09.2023 | 25‰ | 25.10.2023 | 9‰ |
| Irrigation | 16‰ | 7‰ | 22.09.2023 | 29‰ | 25.10.2023 | 9‰ |

This statistical result aligns with the visual analysis of Figure 9, where the differences between the sample types are usually lowest for the control site. The rmANOVA applied to the control, irrigation and irrigation stop site individually performs again worse than the LME regarding the residual based post-analysis. For irrigation and control the residual distribution has a clear tendency (the residuals are not randomly distributed) but the distribution is far better than for irrigation stop. For irrigation, the rmANOVA confirms the LME result whereas for control no significance was yielded between sample types. For $\delta^2$H, in comparison to the $\delta^{18}$O results, the control site also yields a highly significant difference between sample types ($p < 0.0001$ LME). The rmANOVA shows the same problematic residual distribution as for $\delta^{18}$O. However, the rmANOVA supports the analysis by LME and yields a significant difference between sample types for control ($p = 0.03$). For the irrigation treatment the rmANOVA supports the high significant difference found by the LME approach ($p < 0.0001$).

When only comparing the two xylem water extraction methods, the LME yields a highly significant influence from the treatment and sample type on the measured $\delta^{18}$O and $\delta^2$H values ($p < 0.0001$), which is also apparent from the statistical values in Table 1. For $\delta^2$H, unlike the whole dataset which also includes soil, there is no significant interaction between the treatment and the sample type present. The two xylem extraction methods were also compared for each treatment individually. Thereby, all treatments show a highly significant difference in the stable water isotopic composition between xylem water extraction methods for $\delta^2$H and $\delta^{18}$O ($p < 0.0001$ LME). The rmANOVA, again performing worse than the LME approach, supports these results largely but yields only a low-level significance for the control treatment. But the rmANOVA for the xylem samples shows for $\delta^{18}$O and $\delta^2$H that the sample type has the much stronger influence on the stable water isotopic composition compared to treatment ($p < 0.0001$ compared to $p < 0.05$).

A similar comparative analysis was performed to compare xylem sample types to the soil samples. For the $\delta^2$H measurements, xylem-SPB samples compared to the soil-CVD samples only yielded a low-level significant difference for the irrigation-stop site, at irrigation and control site no statistical difference was found. For $\delta^2$H xylem-CVD samples, in general and in all treatments individually the differences towards soil-CVD samples were significant. But the control site samples showed a lower significance ($p = 0.025$ LME) compared to irrigation and irrigation-stop ($p < 0.0001$ LME). For $\delta^{18}$O, comparing soil-CVD to xylem-SPB samples, the difference was not significant for all the treatments and each treatment individually, including irrigation-stop. Regarding the comparison between xylem-CVD samples and soil-CVD samples, the difference for irrigation and irrigation stop is highly significant ($p < 0.0001$ LME) but for control the difference is not significant. The summary statistics can be found in Appendix 2 and 3.

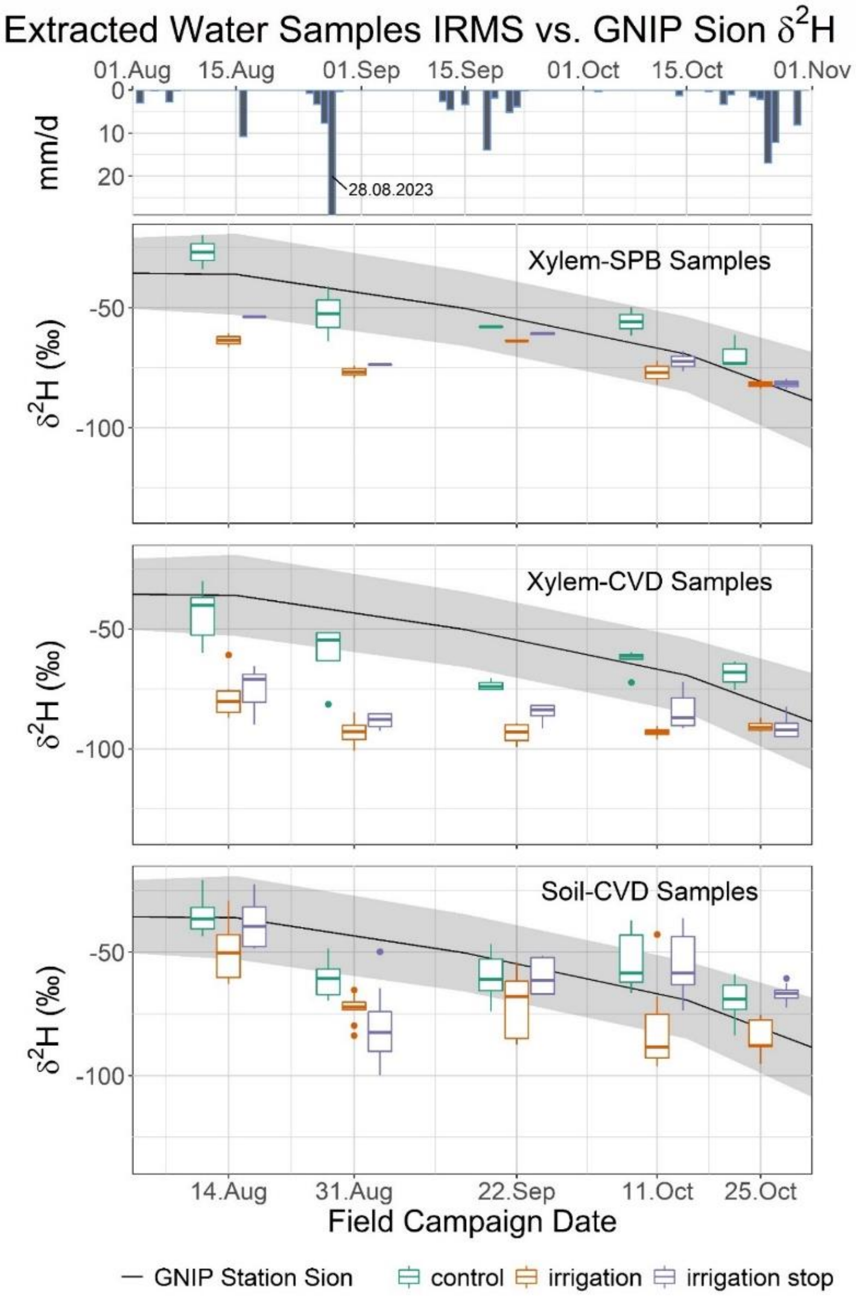


*Figure 10: Temporal evolution of the $\delta^2H$ values of the different samples in comparison to the long-term mean from the GNIP station in Sion. The standard deviation from the mean is shaded in grey. The GNIP Sion annual mean $\delta^2H$ value is -77‰.*

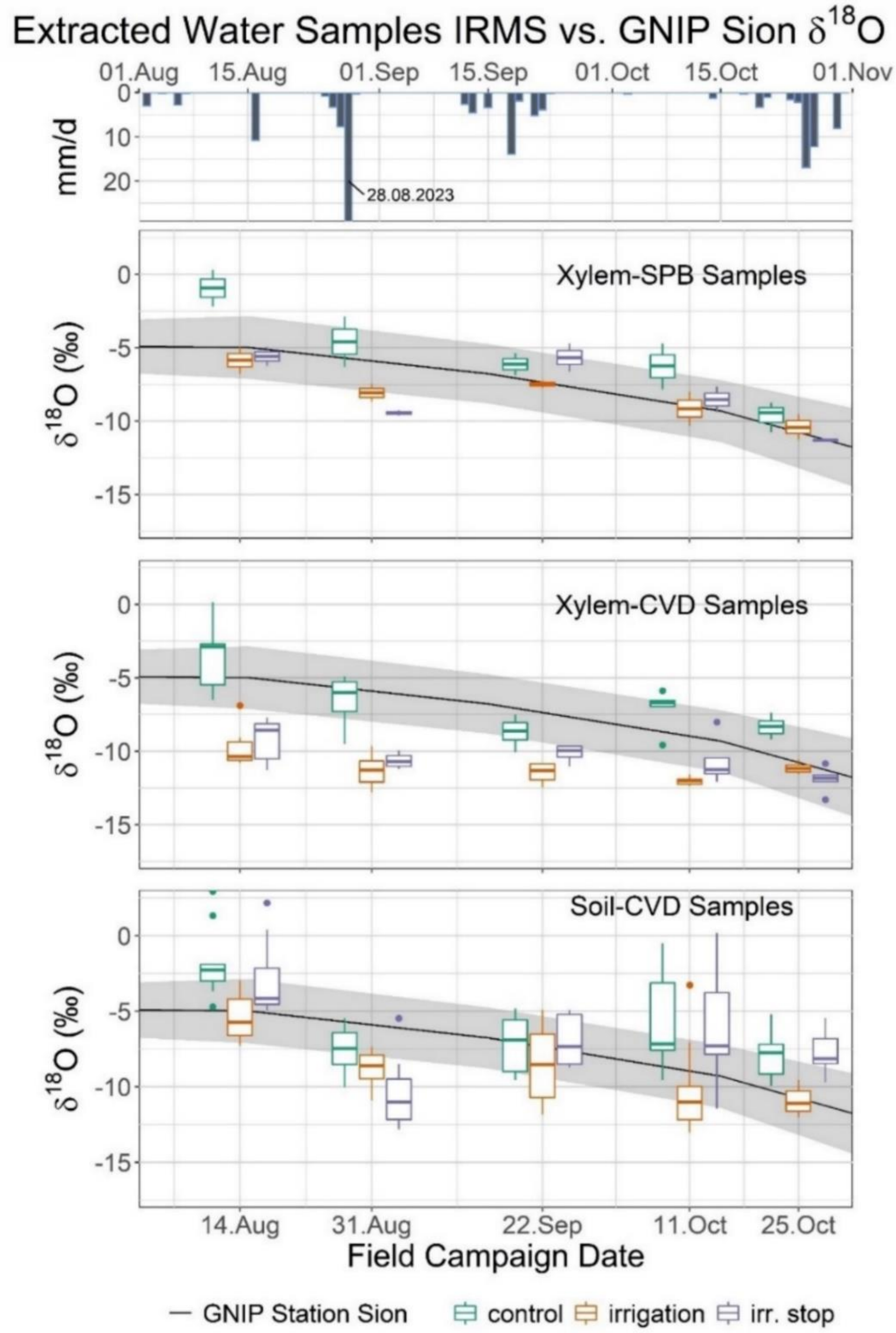


*Figure 11: Temporal evolution of the samples δ*$^{18}$*O values in comparison to the GNIP Sion station mean values. The grey shaded are indicates the standard deviation from the GNIP mean. The GNIP Sion annual mean δ*$^{18}$*O is -10.1‰.*

### 3.1.3 Qualitative Comparison between In-Situ and Destructive Sample Data

The in-situ soil data contains measurements to a depth of 2m compared to the top 50cm for the desctructive samples, but the soil-probes for the in-situ sampling are only placed in one vertical profile, i.e. no horizontal spatial variation is assessed. The stable water isotopic value for the in-situ extracted and IRLS measured soil is usually more negative than the stable water isotopic value from the destructive samples and follow less the GNIP registered seasonal cycle (Appendix 4 & 5, Figure 10-12). The in-situ equilibrium vapor extracted water (xylem-EV) follows for $\delta^{18}$O largely the GNIP station stable water isotopes. This is also the case for $\delta^{2}$H, but less pronounced. For $\delta^{2}$H, the irrigation-stop site xylem-EV samples usually shows the heaviest isotopic composition (highest values), with irrigation in the middle and control with the lightest isotopic composition. The $\delta^{18}$O xylem-EV values show a different characteristic with irrigation being the heaviest, followed by irrigation-stop and control, whereby the placing between control and irrigation-stop is not very clear. The xylem-EV samples show a much greater variability compared to the destructive xylem samples (Appendix 4 & 5, Figure 10-12). Furthermore, it seems that the variability increases towards the end of the growing season.

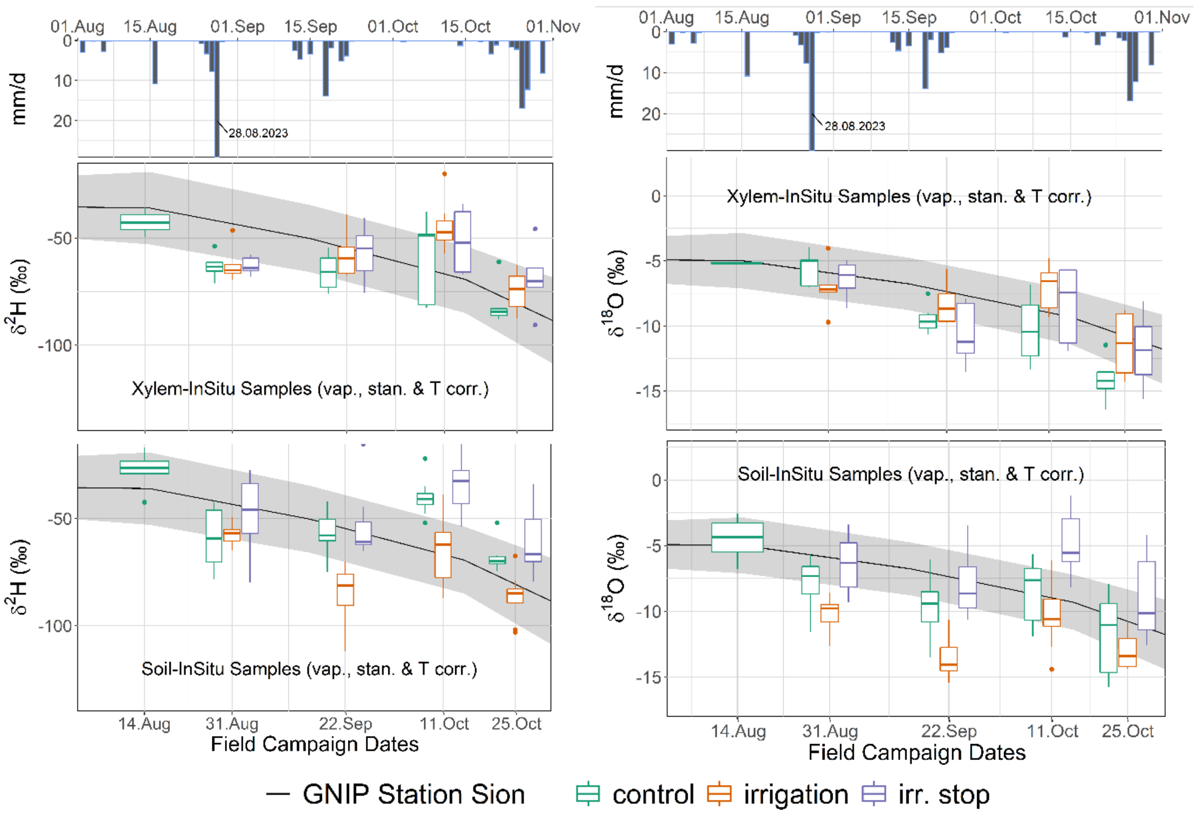


*Figure 12: Stable water isotope values extracted with the equilibrium vapor method and measured in the field. The in-situ data is in some cases from up to 4 days later or earlier compared to the destructive measurement due to measurement gaps. The in-situ data is corrected based on the vapor content, standards and temperature. The correction for the in-situ data was performed by Dr. Elham Freund (UZH), Dr. Katrin Meusburger (WSL), Dr. Marco Lehmann (WSL) and Dr. Zhaoyong Hu (WSL).*

## 3.2 IRMS vs. IRLS Measurements

### 3.2.1 Comparison and Relation to Analytical Precision

In total, 240 samples were measured with the IRMS and IRLS. To analyse the difference in the isotopic values between the two measurement methodologies statistically, the IRMS value was simply subtracted from each IRLS measurement for the same sample according to *Eq. 5*, which is like the approach chosen in Martín-Gómez et al. (2015) and West et al. (2010). The formula shows only the calculation for $\delta^2$H, but it was done the same way for $\delta^{18}$O.

$$\Delta\, \delta^2 H_i = IRLS\, \delta^2 H_i \,-\, IRMS\, \delta^2 H_i \qquad (Eq.\ 5)$$

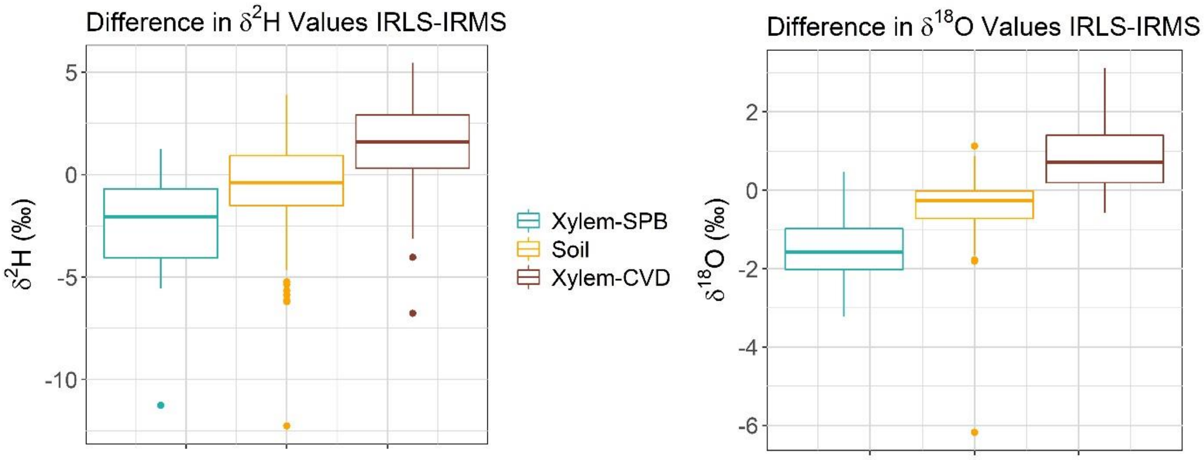


*Figure 13: $\delta^{18}$O and $\delta^2$H boxplots of the difference between IRLS and IRMS measurements calculated based on Equation 4. The differences are grouped by extraction-type (soil, xylem-SPB and xylem-CVD).*

From Figure 13, one can derive that xylem-CVD values are overestimated by the IRLS (lower IRMS than IRLS values) and xylem-SPB values seem to be underestimated by the IRLS (lower IRLS than IRMS values). For interpretation it is important to be aware that the subtraction in *Eq. 5* is usually between two negative values. The soil samples are distributed around the zero line or slightly below for the $\delta^{18}$O values (Figure 13), and there is no difference with depth between the IRLS and IRMS measurement (Appendix 7). Furthermore, a larger variance can be observed for $\delta^2$H compared to $\delta^{18}$O for all sample types. The boxplots for the difference in $\delta^2$H have additionally a higher overlap compared to the boxplots for the difference in $\delta^{18}$O. But it is important to consider the accuracy and precision of the measurement systems. For the IRLS measurements, the accuracy is 0.1-0.3 ‰ for $\delta^{18}$O and 0.6-1 ‰ for $\delta^2$H and the precision is <0.2 ‰ for $\delta^{18}$O and < 0.5 for $\delta^2$H. For the IRMS measurements the laboratory at WSL reported an accuracy of < 0.2 ‰ for $\delta^{18}$O and < 0.5 ‰ for $\delta^2$H and a precision of <0.2‰ for $\delta^{18}$O and < 0.6‰ for $\delta^2$H (personal communications with Dr. Marco Lehmann). Therefore, the deviation from zero for soil samples in general and additionally xylem-CVD samples for $\delta^2$H does not exceed the deviation possible due to the measurement precision (i.e. the SD). The river samples show as expected no difference from the zero line apart from precision based small variations (Appendix 8).

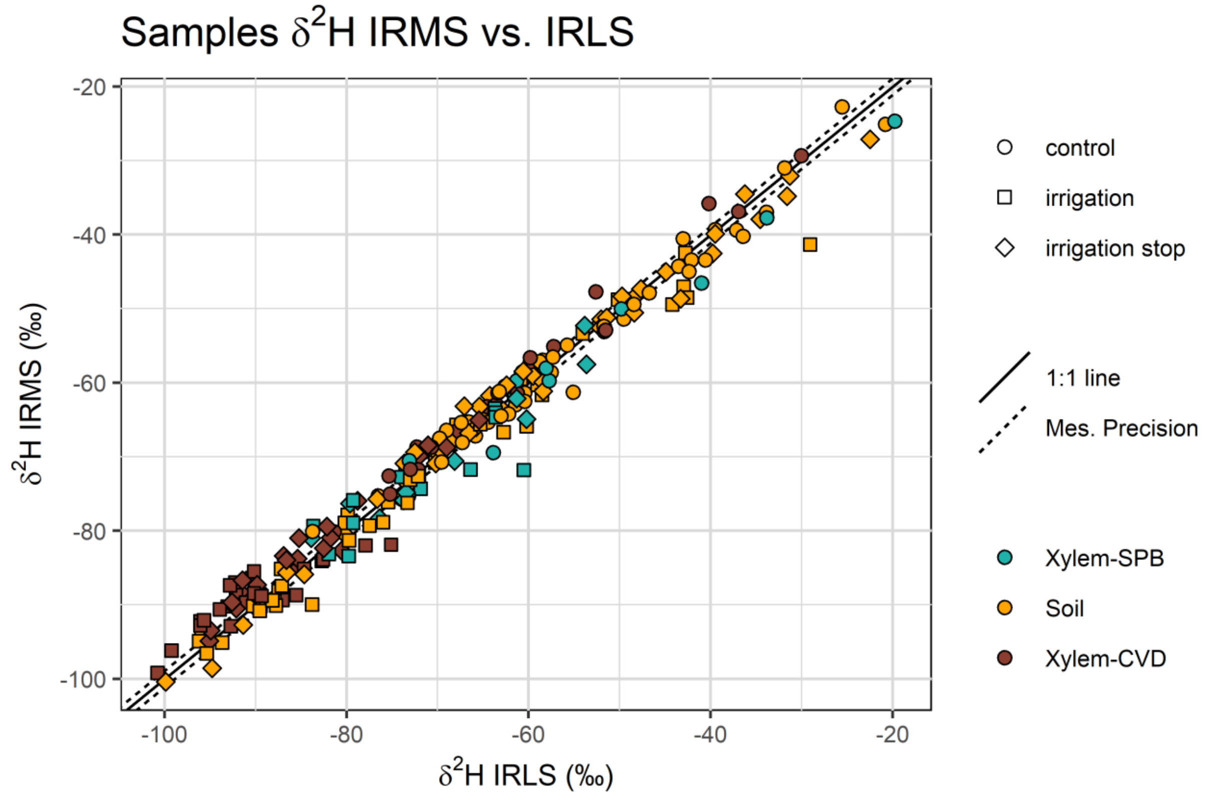


*Figure 14: $\delta^2$H IRMS and IRLS values grouped by sample type shown in reference to the 1:1 line and the SD (precision) acquired by the standard measurements (equal IRMS and IRLS value).*

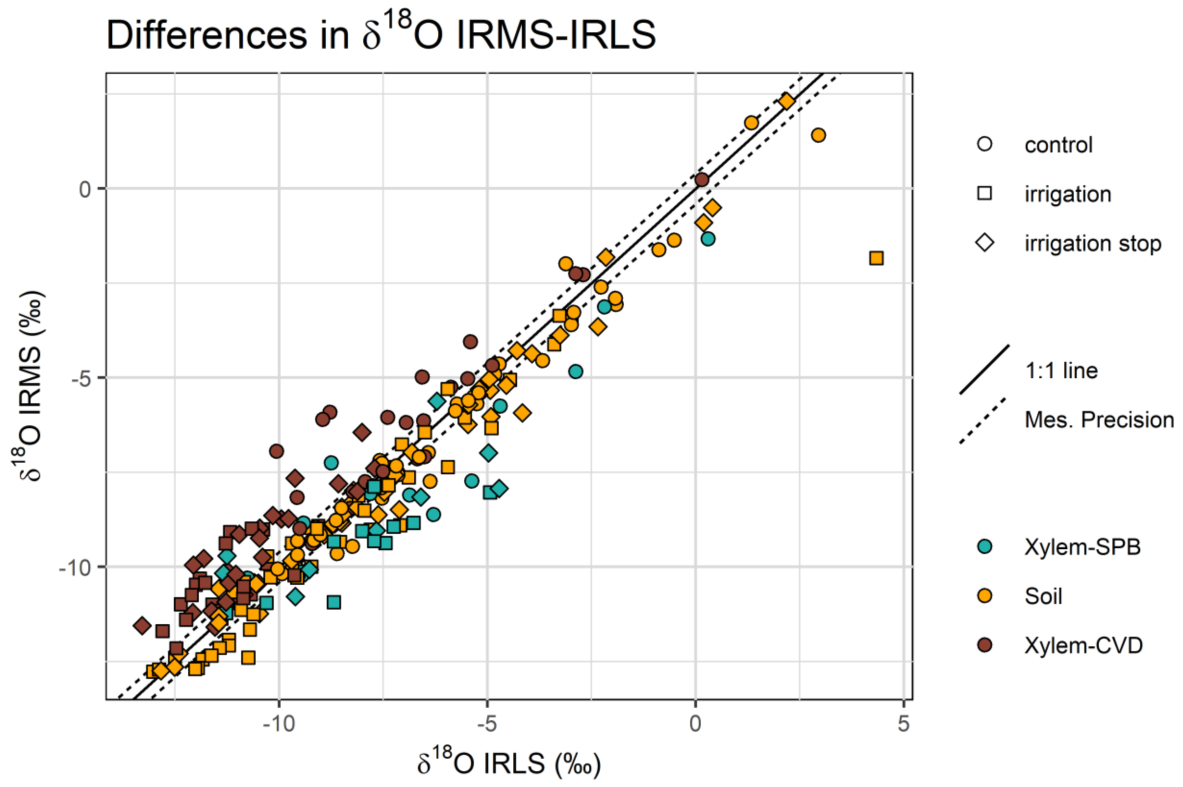


*Figure 15: $\delta^{18}$O values of the IRMS and IRLS measurements grouped by sample type and in reference to the 1:1 line and the SD (precision) acquired by the standard measurements.*

Figures 14 & 15 support the general trends seen in Figure 13. For $\delta^{18}O$ in Figure 15 the grouping of xylem-CVD samples above the 1:1 line and the xylem-SPB samples below it is evident. The difference in the IRLS and IRMS values is furthermore significantly different between sample types ($p < 0.0001$ with rmANOVA and LME) but not between treatments ($p > 0.3$ with rmANOVA and LME). However, Figure 14 does not show a grouping as clear as before for $\delta^{2}H$ values anymore. Tendencies seem to still be present, but the groups are not as distinct as they are for $\delta^{18}O$. However, the statistical tests (rmANOVA and LME) show significance regarding $\delta^{2}H$ differences between IRLS and IRMS for distinct sample types ($p < 0.0001$) but not for treatments ($p > 0.05$). Regarding the soil-CVD samples, they seem to follow roughly the 1:1 line with some outliers. There is no clear difference between treatments or over time visible when the data is plotted (Appendix 6). Furthermore, no trend with the soil depth is visible (Appendix 7).

### 3.2.2 Deuterium-Excess Differences between IRLS and IRMS

The opposite direction concerning the measurement-system induced bias in xylem-CVD (positive deviance from the 1:1 line) and xylem-SPB (negative deviance from the 1:1 line) samples is also apparent when the deuterium excess values are analyzed (Figure 16). The soil-CVD samples cluster again around the 1:1 line but have a larger spread of $d_{excess}$ values in the IRMS and IRLS measurement. The xylem-CVD samples however show higher IRMS $d_{excess}$ values compared to the IRLS measurement $d_{excess}$. The opposite is the case for xylem-SPB samples.

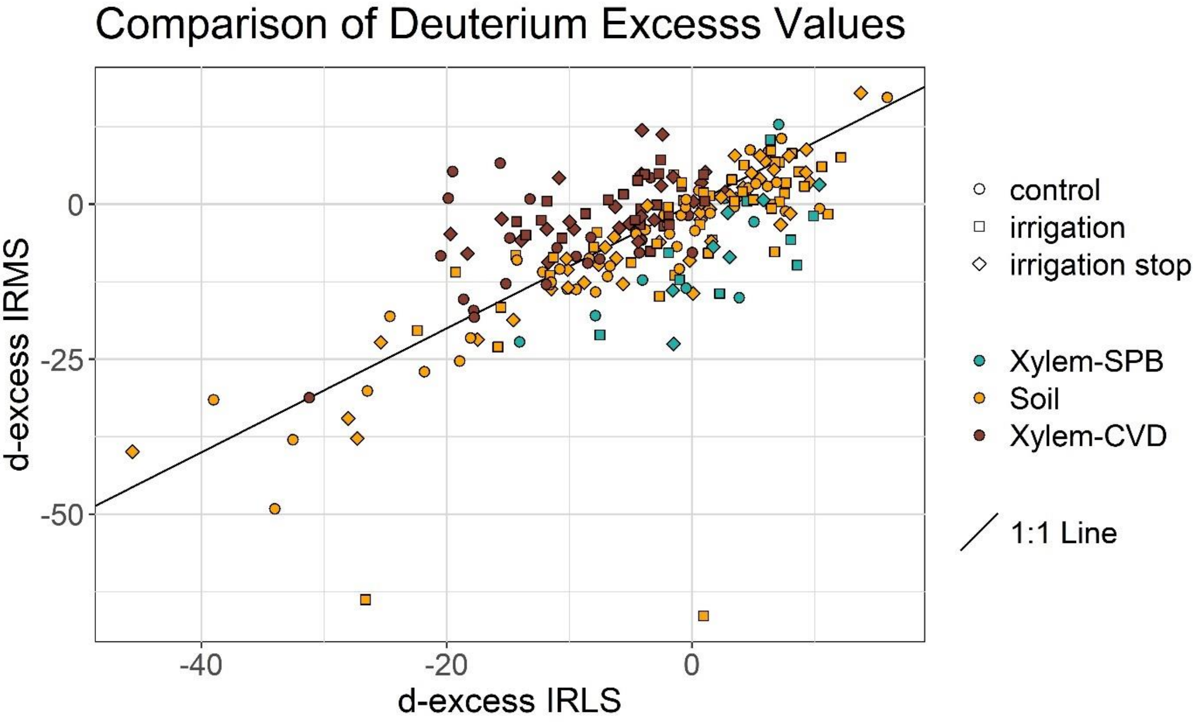


*Figure 16: Deuterium excess values from the IRLS and IRMS measurement compared. For calculation see Equation 4 section 1.3.*

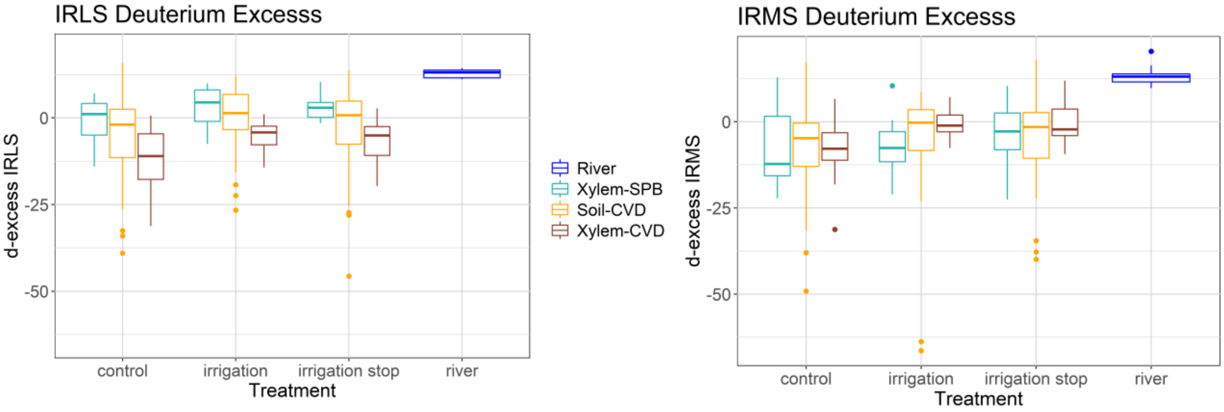


*Figure 17: Comparison of the deuterium excess values between the IRLS and IRMS measurement.*

From Figure 17 it is visible that the trends in the deuterium excess between the IRLS and IRMS are opposite. While for the IRLS measurements the xylem-SPB values are the highest, followed by soil and then below the xylem-CVD samples, such a trend is not visible anymore for the IRMS measurement deuterium excess calculation. The soil values are mostly stable between the IRLS and IRMS measurement with small deviances. The IRLS measurement was always done first and afterwards the IRMS measurement was performed.

### 3.2.3 IRMS-IRLS Statistical Deviations from Zero

To test whether the stable water isotope differences between IRLS and IRMS are significantly different from zero for each treatment, a one-sample test is used. $\delta^{18}$O shows for xylem-SPB and soil-CVD a normal distribution, therefore the one-sample t-test is used with the assumed mean to be zero as the IRMS and IRLS should have the same measured $\delta^{18}$O value. Xylem-CVD shows a small deviation from normality when the Shapiro-test is used. Therefore, the Wilcoxon-test is applied instead of the one sample t-test. A similar small deviation from normality is found for the $\delta^{2}$H values in all sample types, leading to the use of the Wilcoxon-test to investigate the difference in the $\delta^{2}$H values between the IRLS and IRMS. All tests show a significant deviation from zero, indicating that even for soil samples, there is a difference between the IRLS and IRMS measurement. However, the mean and 95-percentile confidence interval (Table 2) indicate that only xylem-samples have a larger deviation from zero (positive or negative) concerning the measurement precision for $\delta^{18}$O (>0.4 ‰) and $\delta^{2}$H (> 1.1 ‰). Therefore, these statistical results need to be interpreted with caution (see Discussion section 4.2).

*Table 2: 95-percentile confidence interval for the difference between IRLS and IRMS by sample type. All the values are given as IRLS-IRMS $\delta^{18}$O [‰] resp. $\delta^{2}$H [‰] values.*

| **$\delta^{18}$O [‰]** | 95% confidence interval | | | **$\delta^{2}$H [‰]** | 95% Confidence Interval | | |
|---|---|---|---|---|---|---|---|
| | lower | upper | mean | | lower | upper | mean |
| Xylem-CVD | 0.66 | 1.05 | 0.85 | Xylem-CVD | 0.96 | 2.03 | 1.5 |
| Xylem-SPB | -1.86 | -1.1 | -1.48 | Xylem-SPB | -3.69 | -1.38 | -2.53 |
| Soil-CVD | -0.44 | -0.26 | -0.35 | Soil-CVD | -0.86 | -0.14 | -0.5 |

### 3.2.4 Comparison of the $\delta^2H$ and $\delta^{18}O$ IRMS-IRLS Bias

To see whether the same samples are biased for the $\delta^2H$ and $\delta^{18}O$ values, the Kendall correlation test was applied on the normalized stable water isotope values differences between the IRLS and IRMS measurement. The Kendall's rank correlation test (tau) allows to test the similarity of ranking between two variables (Kendall 1938) and is therefore suitable for the data presented here. For the xylem-SPB samples, the bias between the IRLS and IRMS measurement does not seem to be related between $\delta^2H$ and $\delta^{18}O$ ($p >> 0.05$). For the xylem-CVD samples, the Kendall test yields a correlation between the $\delta^2H$ and $\delta^{18}O$ bias ($p \approx 0.002$, $tau \approx 0.25$). The soil-CVD samples were also tested. No relationship was expected as the bias is generally below the precision of the measurement instrument. This expectation was confirmed by the Kendall rank correlation test ($p >> 0.05$).

## 3.3 Drought Dependency of the Xylem Stable Water Isotopes

The leaf water potential was measured for all the field campaigns pre-dawn and midday. However, due to measurement instabilities of the Scholander pressure bomb device used for the first field campaign, the leaf water potential measurements reported with this device during the first field campaign were excluded from analysis. Regarding the measurements on the 01.09.2023 and 22.09.2023 in Figure 18, there were precipitation events short before the field campaign, which has led to only little difference between the pre-dawn and midday leaf water potential (LWP). The rainfall-effect is especially pronounced on the 25.10.2023, with rainfall during the field campaign. No difference can be seen between the pre-dawn and midday leaf water potential on that day (Figure 18). Only the field campaign on the 11.10.2023 was during a drought event, therefore it is the only measurement point of relevance to investigate whether water stress in trees has an important impact on the stable water isotope measurements.

Nevertheless, a rmANOVA was performed for each treatment separately to see whether the water availability condition had a measurable effect on the registered stable water isotope signal. The treatments were analysed regarding the leaf water potential (water availability) effect separately to better distinguish the two influencing factors, as the treatment itself already has a significant influence on the leaf water potential according to a performed rmANOVA test. The test was performed with the sample type to see if there is a significant interaction between them which could influence the $\delta^{18}O$ and $\delta^2H$ values. However, the test results show no significant influence of the leaf water potential on the measured stable water isotopes in tree xylem. Only the pre-dawn leaf water potential has a p-value close to 0.05 for the irrigation stop and the control treatment regarding its effect on the $\delta^2H$ values, but it is not enough to address it as a significant influence, as a clearer result is need if processes in ecology are analyzed according to Zuur et al. (2009) and Jeon et al. (2022). Furthermore, when Figure 18 is investigated visually in comparison to Figure 9-11 (section 3.1), no relationship between drought effects for the xylem samples can be detected.

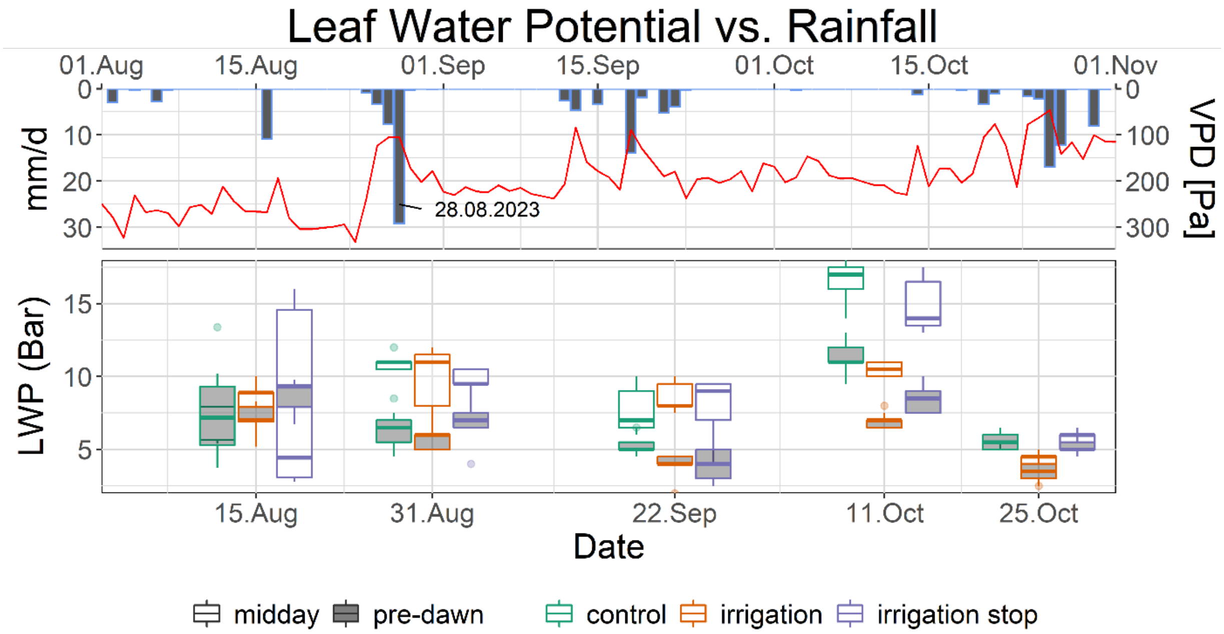


*Figure 18: Midday and pre-dawn leaf water potential grouped by treatment and field campaign. On top, the daily precipitation and vapor pressure deficit (VPD, red line) is plotted for comparison.*

# 4. Discussion

## 4.1 $\delta^{18}O$ and $\delta^{2}H$ Differences between Sample Types and Treatments

A significant difference between the soil-CVD, xylem-CVD and xylem-SPB extracted water stable isotopic composition has been found. Furthermore, a significant difference in the stable water isotopic values between the three treatments was observed for all the sample types. These results are based on the IRMS measurements and are assumed to be free of the possible IRLS induced bias (chapter 4.2).

### 4.1.1 Soil Stable Water Isotope Difference between Treatments and Methods

When looking at the soil data in Figure 10 and 11, one can see that the stable water isotopic composition at the control and irrigation-stop site soil is usually similar, i.e. have a similar mean, which is supported by the in-situ measurements. Irrigation site soil shows a lower isotopic composition in comparison. This is not surprising, as the irrigation water comes from the nearby channel (labelled river in Figure 9), which has a far lower isotopic value compared to the control site soil and precipitation at the Pfynwald site. Generally, the soil sample stable water isotopic values follow the GNIP $\delta^{18}O$ and $\delta^{2}H$ long term average values for the soil-CVD samples. The expected trend in the soil isotopic value with depth (cf. Chapter 1.3) is supported by the destructive soil samples apart from dates where rainfall changed the soil water isotopic values near the surface so that it became more negative than the lower soil layers (e.g., 31.08.23 and 25.10.23 in Appendix 1).

The pattern from the destructive samples is largely supported by the in-situ data, even though the variations of the in-situ data are higher in comparison. The in-situ data shows a slightly lower isotopic average values compared to soil-CVD samples. This is not surprising as deeper soil layers are supposed to have lower isotopic values, which are more stable over time (Bertrand et al. 2014; Dubbert & Werner 2019; von Freyberg et al. 2020) and the in-situ probes range from -20 to -200cm compared to the destructive samples which are taken between -5 and -50cm (cf. Appendix 9). Therefore, mainly the upper soil water stable isotopic values are supposed to follow closer to the seasonality whereby the signal in the depth can be expected to be diluted. A further effect which influences the difference between the two methods comes from the time-lag. First, it takes a while until an isotopic signal is transmitted to the depth which is dependent on the infiltration speed of the water which is again dependent on soil type and vegetation (Dubbert & Werner 2019; von Freyberg et al. 2020; Sprenger et al. 2016), whereby a transect over a larger soil column will likely lead to a higher heterogeneity in the soil water stable isotopic values, as the isotopic value changes with depth (Dubbert & Werner 2019; von Freyberg et al. 2020; cf. chapter 1.3). Furthermore, there is a time lag between measurements, leading to up to several hours in between measurements, which can lead to differences in the measurements due to a different infiltration depth of fresh precipitation or evaporative effects, which alter the isotopic signal. What is not incorporated in the in-situ data is the spatial variability of the soil isotopic composition, which is visible in Appendix 9. Furthermore, the irrigation-stop in-situ soil probe seems prone to overestimating the isotopic value compared to the control site and the destructive irrigation-stop soil samples (Appendix 9).

The soil-CVD water stable isotopic values from the field campaign on the 31.08.2023 show an exceptional pattern in comparison to the whole dataset. The stable water isotopic values are more negative compared to the GNIP station, i.e. have a greater deviance to the mean and SD compared to the stable water isotopic values from the other field campaigns. Furthermore, irrigation stop has a more negative (i.e. lighter) isotopic composition than the control and irrigation site soil water. The overall lower stable water isotopic values could be connected to the rainfall from the 28.08.2023, where the strongest rainfall event over the whole data series occurred. It has been reported that storms generally have a lower stable water isotopic composition compared to warm frontal systems (Allen et al. 2022), explaining the observation of lower soil water stable isotopic values on the 31.08.2023, as well as making sense with the vertical isotopic signal in the soil on that date (Appendix 1).

The difference in the stable isotopic signal between the irrigation-stop destructive soil cores stable water isotopic signal on the 31.08.2023, where two soil cores do not fit the general trend, could be connected to a heterogenous throughfall or infiltration pattern. First, regarding the throughfall effect, the irrigation-stop site in-situ measurement station, where close by the destructive soil cores were sampled, is characterized by a low canopy density. Hence throughfall effects, which are reported to change $\delta^{18}O$ values up to 5‰ (reported mean by literature: 0.6‰, Allen et al. 2017), could have had a certain influence on the differences between soil cores at the irrigation-stop site on the 31.08.2023 field campaign. Second, the infiltration pattern could have led to the distinct isotopic signal due to a difference in the preferential flow path system between the cores. The two soil cores which do not align with the others could have smaller pores or less macropores, so that more of the recent rainfall is still present in shallower soil and not yet passed on to deeper soil layers. A high heterogeneity of infiltration rates in forests and a general higher infiltration rate under tree stands was found by previous studies (e.g., Archer et al. 2016; Alaoui et al. 2011; Dubbert & Werner 2019; Sprenger et al. 2016). Therefore, a combined effect of a distinct infiltration rate depending on the tree cover and the difference from throughfall and open precipitation could have led to the distinct stable water isotopic signal in two soil cores from the irrigation-stop site.

### 4.1.2 Tree Water Uptake – Xylem-SPB and Soil-CVD Stable Water Isotope Comparison

For all treatment sites, the xylem-SPB and soil-CVD stable water isotopic values are in agreement while the xylem-CVD extracted water shows distinct isotopic values. The significant differences between the stable water isotopic signal of sample types appears to originate from the offset of xylem-CVD extracted water to the other sample types (soil-CVD and xylem-SPB). Therefore, to analyse tree water uptake, the comparison between xylem-SPB and soil-CVD stable water isotopic values seems most reasonable. Using the extraction method to analyse tree water uptake which yields the lowest difference towards the source water isotopic composition is furthermore in accordance with previous research (e.g., Barbeta et al. 2022).

For the control site, the differences between the isotopic values of the soil-CVD, xylem-SPB and xylem-CVD are the smallest. The difference between the soil-CVD and xylem-SPB isotopic values is not statistically significant. The stable water isotopic composition furthermore follows in all cases (soil and xylem) the GNIP seasonal trend in stable water isotopes in precipitation. This implies that the trees at the control plot take up water from the top 50cm of soil. The main use of shallow water sources has been shown in previous research for Scots pine in the Valais when the soil dries up (Bertrand et al. 2014). Bertrand et al. (2014) found this dynamic mainly towards the end of the growing season, which aligns with the observations made here which covers the growing season from August to October. More generally, Bachofen et al. (2024) concluded that trees in arid regions primarily take up shallow soil water when the groundwater cannot be reached, which aligns with Bertrand et al. (2014).

At the irrigation site, no significant difference was found between the soil-CVD and xylem-SPB samples isotopic composition, implying that trees take up water preferably from the upper soil layers. Compared to the control site, the xylem-SPB values at the irrigation site are more stable over time and more negative, especially during summer. This is caused by the irrigation water which has a lower stable water isotopic value. However, the xylem-SPB and soil-CVD isotopic value is higher than the pure irrigation water isotopic value on average due to mixing with rainfall water and enrichment in heavy isotopes under evaporation. Whereby on the 14.08.2023 the evaporation enrichment and on the 22.09.2023 the mixing with rainfall was probably the cause for the higher isotopic values in the soil water compared to the irrigation water at the irrigation site (Figure 10 & 11, Appendix 1). On the 14.08.2023, the isotopic values for $\delta^2H$ decreases with depth (Appendix 1). On the same date (14.08), the xylem-SPB $\delta^2H$ values are lower than the top 50cm of the soil (Figure 9). This indicates that the trees could access deeper soil layers with their roots which contain more water which has a lower isotopic value than the near surface water (cf. chapter 1.3) to meet the high transpirational demand. This pattern aligns with previous research (e.g., Bachofen et al. 2024; Dubbert & Werner 2019). That trees take up water preferably from the most saturated subsurface area was also proposed by Bachofen et al. (2024). Therefore, it can be assumed that the irrigation site trees probably take up preferably near surface soil water but are able to access easily deeper soil water when the surface soil dries up.

At the irrigation-stop site, the xylem-SPB stable water isotopic compositions are often below the soil water stable isotopic composition, i.e. xylem-SPB stable water isotopic composition is more negative than the soil-CVD (top 50cm) stable water isotopic composition. This is especially the case after a dry period (Figure 9-11). For $\delta^2H$, the difference between the xylem-SPB and soil-CVD stable water isotopic composition is furthermore statistically significant for the irrigation-stop site (Figure 10). The irrigation-stop site xylem-SPB stable water isotopic composition is additionally more stable over time compared to the control site and similar to the irrigation site xylem-SPB stable water isotopic composition. This indicates that during drought phases the irrigation-stop site trees can access deeper water reservoirs (e.g. on the 11.10.2023), because deeper soil water reservoirs are assumed to have lower isotopic values than the near surface soil water and have less variation over time (Brinkmann et al. 2018; von Freyberg et al. 2020; Figure 9-11). This observation favors the hypothesis that the irrigated trees developed a deeper rooting network during the irrigation phase, enabling them to access water stored deeper in the soil, especially under dry conditions.

An exception to this pattern is the field campaign on the 22.09.2023, where irrigation-stop and control site have a similar xylem-SPB extracted water stable isotopic composition while irrigation is only slightly more negative (Figure 9). On this date, at the irrigation-stop site the xylem-SPB extracted water stable isotopic composition follows neatly the soil-CVD extracted water stable isotopic composition (i.e. the water stable isotopic composition in the top 50cm), which is unique in the dataset. The phase of rainfall before this field campaign probably led to sufficient moisture being available in the upper soil (cf. Figure 10 & 11). In accordance with Bachofen et al. (2024) the trees took up preferably water from the upper soil layers on the 22.09.2023, as it was sufficient to supply their transpirational demand. This pattern during a wet phase furthermore supports that the irrigation and irrigation-stop site trees have access to additional water resources during drought while this is not the case for the control site.

### 4.1.3 Xylem-SPB and Xylem-CVD Water Extraction Method Comparison

According to the statistical analysis and visible in Figures 9-11, the two destructive xylem water extraction methods show significant differences. The xylem-CVD method always yields significantly lower (more negative) stable water isotopic values than the xylem-SPB extracted water. Even though there is a clear difference in the isotopic composition between the two water extraction methods, the presence of a seasonal pattern at the control site and its absence at the irrigation and irrigation-stop site is similar between the xylem-SPB and xylem-CVD extracted water stable isotopic value. However, the difference in the stable water isotopic value between control site compared to irrigation and irrigation-stop is smaller for the xylem-SPB samples compared to the xylem-CVD samples for all sample dates (Figure 9). The differences in the stable water isotopic value between xylem-CVD and xylem-SPB extracted water is similar between the irrigation and irrigation-stop treatment but nearly double of the offset observed for the control site xylem samples (Table 1).

The offset between different xylem water extraction methods aligns with previous studies (e.g. Barbeta et al. 2020 & 2022; Chen et al. 2020; Duvert et al. 2023; Zuecco et al. 2022). It was furthermore found that the offset is of different magnitude between different tree species (Duvert et al. 2023; Zuecco et al. 2022). Zuecco et al. (2022) found an offset of 2-16% for $\delta^2H$ and 0.2-2‰ for $\delta^{18}O$ for different broadleaf tree species when they compared the SPB extraction method to CVD extracted samples. The differences found for Scots pine trees here between the xylem-CVD and xylem-SPB method ranged from 0.5-29‰ for $\delta^2H$ (mean 14.3‰) and 0.3-4.5‰ for $\delta^{18}O$ (mean 2.4‰, Table 1). The wider range of differences found in the data presented in this study is probably connected to a larger spectrum of environmental conditions covered. Zuecco et al. (2022) sampled exclusively during times of strong water deficits during the summer while the data presented here covers different water conditions and a longer temporal period. Another possible reason for the different range of offsets found in the Scots pine trees in this study compared to Zuecco et al. (2022) could be because different species are investigated. Previous studies found that such species-specific offset differences occur (Duvert et al. 2023). Wen et al. (2023) who applied pressure based (like SPB) and CVD extraction methods on potted one year old apple trees and found a much smaller offset for $\delta^2$H and did not find a significant offset for $\delta^{18}$O which could again be due to the different environmental conditions or species specific. Barbeta et al. (2022) found an offset which is comparable to what was found in this study. They used the Cavitron-centrifugal extraction method (other pressure-based method) and the CVD extraction method on other tree species from the Pinaceae family (Pinus pinaster) under environmental conditions. Therefore, distinct offsets between studies regarding the CVD extraction method and the source water or xylem water extracted with other methods is probably dependent on the tree species and environmental factors.

In the study of Barbet et al. (2022) they found furthermore that the Cavitron-centrifugal extraction method extracts water with no statistically significant difference to the isotopic composition of the trees source water and therefore conclude that the Cavitron-centrifugal extraction method is suitable to extract the water taken up by a tree. Similarly, my dataset shows only a marginal offset between the trees source water (soil-CVD) and the SPB extracted xylem water isotopic composition, implying that the SPB extraction method is a reliable method to extract the trees xylem water to analyze tree water uptake and is comparable to other pressure-based methods.

The two water world hypothesis as a possible reason for the isotopic differences between the soil water (tree source water) and the xylem-CVD extracted water (tree water) is contradicted by the data presented here. At the Pfynwald study site, only marginal differences between the bulk soil water isotopic composition (extracted with the CVD method) and the xylem water stable isotopic composition (extracted with the SPB method) were observed for the irrigation-stop site and no difference at all was found for the other treatments. Furthermore, no difference between the in-situ (equilibrium vapor extracted) soil water and tree xylem water was detected. Therefore, no difference between the trees source water and the trees xylem water indicates that the bulk soil water represents the source from which the trees take up water with no distinction within the soil. This interpretation aligns with previous research applying an in-situ vapor equilibrium method (e.g., Gessler et al. 2022) or a variety of different pressure-based extraction methods (e.g., Barbeta et al. 2020 & 2022; Wen et al. 2023), source water labelling experiments with water of a known isotopic composition (e.g., Vargas et al. 2017) or by experiments on the isotopic difference between the bulk and mobile soil water (e.g., Sprenger et al. 2018).

These results furthermore contradict the second hypothesis, which states that root water fractionation causes the offset between the trees source water (soil water) and the CVD extracted tree xylem water (Vargas et al. 2017). The root water-uptake fractionation was already opposed by previous research (e.g., Barbeta et al. 2020 & 2022; Wen et al. 2023). The isotopic signal between the xylem-SPB and soil-CVD extracted water would be significantly different if root water fractionation processes would occur, which is not the case. Furthermore, there are small insignificant differences occurring between the xylem-SPB and soil-CVD extracted water at the control plot with contrasting directions between sample dates. If root water uptake would fractionate against the heavier or lighter isotopes, the direction of the marginal differences between soil-CVD and xylem-SPB stable water isotopic signal would be expected to be always in the same direction (e.g., a fractionation against the heavy isotope would lead to a constant more negative isotopic value in the tree xylem), which is not the case.

The third hypothesis after Chen et al. (2020), that the offset is created by a dynamic exchange of deuterium between the xylem water and organic structures (e.g., the cell wall) under CVD extraction (cf. chapter 1.1), is also contradicted, because the data presented here shows an offset between the CVD and SPB extracted water for $\delta^{18}O$ and $\delta^{2}H$. Already Barbeta et al. (2022) challenged the hypothesis from Chen et al. (2020) because the deuterium exchange could not explain the magnitude of the offset between the CVD and Cavitron-centrifugal extraction method observed in their study.

The fourth hypothesis by Barbeta et al. (2022) and Zuecco et al. (2022) states that storage effects might be the cause for the offset in the stable water isotopic composition between xylem water extraction methods. They argue in their studies that the pressure-based methods (Cavitron-centrifugal and SPB) extract mobile water, which is used for transpiration, while the CVD method extracts water trapped in living cells alongside the mobile water. Barbeta et al. (2022) argue that the difference in the isotopic signal between the xylem water and symplastic water (water in storage within cells) comes from fractionation processes when water from the xylem enters the cell through aquaporins. The latter argument is supported by other studies (e.g., Zhao et al. 2016). This hypothesis furthermore aligns with the findings from Chen et al. (2020), where after rehydration of a piece of wood with labelled water an offset between source water and CVD extracted water still occurred. Therefore, during the rehydration where water moved through the xylem and again into the storage through aquaporins, fractionation could have occurred, so that after another extraction a CVD offset compared to the source water was still visible. It furthermore aligns with the findings of Fabiani et al. (2022). They found a difference in the isotopic signal when extracting sapwood water and heartwood water with the CVD method, which is dependent on whether the tree is a coniferous or deciduous species. Fabiani et al. (2022) found a larger offset for coniferous tree species, which supports the previous interpretation that studying a different species (coniferous) caused a difference in the offset presented here between the SPB and CVD extraction compared to Zuecco et al. (2022), who used deciduous trees. It is likely that in their study the heartwood contains mainly storage water while the sapwood water consists of storage water within the sapwood cells together with the freshly taken up flowing water (cf. chapter 1.4.1).

These effects seem to be able to explain the pattern observed at the control plot. The stable water isotopic offsets between the two xylem water extraction methods are similar compared to the ones found in other studies (e.g., Barbeta et al. 2022; Zuecco et al. 2022). Furthermore, the temporal pattern fits these explanations. In times of drought, the xylem-CVD value seemed to be higher (less negative) compared to the previous date (11.10), whereas if rainfall occurred between sampling dates (e.g., 15.08 to 31.08 and 31.08 to 22.09) the xylem-CVD values are lower (more negative). It can be assumed that a depleted storage on the 11.10 leads to only a small contribution of storage water to bulk stem water, decreasing the difference between the SPB and CVD extracted xylem water stable isotopic composition.

However, the storage-fractionation-effect explanation alone fails to address the large differences between xylem-SPB and xylem-CVD extracted water stable isotopic compositions nor its temporal pattern. If fractionation during cell water uptake during water movements through the aquaporins was the only mechanism at work, one would expect a similar offset and temporal evolution between all treatments, especially between irrigation-stop and control. Whereby the physical functionality between treatments should not change significantly between cells of the same tree species. But according to the statistical results, there is a significant difference in the offset between treatments and a temporal pattern is not seen in either the irrigation nor irrigation-stop site isotopic composition.

The diurnal cycle of cellular water storage build-up during the night and depletion over the day could play an additional role (e.g., Liu et al. 2021; Zweifel et al. 2021) could play an additional role at the irrigation-stop site. As previously established (chapter 4.1.2), the irrigation-stop site trees are likely able to access deeper soil water compared to the control site trees. Therefore, the water stored in tree cells which can be used to meet the trees' transpirational demand during the day and is replenished during the night (Zweifel et al. 2021), probably comes at the irrigation-stop site from deeper water layers. These are of a stable and lower isotopic (more negative) composition (e.g., Allen et al. 2019). The water uptake from deeper soil layers is supported by the lower LWP during the night, forcing the water taken up to come from a water pool with a lower potential (i.e., more water saturated soil layers, cf. chapter 1.4.2), which is assumably the water the deeper soil. The possibility to replenish the water storage within the tree cells with water of a more negative isotopic composition probably amplifies the isotopic fractionation effect when water moves from the xylem into the cells and increases the contribution of storage water to the CVD extracted xylem water, leading to a larger difference between the CVD and SPB extracted xylem water isotopic composition.

At the irrigation site, the tree cellular water storage is probably never fully depleted. Only from the first to the second field campaign, there is a slight reduction in the CVD extracted xylem water stable isotopic composition (Figure 10 & 11). There, high atmospheric demand (high VPD) between the first and second field campaign could have led to some storage water being used to meet transpirational demand (Figure 18). The low (more negative) isotopic values of the source water after the intense rainfall on the 28.08.2023 (Figure 18), which was likely used to replenish the cellular water storage, probably amplified the effect from fractionation when water moves from the xylem into the tree cells, leading to a lower isotopic value of the water extracted with the CVD method. As the VPD in the consecutive days never were on a level as between the 14th and 31st of August 2023, the irrigation site tree probably never had to use cell storage water again to meet the atmospheric water demand. Thus the cell water storage contributed substantially to the total CVD extracted xylem water on consecutive sampling campaigns, leading to larger offset towards the SPB extracted xylem water isotopic composition compared to the control site trees.

Therefore, the temporal variation in the amount of water stored in the tree cells and the diurnal variation in the water uptake depth, which impacts the stable water isotopic composition of the water used by the trees to replenish the tree cell water storage, is proposed to strongly affect the offset between the stable water isotopic composition of xylem water extracted with the CVD method and other xylem water extraction methods as well as compared to the trees source water.

However, the data presented here does not cover the full growing season and only one really dry sampling date. Therefore, future research is needed to verify these patterns. Furthermore, the hypothesis of a possible diurnal variation of tree water uptake depth and storage dynamics should be part of future research, where the isotopic composition offset between methods during the day should be compared to the offset during the night. It further highlights the need of isotope analysis in tree xylem with a high temporal resolution to answer questions on the water dynamics in the soil-biosphere-atmosphere continuum.

### 4.1.4 Comparison of In-Situ and Destructive Xylem Water Extraction Methods

The in-situ xylem isotopic data does not compare well to either of the two destructive sampling approaches. First, the variability of the in-situ stable water isotopic data is much larger compared to the xylem-SPB and xylem-CVD sampled water stable isotopic values. This is the case over the whole dataset but also for the variability of each sampling date individually. Second, the in-situ xylem-EV (equilibrium vapor) method does not show a pattern similar to the other methods regarding the similarity between irrigation and irrigation-stop site nor regarding their relationship towards control site samples. The only thing that is similar between all methods is that the control site xylem samples follow roughly the GNIP Sion precipitation stable water isotopic signal. Previous research found differences between destructive and in-situ sampling methods for xylem stable water isotopes mainly for labelling experiments (e.g., Kübert et al. 2020; Mennekes et al. 2021) but measurements with natural abundances of isotopes usually yielded no significant differences (e.g., Gessler et al. 2022; Kübert et al. 2020; Volkmann et al. 2016). A possible reason for the differences is that not the same trees are sampled with the destructive and the in-situ method due to the tree crown accessibility. However, the use of different trees is not able to explain the higher variability of the in-situ xylem extracted water compared to the destructive sampled xylem water isotopic value.

The high variability within the in-situ measurements exceeds the tree-stand variability reported by literature of 0.2-3‰ for $\delta^{18}O$ and 1-8‰ for $\delta^{2}H$ (Bernhard et al. 2024; Goldsmith et al. 2018), whereby the destructive methods seldom exceed it (Figure 10-12). The comparison between the in-situ and CVD extraction by Mennekes et al. (2021) found in a labelling experiment on potted trees an opposite trend, where the CVD extraction yielded a higher variation. The contrary high variability of the xylem in-situ measurements in this study could be due to the time-lag between measurements as several hours are needed for a complete measurement of a site. Several studies have shown short term changes (sub-daily to daily) in the xylem water stable isotopic composition, due to a change in the relative contribution of different soil depths to the tree water uptake (De Deurwaerder et al. 2020; Dubbert & Werner 2019; Gessler et al. 2022), the input of new precipitation (Gessler et al. 2022; Mennekes et al. 2021) or hypothesized storage effects could affect the xylem isotopic values over time (De Deuwaerder et al. 2020). Therefore, the high variability of in-situ measurements in this study are likely to be explained by the time-lag between the individual trees in-situ measurements. Previous studies also point out that variability of stable water isotopes between trees decreases with increasing sampling height (Mennekes et al. 2021; Treydte et al. 2021; Volkmann et al. 2016). A reduced variability with tree height could be a reason why the in-situ xylem water stable isotopic measurements show such a hight variability compared to the destructive xylem water stable isotopic composition (Appendix 4 & 5). One reason for the reduced variability with tree height could be the mixing of water inside the tree from different sources or different flow path lengths inside the tree (Mennekes et al. 2021; Treydte et al. 2021).

An additional effect which could lead to the difference between the xylem-EV isotopic values and the xylem-SPB values is that the tree releases storage water from the heartwood and wood cells in general, which mix continuously with the freshly taken up water, which was already hypothesised by De Deurwaeder et al. (2020). Therefore, during times of high-water demand, the xylem-EV isotopic value should be above the xylem-SPB isotopic value when storage water is released. This is caused by the lower isotopic value of storage water in tree cells, as observed by several studies (e.g., Barbeta et al. 2022; Fabiani et al. 2022). Whereby the heartwood has been identified as especially depleted in heavy isotopes (Fabini et al. 2022), which is an important water storage compartment (Hu et al. 2018). For irrigation and irrigation-stop, the xylem-EV isotopic value is indeed remarkably higher on the most drought affected day, on the 11.10.2023, and no difference between the xylem-EV and xylem-SPB value can be seen on the day with assumably moist soil, on the 22.09.2023 (Appendix 4 & 5). Such a pattern is largely absent at the control site trees. This could be due to the failure of recharging their storage during the night, aligning with the findings from the differences in the isotopic offset between the destructive extraction methods.

It is also visible from Appendix 4 & 5, that the irrigation site in-situ measured xylem stable water isotopic composition is closer related to the destructive soil water samples stable water isotopic composition compared to the in-situ soil stable water isotopic measurements. This indicates that the irrigation-site trees take up if available shallow soil water. This assumption aligns with the previous research and the interpretation derived from the destructive samples, indicating that trees preferably take up shallow water (Bachofen et al. 2024). An exception is the 11.10.2023. One possible explanation could be that the trees can still access sufficient water near the surface, but the water near the surface got enriched in heavy isotopes.

For the irrigation-stop site trees, there is on certain dates an offset between the soil in-situ measured water isotopic composition and the in-situ measured tree xylem-EV extracted water isotopic composition, whereby the xylem-EV isotopic values are lower (more negative) during drought (11.10.23) and equal to the in-situ soil water during times of enough near surface soil moisture (22.09.23, Appendix 4 & 5). This supports the previous assessment based on the destructive samples that the irrigation-stop site trees were able to grow a different rooting network during the time of irrigation to access deeper water layers in times of water stress. Therefore, even if certain differences between the destructive samples and the in-situ measured isotopic composition of source and xylem water occur, the fundamental interpretations do not change. Even if the in-situ water isotope measurement techniques are still in development and face certain challenges (e.g., Volkmann et al. 2014 & 2016; Bachofen et al. 2024), based on the qualitative comparison to destructive measurements in the study presented here they seem to be a promising tool to estimate the tree water uptake depth.

## 4.2 Difference in Stable Water Isotopes between the IRLS and IRMS Measurement

Even though a significant bias between the IRLS and IRMS for the xylem-SPB and xylem-CVD samples was measured, the offset is much smaller than the difference between the xylem-SPB and xylem-CVD stable isotopic composition for the same trees. The IRMS values are assumed to be without a major bias, as previous studies found that only very high concentrations of contaminants lead to major impacts on the measured stable water isotopic values (e.g., Brand et al. 2009; Martín-Gómez et al. 2015; West et al. 2010). Furthermore, taking the IRMS as the “true” value for comparison of the measurement method aligns with previous studies (e.g., Cui et al. 2021; Martín-Gómez et al. 2015; West et al. 2010). For the IRLS measurement, only a flagging tool and no combustion module was used. No influence from the treatment on the difference between the IRLS and IRMS measurement from the treatment was detected. All treatments show the same pattern of sample type induced bias for the IRLS measurement.

### 4.2.1 Soil Stable Water Isotopes – Measurement System induced Bias

The IRLS measurements of the CVD-extracted soil water samples deviate marginally negatively from the IRMS values. This means that the IRLS water stable isotopic measurements are marginally more negative compared to the IRMS measurements (Figure 13-15). The direction of the deviation is in accordance with the findings from Martín-Gómez et al. (2015). However, the deviation from zero is for the soil values smaller than the measurement systems precision. Therefore, even if statistically significant differences occur, a measurement system induced precision error for soil-CVD extracted water samples cannot be differentiated with certainty from measurement system introduced biases. That the measurement system precision plays a role is also partially supported by the missing link between the soil $\delta^{18}O$ and $\delta^{2}H$ bias for the IRLS-IRMS comparison. The direction of the very small bias for soil is the same as for xylem-CVD-extracted samples, leading to the assumption that the same chemicals would interfere with the IRLS measurement for both sample types. Therefore, the link between the $\delta^{18}O$ and $\delta^{2}H$ bias would be expected to be the same for soil- and xylem-CVD samples. However, this is not the case. Therefore, the work presented here fails to reject the 0-hypothesis with confidence, indicating that there is no difference in the stable water isotopic values between the IRLS and IRMS measurement for soil-CVD samples at the Pfynwald research site. This falls in line with the more recent research of Jeon et al. (2024), who did not find a difference in the soil stable water isotopic composition between IRLS and IRMS measurement methods when extracting water with suction cups.

But these results for the soil are of limited transferability to other sites. Martín-Gómez et al. (2015) suggested that location specific soil properties probably affect the sample contamination with organic compounds, as their study shows high measurement system induced biases compared to other studies (e.g. compared to West et al. 2010). Martín-Gómez et al. (2015) hypothesize that this could be connected to the geomorphology of the area, as they found the biggest discrepancies between IRLS and IRMS soil water stable isotope measurements at the valley bottoms. They argue that water soluble organic compounds accumulate along the flow path of water, therefore leading to an enrichment in organic contaminants for the IRLS at the valley bottom (referred to as accumulation effect in this thesis). The Pfynwald, positioned on an alluvial fan, does not show a geomorphological structure which enables confluence and accumulation of water in a certain location. Accumulation could only happen within very small geomorphological features and never on a scale as described in Martín-Gómez et al. (2015). Therefore, the absence of a bias between the IRLS and IRMS measurement for the soil aligns with their interpretations and conclusion. However, there are two further aspects which could affect the presence of organic substances which contaminate the IRLS measurements.

First, the differences in the soil-CVD measurement bias between different studies could be due to a different level in the production of organic contaminants within the soil by microorganisms (referred to as production effect). As the accumulation effect, the within soil production effect could also be related to the topography. According to Insam & Seewald (2010), the production of contaminants like alcohols are related to the aerobic conditions of the soil. Thereby, anaerobic conditions promote the formation of organic substances like alcohols (Insam & Seewald 2010; Rissanen et al. 2020), which interfere with the IRLS measurement. As wetter conditions are expected in valley bottoms where water accumulates, more anaerobic conditions are expected there as well, which would align with the findings from Martín-Gómez et al. (2015). It also aligns with the data presented in this thesis, as the soil in Pfynwald is rather dry and aerobic. Therefore, the soil at Pfynwald is expected to have a low or absent production of organic components relevant for the IRLS measurement.

The second different influence from what Martín-Gómez et al. (2015) suggested is the possible influence from litter input (referred to as input factor), which is compared to the other two factors topography independent. West et al. (2010) found different levels of organic substances which interfere with the IRLS for the leaves of different species. Therefore, the mixture of the litter containing different residuals from different plants and tissues of a plant species might influence the level of organic contamination in the soil. However, Cui et al. (2021) found no contamination for the soil samples but high contamination levels for the leaves growing above the soil. Therefore, also with regard to the spatial pattern observed by Martín-Gómez et al. (2015), it is more likely that the bias between IRLS and IRMS is due to the accumulation or production factor.

A recent study relating the difference in the stable water isotopic values between IRMS and IRLS measurement techniques to soil properties did not find a significant relationship (Jeon et al. 2024). However, in their study Jeon et al. (2024) sampled only soil columns with similar topographical features. All their samples were taken near a crest of a hill, so that no water or organic contaminants could accumulate. Furthermore, the range of soil properties was very small in their study, as they compared only three soil columns from the same area. Therefore, the hypotheses from Martín-Gómez et al. (2015) or introduced here are not entirely contradicted. Additionally, larger differences between soil properties could still influence the differences between IRLS and IRMS measured stable water isotopes. This could for example be the case, if the soil properties make it more prone to waterlogging or retaining organic substances like alcohol through absorption onto soil particles.

Future experiments to investigate the relative importance of the accumulation or production factors could contain a mixed approach between a greenhouse and nature setting. In the greenhouse, one could arrange pots with trees containing different levels of soil water saturation. The stable water isotope bias from water samples extracted from the soil can then be compared to microbial activity, root area oxygen content and water saturation to determine the production factor. This experiment can be extended by observing the change in the stable water isotope measurement bias between the IRLS and IRMS along a catena with stable levels of water saturation along it. The first experiment should be easier to do and would help determine if there is a production factor present. However, the second experiment would be needed to assess the relative importance of the two factors.

### 4.2.2 Xylem Stable Water Isotope – Measurement System induced Bias

In the case of the CVD extracted xylem water, several previous studies point out that organic substances are extracted with the water (Brand et al. 2009; West et al. 2010). In the study of West et al. (2010), the CVD extracted water in their study showed a milky-whitish colorization and a typical plant odor. This was also the case for the CVD-extracted Scots pine samples used here, but after filtering the white color wasn't present anymore and the pine-odor was reduced substantially in the sample. Still, as visible from the data presented in this study, organic contaminants were still present, which interfered with the IRLS measurement. The xylem-CVD samples deviated for $\delta^{18}O$ and $\delta^{2}H$ visually and statistically from zero, where zero indicates no difference between the IRLS and IRMS measurement (i.e. IRMS-IRLS equals zero, Figure 13). However, the xylem-CVD deviation for $\delta^{2}H$ is below the International Atomic Energy Agency (IAEA) maximum accepted bias (< 6‰, Martín-Gómez et al. 2015). For $\delta^{18}O$ the maximum accepted bias of 0.8‰ is surpassed (Martín-Gómez et al. 2015). Furthermore, the deviance from zero surpasses the measurement precisions for the xylem-CVD extracted water $\delta^{18}O$ and $\delta^{2}H$ (cf. Table 2).

The SPB extracted xylem samples show a stronger bias compared to the xylem-CVD samples. The bias for the xylem-SPB samples exceeds the measurement precision for $\delta^{18}O$ and $\delta^{2}H$, but again only the $\delta^{18}O$ bias exceeds the IAEA maximum acceptance (for acceptance levels see Martín-Gómez et al. 2015). Furthermore, the bias between the IRLS and IRMS isotopic measurement shows a different direction for the xylem-SPB and xylem-CVD samples. While IRLS overestimates the stable water isotopic values for xylem-CVD extracted water samples, it underestimates the IRLS values for xylem-SPB extracted water samples (i.e. lower IRLS than IRMS values for xylem-SPB extracted water samples).

In comparison to the results presented here, West et al. (2010) found no bias between the IRLS and IRMS technique for CVD extracted water from leaves for Pinus radiata trees (Monterey pine, another species from the Pinaceae family). However, on one hand they observed the interference in stable water isotopic measurements from needles and on the other hand it was a different subspecies, leading to a limited transferability of their results to the ones presented here. This especially as they found highly different measurement biases between IRLS and IRMS for different tree species. Additionally, West et al. (2010) found different directions of the bias (positive and negative deviations) depending on the IRLS methodology used. However, the results presented here used the same IRLS technique to analyse the isotopic composition of xylem-CVD and xylem-SPB extracted water composition. Therefore, the different direction of the isotope bias in the SPB and CVD extracted water presented here has a different origin compared to West et al. (2010).

The results from Martín-Gómez et al. (2015) are more comparable to the results presented in this thesis, as they also used CVD extraction and sampled twig xylem rather than leaves. However, they sampled far fewer trees per species than were used for the analysis presented here. Martín-Gómez et al. (2015) sampled four Scots pine trees and two additional Aleppo pine trees, which are both from the Pinaceae family. While they found a slightly higher bias for $\delta^2H$ (1.7‰ in their study compared to 1.5‰ reported here), their measured bias for $\delta^{18}O$ was far lower (0.42‰ in their study compared to 0.85‰ reported here). Thus, they did not exceed the maximum bias accepted by the IAEA (Martín-Gómez et al. 2015). However, the standard deviation for their Scots pine data is much higher for $\delta^2H$ and only marginally lower for $\delta^{18}O$ compared to this study. In their small dataset, outliers probably played a major role in the outcome for Scots pine trees. Martín-Gómez et al. (2015) found for other tree species a maximum discrepancy between the IRLS and IRMS measurement method for xylem-CVD samples at 8‰ for $\delta^{18}O$ and 92‰ for $\delta^2H$. West et al. (2010) found for leaves for other tree species a discrepancy of 12‰ for $\delta^{18}O$ and 35‰ for $\delta^2H$. Therefore, even if the measurement system bias for water extracted from Scots pine xylem is significant for $\delta^{18}O$, compared to other tree taxonomic families the measurement system induced bias due to organic contaminants is still very low.

Based on Martin-Gomez et al. (2015) and West et al. (2010), this difference between tree species is most likely due to different within-tree production levels of organic contaminants. However, each plant itself seems to have different alcohol production levels in different parts of the plant. This is derived from comparing the data from West et al. (2010) for leaves and Martin-Gomez et al. (2015) for twig xylem for the same tree families. It is further supported by Cui et al. (2021), who found differences in the measured water stable isotope bias between IRLS and IRMS for the stem and leaves of the same tree. An additional difference could come from the uptake of alcohol through the roots. According to Rissanen et al. (2020), Scots pine trees could take up methanol from the rooting area if they are under anoxic conditions and favour the production of alcohol. As tree species tend to inhabit specific niches of soil water availability (Bachofen et al. 2024), the differences in contamination content in xylem between species could also come from the specific niche they inhabit based on whether it is mainly dry or wet soils.

Regarding the reason behind the difference in the direction of the bias for CVD and SPB extracted samples a few options are plausible. One possibility is that the xylem-SPB samples faced evaporation between the measurements, leading to an isotopic offset due to the small volume of extracted water whereby evaporation free SPB-extracted water would not have a bias at all. This is a possible explanation for the xylem-SPB bias between measurement methods, as the IRMS (the second measurement) $d_{excess}$ is always lower in the stable water isotopic value than the IRLS (first measurement, Figure 16 & 17). However, some sort of enrichment in the heavier isotopes even if of lower magnitude would be expected for the soil samples when major evaporation occurs during measurement inside the vials, even though the larger samples would make its impact smaller. But this is largely not the case. Few soil outliers could be explained by the breaking of the vial and some evaporation occurring during saving of the liquid into a new vial. An evaporation offset between measurements can be excluded for the xylem-CVD samples, as they are less enriched in the heavy isotopes for the IRMS measurements, i.e. the second measurement. For the IRLS measurement (the first measurement, Figure 17) the xylem-CVD samples are more enriched in heavy isotopes, i.e. have a lower $d_{excess}$ value. Therefore, it can also be excluded that the xylem-SPB samples would show the same offset as xylem-CVD extracted water samples if there were no evaporation, as their $d_{excess}$ values would be expected to be even lower for the first measurement compared to the xylem-CVD samples (Figure 17).

Other possible explanations for the different direction of the offset are that there are different chemicals or different relative concentrations of chemicals extracted by the two methods. Johnson et al. (2017) compared induction extracted samples to CVD extracted samples isotopic composition offset for leaf, soil and xylem material over different IRLS measurement devices. They found that the relative abundance of methanol and ethanol changes the measured isotopic offset between positive and negative for certain IRLS measurement devices, which they argue originates from the distinct wavelength used in the device. Should the xylem-SPB or xylem-CVD samples contain different relative abundances of these contaminants, it would explain why a different offset direction is seen between these sample types. That different contaminants could play a role is supported by the different colouring of the xylem water sample when extracted with the CVD or SPB method, whereby the CVD extracted xylem water had a whitish colour. This was removed through filtering, but some residuals possibly remained. The main factor causing the different direction of the offset cannot be determined with the data presented here.

The observation of the different IRMS-IRLS measurement offsets between the xylem-CVD and xylem-SPB extracted water supports the previous established interpretation that the two xylem water extraction methods access a different water pool (cf. chapter 4.1.3). Furthermore, it additionally contradicts hypothesis three from Chen et al. (2020, cf. chapter 4.1.3), that the exchange of H atoms with the cell structure is the main cause the isotopic offset between xylem water extraction methods. If this were the case, no difference between the offset of the IRLS and IRMS isotope measurement for the two xylem water extraction methods would be expected, as both methods (SPB and CVD) would access the same xylem water pool with the same contaminants (e.g., alcohols) but only the CVD method additionally exchanges some deuterium atoms with the cell structure. However, how can it be excluded that both methods extracted the same water pool and only one method (CVD) is able to extract contaminants (like alcohols) alongside? This can be excluded as relevant VOCs are water soluble (Rissanen et al. 2020). Therefore, if the same water pool were sampled by xylem-CVD and xylem-SPB methods, the same alcohols which interfere with the IRLS measurement would be extracted. Furthermore, according to Rissanen et al. (2020) most alcohol production occurs within the cell. Therefore, when water is extracted from within the cell (cell water storage), alcohols are likely extracted alongside of it.

In a future step the relative concentration of methanol and ethanol in the SPB and CVD extracted samples should be measured to see whether there is a difference in their relative abundance. Furthermore, one should reverse the measurement cycle for samples, i.e. that the IRMS measurement comes first and then the IRLS measurement. If the offset is reversed, evaporation between the measurements could be the cause of the offset between the measurement methods. If these experiments yield no explanatory results, one could test for other possible contaminants of the water samples which could alter the IRLS stable water isotopic measurement. However, due to the relatively small differences between the measurement's methods for Pinus sylvestris in comparison to the offset created by water extraction methods, its influence on water uptake research is probably small, i.e. one can still yield reasonable accurate results regarding the water uptake depth when measuring the isotopes with the IRLS.

## 4.3 The Influence from Drought on the Xylem Stable Water Isotopic Composition

The statistical analyses did not yield a significant relationship between the LWP, either pre-dawn or midday, and the isotopic values. However, only one field campaign yielded a LWP under very dry conditions. Therefore, the statistical results could be due to the absence of drought conditions during the study period and thus the dataset. Furthermore, the first field campaign LWP had to be excluded due to a SPB failure (Figure 18). On one hand, the results yielded with the first SPB are not reasonable due to the absence of differences between pre-dawn and midday LWP during a day with high VPD (high evaporative demand). Second because they yielded a much larger variability, which is absent in the data from the other field campaigns. Therefore, it can be assumed that there were issues with the SPB that was used on the first field campaign. The device was replaced in the consequent field campaigns. Previous research from Vargas et al. (2017) and Barbeta et al. (2020) focused their drought experiments on potted and irrigated plants, i.e. not on plants in the natural environment. However, Duvert et al. (2023) tested this hypothesis on trees in their natural environment and did not find an impact from the plant water status on the plant isotopic offset. However, as they found a major difference in the offset between plant species, this offset could be related to the water storage mechanisms within the plant and thus species-specific differences.

Even though no significant influence from the plant water status (LWP) on the isotopic offset was found, it is notable that on the 22.09.2023, where the soil in the upper layers provided enough moisture to meet the evaporative demand, the offset between the xylem water CVD and SPB extraction method is largest for most treatments (Table 1). Furthermore, between the first and second field campaign, where the atmospheric water demand (VPD) and thus assumably transpiration was highest, the only change in the CVD extracted xylem water isotopic composition at the irrigation site was observed (Figure 10-11 & 18). Therefore, an influence from the plant water status or plant water availability cannot be excluded and more data is needed to assess the connection between the plants water demand and the isotopic offset between xylem water extraction methods. In the future, experiments including rain exclusion shelters could help to maintain dry conditions to observe this dynamic. Additionally, as Duvert et al. (2023) only tested this hypothesis on tropical trees, future experiments which cover a wider range of species and climates should be assessed.

# 5. Conclusion

This thesis shows the methodological implications from the xylem water extraction method (cryogenic vacuum distillation extraction, Scholander pressure bomb extraction and equilibrium vapor extraction) as well as from the isotope measurement method (mass spectrometer and laser spectrometer) on isotope-based tree water uptake investigations. The study focused on Scots pine trees under different water availability conditions at the Pfynwald long term drought monitoring site.

## 5.1 SPB & EV Xylem Water Extraction are Reliable Tools to Study Tree Water Uptake

The Scholander Pressure Bomb extraction of tree xylem water was found to be a reliable tool to assess tree water uptake, because no isotopic difference between the SPB extracted xylem water and the source (soil) water stable isotopic composition was found. The in-situ equilibrium vapor extracted and SPB extracted xylem water showed a qualitative agreement in their water stable isotopic composition, highlighting the potential of the equilibrium-vapor extraction method to reliably investigate the flow of water through the soil-biosphere-atmosphere continuum.

## 5.2 Tree Water Uptake Depth depends on the Treatment

It was observed that trees at the irrigation-stop site can access water from deeper soil layers (<-50cm) under dry conditions (irrigation was terminated in 2013), while the control site trees depend on shallow soil, leading to the assumption that the irrigation period (2003-2013) helped the trees at the irrigation-stop site to develop a deeper rooting network. The irrigation site trees (600mm added irrigation water per growing season), probably have such a rooting network as well, but due to the irrigation water which reduces the vertical gradient of stable water isotopes in the soil, at the irrigation site the water uptake depth is difficult to distinguish.

## 5.3 Xylem Water Stable Isotopic Composition Differs between Extraction Methods

The water samples obtained with the widely used CVD xylem water extraction method showed a different stable water isotopic composition compared to the trees water source (soil water) or the SPB extracted xylem water. Therefore, it can be excluded that there is no stable water isotopic difference between the xylem water extraction methods and the null hypothesis of the first research question raised in this thesis is rejected. That the CVD xylem water extraction leads to a water isotopic offset compared to the trees water source or other xylem water sampling methods was observed in previous research and several hypotheses were proposed to explain its cause. The first and second hypothesis from literature, that two water pools of distinct isotopic signatures exist in the soil and the trees take up water only form one (two-water-world hypothesis, Brooks et al. 2010) or that isotopic fractionation occurs during root water uptake (Vargas et al. 2017) are contradicted, since the SPB and EV extracted xylem water stable isotopic composition are not significantly different from the source water (soil water) isotopic composition. The third hypothesis from literature proposed that an exchange of deuterium atoms between the xylem water and the cell structure (e.g., cell wall) occurs when the CVD xylem water extraction method is used, creating the observed methodological offset (Chen et al. 2020). However, the research presented here clearly shows that an isotopic offset between xylem water extraction methods occurs also for $\delta^{18}O$ and not only for $\delta^{2}H$, contradicting the completeness of this hypothesis.

Therefore, the research presented here supports the fourth hypothesis proposed by Barbeta et al. (2022), stating that the CVD xylem water extraction method extracts water stored within the tree cells alongside the xylem water, whereby the water inside of the tree cells have a different isotopic composition because of isotopic fractionation occurring when water moves into the cells, creating the stable water isotopic offset between xylem water extraction methods. However, this hypothesis alone fails to explain why the offset between the SPB and CVD extracted xylem water isotopic value was observed to be different between treatments. The described offset was much larger at the irrigation and irrigation-stop site compared to the control site. This leads to the proposition, that the trees at irrigation and irrigation-stop site are able to fully replenish their cell water storage leading to a larger contribution of cell storage water to the total CVD extracted xylem water, increasing the isotopic offset between xylem water extraction methods. The larger offset at the irrigation and irrigation-stop compared to the control site is probably amplified by the lower isotopic value of the source water (soil water) during the times when the tree cell storage water is replenished. In this study, at the irrigation site such a possible effect was observed when VPD (atmospheric water demand) decreased and heavy rainfall reduced the water isotopic value in the soil. At the irrigation-stop site, it is assumed that a diurnal change in the water uptake depth furthermore strengthens this pattern, whereby during the night the tree cell water storage is refilled with deeper soil water, which has a more negative isotopic value. As these interpretations rely to some extent on singular field campaigns, further research is needed to assess changes in the dynamic of the contribution from cell storage water to the isotopic offset by the CVD xylem water extraction method and investigate this pattern in other tree species.

## 5.4 Tree Water Status shows no Clear Relationship to its Xylem Water Isotopic Value

Concerning the possible relationship between the plant water status (pre-dawn and/or midday LWP) and the isotopic offset between the xylem water extraction methods, no statistically significant correlation was found. Therefore, the second null-hypothesis cannot be rejected, and the plant water status is assumed to have no impact on the stable water isotopic values or offsets between water extraction methods. However, this result faces the limitation that LWP measurements from only one field campaign under dry conditions were available and only one field campaign was performed during a period of high VPD, whereby on the sample date with high VPD the LWP measurements had to be discarded. However, the VPD seemed visually to affect the CVD extracted xylem water isotopic value. Furthermore, the treatment, and thus the trees water availability, had a notable influence on isotopic offset between xylem water extraction methods. Therefore, future research is needed to assess the effect of the plant water status on the isotopic offset between xylem water extraction methods. Here, special attention should be given to extremes in the atmospheric water demand (VPD) and soil water availability to restore the tree cell water storage.

## 5.5 Xylem Water Isotopic Values Differ between the IRLS and IRMS Measurement

The third null-hypothesis investigated in this thesis, that no difference in the measured stable water isotopic values between the IRLS and IRMS exists, is partially rejected, because a significant difference between the IRLS and IRMS measurement was found for xylem extracted water samples but not for soil extracted water samples. The treatment did not have an influence on the offset between the IRLS and IRMS measurement. However, the differences are small compared to the general uncertainties faced in tree water uptake studies and are thus not expected to influence the tree water uptake assessments significantly, even if the measurements were done with a IRLS device. But notably, the offset between the IRLS and IRMS measurement shows a different direction for the CVD and SPB extracted xylem water, with the IRLS overestimating the xylem-CVD and underestimating the xylem-SPB water stable isotopic values. The IRLS bias for the xylem-CVD extracted water stable isotopes has been observed and analysed by previous research. For the xylem-SPB samples, evaporation between measurements, different contaminants or different relative amounts of contaminants could lead to a different offset compared to the xylem-CVD extracted water. Future research should address the possible reasons for the distinct offsets between the IRLS and IRMS measurement for the xylem-SPB and xylem-CVD extracted water.

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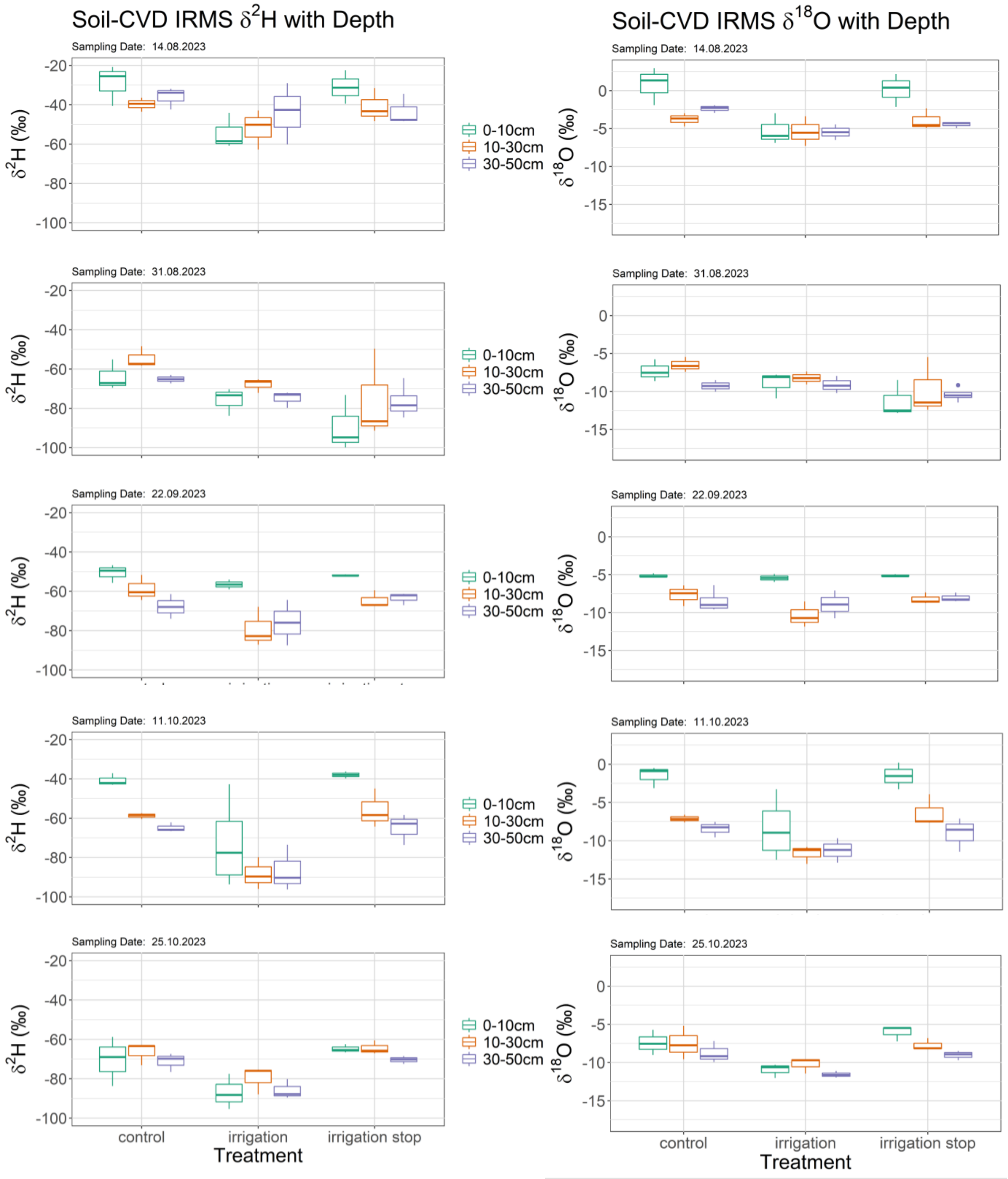
# Appendix



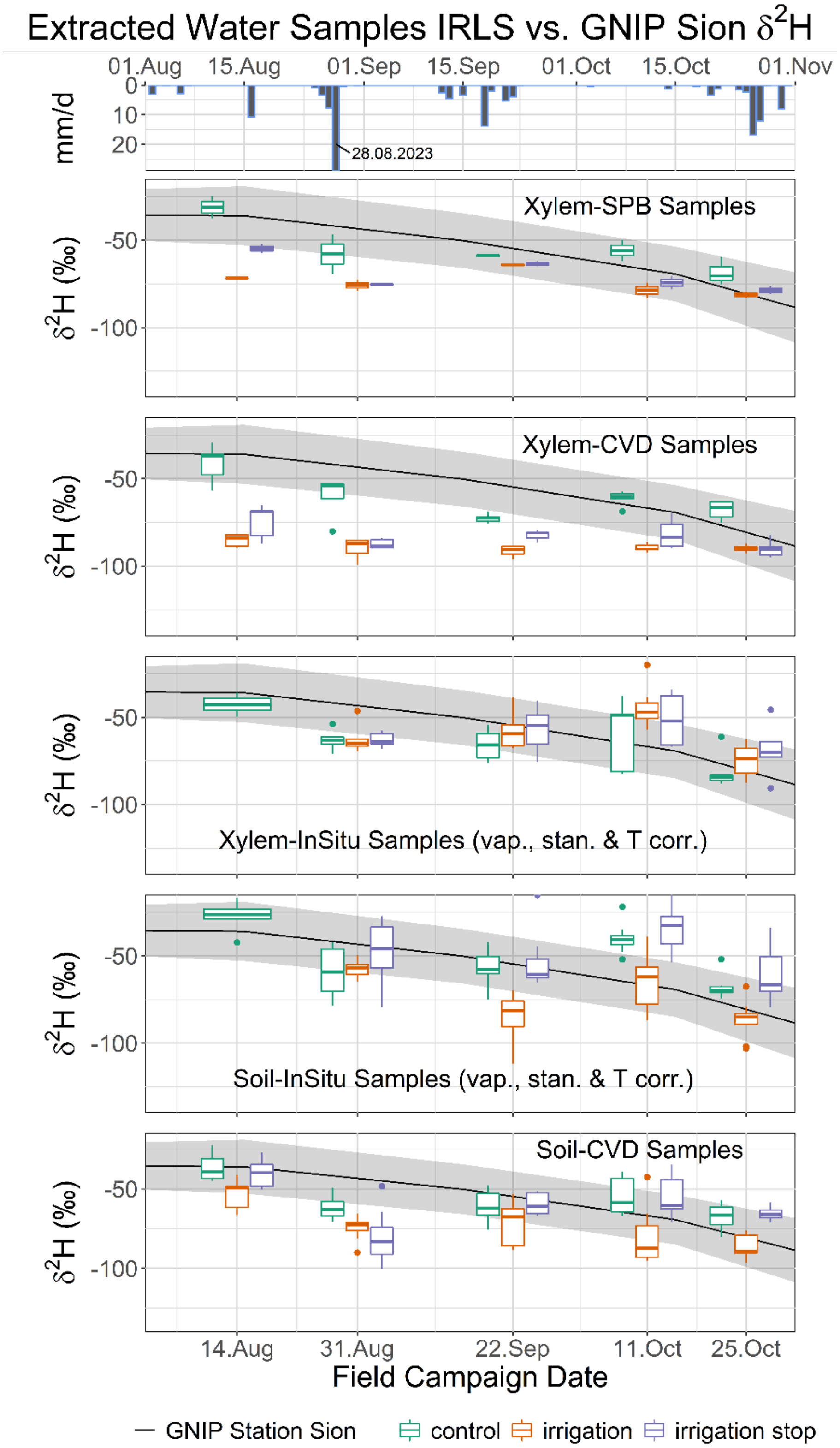
*Appendix 1: Soil-CVD stable water isotopic composition with depth over the different field campaigns.*

*Appendix 2: $\delta^2H$ mean and standard deviation over the different groups which were subject to the statistical analysis.*

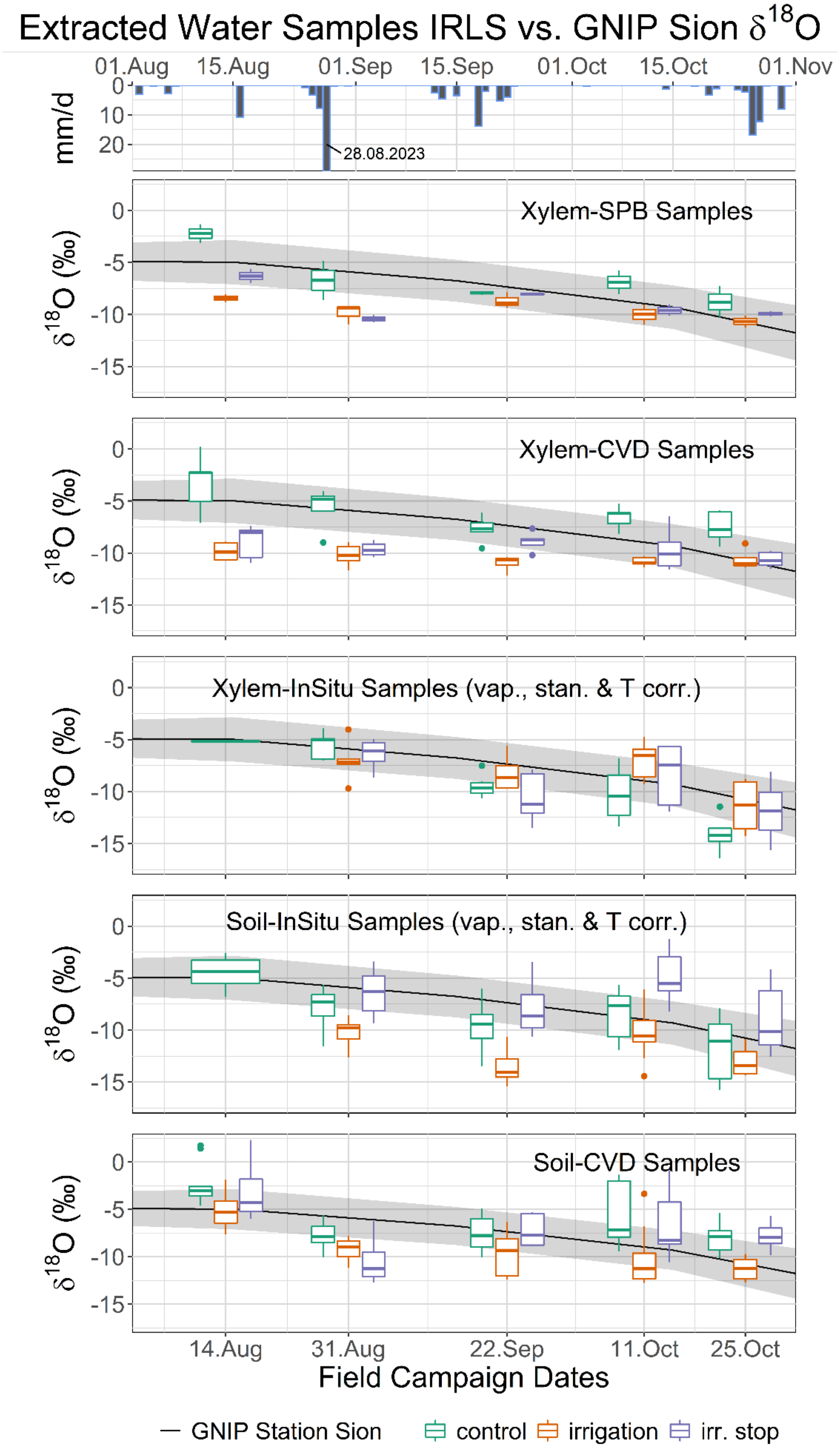
| **Summary Statistics $\delta^2H$ IRMS** | Mean [‰] | Standard Deviation [‰] |
|---|---|---|
| Xylem-SPB | -65 | 15 |
| Soil-CVD | -63 | 17 |
| Xylem-CVD | -79 | 16 |
| Control | -57 | 14 |
| Irrigation | -78 | 16 |
| Irrigation-stop | -69 | 18 |
| 14.08.2023 | -50 | 19 |
| 31.08.2023 | -74 | 15 |
| 22.09.2023 | -69 | 14 |
| 11.10.2023 | -70 | 18 |
| 25.10.2023 | -77 | 11 |
| Xylem-SPB control | -54 | 17 |
| Soil-CVD control | -56 | 14 |
| Xylem-CVD control | -62 | 13 |
| Xylem-SPB irrigation | -72 | 9 |
| Soil-CVD irrigation | -72 | 17 |
| Xylem-CVD irrigation | -90 | 6 |
| Xylem-SPB irrigation-stop | -68 | 11 |
| Soil-CVD irrigation-stop | -61 | 17 |
| Xylem-CVD irrigation-stop | -85 | 8 |
| River / Irrigation Water | -101 | 3 |

*Appendix 3: $\delta^{18}O$ mean and standard deviation for the different groups which were subject to the statistical analysis.*

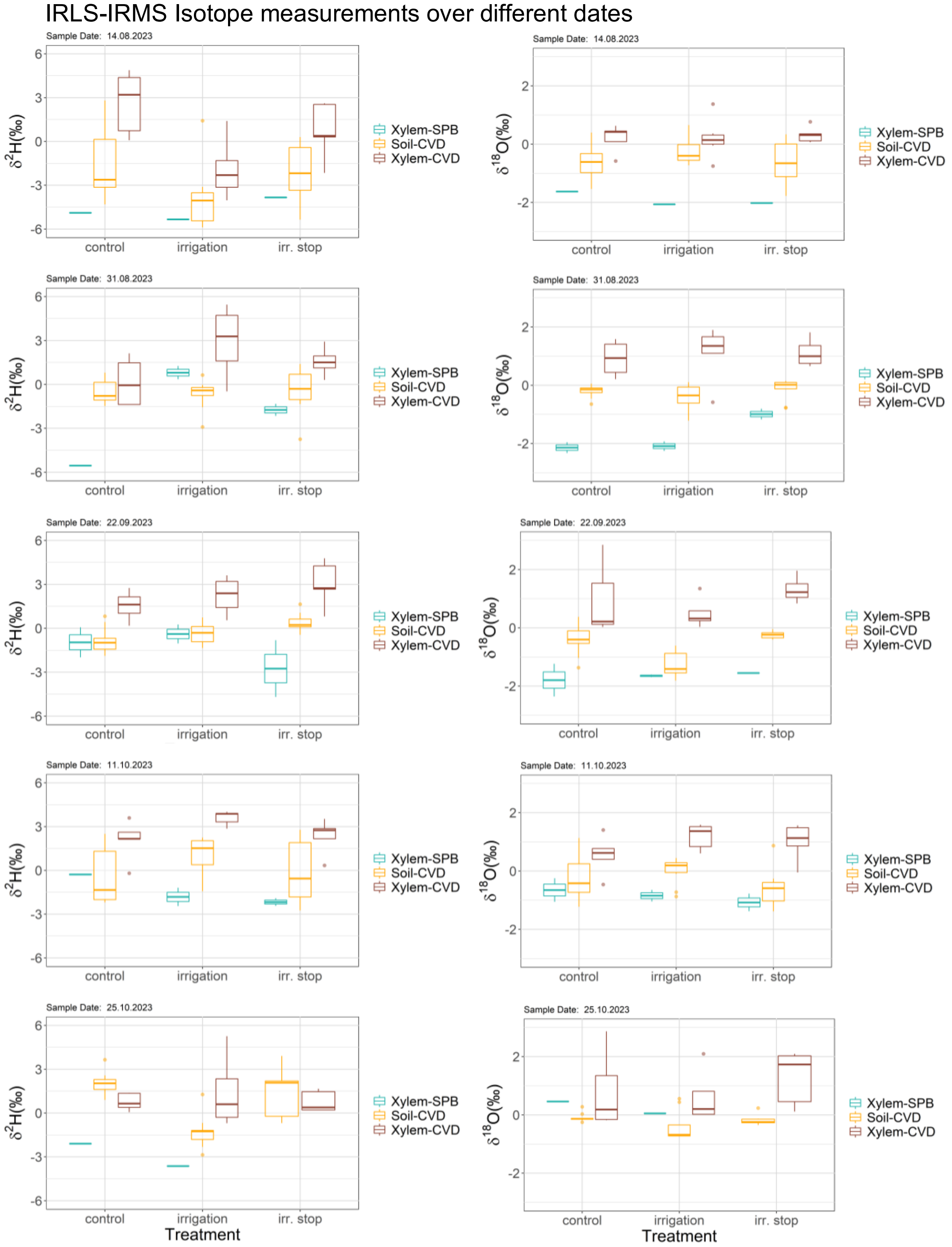
| **Summary Statistics $\delta^{18}O$ IRMS** | Mean [‰] | Standard Deviation [‰] |
|---|---|---|
| Xylem-SPB | -7.3 | 2.8 |
| Soil-CVD | -7.2 | 3.5 |
| Xylem-CVD | -9.5 | 2.6 |
| Control | -6.2 | 3 |
| Irrigation | -9.4 | 2.9 |
| Irrigation-stop | -8.2 | 3.2 |
| 14.08.2023 | -4.6 | 3.7 |
| 31.08.2023 | -9 | 2.3 |
| 22.09.2023 | -8.1 | 2.2 |
| 11.10.2023 | -8.3 | 3.4 |
| 25.10.2023 | -9.5 | 2 |
| Xylem-SPB control | -5.9 | 3.3 |
| Soil-CVD control | -6 | 3.1 |
| Xylem-CVD control | -6.8 | 2.5 |
| Xylem-SPB irrigation | -8.2 | 1.8 |
| Soil-CVD irrigation | -8.6 | 3.4 |
| Xylem-CVD irrigation | -11.2 | 0.9 |
| Xylem-SPB irrigation-stop | -8.1 | 2.4 |
| Soil-CVD irrigation-stop | -6.9 | 3.4 |
| Xylem-CVD irrigation-stop | -10.5 | 1.4 |
| River / Irrigation Water | -14.3 | 0.3 |



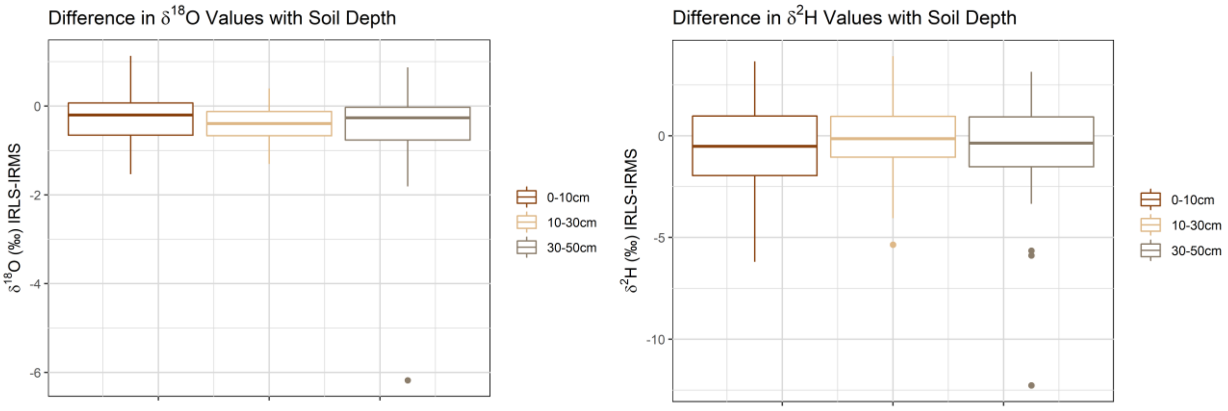
*Appendix 4: IRLS measurements of the destructive samples compared to the in-situ data (vapor equilibrium extracted) and rainfall data. The in-situ data is in some cases from up to 4 days later or earlier compared to the destructive measurement due to measurement gaps. The in-situ data is corrected based on the vapor content, standards and temperature. The correction for the in-situ data was performed by Dr. Elham Freund (UZH), Dr. Katrin Meusburger (WSL), Dr. Marco Lehmann (WSL) and Dr. Zhaoyong Hu (WSL).*



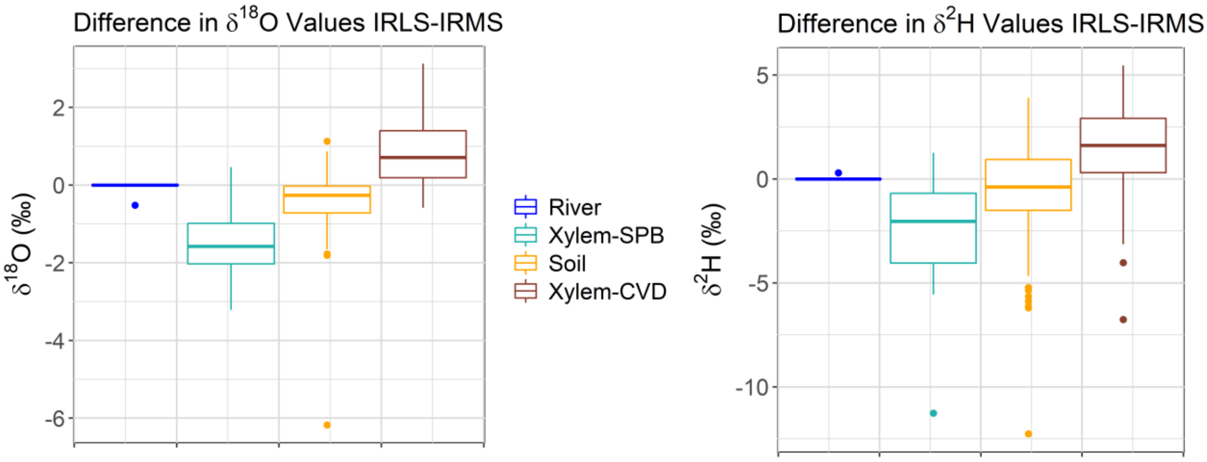
*Appendix 5: IRLS measurements of the destructive samples compared to the in-situ data (vapor equilibrium extracted) and rainfall data. The in-situ data is in some cases from up to 4 days later or earlier compared to the destructive measurement due to measurement gaps. The in-situ data is corrected based on the vapor content, standards and temperature. The correction for the in-situ data was performed by Dr. Elham Freund (UZH), Dr. Katrin Meusburger (WSL), Dr. Marco Lehmann (WSL) and Dr. Zhaoyong Hu (WSL).*



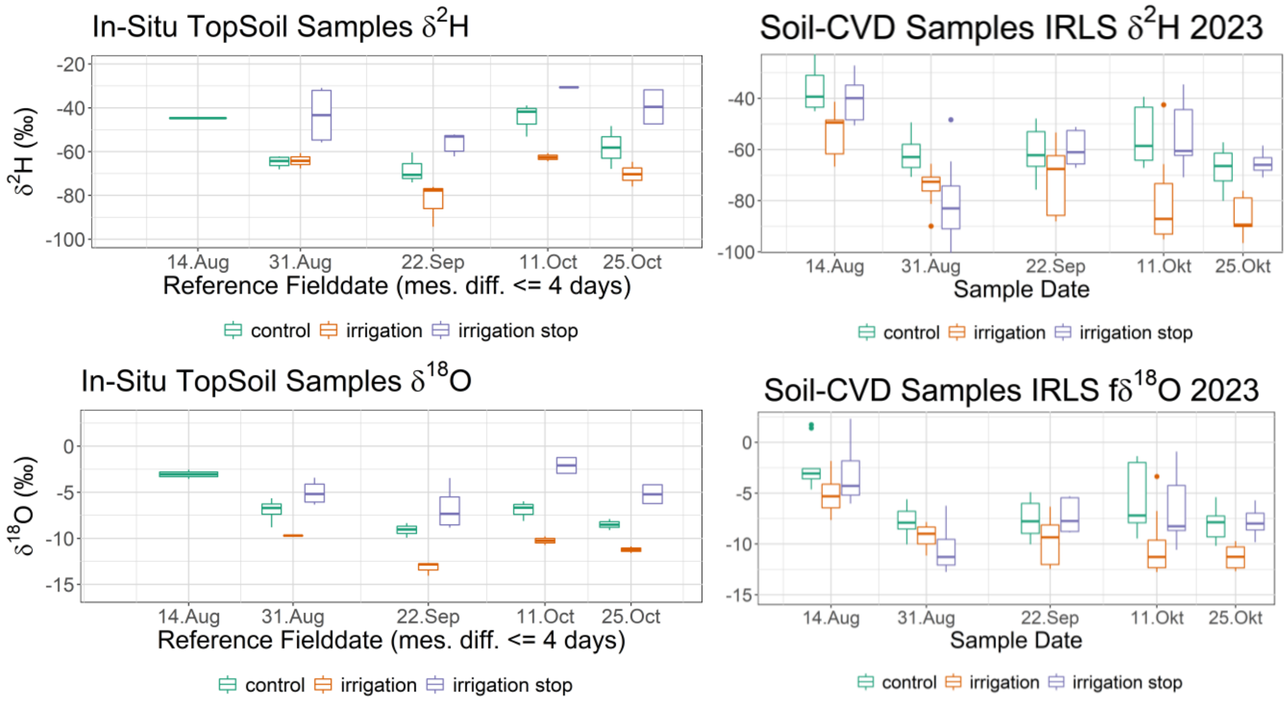
*Appendix 6: Difference between IRMS and IRLS stable water isotope measurements grouped by sample type and displayed over the different field campaigns.*



*Appendix 7: Difference of the IRMS and IRLS stable water isotope measurements with soil depth.*



*Appendix 8: Differences in stable water isotopic values including the river samples.*



*Appendix 9: Comparison of most shallow in-situ measurements and the destructive soil-CVD measurements. The in-situ data contains the 20 and 40cm deep probe. Notable is the large spatial variability for the top 50cm, which is only incorporated in the destructive samples but not in the in-situ measurements.*

# Declaration of Independence

Personal declaration: I hereby declare that the submitted Thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the Thesis.

Author: Maurus N. Villiger Date: 31.07.2024

Signature: