

Seasonal Variation in Tree Water Uptake in a Pre-Alpine Catchment (Alptal, CH): A Stable Isotope Study

GEO 511 Master's Thesis

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Abstract

The transpiration of forests is a major component of the water cycle and is highly sensitive to climate change. The interactions between soil moisture contents and tree water uptake are complex, influencing not only local water dynamics but also the health and resilience of the ecosystem. This thesis investigates the spatial and temporal variability in water uptake depths (WUD) of Norway spruce (Picea abies) trees in a headwater catchment in the pre-Alpine Alptal valley. Twig xylem water, stem core xylem water, bulk soil water, and mobile soil water were sampled at five study sites on five days between July and October 2024. δ^2 H and δ^{18} O were analysed in reference to the isotopic composition of precipitation, soil moisture contents, weather and groundwater data, as well as study site and tree characteristics. The contribution of shallow (5-20 cm) and deeper (25-50 cm) soil water to tree water uptake was calculated using the MixSIAR model. All water sources were enriched in heavy isotopes in August, which primarily reflected the impact of evaporation in summer and snowfall in autumn on the isotopic composition of precipitation. The significant differences in the isotopic composition of soil water between study sites as well as between elevation zones could not be attributed to particular study site characteristics. Twig xylem water samples had a positive lc-excess, which potentially resulted from the uptake of dew or fog drip from the unsampled topsoil. Contrarily, stem core xylem water samples had a negative lc-excess, indicating a high within-tree variability in water isotopes and high model uncertainty. The modelled WUD was primarily a mixture of shallow and deep bulk soil water; no temporal or spatial trends and no impact of soil moisture on the WUD could be detected. The high uncertainty of the model results can be attributed to the small sample size and limited number of study sites, combined with the high number of variables affecting the WUD and the unexpected isotopic composition of tree xylem water. Additionally, soil moisture was relatively high throughout the entire study period, especially at greater soil depths, which was responsible for a less distinct soil profile and a smaller range in soil moisture conditions. This thesis contributes to research by discussing key factors that influence the results of WUD studies in wet regions.

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Abbreviations

a.s.l. Above sea level d-excess Deuterium excess

 $\delta^2 H$ Deuterium (%) $\delta^{18} O$ Delta-18-O (%)

GMWL Global Meteoric Water Line

GNIP Global Network of Isotopes in Precipitation

IAEA International Atomic Energy Agency

L1-L5 Study site 1-5

lc-excess Line-conditioned excess
LMWL Local Meteoric Water Line

MeteoSwiss Swiss Federal Office of Meteorology and Climatology

TWI Topographic Wetness Index

VSMOW Vienna Standard Mean Ocean Water

VWC Volumetric water content

WSL Swiss Federal Institute for Forest, Snow, and Landscape Research

WUD Water uptake depth

1.Introduction

1.1. Objective

The global water cycle shapes ecosystems and has major implications for natural hazards and agriculture (Kirchner et al., 2023). The role of plant transpiration in the water cycle has attracted considerable attention as it accounts for approximately 61% of global evapotranspiration, with about 39% of terrestrial precipitation being transpired (Schlesinger & Jasechko, 2014). In particular, forests play a significant role in the water cycle through transpiration, interception, and the transportation of water into the soil (Sheil, 2018). Soil water storage impacts various components of the water cycle, including root water uptake, evaporation, and groundwater recharge. Therefore, research on soil water storage and transpiration is important for a better understanding of hydrological processes and a more accurate hydrological modelling (Sprenger et al., 2016).

Climate change affects various components of the water cycle, leading to changes in the frequency and seasonality of droughts (Brunner et al., 2023) and an increase in potential evapotranspiration of about 20% (Calanca et al., 2006) in the Alps. In northeastern Switzerland, a decrease in summer precipitation is predicted if no effective mitigation measures are implemented (Fischer et al., 2012). Therefore, understanding the response of the water cycle to climate change is indispensable for catchment hydrology and the adaptation of sustainable forest management (Scandellari et al., 2024). The water uptake of trees affects water storage and fluxes and the catchment response to precipitation events (Sprenger et al., 2016), raising the question of how tree water uptake will respond to future changes in water availability. Research on the response of trees to drought is particularly relevant because forest drought resilience again has significant feedbacks on the water cycle (Bachofen et al., 2024). However, tree vulnerability to drought is not yet well understood, as it depends on various factors, including tree hydraulic traits such as the rooting depth (Choat et al., 2018).

Previous research has shown that trees can adjust their water uptake depth (WUD) in response to seasonal or long-term changes in moisture availability, which impacts transpiration and susceptibility to drought. Trees typically shift their WUD to deeper, moister soil depths when the water availability decreases under drought conditions (Bachofen et al., 2024). In contrast, some studies have found a decrease in water uptake from shallow soil but no compensation from deeper soil (Gessler et al., 2022), while other studies have detected no significant shifts in WUD in response to drought (Goldsmith et al., 2012). The adaptation of WUD depends on various factors such as plant species, climate, season, region, and local competition and is limited by the soil and rooting depth (Bachofen et al., 2024; Grossiord et al., 2017; Scandellari et al., 2024). Consequently, research findings cannot be transferred to other sites without consideration. Moreover, the interaction of WUD adaptation strategies and other drought responses of trees is not yet fully understood (Bachofen et al., 2024).

Studies on the response of the tree WUD to water availability at more diverse sites are essential (Bachofen et al., 2024). While studies have been conducted in the Central Alps due to more significant changes in drought occurrence and seasonality (Brunner et al., 2023), little to no research has been done in the pre-Alpine region because of the high annual precipitation ranging from 1500 to 2500 mm (NCCS, 2023). However, Allen et al. (2019) reported that trees in Swiss high-precipitation regions rely on summer precipitation, whereas trees in most other regions mainly use winter precipitation. Therefore, particularly spruce trees growing in wet regions such as the pre-Alps could be vulnerable to summer drought (Allen et al., 2019). Moreover, the vulnerability of spruce trees to drought could be further enhanced by their typically shallow roots limiting the WUD (Brinkmann et al., 2019).

In the pre-Alps, temperatures are expected to rise by 2 to 3.3°C by mid-century compared to today. Without strong climate change mitigation measures, precipitation is forecasted to increase by 2 to 27% in winter and to decrease by up to 27% in summer and autumn (NCCS, 2023). Additionally, the snow cover in the pre-Alps is sensitive to changes in temperature, so a temperature increase directly affects hydrology (Stähli et al., 2021). Furthermore, evapotranspiration in the Alps may be enhanced as plants respond to rising temperatures, which further reduces the availability of soil and surface water (Mastrotheodoros et al., 2020). It can be concluded that research on the adaptation of tree WUD to changes in water availability in the pre-Alps is crucial for sustainable water and forest management.

1.2. Research Questions

Based on the research gaps addressed above, this thesis investigates the spatial and temporal variability in water uptake by trees in the pre-Alpine region. Two main research questions and three hypotheses (H) are discussed:

1. How does the temporal variability in soil moisture affect the water uptake depth of trees?

H1: The main source of tree water uptake is deeper during periods of lower soil moisture.

2. How does the water uptake depth of trees vary between the study sites?

H2: The water uptake depth is deeper at drier sites.

H3: The water uptake depth depends on slope and elevation

2. Theoretical Background

2.1. Stable Water Isotopes

2.1.1. Water Isotopes

Atoms consist of positively charged protons, negatively charged electrons, and neutrons with no charge. Electrons have a relatively low mass compared to protons and neutrons, which have similar masses. Therefore, the mass of an atom is determined by the total number of protons and neutrons. Isotopes are atoms of the same element that have the same number of protons but a different number of neutrons. The slight difference in mass between isotopes affects the strength of bonds with other elements and, consequently, their behaviour in chemical and physical reactions (Sharp, 2017).

There are three natural hydrogen isotopes and three stable oxygen isotopes (Table 1). 1 H (protium) and 2 H (deuterium) are stable hydrogen isotopes, while 3 H (tritium) is radioactive with a half-life of 12.3 years due to the instability caused by the additional neutron (Sharp, 2017). The three stable oxygen isotopes are 16 O, 17 O, and 18 O, with 17 O having a relatively low abundance (Mook, 2000). The ratio of 2 H/ 1 H varies between -45% and +100%, while the ratio of 18 O/ 16 O varies between -50% and +50% (Gat et al., 2001). The hydrogen isotope ratio varies more than the oxygen isotope ratio because the relative mass difference between the hydrogen isotopes is larger (Mook, 2000).

Table 1: The atomic number, neutron number, mass number, and the abundance of the hydrogen isotopes ¹H, ²H, and ³H and the oxygen isotopes ¹⁶O, ¹⁷O, and ¹⁸O, data from Gat et al. (2001) and Mook (2000).

	Symbol	Atomic	Neutron	Mass	Abundance
		number Z	number N	number M	(%)
Hydrogen	¹ H	1	0	1	99.985
	² H	1	1	2	0.0155
	³ H	1	2	3	< 10 ⁻¹⁷
Oxygen	¹⁶ O	8	8	16	99.759
	¹⁷ O	8	9	17	0.037
	¹⁸ O	8	10	18	0.204

The ratio of heavy to light isotopes is typically expressed in δ (‰) as calculated in Equation 1. The isotope ratio R is the abundance of the heavier isotope divided by the abundance of the lighter isotope, such as ${}^2H/{}^1H$ or ${}^{18}O/{}^{16}O$, of the sample (R_{sample}) and the standard (R_{standard}) (Craig, 1961).

$$\delta \, [\%_0] = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$
 Equation 1

The commonly used standard is the Vienna Standard Mean Ocean Water (VSMOW). The International Atomic Energy Agency (IAEA) initiated the mixing of waters from selected locations worldwide, which were then calibrated in laboratories and named VSMOW (Aggarwal et al., 2011). Consequently, a negative δ indicates a depletion of heavy isotopes in the water sample compared to the VSMOW, which is typical for meteoric water. Water samples that have undergone evaporation are enriched in heavy isotopes and have a less negative or positive δ value (Gat, 1996).

2.1.2. Isotope Fractionation

The mass difference between heavy and light isotopes leads to distinct chemical and physical properties (Sharp, 2017). Heavy isotopes are less mobile, have slower diffusion velocities, and reduced collision frequencies with other molecules. Additionally, heavy isotopes have higher binding energies, which causes them to favour stronger bonds, such as those present in the solid phase (Mook, 2000). Consequently, phase changes can lead to one water source becoming enriched while the other water source becomes depleted in heavy isotopes (Sodemann, 2006).

There are two types of isotope fractionation: equilibrium fractionation and kinetic fractionation. Equilibrium fractionation is a reversible process that occurs within a chemical equilibrium. For example, if solid and liquid water coexist, isotopes are exchanged until they are in an equilibrium (Dütsch, 2016). In contrast, kinetic fractionation is irreversible because the two water sources are isolated after a phase change. One example is the evaporation of water vapour from the ocean surface. Lighter water isotopes evaporate more quickly, resulting in the vapour being depleted in heavy isotopes. If the water vapour is transported away, an equilibrium can no longer be achieved (Sodemann, 2006). Meteoric waters are depleted in heavy isotopes by about 10‰ in δ^{18} O on average compared to the VSMOW due to kinetic fractionation during the evaporation of ocean water (Gat et al., 2001).

Several studies have found no isotopic fractionation during plant water uptake (Amin et al., 2021; Chen et al., 2020; Dawson & Ehleringer, 1991; Washburn & Smith, 1934). However, fractionation can occur under specific climatic conditions and for particular plant species, plant growth states, and soil types (Dawson & Ehleringer, 1993; Poca et al., 2019; Vargas et al., 2017; Zhao et al., 2024). In particular, isotope fractionation is often observed during the water uptake of plant species adapted to saline soils (Ellsworth & Williams, 2007).

2.1.3. Global and Local Meteoric Water Line

The isotopic composition of meteoric waters globally was first analysed by Craig (1961), who calculated the linear relationship (Equation 2) between hydrogen and oxygen isotopes of meteoric waters that have not undergone evaporation.

$$\delta^2 H [\%_0] = 8 \times \delta^{18} O [\%_0] + 10$$
 Equation 2

This linear relationship is known as the global meteoric water line (GMWL) (Gat, 1996). Since the relationship between hydrogen and oxygen isotopes varies spatially and temporally, a local meteoric water line (LMWL) is often used to describe the relationship between water isotopes in precipitation at a specific location. The slope and intercept of the GMWL (Equation 2) are adjusted to local climate conditions using long-term data on the isotopic composition of precipitation. For example, a flatter slope indicates a warmer, drier climate with higher evaporation rates (Putman et al., 2019; Rozanski et al., 1993). However, the duration of data collection required to compute a LMWL depends on the processes being analysed and the scope of the research project. For analyses of processes with shorter residence times, such as plant tissues, it may be beneficial to compute a LMWL based on data from a shorter study period (Putman et al., 2019).

Deuterium excess (d-excess) is a measure to quantify the deviation of the isotopic composition of a water source from the GMWL and can be calculated using Equation 3 by Dansgaard (1964).

$$d$$
-excess = $\delta^2 H - 8 \times \delta^{18} O$ Equation 3

Additionally, the deviation of the isotopic composition of a water source from the LMWL can be quantified using the line conditioned excess (lc-excess) as calculated in Equation 4, with a and b representing the slope and intercept of the LMWL (Landwehr & Coplen, 2006).

$$lc\text{-}excess = \delta^2 H - a \times \delta^{18} O - b$$
 Equation 4

A d-excess or lc-excess of 0 indicates that there is no difference between the isotopic composition of the water sample and that of the global or local precipitation, respectively (Landwehr & Coplen, 2006).

2.2. Spatial and Temporal Variability of Soil Water Isotopes

The isotopic composition of soil water is affected by various processes that vary spatially and temporally, such as precipitation, transpiration, interception, and evaporation (Figure 1) (Sprenger et al., 2016). Additionally, the isotopic composition of water sources in a forest is influenced by non-fractionating processes such as root WUD, foliar water uptake, as well as soil water storage and percolation (Allen et al., 2022).

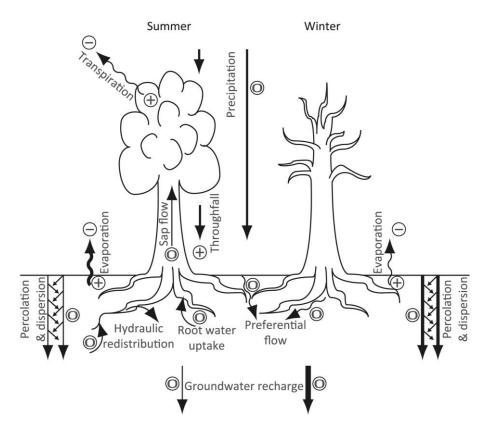


Figure 1: Processes affecting the isotopic composition of forest soils in a temperate climate in summer (left) and in winter (right). Plus signs indicate an enrichment and minus signs indicate a depletion of heavy isotopes. Zero signs indicate processes where no isotope fractionation is expected to occur (Sprenger et al., 2016, p. 676).

2.2.1. Precipitation

The isotopic composition of precipitation varies with temperature, latitude, altitude, continentality, season, climate, and precipitation amount (Figure 2). A key factor in some of these effects is temperature. At warmer temperatures, more evaporation affects water drops during their fall to the ground, which leads to an enrichment of heavy isotopes (Dansgaard, 1964). The global variability (Figure 3) is visible when looking at δ^{18} O in precipitation measured by the Global Network of Isotopes in Precipitation (GNIP) (Bowen & Wilkinson, 2002).

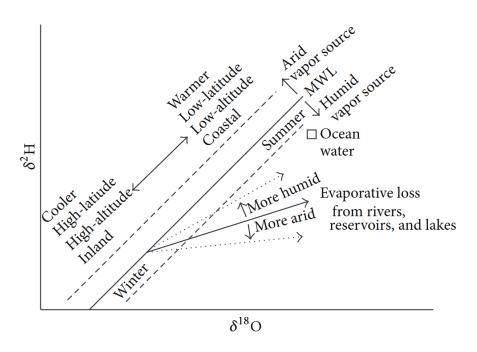


Figure 2: Spatial and temporal variability of the isotopic composition of precipitation with temperature, latitude, altitude, continentality, season, and climate (Xi, 2014, p. 4).

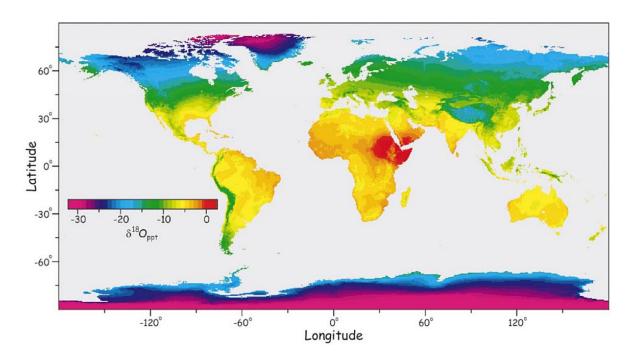


Figure 3: Map of modelled $\delta^{18}O$ based on data from GNIP measurement stations (Bowen & Wilkinson, 2002, p. 317).

The latitude effect results in precipitation being more depleted in heavy isotopes at higher latitudes, with a decrease of approximately 0.6% in δ^{18} O per degree of latitude in Europe and the USA. In Antarctica, the decrease can be as much as 2% δ^{18} O per degree of latitude. This effect is caused by the decrease in mean annual air temperature towards higher latitudes (Gat et al., 2001). The latitude effect is well visible in North America, Russia, and near the poles (Figure 3).

The altitude effect causes precipitation to be more depleted in heavy isotopes at higher altitudes and is linked to changes in pressure and temperature with altitude. At higher altitudes, condensation occurs at lower temperatures due to the lower pressure. Additionally, temperatures typically decrease with altitude (Gat et al., 2001). In the Alps, δ^{18} O in precipitation decreases on average by about 0.2% per 100 m of elevation (Schürch et al., 2003). The altitude effect is visible especially in the Tibetan plateau, the Andes, and the Alps (Figure 3). The relationship between the isotopic composition of precipitation and altitude on a smaller scale is often more complex due to various processes such as moisture transport and topography (Fischer et al., 2017b).

The continentality effect causes precipitation near the ocean to have a similar isotopic composition to that of the ocean water and then to become progressively more depleted in heavy isotopes as water vapour is transported further inland. This effect can be explained by the progressive formation of precipitation with distance from the ocean, a process affected by fractionation (Gat et al., 2001). The continentality effect can be observed at the northeastern coast of Russia and the western coast of Alaska (Figure 3). These regions are less depleted in heavy isotopes compared to regions further inland that have similar altitudes and latitudes.

The seasonal effect in temperate climate zones typically leads to precipitation being more depleted in heavy isotopes during winter than in summer (Mook, 2000). This effect is mainly driven by seasonal variations in temperature and humidity. Additionally, the isotopic composition of precipitation varies seasonally with changes in meteorological conditions and the source region of water input (Gat et al., 2001). Lastly, the amount effect causes heavy precipitation events to be more depleted in heavy isotopes (Dansgaard, 1964). Smaller precipitation events are more enriched in heavy isotopes due to enhanced evaporation from the raindrops (Gat et al., 2001).

2.2.2. Transpiration and Interception

Precipitation in a forest can be transpired, intercepted, and evaporated, or reach the soil as throughfall or stemflow. Therefore, tree cover affects the isotopic composition of the water input into the soil as well as the soil water storage (Gat et al., 2001).

Transpiration has an impact on the isotopic composition of soil water even without isotope fractionation during plant water uptake (Dawson & Ehleringer, 1991) and the back-diffusion of enriched leaf water through the stem towards the roots (Dawson & Ehleringer, 1993). Seasonal variations in transpiration rates and WUDs lead to certain precipitation events being mainly transpired, while other events recharge the soil water storage. At the same time, the isotopic composition of different precipitation events varies. Consequently, the temporal variability in transpiration affects the isotopic composition of precipitation stored in the soil (Gat et al., 2001), and changes in land use or vegetation cover influence the isotopic composition of soil water. Additionally, fractionation may affect leaf water during transpiration, which has an impact on the isotopic composition of atmospheric humidity (Simonin et al., 2014).

Interception affects the amount of precipitation reaching the soil, and the isotopic composition of throughfall is affected by evaporation (Sprenger et al., 2016). Fog drip onto the soil is typically

enriched in heavy isotopes due to evaporation from the plant surface (Liu et al., 2006). The proportion of intercepted precipitation that reaches the soil as stemflow or throughfall varies with numerous factors, including tree species and age, season, and precipitation intensity. In a spruce forest in Zurich, 61% of the precipitation that reached the ground was intercepted on an annual average, with most precipitation reaching the soil in the form of throughfall (Grundmann et al., 2024). Moreover, the interception of snow causes snow or snowmelt on the ground to be more enriched in heavy isotopes due to sublimation from the plant surface (Koeniger et al., 2008). However, the isotopic composition of snowmelt is affected by various processes causing fractionation, such as preferential water flows (Zhou et al., 2008) or isotopic exchange between water and ice (Lee et al., 2010).

2.2.3. Evaporation

The isotopic composition of soil water varies throughout the soil profile and is typically more enriched and variable in shallow soil than in deep soil (Sprenger et al., 2016; von Freyberg et al., 2020a). Evaporation at the soil surface causes an enrichment of heavy isotopes in shallow soil water (Goldsmith et al., 2012; Sprenger et al., 2016), with a smaller effect if precipitation amounts are high (Allen et al., 2022). In regions with frequent precipitation events, an isotopic enrichment may not be visible even in the shallowest soil water (Rossatto et al., 2011). Consequently, the isotopic composition of shallow soil water responds more to temperature increases and has a higher temporal variability, which leads to a more pronounced depth profile during drought conditions (Gessler et al., 2022).

The isotopic composition of deeper soil water is more constant as recent water inputs mix with older water pools, which have an isotopic composition that resembles a long-term average of previous precipitation events (von Freyberg et al., 2020a). The enriched isotope signal resulting from evaporation at the soil surface disappears as water percolates down through the soil profile because the relatively small volume of the shallowest soil water becomes insignificant. The depth to which the enriched isotopic signal is visible depends on various factors such as soil texture and climate (Sprenger et al., 2016).

The isotopic composition of soil water does not necessarily change continuously with soil depth. Firstly, the isotopic composition of precipitation inputs can fluctuate with weather conditions. Additionally, the evaporative enrichment at the soil surface and the percolation of water through the soil depend on short-term weather conditions (von Freyberg et al., 2020a). Secondly, soils consist of pores that vary in size, resulting in distinct isotopic compositions of more mobile and more bound water pools. Mobile soil water is typically younger and has a higher temporal variability than bound soil water (Sprenger et al., 2019). At the same time, water infiltrates more quickly through preferential flow pathways, resulting in mobile soil water being less affected by evaporation (Goldsmith et al., 2012). The exchange between mobile and bound water depends on the soil type (Vargas et al., 2017). Even in relatively wet soils, the exchange between mobile and bound water can be minimal (Sprenger et al., 2019).

2.3. Tree Water Uptake Depth

2.3.1. Tree Water Sources

By using stable water isotopes, soil water pools can be distinguished based on soil depths, seasonality, such as summer and winter precipitation inputs, or mobility, such as bulk and mobile soil water (Figure 4) (Floriancic et al., 2024).

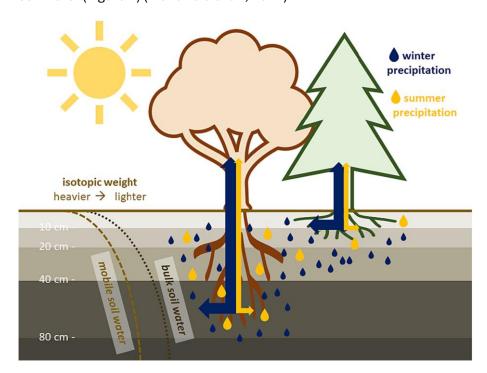


Figure 4: Potential water sources of trees vary in their isotopic composition with soil depth, mobility, and seasonality of precipitation inputs (Floriancic et al., 2024, p. 13).

The abundance and contribution of different water sources to tree water uptake vary both spatially and temporally. Local precipitation and snowmelt stored as soil water are usually the main water source of plants, while some trees also use shallow groundwater (Allen et al., 2022). At the same time, trees may prefer soil water over groundwater or surface water (Dawson & Ehleringer, 1991). Furthermore, trees typically prefer to transpire less mobile soil water as it is a more reliable water source than mobile water, especially during the growing season (Kirchner et al., 2023). Floriancic et al. (2024) found that beech and spruce trees in Zurich preferentially used winter precipitation even in summer. On a global annual average, recent precipitation accounts for approximately 70% of plant transpiration, while past precipitation stored in deeper unsaturated soils and rocks accounts for about 18%, and groundwater contributes about 11% to transpiration (Miguez-Macho & Fan, 2021). Another locally relevant water source can be dew or fog drip taken up by shallow roots (Dawson, 1998; Muñoz-Villers et al., 2025) or through foliar water uptake (Limm et al., 2009).

The rooting depth limits the WUD. Trees adjust their root distribution to water and nutrient availability (Goldsmith et al., 2012). At the same time, the root distribution restricts the range of accessible water sources and their contribution to water uptake (Bachofen et al., 2024). The

maximum rooting depth is particularly relevant under drought conditions (Choat et al., 2018). The rooting depth of trees varies with climate, with generally deeper, narrower root systems in arid climates and shallower but wider root systems in humid climates (Tumber-Dávila et al., 2022). Additionally, the fine-root mass and length can vary between soil types. For example, fine roots can grow faster and have a larger mass in sandy than in clay soils (Weemstra et al., 2017).

Water availability affects the root distribution (Allen et al., 2022). Firstly, roots are typically shallower than the groundwater level to avoid oxygen stress. Secondly, the rooting depth depends on the water infiltration depth in well-drained soils. Consequently, the rooting depth also depends on climate, soil type, and local topography, which can lead to small-scale variability in rooting depths. While the soil may be well drained at an upslope site, it can be waterlogged in the depression below (Figure 5) (Fan et al., 2017). In a meta-study, a mean maximum rooting depth of about 2 m was calculated, with most trees having roots extending down to 1.5 to 2.5 m (Tumber-Dávila et al., 2022). However, roots are not necessarily evenly distributed across the soil profile. They can follow macropores (Stewart et al., 1999) or be concentrated in the uppermost 10 cm of the soil due to nutrient availability (Muñoz-Villers et al., 2025). Additionally, some roots may be more relevant to the water uptake than others (Allen et al., 2022).

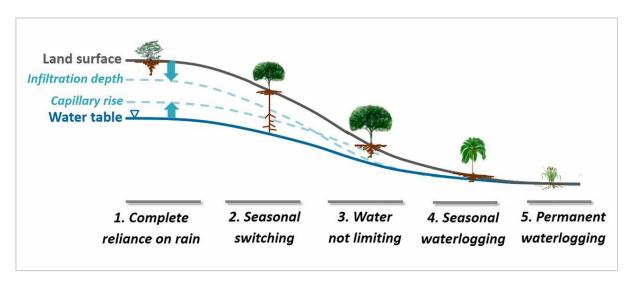


Figure 5: Small-scale variability in root distribution caused by topography (Fan et al., 2017, p. 10573).

2.3.2. Spatial Variability

The tree WUD varies spatially with climate, topography, and soil characteristics. Firstly, climate affects the WUD, with generally lower WUDs in deserts and temperate grasslands than in tropical rainforests (Bachofen et al., 2024). Furthermore, the contribution of groundwater as a water source varies between climate zones, generally increasing with aridity (Evaristo & McDonnell, 2017). Under high soil moisture conditions, recent water sources are more relevant, whereas winter precipitation can be the main water source of trees at drier sites (Allen et al., 2019). Secondly, local topography affects the WUD due to varying soil water availability and root distribution, as discussed in the previous section (Figure 5). The groundwater level is lower at

upslope sites, allowing deeper roots and, consequently, a broader range in WUDs. In contrast, the WUD of trees in depressions is limited by the shallow groundwater table that restricts the rooting depth (Rossatto et al., 2011). Lastly, soil characteristics affect the WUD because the root water uptake depends on the hydraulic conductivity of the soil (Bachofen et al., 2024) and because the root growth varies with soil type (Weemstra et al., 2017).

The WUD varies between tree species due to their distinct water uptake strategies. In a global comparison, conifers have a shallower mean WUD of 47 cm, deciduous broadleaved trees have a moderate mean WUD of 57 cm, and evergreen broadleaved trees have the deepest mean WUD of 62 cm (Bachofen et al., 2024). The water uptake strategy also affects the response of trees to drought. For example, beech trees have a shallower root system than oak trees, so beech trees depend on shallower, less reliable water sources (Fabiani et al., 2022). Compared to beech and oak trees, spruce trees use more mobile soil water and rely more on summer precipitation, which makes them more vulnerable to summer droughts (Allen et al., 2019).

Tree age, size, and local competition can also affect the WUD. For example, Dawson & Ehleringer (1991) found that young trees growing at a streamside relied on shallow soil or surface water, while adult trees used more constant, deeper water sources. Moreover, larger trees may better adapt their WUD to drought, for example, by increasing the use of groundwater as a water source (Muñoz-Villers et al., 2025). At the same time, no correlation between WUD and tree size may be detected because other factors, such as nutrient availability, also affect the root distribution (Goldsmith et al., 2012). Furthermore, local competition between plant species can affect the WUD. For example, trees can increase their WUD to reduce competition with shrubs and herbs that have shallower roots (Dawson & Ehleringer, 1991). Another study revealed that the WUD of oak trees was shallower in pure stands than in mixed stands, where they used more reliable water sources and were less vulnerable to drought (Bello et al., 2019). Moreover, the shallow root distribution of Norway spruce trees can lead to reduced water availability if grown in a mixed stand with European beech (Goisser et al., 2016).

2.3.3. Temporal Variability

The WUD can vary over time with transpiration and water availability (Bachofen et al., 2024). The WUD is typically deeper during periods of low soil moisture, while plants transpire more shallow soil water under wet conditions (Dawson & Pate, 1996; Miguez-Macho & Fan, 2021). Temporal changes in WUD depend on various factors such as plant species, climate, season, region, and local competition and are limited by the soil and rooting depth (Bachofen et al., 2024; Scandellari et al., 2024). Therefore, some studies found no significant change in WUD and root distribution (Goldsmith et al., 2012) or detected a decrease in water uptake from the shallow soil but no compensation with deeper soil water (Gessler et al., 2022) as a response to drought.

However, most trees in temperate seasonal forests have a deeper WUD during the dry season than during the wet season (Bachofen et al., 2024). For example, increased temperatures in the Swiss Pfyn forest caused soil drought and enhanced groundwater uptake by trees (Bertrand et al.,

2014). During dry summer months, winter precipitation can be the primary water source of trees, while trees use more recent water sources during winter. This shift can be explained by summer precipitation being more affected by evaporation, interception, and transpiration by forest floor vegetation (Allen et al., 2019; Floriancic et al., 2024).

2.4. Using Isotopes to Trace the Tree Water Uptake

2.4.1. Applications for Stable Isotopes

Isotope analyses are applied in diverse research fields, from planetary science and petrology to palaeontology, paleoclimate, and hydrology. The stable isotopes of hydrogen, oxygen, carbon, nitrogen, and sulphur have been used in diverse geochemical research projects due to their abundance in most natural materials (Sharp, 2017).

In hydrology, the stable water isotopes of hydrogen and oxygen are especially valuable as they are natural tracers (Orlowski et al., 2013). Stable water isotopes are measured in precipitation, groundwater, as well as in soil water, plant xylem water, and surface water to improve the understanding of the terrestrial and atmospheric water cycle and hydrological modelling (Aggarwal et al., 2011; Scandellari et al., 2024). Isotope data is especially valuable in combination with data on water fluxes and potentials (Kirchner et al., 2023). The application of stable isotopes in hydrology was first promoted by the IAEA, which was established in 1957 (Aggarwal et al., 2011). This field of research has been rapidly growing, with many more applications yet to be discovered and developed (Goldsmith et al., 2012; Scandellari et al., 2024).

Hydrogen and oxygen isotopes have been used for decades to study plant water uptake. The isotopic composition of soil water and tree xylem water has been used to analyse the variability of tree water uptake in various research projects (Bello et al., 2019; Bertrand et al., 2014; Dawson & Ehleringer, 1991). δ^{18} O can be measured more precisely and provide a clearer signal than δ^2 H (Bernhard et al., 2024). At the same time, δ^2 H is more affected by evaporation and, therefore, can be more informative when distinguishing between deep and shallow soil water. Most hydrological studies include both oxygen and hydrogen isotopes and additionally evaluate the d-excess or the lc-excess (Sprenger et al., 2016). Specific analyses focus on only one isotope, for example, because δ^{18} O in the cellulose of tree rings preserves information better than δ^2 H (Diao et al., 2025).

The availability of stable water isotope databases has been increasing. Scandellari et al. (2024, p. 3) summarised various large stable water isotope databases. The GNIP is a long-term monitoring network of hydrogen and oxygen isotopes in precipitation, established in 1960 by the IAEA and the World Meteorological Organisation. At that time, the database included approximately 100 sites worldwide with monthly data collection. Various early studies on the isotope composition in the water cycle are based on GNIP data (Dansgaard, 1964; Gat et al., 2001; Rozanski et al., 1993). Additionally, the Global Network of Isotopes in Rivers (GNIR) was developed in 2002. GNIP and GNIR data can be downloaded from the WISER portal (IAEA, 2025a, 2025b). In Switzerland, stable

water isotope data has been collected since 1980 by the ISOT module of the National Groundwater Monitoring NAQUA. The network includes 13 precipitation and nine watercourse sampling sites, and additionally, 50 groundwater sampling sites between 2006 and 2013 (FOEN, 2025; Schürch et al., 2003).

2.4.2. Calculation of the WUD

The natural variability in the isotopic composition of water sources is used to distinguish them and estimate their contribution to plant water uptake. The isotopic composition of all potential water sources, such as groundwater and shallow and deep soil water, is compared to the isotopic composition of the vegetation xylem water, which is considered to be a mixture of all water sources (Bertrand et al., 2014; Rossatto et al., 2011). The isotopic composition of a mixture (δ_M) consisting of two water sources can be calculated using a mass balance equation if the fraction (f) and the isotopic composition (δ) of both water sources are known (Equation 5) (Mook, 2000).

$$\delta_{\rm M} = f_1 \delta_1 + f_2 \delta_2$$
 Equation 5

The relative contributions of the different water sources are typically determined using a mixing model (Bachofen et al., 2024). Many mixing models are based on Equation 6, which is a reformulation of Equation 5 that accounts for any number of isotopes and water sources. Y_j represents the isotopic composition of the mixture of all isotopes j used as tracers. k is the source isotope, p_k is their contribution to the mixture, and μ^s_{jk} is the mean isotope value of each source (Stock et al., 2018).

$$Y_i = \sum_k p_k \mu_{ik}^S$$
 Equation 6

The two types of mixing models that are most commonly used for isotope analyses in hydrology are linear and Bayesian mixing models. Linear mixing models calculate the contribution of water sources using a mass balance equation (see Equation 6). The calculation is limited to a maximum of n+1 sources with n isotope groups. Otherwise, the system is mathematically underdetermined, as in the case of two equations but three unknowns. IsoSource is a well-known linear mixing model that was developed as an alternative for situations where the number of sources exceeds n+1. The IsoSource model calculates the range of possible contributions of each source by testing for all combinations of source contributions if they add up to the isotopic composition of the mixture (Phillips & Gregg, 2003).

The second type of mixing model is the Bayesian mixing model, which has the advantage of computing probability distributions while accounting for uncertainties and variability in isotope data (Stock et al., 2018). The first widely used Bayesian mixing model in stable water isotope studies was MixSIR. This model computes probability distributions of source contributions to the mixture while considering measurement and fractionation uncertainties as well as data variability in source and mixture isotope data (Moore & Semmens, 2008). Subsequently, MixSIR was further developed into the SIAR model by Parnell et al. (2010). SIAR is especially suitable for underdetermined systems, accounts for data uncertainty and variability, and can include a

concentration dependence (Parnell et al., 2010). SIAR was then further developed into the MixSIAR model, which is frequently used today. MixSIAR is very flexible and has the advantage of including fixed and random effects as covariates, as well as multiple sources (Stock et al., 2018). More details are described in Chapter 2.4.3. Mixing models are continuously improved and adjusted to specific fields of research. For example, CrisPy is a more recently developed root water uptake isotope mixing model, which has the advantage of being more accurate and robust (Fu et al., 2024).

Other methods besides mixing models can be used to analyse the water uptake of plants. For example, a simple linear interpolation can be used to calculate a single WUD. Although this method neglects the mixing of water sources, it can be useful as input for subsequent statistical analyses (Prechsl et al., 2015). Furthermore, other approaches, such as the seasonal origin index, can be used to quantify the contribution of different water sources (Allen et al., 2019).

2.4.3. MixSIAR

MixSIAR is an open-source R software package that was published in 2018 and has become a commonly used model in WUD studies. The model is applied in various research fields due to its flexibility and accessibility for users at different experience levels. Users can choose between a code script and a graphical user interface to load data files and specify model options. Furthermore, MixSIAR can generate outputs for underdetermined systems but with a high uncertainty in source contributions (Stock et al., 2018).

Model input data can be either the raw isotope data of each source or a summary statistic that includes the sample size and the mean and variance of the isotope values for each source. When using the summary statistic as input data, tracers are assumed to be independent of each other. When using raw isotope data, multivariate normality is assumed, and a variance-covariance matrix is calculated for each source. The model writes a JAGS (Just Another Gibbs Sampler) model file and runs it to produce diagnostics, posterior density plots, and summary statistics with uncertainty intervals. The model output shows the effects of covariates on the variability in source contributions (Stock et al., 2018).

A key advantage of MixSIAR is that fixed and random effects can be specified. Fixed categorical effects are used in cases where the mixtures are grouped, and the differences in source contribution between these groups are analysed. Fixed continuous effects are used if there is an additional numerical measure characterising the mixtures, and the effect of this measure on the source contributions is analysed. Random effects are defined if mixtures and sources have certain similarities, such as the region of origin, which could explain similarities between the source contributions to certain mixtures. All occurring effects must be specified. An alternative would be to filter the data and run the model separately for each group. Furthermore, discrimination factors can be specified to account for systematic shifts in tracer values due to biological or chemical processes during the mixing process, such as fractionation (Stock et al., 2018). The discrimination factors are typically set to 0 when studying plant water uptake based on

the assumption of no fractionation during root water uptake (Fu et al., 2024). However, it should be considered that the model is highly sensitive to discrimination factors, which are difficult to determine (Stock et al., 2018).

2.4.4. Limitations of WUD Research

Some limitations must be considered in WUD studies, as plant water uptake is a complex process affected by various factors. Firstly, mixing models rely on significant differences between the isotopic compositions of all potential water sources (Stock et al., 2018). If the sources cannot be distinguished, their contribution to water uptake cannot be calculated. This problem can be avoided by including other isotopes, which makes the research more expensive (Scandellari et al., 2024), or by labelling certain water sources, for example, with deuterium-enriched water (Grossiord et al., 2014).

Secondly, mixing models are based on the assumption that all potential plant water sources are included as input variables (Stock et al., 2018) and that there is no fractionation during root water uptake (Fu et al., 2024). Furthermore, mixing models assume that the isotopic composition of both the mixture and the sources is known and invariant (Stock et al., 2018). Consequently, the sampling design must be suitable for the research topic, study site, and sampling period. For example, fluctuations in the isotopic composition of water throughout the soil profile might be missed if the spatial resolution of sampled soil depths is insufficient, which could lead to misinterpretations (von Freyberg et al., 2020a). Moreover, the sampling method affects whether the isotopic composition of mobile soil water or bulk soil water is measured (Sprenger et al., 2016). Additionally, there may be a time lag between root water uptake and water present in the branches, depending on the sap-flow velocity, which should be considered in the sampling design (Bernhard et al., 2024).

Lastly, conclusions are limited because mixing models only indicate the estimated contribution of the water sources to plant water uptake. However, the importance of the different water sources may vary, as plants do not necessarily rely on the water source with the highest contribution (von Freyberg et al., 2020a). Additionally, trees may not have equal access to each water source due to their root distribution (Stewart et al., 1999). Especially when analysing the response of WUD to drought, it must be considered that water is not always the limiting factor. For example, nutrient availability could affect the root distribution (Goldsmith et al., 2012). Generally, the transpiration response to drought and tree physiology is complex, and further research is required to better understand the WUD response to drought (Carminati & Javaux, 2020).

3. Study Site

3.1. Study Site Description

3.1.1. Location and Topography

The Alptal is a valley in the Swiss pre-Alps, located about 40 km southeast of Zurich in the Canton of Schwyz (47°2'20.5"N, 8°43'19.0"E) (Figure 6). The catchment of the river Alp has a size of 46.4 km² and an altitude ranging from 840 to 1899 m a.s.l.. The Alp flows into the Sihl and then via the Limmat and the Aare to the Rhine and finally into the North Sea (Ragettli et al., 2021; Stähli et al., 2021). The steep headwater catchments are characterised by a complex terrain affected by soil creep and landslides. The headwater streams are mostly shallow, with only larger streams incised by more than 0.5 m (van Meerveld et al., 2018).

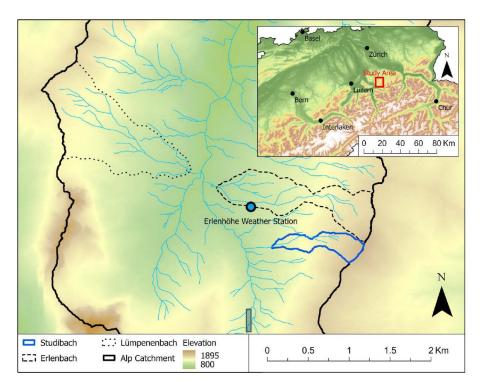


Figure 6: Map of the Studibach headwater catchment and the Erlenhöhe weather station in the Alp catchment and their location in Switzerland (van Dijk, 2024, p. 4).

3.1.2. Climate

The Alptal has a humid temperate climate. The weather station of the Swiss Federal Office in Meteorology and Climatology (MeteoSwiss) in Einsiedeln, located at 912 m a.s.l., recorded a mean annual air temperature of 7.3°C from 1995 to 2024. Average monthly temperatures varied between 16.2°C in July and -1.5°C in January over these 30 years (MeteoSchweiz, 2025b). The mean annual air temperature increased by about 2°C over a 50-year period, with the highest temperature increase in April and June (Stähli et al., 2021). The annual precipitation is relatively high, with 1694 mm measured in Einsiedeln on average from 1995 to 2024. Most precipitation falls

in summer, with an average of 199 mm in August, while the least precipitation falls in February, with 100 mm on average (MeteoSchweiz, 2025b). A significant change in precipitation over time could not be detected (Stähli et al., 2021). The Erlenhöhe weather station, located about 500 m from the Studibach catchment at 1210 m a.s.l., measured a long-term mean annual precipitation of 2266 mm and a mean annual air temperature of 5.7°C between 1969 and 2019 (Stähli et al., 2021).

A continuous snow cover usually persists only at higher altitudes between December and April, with snow depths of up to 2 m (Stähli et al., 2021). At high altitudes, snowfall can occur even during summer months, but it typically melts quickly (van Meerveld et al., 2018). The modelled maximum snow water equivalent is about 50% lower in forested areas compared to open areas such as meadows (Stähli & Gustafsson, 2006). Due to climate change, the duration of snow cover has decreased, and snowmelt has shifted towards earlier months, causing reduced runoff in April (Stähli et al., 2021).

In the headwater catchments, runoff reacts to precipitation events within minutes (Hagedorn et al., 2000). The contribution of precipitation to runoff increases with precipitation during an event, while runoff following low precipitation events mainly consists of pre-event water sources (Fischer et al., 2017a). Flow pathways can significantly change under storm conditions (Hagedorn et al., 2000). High-conductivity soil layers near the soil surface are important for runoff generation (van Meerveld et al., 2018). Surface runoff increases with precipitation amount and intensity and occurs mainly in meadows and wetlands due to saturated overland flow and on bare soils or infiltration-excess overland flow (Sauter, 2017).

3.1.3. Vegetation

In the Studibach headwater catchment, the vegetation cover is highly heterogeneous. The catchment is covered by about 53% forest, 28% wetland, and 14% meadows. Steeper slopes are mostly forested, whereas flatter areas are more commonly wetlands or meadows (Stähli et al., 2021; van Meerveld et al., 2018). The forest is primarily composed of Norway spruce (Picea abies) and silver fir (Abies alba), with an understory of blueberries (Vaccinium sp.) and horsetail (Equisetum sp.) (Feyen, 1998; Ragettli et al., 2021).

The Alptal valley was affected by deforestation for agriculture and fuel production until reforestation and protection measures were introduced in the 19th century (Hegg et al., 2006). Today, part of the Alptal, including the Studibach headwater catchment, is located within the nature reserve Ibergeregg, which is characterised by high biodiversity and large wetlands. It provides a habitat for endangered species such as the western capercaillie (Tetrao urogallus), the cranberry fritillary (Boloria aquilionaris), and felwort (Swertia perennis). Hiking and winter tourism are permitted in restricted areas and on pathways, especially between December and July (Umweltdepartement Kantons Schwyz, 2008). In summer, the meadows at higher altitudes are used for cattle grazing (Rinderer et al., 2016).

3.1.4. Soil and Bedrock

Soils in the headwater catchments are characterised as Gleysol, known for their low permeability (Hagedorn et al., 2000). The soils are mostly shallow, with depths of less than 1 m. However, the soil depths range from 0.5 m on ridges to 2.5 m in depressions (van Meerveld et al., 2018). The bedrock in the Alptal mainly consists of Flysch. In the Studibach headwater catchment, the parent rock material is Wägitaler Flysch at the lowest altitudes, Wild Flysch at moderate altitudes, and Schlieren Flysch at the highest altitudes (van Meerveld et al., 2018). Flysch has a low permeability and consists of calcareous sandstones, argillite, and bentonite schists (Hagedorn et al., 2000; van Meerveld et al., 2018).

The low permeability of the Gleysol soil and Flysch bedrock prevents deep water infiltration, leading to generally shallow groundwater levels (Hagedorn et al., 2000; Kiewiet et al., 2019; Ragettli et al., 2021). Furthermore, soil moisture contents are relatively high due to frequent heavy precipitation events combined with the low permeability of the soil and the bedrock. At the same time, there is a high spatial variability in soil moisture within the catchment due to variations in topography and vegetation (van Meerveld et al., 2018).

3.2. Study Sites

The study sites are in the Studibach catchment, a steep headwater catchment on a south-facing slope in the northwest of the Alptal valley. The Studibach stream drains into the Zwäckentobel and then into the Alp. The catchment has a size of 0.2 km², an elevation range between about 1270 and 1650 m a.s.l., and average and maximum slopes of 21° and 47°, respectively (van Meerveld et al., 2018). The five study plots L1 to L5 are square areas, each measuring 15 m * 15 m, located along a transect with an elevation range from 1267 to 1454 m a.s.l. (Table 2). L1 and L2 are in the low elevation zone, L3 is in the medium elevation zone, and L4 and L5 are in the high elevation zone (Figure 7).

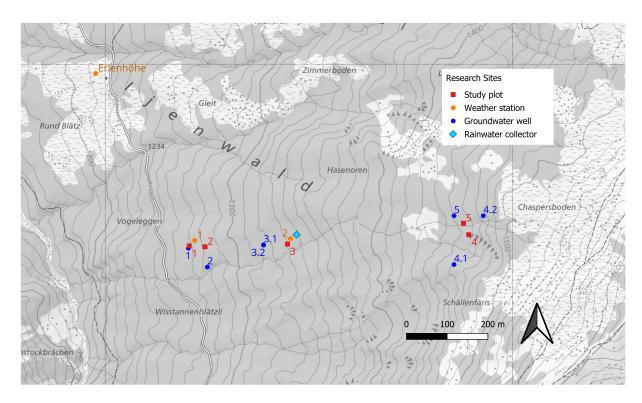


Figure 7: Location of the study plots, weather stations, groundwater wells, and the rainwater collector along the hillslope transect on a national map provided by Swisstopo.

All trees in the study plots are Norway spruce trees, except for two young trees at the edge of L1. L1 and L2 are in a forest stand surrounded by wetlands and meadows. A small stream flows through L1. L3 and L4 are in an open forest stand with several felled trees and fallen branches. At L3, there are relatively young trees, wetland plants, shrubs, moss, and grasses. The centre of L4 is mainly covered by moss and berries, while mainly wetland plants and grasses surround the study site. L5 is in a forest stand with large trees, and a stream runs along the edge of the study plot.

Table 2: Coordinates and characteristics of the study sites L1-L5. The tree cover was calculated by dividing the total tree area within the study plot measured at breast height by the total area of the plot (15 m * 15 m).

Study	Latitude	Longitude	Altitude	Vegetation	Tree	Number
site			(m a.s.l.)		cover (%)	of trees
L1	47°02'20.0"N	8°43'03.7"E	1267	Forest	0.18	23
L2	47°02'19.9"N	8°43'05.5"E	1282	Forest	0.49	38
L3	47°02'20.0"N	8°43'15.1"E	1331	Open forest	0.49	14
L4	47°02'20.5"N	8°43'36.2"E	1458	Open forest	0.54	24
L5	47°02'21.4"N	8°43'35.6"E	1454	Forest	0.73	10

The soil profiles (Table 3) are highly heterogeneous within each study plot. At L1 and L2, the A_h horizon is very porous and has a depth of about 25 cm. Below this, a reduced gleyic horizon (G_r)

can be found (Figure 8). The soil profile at L3 is similar to that at L1 and L2, but the G_r horizon is drier, and the clay content appears to be lower. At L4 and L5, the A_h horizon is about 15-20 cm deep. In contrast to all other sites, there is a reduced-oxidised horizon (G_{ro}) (Figure 9). At L4, the organic surface layer (L) is the deepest, with typical depths of 5-10 cm but maximum depths of up to 20 cm.

Table 3: Description of a typical soil profile at each study site down to a depth of 50 cm.

	L1	L2	L3	L4	L5
L	2-5 cm	2-5 cm	2-8 cm	5-10 cm	2-8 cm
Ah	20-30 cm	20-30 cm	30-40 cm	15-25 cm	10-20 cm
G_{ro}	-	-	-	>30 cm	>30 cm
G r	>30 cm, very	>30 cm, very	>40 cm, less	-	-
	clayey and	clayey and	clayey, drier,		
	wet	wet	more porous		



Figure 8: Soil profile at L2 with a depth of 45 cm.



Figure 9: Soil profile at L5 with a depth of 35 cm.

The slope within and around L1 is moderate. L2 is steeper, with a flatter area uphill and a steeper slope downhill. L3 has a moderate slope, but the topography within and around the study site is very heterogeneous due to temporary stream channels and dead wood. L4 is located on a flatter area between steep slopes. L5 has a moderate slope, but it is slightly steeper, both uphill and downhill. Slope, aspect, and TWI (Table 4) were calculated in QGIS by Charmaine Bassfeld. The DEM SwissALTI3D (Swisstopo, 2024) with a resolution of 0.5 m was used, and an area within a radius of 5 m around the centre of the study plots was included. The TWI was calculated using the r.watershed tool by GRASS and default settings, and default operations were used to compute the slope and aspect. Additionally, the slope was measured in the field between the lysimeters and at five locations across the study plots. The field measurements and the calculated slopes have a similar range. Therefore, the slopes calculated in QGIS were used for further analysis.

Table 4: Slope, aspect, and TWI at the study sites L1-L5 that were calculated using QGIS and the slope measured in the field for comparison.

	Field estimates		Calculated using QGIS						
	Slope (°)		Slope (°)		Aspect (°)		TWI		
Study	Mean	Between the	Mean	Range	Mean	Range	Mean	Range	
site		lysimeters							
L1	16	16	16.22	29.36	261.03	355.76	3.75	9.28	
L2	25	20	17.94	30.60	274.52	348.72	4.42	8.74	
L3	17	18	19.96	33.73	261.70	145.81	2.65	5.56	
L4	33	11	17.73	54.53	222.76	355.15	1.99	9.64	
L5	8	5	15.66	36.67	223.91	359.19	4.07	10.30	

3.3. Research in the Alptal

The Alptal is hydrologically interesting because of the high precipitation rates combined with the low permeability of soils and bedrock, which leads to fast runoff responses (Stähli et al., 2021). Particularly, the heterogeneous land cover (Stähli et al., 2021) and topography as well as the steep slopes (van Meerveld et al., 2018) make research in the headwater catchments interesting. Furthermore, early research was conducted due to the flood risk affecting villages in the valley (Stähli et al., 2021).

Research started in 1963 with a focus on water quality, floods, and forest management (van Meerveld et al., 2018). The Swiss Federal Institute for Forest, Snow, and Landscape Research (WSL) has been using the Alptal as a research site since 1965 for long-term monitoring of runoff and analysing the impact of forests on flood events (Hegg et al., 2006). Since the 1980s, geomorphology, biogeochemistry, and ecohydrology have been studied in the headwater catchments. Today, climate impacts on water supply and runoff generation have attracted considerable attention (Stähli et al., 2021). Since 2009, the University of Zurich has been conducting research on hydrological processes that cause rapid runoff responses, and the variability of groundwater levels, the isotopic composition and chemistry of streamflow, and their responses to precipitation events have been studied (van Meerveld et al., 2018).

MeteoSwiss operates a meteorological station in Einsiedeln (MeteoSchweiz, 2025a), and the WSL runs the Erlenhöhe meteorological station (WSL, n.d.). Since 2009, 51 groundwater wells have been installed in the Studibach catchment (van Meerveld et al., 2018). Various research projects have analysed runoff generation processes (Fischer et al., 2017a; Hagedorn et al., 2000; van Meerveld et al., 2018), stream network dynamics and flow pathways (van Meerveld et al., 2019), groundwater (Kiewiet et al., 2019, 2020; Rinderer et al., 2016), flood frequencies (Ragettli et al., 2021), and sediment transport (Rickenmann et al., 2012; van Dijk, 2024).

The isotopic composition of streams, precipitation, and groundwater has been measured in five research projects. Firstly, Fischer et al. (2017a) analysed the contribution of pre-event water to runoff using isotope hydrograph separation. δ^2H and $\delta^{18}O$ of stream water and precipitation were measured during 13 precipitation events in the snow-free period of 2010 and 2011. Subsequently, Fischer et al. (2017b) analysed the spatiotemporal variability in the isotopic composition of precipitation and its effect on isotope hydrograph separation. Additionally, the isotopic composition of stream water in five sub-catchments was sampled weekly between 2015 and 2019 (Staudinger et al., 2020), and daily stable water isotope data of precipitation and stream water was collected between 2015 and 2019 (von Freyberg et al., 2022). Furthermore, Kiewiet et al. (2019) studied the spatiotemporal variability in hydrochemistry, including stable water isotopes of shallow groundwater from 34 to 47 wells. Subsequently, Kiewiet et al. (2020) analysed the spatial variability in groundwater isotopes and their effects on hydrograph separation. Lastly, stable water isotopes of the bulk snowpack, throughfall, and open precipitation were measured seven times in the winter of 2018 to analyse the influence of canopy cover on snowpack accumulation (von Freyberg et al., 2020b).

4. Methods

4.1. Sampling Design

Fieldwork was conducted between 27.06.2024 and 06.10.2024. Isotope samples were collected on five days, with breaks of about three weeks between the sampling days, on 17.07.2024, 04.08.2024, 28.08.2024, 15.09.2024, and 03.10.2024. Manual soil moisture measurements and rainwater sampling were done weekly. Study sites with a range in elevation and soil moisture conditions were selected along a transect in the Studibach catchment, considering data availability, vegetation, and suitability for sample collection. From June to October, soil moisture was measured manually at all sites and sap flow was analysed at three sites for certain trees for other research projects. Furthermore, data from groundwater wells, weather stations, and humidity and air temperature loggers were available for several study plots. Contamination of the samples with sunscreen or bug repellent was prevented, and all samples were transported in a cooling bag. Additional photos of the study sites and measurement instruments can be found in Appendix C.

At each study site, three healthy and sufficiently large Norway spruce trees with low branches were selected for sampling. The approximated tree age varies between 51 and 137 years, and the circumference at a breast height of 1.5 m ranges from 0.66 to 2.80 m (Table 5). Stem cores were collected after the last sampling day for an age estimation. All cores were dried before analysis at the laboratory of the WSL in Birmensdorf, Zurich. The cores were prepared using a cutting machine and then ground before high-resolution images were taken with Skippy (WSL, 2025). The CooRecorder software (Maxwell & Larsson, 2021) was then used to count tree rings and measure tree ring widths (Appendix A.1).

Table 5: Tree sizes (circumference at breast height) and approximated tree ages at each study site.

Study	Tree 1		Tree 2		Tree 3		Mean	
site	Size (m)	Age (yr)						
L1	1.00	NA	0.66	NA	1.28	73	1.0	73
L2	0.92	117	0.76	NA	1.93	134	1.2	125.5
L3	0.75	137	1.16	NA	0.79	124	0.9	130.5
L4	0.90	NA	1.75	99	1.69	101	1.5	100
L5	2.80	51	2.76	109	1.77	71	2.4	77

4.2. Hydrometric Measurements

4.2.1. Precipitation and Temperature

The Erlenhöhe weather station is located about 500 m northwest of the lowest study site at an altitude of 1210 m a.s.l. (Figure 6). It provides data on air temperature, precipitation, wind speed and direction, humidity, radiation, and air pressure at 10-minute intervals. The precipitation is measured using a heated tipping bucket and a totaliser (WSL, n.d.). Additionally, two temporary weather stations were installed next to L1 and within L3 by Charmaine Bassfeld, providing data on precipitation, air temperature, relative humidity, solar radiation, lightning activity, and wind at 10-minute intervals.

4.2.2. Soil Moisture

Manual soil moisture measurements were done weekly between August and October at five marked locations within each study site. In June and July, soil moisture was measured manually at all sites by Charmaine Bassfeld and Belle Holthuis. Soil moisture was measured in the topsoil using a Moisture Meter type HH2 with a ThetaProbe ML3 sensor, which uses the dielectric constant as an indicator of soil moisture (Delta-T Devices Ltd., 2017). Additionally, soil moisture, humidity, and air temperature loggers were installed by Charmaine Bassfeld. Soil moisture data was recorded continuously throughout the entire study period at depths of 5, 15, and 30 cm at L1, L2, and L3 and also at a depth of 45 cm at L3.

4.2.3. Groundwater

Since 2009, 51 groundwater wells have been installed in the Studibach catchment (van Meerveld et al., 2018), ideally providing continuous data on groundwater levels every 10 minutes. Near L1, L2, and L5, groundwater data is available between November 2023 and October 2024. At L3 and L4, two loggers located further from the study sites were used to approximate the groundwater level and variability over the summer of 2024. At L1, a pressure data logger (KELLER Pressure) measured the groundwater level, while all other groundwater levels were measured by Odyssey capacitance water level loggers (Dataflow Systems Ltd).

4.3. Sampling

4.3.1. Lysimeters

Mobile soil water was extracted using suction lysimeters, which consisted of a plastic tube with a diameter of 3 cm and a porous ceramic cup at the bottom. The upper end of the tube was closed with a rubber stopper, which was connected to a plastic hose that could be closed with a clamp. When a negative pressure is applied to the suction lysimeter, soil water flows into the porous cup.

Four vertical lysimeters were installed in the centre of all five study plots at depths of 10, 20, 30, and 50 cm. Firstly, a hole with a specified depth was dug using a soil auger that had the same diameter as the lysimeter (Figure 10). A lysimeter was then inserted into the hole, and the correct depth was confirmed and labelled. Gaps around the lysimeter were filled with clay from deeper soil horizons to minimise direct contact between the porous cup and air (Figure 11). This was essential to create a vacuum in the tube and prevent direct percolation of water along the tube, causing the mixing of different water sources (Ceperley et al., 2024). All lysimeters were tested and emptied once before the first sample collection. About a week before each sampling day, the lysimeters were emptied completely using a plastic hose connected to a 60 mL syringe. A hand pump was then used to apply a negative pressure of about 70 kPa (Figure 12) before the hose was tightly closed with a clamp. For sampling, water in the lysimeters was collected using the plastic hose and the syringe. If there was enough water in the lysimeters, the syringe and the hose were once flushed before sample collection. 20 mL glass vials were completely filled with water, leaving no space for air, and stored in a refrigerator at about 4°C.



Figure 10: Auger used for lysimeter installation and soil sampling.



Figure 11: Lysimeter closed with a clamp and sealed with clay.



Figure 12: Application of a negative pressure using a hand pump.

On the sampling day on 15.09.2024, the tubes at L5 were covered in snow (Figure 13). Between the first and the second sampling day, a heavy precipitation event caused overbank flow of the stream near L5 (Figure 14). The lysimeters were flooded and reinstalled about 4 m into the forest and at a larger distance from the original stream channel.



Figure 13: Lysimeters at L5 covered in snow on 15.09.2024.



Figure 14: Traces of overbank flow at L5 observed on 04.08.2024.

4.3.2. Soil

Bulk soil samples were collected at all sites at depths of 5, 10, 15, 20, 30, 40, and 50 cm. In consideration of the high spatial heterogeneity, three soil profiles across each site were taken using an auger (Figure 10). For each depth, an equal amount of soil from the three profiles was collected in a plastic cup and mixed thoroughly before it was filled into 12 mL glass exetainers (Labco Ltd, UK). Some pores filled with air were allowed in the exetainers to prevent the glass from breaking when frozen. The soil samples were stored in a freezer at -22°C.

4.3.3. Trees

For xylem water isotope measurements, twig samples of the three selected trees at each study site were collected, with each sample consisting of multiple twigs of the tree. Twigs were cut at least ten nodes away from the tip of the stem to avoid back-diffusion of water affected by isotope fractionation (Dawson & Ehleringer, 1993). The bark and phloem tissue were removed with a knife in the field as they could potentially contain isotopically enriched water (Bello et al., 2019; Ceperley et al., 2024). For comparison, stem wood cores were taken using an increment borer at a height of about 1.5 m. Only sapwood was sampled, and bark and phloem tissue were removed in the field. Stem cores were collected on the last sampling day to reduce tree damage and not disturb other measurements. To collect enough water, as many twigs or cores as possible were sealed in the 12 mL glass exetainers (Labco Ltd, UK) and later stored in a freezer at -22°C.

4.3.4. Precipitation

Precipitation samples were collected weekly from 04.08.2024 until 03.10.2024 at an altitude of 1338 m a.s.l. (47°02'20.8"N 8°43'16.2"E). The rainwater collector was installed at a height of 95 cm in an open area with more than 10 m distance to trees (Figure 15). The collector consisted of a smaller bottle with a funnel that was inserted into a larger bottle, which had a larger funnel attached to it. This larger funnel was then covered with a small ball (Figure 16). The sample was taken from the smaller inner bottle, from which rainwater was able to overflow into the larger bottle. This installation method minimised the water surface area of the sampled water, and the ball in the larger funnel additionally reduced evaporation, which is important to limit isotope fractionation. Rainwater samples were collected in 20 mL glass vials and stored in the refrigerator at about 4°C.



Figure 15: Installation of the rainwater collector in an open area.



Figure 16: Components of the rainwater collector. The sample was taken from the smaller inner bottle with the green lid.

4.4. Laboratory Analysis

4.4.1. Water Extraction

Twig xylem water, stem core xylem water, and bulk soil water were extracted using cryogenic vacuum extraction at the laboratory of the Department of Environmental System Science at ETH Zurich with the help of Dr. Marius Floriancic and Fabian Strittmatter. In each batch, 20 samples in exetainers were attached to U-tubes above 80°C warm water. The U-tubes were cooled with liquid nitrogen to collect the evaporated water in the form of ice, while a negative pressure between 0.11 and 0.22 mbar was applied to the extraction line. Vegetation and soil samples were extracted for 2 and 3 hours, respectively. Thawed water in the U-tubes was mixed before filtration (13 mm Syringe Filter, PTFE (Hydrophobic), 0.45 μ m). Water was stored in 1.5 mL glass vials in the refrigerator until isotope analysis was performed.

4.4.2. Isotope analysis

Isotope analysis of all samples was conducted at the laboratory of the University of Freiburg by Dr. Barbara Herbstritt between 07.01.2025 and 03.02.2025. $\delta^2 H$ and $\delta^{18} O$ were analysed simultaneously using two cavity ring-down spectroscopy devices, which are cross-validated to guarantee identical results (Herbstritt et al., 2024). For the isotope measurements of bulk soil water, mobile soil water, and precipitation water, the Picarro L2130-i was used. Xylem water isotopes were measured using the Picarro L2140-i device in $i^8 O$ -mode with a Micro-Combustion ModuleTM, which oxidises organic compounds in the vegetation samples to prevent spectral contamination from organic compounds (Picarro, Inc., 2015). Results included $\delta^{18} O$ and $\delta^2 H$ expressed as $i^8 O$ relative to the VSMOW with an accuracy of +/- 0.16 $i^8 O$ and +/- 0.6 $i^8 O$ and +/- 0.6 $i^8 O$ and +/- 0.6 $i^8 O$ in $\delta^2 H$ according to manufacturer specifications.

4.5. Data Analysis

4.5.1. Statistical Analysis

Statistical analyses were performed using R version 4.4.3 (2025-02-28 ucrt). A p-value lower than 0.05 was considered statistically significant for all tests. Pearson correlation tests were performed for correlation analyses as they test the linear relationship between two variables and assume normally distributed data. Two-sided tests with a 95% confidence interval were applied. Kruskal-Wallis tests were conducted to test for significant differences between groups such as study sites, elevation zones, or water sources.

4.5.2. MixSIAR

The Bayesian stable isotope mixing model MixSIAR (see Chapter 2.4.3) was used to calculate the contribution of water sources to the water uptake of the trees (Stock et al., 2018). The model was performed for each sampling day separately, and the model run length was set to long (chainLength=300000, burn=200000, thin=100, chains=3, calcDIC=TRUE). Discrimination factors of 0 were specified based on the assumption of no fractionation during plant water uptake.

For the mixture input data, the mean isotopic composition of the twig xylem water from each elevation zone was used. Consequently, the mean isotope values of each elevation zone included data from 3 (medium elevation zone) or 6 (low and high elevation zone) trees. Additionally, the model was run using the isotopic composition of the 12 stem core xylem water samples collected on 03.10.2024 as a comparison. The elevation zones were included as a random effect in the input data.

The isotopic composition of the bulk soil water samples was used as the source input data, and the elevation zones were included as a source factor. The two potential tree water sources were shallow (5-20 cm) and deep (25-50 cm) soil water. The data type was set to "means", and the mean and standard deviation were computed per elevation zone and water source. The model was run with bulk soil samples on 04.08.2024, 15.09.2024, and 03.10.2024. Two sampling days were excluded because data was only available at L1 (17.07.2024) or in the shallow soil (28.08.2024).

In separate model runs, the isotopic composition of the mobile soil water that was sampled using lysimeters was used as source input data. The medium elevation contains only data from L3 and had to be excluded because no or only one shallow or deep sample was available on all sampling days. Using mobile soil water samples, the model was run for all sampling days.

5. Results

5.1. Overview of the Study Period

5.1.1. Precipitation, Temperature and Humidity

The precipitation measured at the Erlenhöhe weather station by the heated tipping bucket was generally higher than that recorded by the totaliser, particularly during heavy precipitation and snowfall events. The data from the weather stations at the study sites is less reliable due to higher measurement uncertainty and data gaps caused by the low battery durability and blocked sensors. Consequently, the precipitation measured by the heated tipping bucket and the temperature data from the Erlenhöhe weather station were used for further analysis. Continuous data is available, except for the data gap between 31.07.2024 and 05.08.2024 (Figure 17).

Between May and October, temperatures varied between -0.3°C and 28.9°C. The highest monthly mean temperature of 16.9°C was recorded in August, with temperatures decreasing to 9.2°C in May and to 9.9°C in September. The highest total monthly precipitation of 321 mm was measured in May. Monthly precipitation steadily decreased to 110 mm in August before increasing again to 196 mm in September. The highest daily precipitation amounts were recorded on 31.05.2024 (81.2 mm), 30.05.2024 (65.4 mm), and 31.07.2024 (51.1 mm). During the heavy rainfall event on 31.07.2024, the suction lysimeters at L5 were flooded and partly damaged, and the Erlenhöhe weather station was damaged, causing a data gap. The storm did not damage other study plots, but water overflowed from the rainwater collector.

On 15.09.2024, there were patches of snow at all study sites during sample collection. The suction lysimeters at L5 were covered by snow, but the lysimeter water was not more depleted in heavy isotopes than on the following sampling day (see Figure 22). The precipitation at low temperatures that led to snowfall in mid-September is visible in the weather data. After this snowfall, temperatures increased slightly before dropping again at the end of September. Some snowfall before 03.10.2024 is possible, but no snow was observed in the field on the final sampling day.

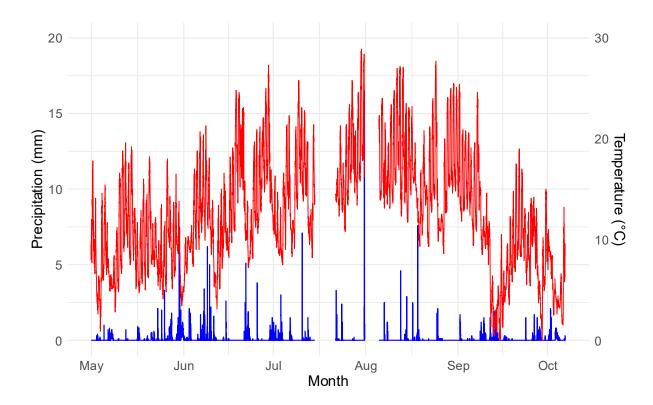


Figure 17: Precipitation and temperature at the Erlenhöhe station from May to October 2024.

The humidity remained relatively high throughout the entire study period, ranging from 31% to 100% at the Erlenhöhe weather station. Mean monthly humidity increased from 85% in May to 90% in September, with a slight recession in August (85%). The relative humidity measured by the weather stations at L1 and L3 is similar to the data measured by the Erlenhöhe station, and there are no clear differences between the two study sites.

In comparison to previous years, precipitation was relatively high. The monthly precipitation amounts recorded by MeteoSwiss in Einsiedeln in 2024 were higher than the mean monthly precipitation over the previous 10 years, except for August and February. Monthly mean temperatures in 2024 were higher from February to April, similar from May to July, and slightly lower in September compared to the 10-year average (MeteoSchweiz, 2025b).

5.1.2. Soil Moisture

The daily mean soil moisture contents per study site and day measured manually and by the soil moisture loggers show a clear temporal and spatial variability (Figure 18). At the same time, the volumetric water content (VWC) is relatively high at all sites, especially at greater soil depths. Throughout the entire study period, manual measurements indicate that L4 and L5 in the high elevation zone have the highest mean VWC of 0.42 and 0.45 m³/m³, respectively. L3, located at a medium elevation, is the driest site with a mean VWC of 0.26 m³/m³, while L1 and L2 in the low elevation zone have a moderate mean VWC of 0.32 m³/m³. The 5 cm deep soil moisture loggers measured a similar mean VWC of 0.36 m³/m³ at L2 and 0.21 m³/m³ at L3.

The temporal variability is visible in both the manual data and the logger data. The manual measurements show a decrease in August at all study sites, most pronounced at L4 and L5. The mean VWC in August across all sites is about 0.25 m³/m³, ranging from 0.21 m³/m³ at L3 to 0.28 m³/m³ at L5. Similarly, the mean monthly VWC measured by the logger at a depth of 5 cm decreased in August to 0.18 m³/m³ at L3. It can be concluded that VWC in the topsoil shows a similar spatial and temporal pattern when measured manually and by the loggers, with slightly lower values recorded by the loggers.

Furthermore, the soil moisture loggers indicate an increase in soil moisture and a decrease in variability with soil depth. At L2, the effect of precipitation events and higher temperatures is still visible at a depth of 15 cm. However, the VWC at a depth of 30 cm remains constant and relatively high throughout the entire study period, varying between 0.50 and 0.55 m³/m³. At L3, the VWC reacts to precipitation events and higher temperatures down to a depth of 30 cm. At the same time, the soil at depths of 15 and 30 cm remains relatively moist, with a mean monthly VWC in August of 0.31 and 0.27 m³/m³, respectively. The soil moisture logger at L1 did not record reliable data.

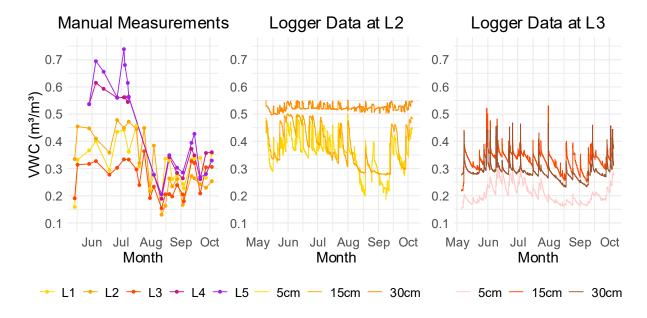


Figure 18: Soil moisture data of manual measurements (left) and soil moisture loggers (middle and right) at the study sites L1-L5 from May to October 2024.

5.1.3. Groundwater

The groundwater level varies spatially, with mean water levels below the surface ranging from 12.2 cm at L3.1 to 98.6 cm at L2 (Figure 19). However, there is no spatial correlation between the groundwater level and the soil moisture content. For example, L3 has the lowest soil moisture content but the highest groundwater levels, while the high elevation zone is characterised by low groundwater levels but high soil moisture contents. This can be explained by the differences in measurement locations for soil moisture and groundwater levels. Additionally, the groundwater level was measured at a specific location, typically outside of the study plots, while the soil

moisture data represents an average of five points across each study plot. Therefore, the high spatial heterogeneity in local topography leads to differences in the data. Groundwater levels can correlate with slope, curvature, and TWI (Rinderer et al., 2014) and are usually shallower in depressions (van Meerveld et al., 2018). Furthermore, the lower groundwater levels at L4 and L5 could be linked to the difference in soil type, as there was a reduced-oxidised horizon instead of a fully oxidised horizon, as at other study sites. Consequently, the soil moisture data is a more accurate representation of the water availability at the study sites.

No clear temporal trend in groundwater levels was detected, although shallow soil moisture decreased in August. This supports the observation that higher temperatures in summer have little to no impact on deeper soil moisture contents. Slightly lower groundwater levels were recorded at L1 and L2 in August. At L3.1 and L3.2, the groundwater levels decreased slightly from June to August. In the high elevation zone, there is little temporal variability in groundwater levels, indicating that there was not much water in the pipes. In contrast, groundwater levels at L1 and L2 react strongly to precipitation events, indicating that the wells are installed near a stream or at a wet location. At L1, the increased fluctuation could be partly explained by the difference in sensor technology, as a KELLER pressure data logger was used.

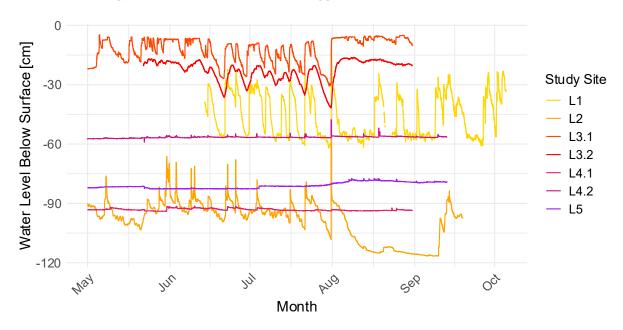


Figure 19: Spatial and temporal variability in groundwater levels measured near the study sites from May to October 2024. Data at L3.1, L3.2, L4.1 and L4.2 are shown, although they are at a greater distance from the study plots because there was no groundwater well close to L3 and L4.

5.2. Overview of Isotope Data

5.2.1. Dual Isotope Plots

The dual isotope plots show the isotopic composition of all water sources per sampling day relative to the LMWL ($\delta^2 H = 7.70 * \delta^{18} O + 9.61$) and its 95% confidence band. The isotope data is presented in the δ notation using the unit ‰ relative to the VSMOW. The opacity indicates the

sampling depth of the soil and lysimeter water (Figure 20). A dual isotope plot showing the differences between the study sites can be found in Appendix A.2. The LMWL was determined by applying linear regression to all ten precipitation samples, and the lc-excess was calculated using Equation 4.

The isotopic composition of the xylem water in the twigs (from here on referred to as twig water isotopes for brevity) plots above the LMWL, outside of the 95% confidence band of the LMWL, with a positive mean lc-excess of 10.3. The offset is not particularly high for any specific sampling day, study site, or tree. $\delta^2 H$ and $\delta^{18} O$ range from -76.1 to -51.0%, and from -13.4 to -8.73%, respectively. Contrarily, the isotopic composition of the xylem water in all stem cores (from here on referred to as stem core water isotopes for brevity) plots below the LMWL, outside of the 95% confidence band of the LMWL. The mean lc-excess is -10.4, and $\delta^2 H$ and $\delta^{18} O$ vary between -81.0 and -57.0%, and -9.42 and -6.36%, respectively. One stem core xylem water sample collected on 15.09.2024 is an outlier, as it is less depleted in heavy isotopes than all stem core xylem water samples taken on 03.10.2024.

The isotopic composition of bulk soil water (from here on referred to as soil water isotopes for brevity) has the broadest range from -114.0 to -20.3\% δ^2 H and from -15.3 to -3.81\% δ^{18} O. Most soil water isotopes plot slightly below the LMWL but remain within or close to the 95% confidence band, with a negative mean lc-excess of -3.54. No consistent depth trend is visible. In September and October, shallow soil water is more depleted in heavy isotopes than deep soil water. The isotopic composition of mobile soil water sampled using suction lysimeters (from here on referred to as lysimeter water isotopes for brevity) plots along the LMWL. The mean lc-excess is -0.70, and lysimeter water isotopes vary between -82.2 and -39.0% δ^2 H and -11.6 and -6.32% δ^{18} O. Shallow lysimeter water is more enriched in heavy isotopes, except for the last sampling day. In comparison to soil and lysimeter water isotopes, the isotopic composition of groundwater sampled in the Studibach catchment by Kiewiet et al. (2019) had a similar but narrower range, with $\delta^2 H$ and $\delta^{18} O$ between -91.1 and -47.1% and -12.9 and -7.6%, respectively. The data of Kiewiet et al. (2019) contains 34 to 47 wells that were sampled during nine campaigns between May and November 2016 and 2017. The isotopic composition of deep soil water and groundwater is often less variable, as deeper water is more mixed with older water sources (von Freyberg et al., 2020a).

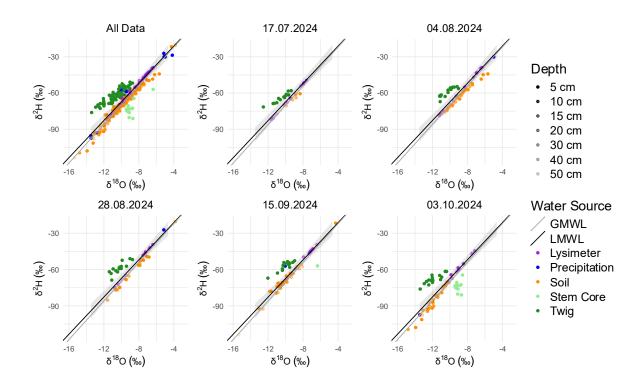


Figure 20: Dual isotope plots of all data and per sampling day with the LMWL ($\delta^2 H = 7.70 * \delta^{18} O + 9.61$) and its 95% confidence band. The opacity indicates the sampling depth.

The isotopic composition of precipitation fluctuates strongly between -97.4 and -27.2‰ δ^2 H and between -13.5 and -4.17‰ δ^{18} O. More negative values in October indicate snowfall and lower temperatures, while less negative values in August indicate higher temperatures. Two samples plot above the LMWL with an lc-excess of up to 10.6. This could be attributed to the natural variability of weather conditions and, consequently, varying source regions of the water vapour (Gat et al., 2001). Other LMWLs calculated for headwater catchments of the Alptal valley vary due to differences in sampling periods, locations, methods, and sample sizes, but all LMWLs plot close to the GMWL (see Appendix A.3). Evaporation from the precipitation collector is assumed to be minimal, as evaporation would lead to a negative d-excess.

The isotopic composition of precipitation is within the range of isotope data previously recorded in and near the Alptal. The closest GNIP station that is located at a comparable altitude of 1055 m a.s.l. and with sufficient data is the Guttannen station at a distance of 54 km (IAEA, 2025a). Monthly composite samples collected from June to October over a 10-year period (2014-2024) found δ^2 H ranging from -103.6 to -23.1‰ and δ^{18} O ranging from -14.4 to -4.32‰ (IAEA/WMO, 2025), which is similar to the isotopic composition of precipitation measured in this study. In the Alptal valley, Fischer et al. (2017a) analysed the isotopic composition of 13 precipitation events during the snow-free period of 2010 and 2011, which all plotted along the GMWL. δ^2 H varied between -153.7 and -17.3‰, and δ^{18} O varied between -20.4 and -2.36‰. Additionally, von Freyberg et al. (2022) collected daily isotope data of precipitation and stream water between 2015 and 2019. δ^2 H ranged from -194.55 to 4.65‰ and δ^{18} O ranged from -25.39 to -0.01‰, with precipitation water being more enriched in heavy isotopes in summer than in winter. It can be

concluded that the isotopic composition of precipitation in this study is within the range of data recorded previously in the Alptal. The smaller range can be explained by the smaller sample size and the shorter sampling period, combined with precipitation being less depleted in heavy isotopes during warmer summer months.

5.2.2. Spatial Variability

The isotopic composition of soil water and lysimeter water varies significantly between study sites as well as between elevation zones (Table 6) as indicated by a Kruskal-Wallis test. In contrast, no significant differences in twig water isotopes were observed between sites or elevation zones, which can be attributed to the small sample size of twigs per site and day (n=3). When examining soil water isotopes for each sampling day individually, significant differences between sites or elevation zones were detected on more than half of the sampling days. No tests were conducted for 17.07.2024, as bulk soil samples were only taken at L1, and for 28.08.2024, as there was only one deep bulk soil water sample. When testing lysimeter water isotopes for each sampling day, only three out of 30 tests were significant, which can be explained by the smaller sample size per site and day.

When testing for shallow and deep soil water isotopes and shallow and deep lysimeter water isotopes separately, significant differences were observed between study sites as well as between elevation zones. However, there are two exceptions: shallow soil water isotopes when testing for differences between sites and shallow lysimeter water isotopes when testing for differences between elevation zones. At the same time, the number of days on which differences between sites or elevation zones are significant varies only slightly between shallow and deep samples. The significance between sites and between elevation zones is similar, although the sample size per elevation zone is greater than that per site. Consequently, there must be significant differences between study sites that are classified within the same elevation zone, such as L1 and L2 or L4 and L5.

Table 6: Significance (p-value) of the differences between study sites and elevation zones of $\delta^2 H$ in soil, lysimeter, and twig water isotopes. *Number of sampling days with significant differences when tested individually / total number of sampling days tested individually.

	Differences between study sites			Differences between elevation zones		
	All samples	Shallow	Deep	All samples	Shallow	Deep
Soil	0.0031	0.0648	0.0437	0.0017	0.0328	0.0259
	(4/4*)	(3/4*)	(1/3*)	(3/4*)	(2/4*)	(1/3*)
Lysimeter	0.0005	0.0203	0.0112	0.0012	0.0889	0.0099
	(1/5*)	(0/5*)	(0/5*)	(2/5*)	(0/5*)	(0/5*)
Twig	0.8699			0.6430		
	(0/5*)			(0/5*)		

A comparison of the sites per sampling day (Figure 21) shows how the mean $\delta^2 H$ in soil water varies between the sites, while $\delta^2 H$ in lysimeter water shows a less clear pattern, and $\delta^2 H$ in twig xylem water does not vary significantly between the sites. $\delta^{18} O$ shows a similar pattern (see Appendix A.4), with the main difference being that the stem core xylem water is more enriched in $\delta^{18} O$ but more depleted in $\delta^2 H$ than twig xylem water.

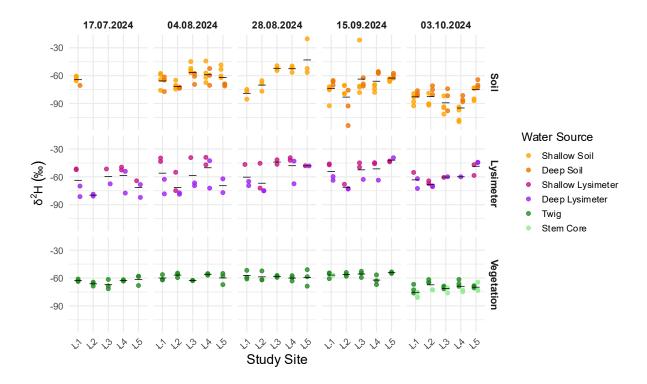


Figure 21: Difference in soil water, lysimeter water, and tree xylem water $\delta^2 H$ between the study sites per sampling day. The horizontal black lines represent the mean values of each water source per day and study site.

Altitude and $\delta^2 H$ or $\delta^{18} O$ in soil water, lysimeter water, and twig xylem water correlate significantly on several sampling days (Table 7), as shown using a Pearson correlation test. The strongest elevation gradient of +13.7% $\delta^2 H$ and +1.69% $\delta^{18} O$ per 100m elevation is observed in soil water isotopes on 28.08.2024. The elevation gradient of soil and lysimeter water isotopes on other sampling days is weaker but consistently positive, if significant, indicating that water is enriched in heavy isotopes at higher altitudes. Twig water isotopes correlate significantly with altitude on 17.07.2024 and 15.09.2024, with an elevation gradient of +1.50 and -1.12% $\delta^2 H$ per 100 m, respectively.

Table 7: Change in $\delta^2 H / \delta^{18}O$ (‰) per 100 m elevation in soil water, lysimeter water, and twig xylem water, with n.sig. indicating non-significant correlations.

	17.07.2024	04.08.2024	28.08.2024	15.09.2024	03.10.2024
Soil	no data	+3.59 / +0.53	+13.7 / +1.69	+6.67 / +0.76	n.sig. / n.sig.
Lysimeter	n.sig. / n.sig	n.sig. / n.sig	n.sig. / +0.98	+7.33 / +0.88	+8.19 / +0.95

Twig	+1.50 / n.sig.	n.sig. / n.sig.	n.sig. / n.sig.	-1.12 / -0.51	n.sig. / n.sig.
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There is no consistent correlation between soil water isotopes and study site characteristics such as slope, aspect, TWI, and tree cover when testing individual days. At the same time, soil water isotopes correlate strongly with aspect and tree cover on two days and with slope on one of four sampling days. Consequently, soil water isotopes correlate negatively with aspect and positively with tree cover when testing over the entire dataset. Nevertheless, more data would be necessary to draw conclusions about these correlations due to the lack of correlation on individual sampling days, combined with the limited number of study sites and the low sample size. Pearson correlation tests were performed, and tests with $\delta^2 H$ and $\delta^{18} O$ had similar results (Appendix B.1).

Table 8: Correlation (p-value) between δ^2H in soil water and study site characteristics for each sampling day and over the entire dataset. The sample sizes are 15 on 28.08.2024 and 35 on 04.08.2024, 15.09.2024, and 03.10.2024. *The correlation between the two variables if the correlation is significant.

	04.08.2024	28.08.2024	15.09.2024	03.10.2024	All data
Mean slope (°)	0.205	0.758	0.921	0.014	0.74
				(-0.413*)	
Mean aspect (°)	0.059	0.015	0.018	0.828	0.01
		(-0.613*)	(-0.397*)		(-0.23*)
Mean TWI	0.003	0.252	0.064	0.000	0.31
	(-0.488*)			(0.561*)	
Tree cover (%)	0.334	0.001	0.134	0.453	0.02
		(+0.763*)			(+0.22*)

5.2.3. Temporal Variability

The temporal variation in δ^2H shows an enrichment of heavy isotopes until August, followed by a depletion of heavy isotopes until October (Figure 22). $\delta^{18}O$ shows a similar pattern (Appendix A.5). The temporal variability in soil and twig water isotopes is more pronounced than in lysimeter water isotopes. On 28.08.2024, soil water is more enriched, with means of -59.4‰ δ^2H and -8.40‰ $\delta^{18}O$, while on 03.10.2024, soil water is more depleted with means of -84.9‰ δ^2H and -11.8‰ $\delta^{18}O$. The mean δ^2H in twig water varies between -57.0‰ on 15.09.2024 and -68.1‰ on 03.10.2024. A clear temporal trend in twig water isotopes is visible due to the limited spatial variability. Mean lysimeter water isotopes vary less over time, with the mean δ^2H ranging from -54.3‰ on 15.09.2024 to -65.5‰ on 17.07.2024. δ^2H in precipitation water fluctuates strongly between -27.2 and -97.4‰, possibly due to early snowfall in mid-September and changes in weather conditions affecting the origin of the water vapour (Gat et al., 2001). However, the isotopic composition of precipitation is generally less depleted in August than in September and October.

In early summer, soil water tends to become more depleted in heavy isotopes with depth, but this trend reverses in September and October. Deeper lysimeter water is typically more depleted, with no clear trend in October. A gap between shallower and deeper lysimeter water isotopes is visible except for 03.10.2024.

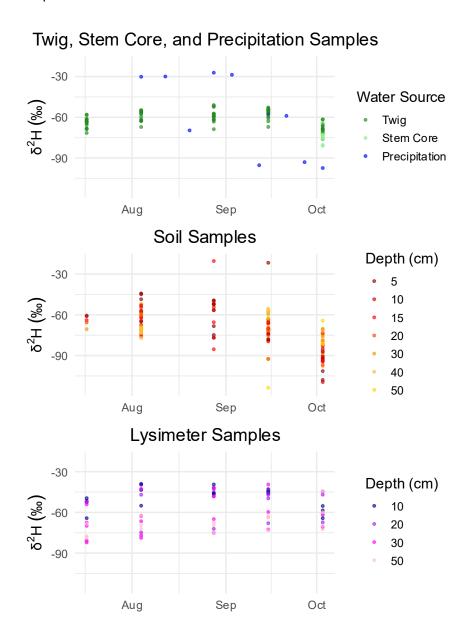


Figure 22: Temporal variability in $\delta^2 H$ of different water sources and soil depths.

5.2.4. Variability with Soil Depth

The isotopic composition of lysimeter water and soil water fluctuates less at greater soil depths (Figure 23) as these are more mixed than shallower soil depths. $\delta^2 H$ and $\delta^{18} O$ show similar trends (Appendix A.6). At L1, L2, and L4, shallow lysimeter water is less depleted in $\delta^2 H$ than shallow soil water. At L3 and L4, the temporal variability in shallow soil water isotopes is the highest, with soil water being the most depleted isotope in September and October.

The depth profile of soil water isotopes is not generally more pronounced than that of lysimeter water isotopes. However, it must be considered that the lysimeter sample size is relatively small, especially when looking at shallow and deep lysimeter samples separately. Additionally, lysimeter samples were collected at only one location per site, unlike bulk soil water samples, which represent an average of three profiles at each study site. Consequently, the larger sample size of bulk soil water compared to lysimeter water leads to a lower uncertainty when calculating the source contribution based on soil rather than lysimeter water isotopes.

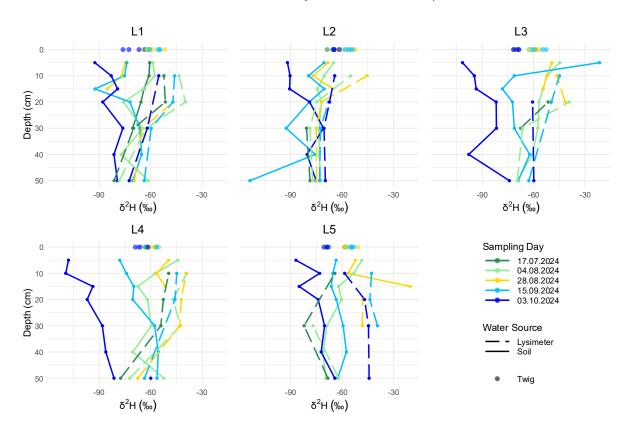


Figure 23: Depth profiles of $\delta^2 H$ in soil water and lysimeter water per study site and sampling day.

5.3. Vegetation Water Isotopes

On 03.10.2024, twig water isotopes have a positive lc-excess of 13.7, while stem core water isotopes have a negative lc-excess of -13.9 (Figure 24). δ^2 H in twig and stem core xylem water have similar mean values of -68.1 and -74.0% and similar ranges of 14.5 and 16.5%, respectively. Furthermore, δ^2 H in both twig and stem core xylem water is most depleted at L1. Contrarily, twig xylem water is significantly more depleted in δ^{18} O, ranging from -13.4 to -10.4%, while δ^{18} O in stem cores has a smaller range between -9.42 and -8.56%. No relationship is visible between δ^{18} O in twig xylem water and stem core xylem water.

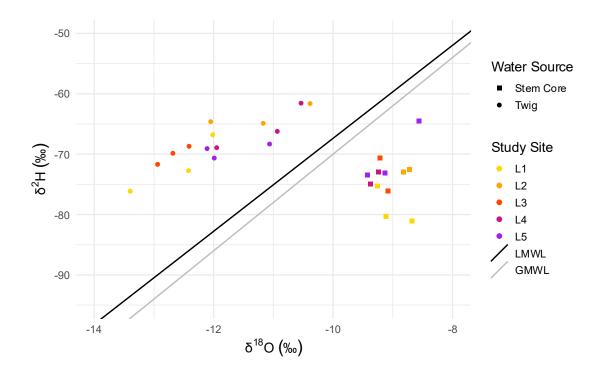


Figure 24: Dual isotope plot of water isotopes in twig xylem and stem core xylem on 03.10.2024 relative to the LMWL and the GMWL.

There is no correlation between twig water isotopes and the characteristics of trees or study sites in the entire dataset, nor is there a consistent correlation when testing individual sampling days (Table 9). More specifically, there is no correlation between $\delta^2 H$ in twig xylem water and tree age, tree circumference, mean aspect, or tree cover over the entire dataset or on individual sampling dates. Two exceptions are the mean slope and mean TWI, which correlate with $\delta^2 H$ in twig xylem water on one of the five sampling days (Appendix A.8). The same correlations exist when testing against $\delta^{18} O$ in twig xylem water, except for the mean aspect being additionally significant (p=0.012) on 15.09.2024 (Appendix B.2).

Table 9: Correlation (p-value) between δ^2H in twig xylem water and tree and study site characteristics for each sampling day and over the entire dataset. The sample size is 15 on all sampling days. *The correlation between the two variables if the correlation is significant.

	17.07.2024	04.08.2024	28.08.2024	15.09.2024	03.10.2024	All
						data
Age	0.371	0.616	0.546	0.971	0.243	0.99
Circumference	0.067	0.341	0.877	0.816	0.770	0.28
Mean slope (°)	0.041	0.570	0.958	0.726	0.558	0.50
	(-0.533*)					
Mean aspect (°)	0.089	0.594	0.569	0.332	0.957	0.90
Mean TWI	0.935	0.959	0.798	0.046	0.960	0.47
				(+0.523*)		
Tree cover (%)	0.751	0.797	0.539	0.744	0.299	0.70

5.4. Comparison of Vegetation and Soil Water Isotopes

As demonstrated in the dual isotope plots, twig water isotopes have a positive lc-excess, stem core water isotopes have a negative lc-excess, and soil water isotopes have a relatively low but negative lc-excess. Therefore, lysimeter water is generally less depleted in δ^2H than twig water, while soil water typically has a similar δ^2H to or is even more depleted in δ^2H than twig xylem water (Figure 25).

Although soil water isotopes plot along the LMWL, their range is the largest. Lysimeter water isotopes have a moderate range, and twig and stem core water isotopes have a relatively small range. Therefore, a comparison of these water sources per sampling day indicates where a shallower or a deeper WUD is expected (Figure 25). $\delta^2 H$ in twig xylem water is generally more similar to $\delta^2 H$ in deep than in shallow lysimeter water, except for 03.10.2024. At L2, the mean twig xylem water $\delta^2 H$ is constantly higher than the mean $\delta^2 H$ of other water sources. This is unexpected and indicates an additional unsampled water source or measurement errors. $\delta^{18} O$ shows a similar pattern to $\delta^2 H$, except for the stem cores xylem water being more enriched in $\delta^{18} O$ than other water sources (Appendix A.7).

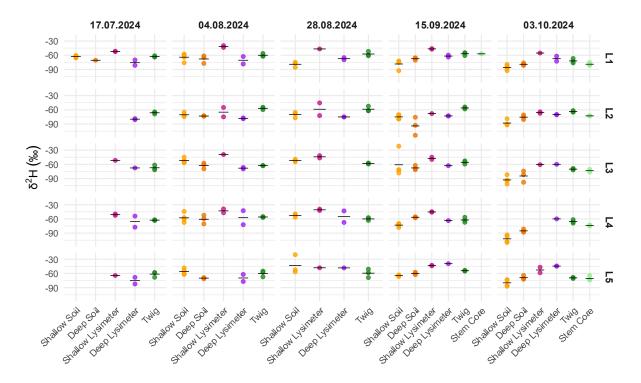


Figure 25: $\delta^2 H$ in stem core xylem water, twig xylem water, shallow and deep soil water, and shallow and deep lysimeter water per sampling day and study site L1-L5.

Significant differences between all water sources over the entire dataset (p=2.97*10⁻¹⁶) as well as on each sampling day were shown by a Kruskal-Wallis test (Appendix B.3). Furthermore, significant differences between $\delta^2 H$ in shallow and deep soil water were detected on one of three sampling days at each elevation zone (Table 10). Between $\delta^2 H$ in shallow and deep lysimeter

water, significant differences were only detected on 04.08.2024 in the low elevation zone (Table 11). On 28.08.2024, no test was performed using lysimeter water isotopes at medium elevation due to insufficient sample size. These differences between shallow and deep soil or lysimeter water isotopes per elevation zone are relevant, as these groups are used as input data in the mixing model. The absence of significant differences between the groups causes model uncertainty.

Table 10: Significance (p-values) of differences between $\delta^2 H$ in shallow and deep soil water when testing for each sampling day and elevation level separately.

Shallow vs. deep soil	Low elevation	Medium elevation	High elevation
04.08.2024	0.366	0.034	0.070
15.09.2024	0.699	0.289	0.002
03.10.2024	0.020	0.289	0.053

Table 11: Significance (p-values) of differences between $\delta^2 H$ in shallow and deep lysimeter water when testing for each sampling day and elevation level separately.

Shallow vs. deep lysimeter	Low elevation	Medium elevation	High elevation
17.07.2024	0.064	0.317	0.077
04.08.2024	0.043	0.221	0.165
28.08.2024	0.157	NA	0.127
15.09.2024	0.157	0.221	1
03.10.2024	0.157	0.317	0.564

5.5. MixSIAR Results

The contribution of shallow and deep bulk soil water to tree water uptake varies spatially and temporally, and the water uptake is typically a mixture of both water sources. One exception is at the medium elevation zone on 04.08.2024 and 15.06.2024, when the contribution of deep soil water is significantly higher than that of shallow soil water. However, the uncertainty in the model results is relatively high, as indicated by the error bar (Figure 26). On 03.10.2024, the model results based on twig water isotopes as mixture input data indicate a shallow water source, while the results based on stem core water isotopes as mixture input data indicate a deep water source. Consequently, the uncertainty caused by the offset of twig and stem core water isotopes from the LMWL must be considered when making conclusions.

The WUD varies over time, but no clear trend can be observed across the elevation zones. Consequently, the effects of temporal changes in soil moisture availability are not visible in the model results. Furthermore, the WUD varies between the three elevation zones, but these differences do not follow a consistent trend throughout the sampling period. Therefore, no

impacts of local soil moisture conditions on the WUD were detected. Although soil moisture is the highest in the high elevation zone and the lowest in the medium elevation zone, no consistent trend in WUD between the elevation zones is visible.

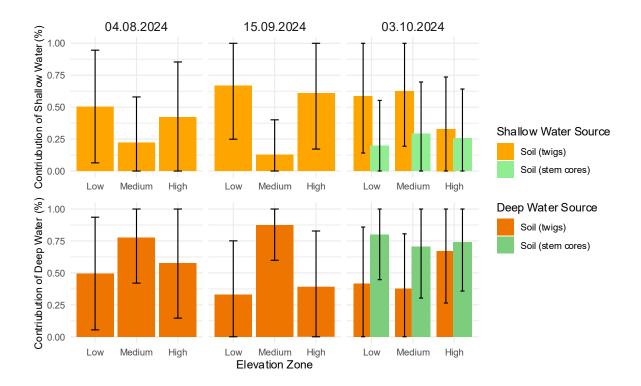


Figure 26: Contribution of shallow and deep bulk soil water to the water uptake of the trees. The box indicates the mean contribution, and the error bar indicates the standard deviation. Soil (twigs) shows the MixSIAR model output if twig water isotopes are used as mixture input data, soil (cores) shows the model output if the stem core water isotopes are used as mixture input data.

When using lysimeter water isotopes as source input data (Figure 27), the model results generally indicate a higher contribution of deep than shallow water to tree water uptake. The only exceptions are in the high elevation zone on 17.07.2024 and 03.10.2024, where the contribution of deep water is higher than that of shallow water. These results differ from those based on soil water isotopes, which generally indicate a shallower WUD. Nevertheless, uncertainties due to the low lysimeter sample size and the lack of significant differences between isotopes of shallow and deep water must be considered.

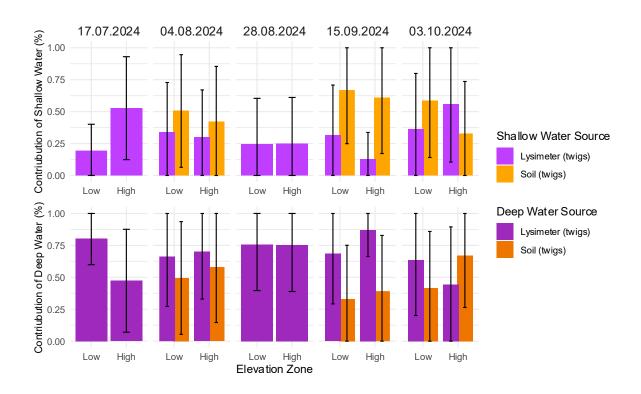


Figure 27: Contribution of shallow and deep bulk soil water and lysimeter water to the water uptake of the trees. The box indicates the computed mean contribution, and the error bar shows one standard deviation.

6. Discussion

6.1. Spatial and Temporal Variability of Isotope Data

6.1.1. Temporal Variability

In August, soil water and twig xylem water are less depleted in heavy isotopes than in colder months. Lysimeter water isotopes show a less pronounced trend. An enrichment of heavy isotopes at higher temperatures is expected due to evaporation affecting water at the soil surface, water vapour in the atmosphere, and water drops of precipitation (Dansgaard, 1964). This effect is not clearly visible in the isotopic composition of precipitation, which is more enriched in July and August than in September and October but fluctuates strongly with short-term weather conditions. The origin and amount of precipitation during individual events vary, which affects the isotopic composition of precipitation (Fischer et al., 2017b). The low correlation between the isotopic composition of precipitation and temperature is attributed to the limited temporal resolution, as only 10 precipitation samples were collected.

These observations agree with isotope data collected previously in the Alptal, where the isotopic composition of precipitation was more enriched in summer than in winter (Fischer et al., 2017a) and shallow groundwater was more isotopically enriched in late summer than in early summer (Kiewiet et al., 2019). Other studies in Switzerland and the Alpine region have identified similar

temporal patterns. For example, Floriancic et al. (2024) found that precipitation, bulk soil water, and mobile soil water were more depleted in heavy isotopes in winter than in summer in a mixed spruce and beech forest in Zurich. Bertrand et al. (2014) observed that tree and soil water samples in the Swiss Pfyn forest were more enriched in heavy isotopes during the warmer months. Moreover, Penna et al. (2013) measured isotopically less depleted soil water in summer and autumn due to changes in the isotopic composition of precipitation in the pre-Alps. Furthermore, precipitation in the eastern Italian Alps was more depleted in spring and autumn than in summer because of lower temperatures and snowfall (Brighenti et al., 2024). This seasonality in the isotopic composition of precipitation was observed at various measurement stations throughout Switzerland, with an average annual amplitude of about 10.6% δ^{18} O at the alpine Grimsel station. In monthly composite data from the Grimsel, δ^{18} O increased by 0.71‰ per 1°C temperature increase (Schürch et al., 2003). With this gradient, the 7°C difference in mean monthly temperature between August and September would cause a difference in $\delta^{18}O$ of 4.97‰. Since the isotopic composition in precipitation fluctuates strongly, the change in δ^{18} O per 1°C temperature increase cannot be precisely determined. However, looking at the temporal variability in δ^{18} O (Appendix A.5), where δ^{18} O fluctuates between -13.5 and -4.17‰, the mean δ^{18} O values of August and September may correspond to a difference of about 4.97%.

The temporal variability in soil water $\delta^2 H$ corresponds to previous groundwater isotope data collected in the Studibach. Mean monthly soil water $\delta^2 H$ varies between -61.9‰ in August and -84.9‰ in October, while Kiewiet et al. (2019) measured $\delta^2 H$ in shallow groundwater to vary between -47.1 and -91.1‰ from May to September. Furthermore, Kiewiet et al. (2019) found temporal and spatial variability to be similar, which corresponds to the results of this study. As observed by Penna et al. (2013) in the Italian pre-Alps, the temporal variability in soil water isotopes is mainly caused by changes in the isotopic composition of precipitation inputs. Shallow soil water isotopes show little to no evaporation effect at the soil surface. This can be attributed to the relatively high soil moisture, even in August. Under wet conditions, an evaporation effect may not be visible even in shallow soil water (Rossatto et al., 2011).

Nevertheless, the temporal variability of water isotopes is higher in shallow than in deep soil water. Shallow soil water isotopes react more to precipitation events, while deep soil water isotopes are a mixture of various water sources and fluctuate less over time. For example, in a sampling campaign across Europe, δ^{18} O in soil water was higher in summer than in spring. At the same time, no significant differences in deeper soil water isotopes were observed between summer and spring (Lehmann et al., 2024). Shallow lysimeter water is slightly isotopically enriched in summer, while the temporal variability in shallow soil water isotopes mainly results from a depletion in heavy isotopes in September and October. This can be attributed to changes in the isotopic composition of precipitation resulting from lower temperatures and snowfall in autumn. Other studies observed the same patterns. For example, Bertrand et al. (2014) found shallow soil water in the Swiss Pfyn forest to be more depleted in heavy isotopes in winter than in summer, whereas deeper soil water isotopes were more constant. This pattern was attributed to

shallow soil water being more susceptible to recent precipitation and evaporation (Bertrand et al., 2014). In shallow, clay loam soils in New Mexico, Grossiord et al. (2017) found that shallow soil water was less depleted in heavy isotopes than in deep soil water on most sampling days. At the same time, shallow soil water being more depleted in spring was explained by the infiltration of precipitation that was depleted in heavy isotopes. These findings further support the observation that soil water isotopes primarily vary with the isotopic composition of precipitation but only slightly due to evaporation from the soil surface.

The temporal variability is the highest for soil water isotopes, slightly lower for twig water isotopes, and the lowest for lysimeter water isotopes. It is expected that the isotopic composition of soil water varies more than that of twig xylem water. Trees typically use a mixture of shallow and deep soil water, and shallow soil water isotopes fluctuate more than deep soil water isotopes. For example, Welp et al. (2008) found a moderate temporal variability in the isotopic composition of precipitation and soil water but only little variability in xylem water isotopes over the summer. In the Swiss Pfyn forest, the isotopic composition of tree xylem water also varied less than that of soil water and precipitation (Bertrand et al., 2014). In a mixed spruce and beech forest in Zurich, Floriancic et al. (2024) found a higher temporal variability in the isotopic composition of precipitation than that of soil water, while vegetation samples were not enriched in heavy isotopes over summer. It can be concluded that the isotopic composition of soil water usually varies more than the isotopic composition of vegetation water, which is consistent with the findings of this thesis.

The temporal variability is less pronounced for lysimeter water isotopes than for soil and twig water isotopes. Unlike shallow soil water, shallow lysimeter water is not depleted in heavy isotopes after snowfall in September and October, which is unexpected. Contrarily, Floriancic et al. (2024) observed a similar temporal variability in mobile and bulk soil water isotopes, with the isotopic composition of precipitation varying more and the isotopic composition of vegetation water varying less. Furthermore, Sprenger et al. (2018) found no significant differences between mobile and bulk soil water isotopes at a depth of 10 cm between May and September. However, mobile soil water isotopes reacted even more than bulk soil water isotopes to recent precipitation events at some study sites (Sprenger et al., 2018), which contradicts the findings of this thesis. One explanation for the stronger impact of snowfall on bulk soil water isotopes than mobile soil water isotopes is the difference in the sampling period. Bulk soil water could contain more water from snowfall events, while mobile soil water consists of precipitation from previous warmer days and water collected over several days. The differences between lysimeter and soil water isotopes caused by the water extraction method are discussed in more detail in Chapter 6.4.

6.1.2. Spatial Variability

There are significant differences in soil as well as in lysimeter water isotopes between study sites and elevation zones, although not on all sampling days when tested separately. This can be attributed to the small sample size per site per day. When testing for differences between shallow and deep water isotopes separately, the shallow soil water isotopes did not vary significantly

between sites, and the shallow lysimeter water isotopes did not vary significantly between elevation zones. The higher significance of differences between deep water isotopes can be explained by the isotopic composition varying more near the surface, which causes less consistent differences between the sites.

The correlation between soil water isotopes and site characteristics is limited; no consistent correlation between soil water isotopes and slope, aspect, TWI, and tree cover could be detected. However, soil water isotopes correlate with aspect and tree cover when testing over the entire dataset. This can be explained by the high spatial heterogeneity within the catchment and the numerous variables that affect the isotopic composition of the soil. For example, aspect and tree cover vary with elevation, which correlates with soil water isotopes (see Appendix A.8).

Furthermore, the tree cover potentially correlates with canopy cover, which can affect soil water isotopes. Von Freyberg et al. (2020b) found a significantly lower snow depth in the forest compared to grassland sites in the Alptal. At the same time, snow is typically more enriched in heavy isotopes with a higher canopy density due to the sublimation of intercepted snow in throughfall (Koeniger et al., 2008; von Freyberg et al., 2020b). Similarly, shallow soil water isotopes at L3 and L4 show the greatest temporal variation, with shallow soil water being most depleted in heavy isotopes in September and October. Consequently, it can be hypothesised that these sites are most affected by snowfall because they are located in the open forest, while all other sites are within a denser forest stand. Although the diameter measured at breast height could predict the leaf surface area of adult Norway spruce trees (Pokorný & Tomášková, 2007), the relatively small size of the study plots and the wide range of tree ages, sizes, and health conditions may lead to a low correlation between canopy cover and tree diameter. Furthermore, other variables such as the tree positions within the forest stand (Pokorný & Tomášková, 2007) can affect this correlation. It can be concluded that the correlation between soil water isotopes and tree cover may not be consistent but could indicate an effect of canopy cover.

There is no visible effect of soil moisture on soil water isotopes and lysimeter water isotopes. While there are differences in soil moisture as well as in soil water isotopes between the elevation zones, the isotope values at the driest study sites are not continuously lower or higher than those at the wettest sites. However, Kiewiet et al. (2019) found that drier sites in the Studibach catchment might be more affected by snowmelt and recent precipitation, and Sprenger et al. (2018) measured larger differences between bulk and mobile soil water isotopes under drier conditions. The generally high soil moisture across all study sites during the sampling period could explain why no effect of soil moisture was observed. Yang et al. (2016) found that soil moisture varied with soil storage capacity and transpiration, while δ^2H changed with precipitation inputs, and both variables were affected by topography, soil properties, and surface cover. Therefore, the relatively wet conditions combined with high spatial heterogeneity and various factors affecting the isotopic composition of soil water could lead to no significant correlation between soil moisture and soil water isotopes.

The isotopic composition of soil and lysimeter water varies significantly between elevation zones, with a significant elevation gradient in soil and lysimeter water isotopes on certain sampling days. The altitude difference between the lowest and the highest study site was 187 m. Unexpectedly, significant elevation gradients for both soil and lysimeter water isotopes were positive, with increases of up to 13.7% δ^2 H and 1.69% δ^{18} O per 100 m, respectively. This is unexpected as precipitation is typically more isotopically enriched at lower elevations (Gat et al., 2001). In the alpine region, δ^{18} O of precipitation samples decreased by about 0.2% per 100 m elevation on average (Schürch et al., 2003). Although changes in the isotopic composition of precipitation is expected to be the main cause of temporal variability in soil water isotopes, an elevation effect is not visible. This relationship between elevation and isotope data can be explained by the spatial variability of other variables, such as transpiration, throughfall, or evaporation (Sprenger et al., 2016) and the mixing of water sources in the soil (von Freyberg et al., 2020a).

On a smaller scale, the isotopic composition of precipitation does not necessarily show an elevation effect due to the combination of topography, atmospheric moisture transport, and rainout processes. Fischer et al. (2017b) detected no correlation between the isotopic composition of precipitation and elevation, rainfall intensity, or rainfall amount in a headwater catchment in the Alptal due to the complex interaction between atmospheric circulation and topography. Other studies in mountainous catchments have confirmed that an elevation effect may not be visible in the isotopic composition of precipitation due to atmospheric moisture transport (Koeniger et al., 2008) and effects of atmospheric conditions, topography and rainout processes (Schürch et al., 2003). Furthermore, a positive elevation gradient of 0.12% δ^{18} O per 100 m elevation in mountainous catchments can be explained by atmospheric moisture processes such as sub-cloud evaporation (Kong & Pang, 2016).

The elevation effect is well visible in large-scale datasets, such as precipitation sampled throughout Switzerland (Schürch et al., 2003), across Europe (Lehmann et al., 2024), or globally (Bowen & Wilkinson, 2002). Contrarily, in small headwater catchments like the Studibach or the Zwäckentobel (Fischer et al., 2017b), other factors might affect spatial variability more than elevation. It can be concluded that the elevation effect on the isotopic composition of precipitation is often well visible in large-scale datasets but not on a smaller scale. Additionally, various variables can lead to differences between the isotopic composition of precipitation and soil water. This finding is supported by the observation that the correlation of soil water isotopes with elevation zones and study sites is similar, although the sample size per elevation zone is higher than that per site.

6.2. Tree Water Uptake Depth

6.2.1. Model Results

The model results indicate a combination of shallow and deep soil water uptake but with a high uncertainty. A mixed or shallow WUD was suggested by previous research. Floriancic et al. (2024) found that spruce trees in Zurich used a mixture of shallow and deep soil water, while they mainly

relied on water stored between depths of 10 and 40 cm. Other studies found shallower WUDs. For example, Norway spruce trees in a podzol soil with a 50 cm deep water table in northern Sweden had mean WUDs between the mor layer and a soil depth of 5 cm (Bishop & Dambrine, 1995). Norway spruce trees in a mixed stand in southern Germany had a mean WUD of 10.8 cm on a wet day in July (Goisser et al., 2016). In contrast, Grossiord et al. (2014) calculated a mean WUD of 40 to 50 cm for Norway spruce trees in a young, mixed, temperate plantation in Germany. In the Italian pre-Alps, beech trees used soil water rather than recent precipitation or groundwater (Penna et al., 2013). However, spruce trees typically have a shallower WUD than other tree species such as Scots pine (Bishop & Dambrine, 1995) or beech (Floriancic et al., 2024; Goisser et al., 2016).

In the relatively wet Studibach catchment, it was expected that trees primarily use shallower soil water consisting of summer precipitation. Diao et al. (2025) found that between May and September, spruce trees used summer precipitation at three forest sites in Switzerland. In contrast, beech and spruce trees in Zurich mainly used winter precipitation even during summer, which was explained by a lack of precipitation reaching the lower soil depths during summer (Floriancic et al., 2024). Across Switzerland, Allen et al. (2019) found that trees mainly used winter precipitation, while spruce trees in high-precipitation regions depended on summer precipitation in summer. Consequently, these contradictory findings can be explained by differences in moisture conditions, with summer precipitation playing a larger role at wetter sites.

The comparison of the water sources per sampling day and site is consistent with the model results. Study sites and days where the twig water isotopes are similar to either deep or shallow soil water isotopes are visible in the model output. For example, at medium elevation (L3) on 04.08.2024, twig water isotopes resemble deep soil water isotopes, resulting in a high contribution of deep soil water computed by the model. Furthermore, δ^2H in twig xylem water is typically more similar to δ^2H in deep than shallow lysimeter water, which is consistent with the model output.

6.2.2. Model Uncertainties

The modelled probability distribution is relatively flat for some model runs, resulting in wide error bars. Consequently, the high model uncertainty must be considered, and the actual WUD may differ from the mean contribution, which typically indicates a combination of shallow and deep soil water (Moore & Semmens, 2008). Several factors contribute to the high uncertainty in the model output. Little uncertainty is caused by the isotope measurements as they have an accuracy of +/- 0.16% in δ^{18} O and +/- 0.6% in δ^{2} H according to manufacturer specifications. Generally, the results of mixing models have a low reliability if the differences between the water sources are smaller than the uncertainties caused by the water extraction, isotope measurements, or modelling (Stock et al., 2018; von Freyberg et al., 2020a).

Firstly, the similarity between shallow and deep water causes uncertainty. Significant differences between shallow and deep soil water $\delta^2 H$ were detected in only one-third of the combinations

when tested by elevation zone and sampling day. Between shallow and deep lysimeter water $\delta^2 H$, few significant differences were identified when tested by elevation zone and sampling day. Therefore, the results based on lysimeter water isotopes are not reliable. Deeper, well-mixed soil water sources can be especially difficult to distinguish (Bishop & Dambrine, 1995). Dry conditions typically lead to a more distinct soil profile due to evaporation at the surface (von Freyberg et al., 2020a), while heavy precipitation events can lead to a less distinct soil profile (Brinkmann et al., 2019). Consequently, the wet conditions leading to a less distinct soil isotope profile may be a key factor causing model uncertainties. To reduce the standard error per water source, the sample size could be increased, and replicates could be taken (Bernhard et al., 2024). Soil samples were taken as a mixture of three profiles to represent an average across the study sites. If the isotopic composition throughout the three profiles was analysed separately, soil sources might be easier to distinguish, and outliers might become visible. Other options to better distinguish the water sources would be to include additional isotopes in the analysis (Scandellari et al., 2024) or to use labelling, such as adding highly deuterium-enriched water (Grossiord et al., 2014).

Secondly, the unexpected positive Ic-excess of twigs causes high model uncertainty (for potential explanations see Chapter 6.3). There were no replicates per tree, but each sample consisted of several twigs. However, increasing the number of sampled twigs could further reduce the uncertainty caused by differences between branches (von Freyberg et al., 2020a). Furthermore, within-tree variability contributes to uncertainty. The model results vary significantly when twig or core samples are used as input data, indicating a high level of uncertainty caused by the choice of tree sampling method. Additionally, potential time lags between root water uptake and twig water, which depend on the sap-flow velocity (Bernhard et al., 2024), were not considered despite their impact on the isotopic composition of xylem water. Time lags due to the transportation of water from the roots to the twigs are neglected by mixing models if the soil samples used as source input data are collected at the same time as the twig samples that are used as mixture data (von Freyberg et al., 2020a). Therefore, the model results are based on the assumption of a relatively low temporal variability in soil water isotopes in comparison to the time lag of tree water uptake and transportation into twigs (Takahashi, 1998).

Thirdly, averaging the soil samples of three profiles as well as across a range of depths, can lead to uncertainty. It is common practice to aggregate soil depths with similar isotopic compositions into a water source, for example Bello et al. (2019), and the sample size and standard deviation are considered by the MixSIAR model (Stock et al., 2018). However, outliers could affect the mean values, causing erroneous results if the sample size is small. However, no strong outliers are visible in the depth profiles. This can be explained by the mixing of three soil profiles, which again could hide outliers. Consequently, the aggregation of different soil depths affects the mean isotope values and increases the standard error in addition to the uncertainty caused by mixing three soil profiles of unknown isotopic composition. Furthermore, the three sampled trees at each study site were combined into one mixture data point. In contrast to source input data, the sample size and standard deviation of the mixture data are not accounted for by MixSIAR. The variability

in the isotopic composition of different trees within a stand is often unknown (Bernhard et al., 2024) and should be considered (von Freyberg et al., 2020a). Although there are no strong outliers in twig water isotopes, small outliers affected the isotopic composition and caused uncertainty in the model output.

Lastly, a cryogenic water extraction bias could affect the model results. For clayey loam soil with a water content of 20%, a mean bias of about -10% δ^2 H and about -0.5% δ^{18} O was detected (Orlowski et al., 2018). However, the cryogenic extraction results of soil water may differ between laboratories (Orlowski et al., 2018), and the bias depends on the extraction procedure and soil type (Allen & Kirchner, 2022). Therefore, it is essential to discuss which water is extracted depending on the extraction temperature, pressure, duration, and soil type, as well as whether plants use more or less mobile water (Orlowski et al., 2013). In a review, cryogenic water extraction of vegetation samples led to an average bias of -6.1 \pm 3.4% δ^2 H, while a bias in δ^{18} O was negligible or not measurable (Allen & Kirchner, 2022). Similarly, Chen et al. (2020) measured very small or no significant δ^{18} O offsets in stem water compared to soil water, but a mean offset of -8.1% δ^2 H ranging from -10.9% to -5.2% δ^2 H. This offset was explained by an exchange between stem organic hydrogen and xylem water (Chen et al., 2020). Contrarily, Diao et al. (2022) found that hydrogen exchange within stem water pools had little impact and explained the enrichment by a fractionation effect of sublimation and evaporation and mixing with water vapour in the laboratory. Consequently, extracting more than 0.6 mL was recommended (Diao et al., 2022). For all twig and soil samples, more than 1 mL of water was extracted, while at least 0.7 mL of water was extracted from stem core samples. Nevertheless, multiple studies might have come to different conclusions regarding the WUD if the xylem water isotope data had been corrected for a cryogenic extraction bias, especially if the differences between the water sources were not large (Allen & Kirchner, 2022), as in this study. Cryogenic vacuum extraction is currently the most commonly used method for water extraction, but alternative methods are being developed and analysed, for example by Kocum et al. (2024).

6.2.3. Model Assumptions

The model results are based on several assumptions that must be considered during evaluation. Firstly, mixing models assume that all possible plant water sources were included as input variables (Stock et al., 2018). Soil depths ranging from 5 to 50 cm were sampled, considering that the sampling depth should reach the groundwater table or the maximum rooting depth (Ceperley et al., 2024). The groundwater table is generally shallow in the Studibach catchment (Kiewiet et al., 2019). However, the groundwater level was deeper than 50 cm in the highest elevation zone and at L2, with the lowest mean depth of 98.6 cm at L2. Consequently, deeper roots and uptake of deeper soil water cannot be excluded at these study sites. At the same time, Norway spruce trees typically have shallow roots (Schmid & Kazda, 2002). In a mixed forest in southern Germany, Norway spruce trees had root tips at a mean depth of 18.9 cm, with a higher fine root surface area in the shallow soil (Goisser et al., 2016). In the central Alps, Norway spruce trees had most of their fine roots in the upper 50 cm of the soil, with only little water uptake from lower depths (Walthert

et al., 2024). Additionally, various factors, such as soil type and local topography, affect the rooting depth (Fan et al., 2017), and deeper roots of Norway spruce trees may have diverse functions (Puhe, 2003). Consequently, water uptake from deeper or shallower soil cannot be excluded since the root distribution was not analysed in this study.

Secondly, it is assumed that the trees use bulk soil water rather than mobile soil water. Especially in the growing season, trees tend to prefer transpiring less mobile soil water, as it is a more reliable water source (Kirchner et al., 2023). However, sampling methods are always less flexible and distributed differently than roots, which causes uncertainty linked to defining soil water sources (von Freyberg et al., 2020a). The model results differ when using lysimeter or soil water isotopes as source input data, indicating that mobile water uptake would lead to erroneous model results.

A third assumption is that there is no fractionation during tree water uptake. This assumption is based on diverse studies that have detected no isotopic fractionation during plant water uptake (Amin et al., 2021; Chen et al., 2020; Dawson & Ehleringer, 1991; Muñoz-Villers et al., 2025; Washburn & Smith, 1934). However, offsets in the isotopic composition have been observed for specific climatic conditions, plant species, plant growth stages, and soil types (Dawson & Ehleringer, 1993; Poca et al., 2019; Vargas et al., 2017; Zhao et al., 2024). Consequently, the possibility of fractionation during root water uptake cannot be excluded, although it is not expected to be significant based on current knowledge.

6.2.4. Research Question 1: Temporal Variability

No consistent trends in WUD over time were detected. Therefore, the primary source of tree water uptake did not change during periods of lower soil moisture, and H1 has to be rejected. This is unexpected, as previous studies in the alpine region had detected an adaptation of tree WUD to lower soil moisture conditions. For example, in the Italian pre-Alps, riparian beech trees possibly used deeper soil water during a drier period (Penna et al., 2013). In the eastern Italian Alps, on shallow sandy loam soils, the WUD was mainly a combination of shallow and deep soil water. At the end of the growing season, the contribution of deep soil water and groundwater increased, while the uptake of shallow soil water decreased. However, shallow soil water remained relevant after precipitation events (Brighenti et al., 2024). Similarly, in the dry Pfyn Forest, the WUD varied seasonally, especially at drier sites. Soil water was the main water source for trees, but groundwater uptake increased when soil moisture contents were low (Bertrand et al., 2014). In a global review, most trees in temperate seasonal forests had a lower WUD during the dry season than the wet season (Bachofen et al., 2024).

These findings support the hypothesis that the soil moisture was never sufficiently low to detect a change in WUD. Soil moisture remained relatively high even in the driest month, especially at greater depths. At the driest site (L3), the mean monthly VWC at 5 cm depth decreased to 0.18 m³/m³, but the VWC was still 0.31 m³/m³ at 15 cm depth even in August. At L2, the temperature increase and reduced precipitation in August were not visible at a depth of 30 cm. With the expected and modelled mean WUD being between shallow and deep soil water, the effect of

decreasing soil moisture content in the topsoil on the tree water uptake is minimal. However, another possibility is that the high model uncertainty and the short sampling period prevented the detection of changes in WUD.

Another reason for detecting no changes in WUD could be that various other factors have an impact on the tree responses to moisture availability, as observed in several studies. For example, Norway spruce trees in the central Alps did not adjust their WUD during drought (Walthert et al., 2024). In the Swiss Lägeren forest, Norway spruce trees did not shift their WUD to deeper soil due to seasonal changes in soil moisture, while other tree species adjusted their WUD. This was explained by the WUD being constrained by a shallow root distribution (Brinkmann et al., 2019). This highlights the importance of tree species and their adaptation of water uptake strategies in response to drought. Furthermore, beech trees in Birmensdorf had a reduced water uptake from shallow soil during drought, but there was no compensation from deeper soil (Gessler et al., 2022). Additionally, in a seasonally dry tropical montane cloud forest in Mexico, no seasonal change in WUD was detected, which was explained by other factors affecting the root distribution, such as nutrient availability (Goldsmith et al., 2012). It can be concluded that various factors, such as the rooting depth or the water uptake strategy, could lead to no shifts in WUDs. Other factors affecting the adaptation of the WUD to moisture availability can be local competition, soil depth, or root distribution (Bachofen et al., 2024; Scandellari et al., 2024).

6.2.5. Research Question 2: Spatial Variability

The WUD varies between the elevation zones, but no consistent differences are observed. The high elevation zone has the highest soil moisture contents, while the medium elevation zone has the lowest soil moisture contents, but this is not visible in the modelled WUD. No effects of study site characteristics such as slope, TWI, and tree cover, as well as elevation and soil moisture on the WUD were detected, and H2 and H3 have to be rejected.

In contrast, several previous studies found a dependence of water availability on the WUD. For example, Allen et al. (2019) found that spruce trees across Switzerland used different water sources depending on water availability. However, the effect of soil moisture on the WUD might be easier to detect on a larger scale. The range of soil moisture conditions might have been too narrow between the study sites throughout the study period, resulting in no visible differences in WUD. Furthermore, the water availability throughout the entire soil profile varies with topography, which affects the root distribution and, consequently, the WUD (Fabiani et al., 2022; Rossatto et al., 2011). In the eastern Italian Alps, bulk soil water isotopes varied significantly between the hillslope site and the wet meadow site, with a less distinct soil profile in the wet meadow. However, the WUD behaved similarly at the sites (Brighenti et al., 2024). Therefore, water availability may affect the WUD, while various other factors can also play a significant role.

Correlations between tree characteristics and the WUD can be excluded because the twig water isotopes did not correlate with site or tree characteristics. However, other studies have detected correlations between the WUD and tree characteristics such as tree size and age. Muñoz-Villers

et al. (2025) found that larger trees adapted better to drought at the end of the dry season by using more groundwater. Bishop & Dambrine (1995) reported that Norway spruce trees with larger circumferences at breast height had a deeper WUD. Furthermore, the WUD of trees in a relatively wet, mixed forest in China depended on leaf and fine root biomass as well as diameter at breast height (Zhang et al., 2020). In the eastern Italian Alps, larger trees had a deeper WUD, which was attributed to a greater rooting depth (Brighenti et al., 2024). Similarly, young and adult Norway spruce trees may have different WUDs (Floriancic et al., 2024). It can be concluded that no significant effect of tree characteristics on the WUD was detected due to the small sample size and numerous other variables affecting the WUD.

Correlations between study site characteristics and the WUD can be excluded due to the lack of correlation between soil water isotopes and site characteristics. Although the soil type varies between the elevation zones, the high elevation zone did not have a consistently lower or higher WUD. Previous studies have detected significant effects of soil characteristics on the tree WUD. In a relatively wet forest in China, the WUD correlated with field capacity, soil bulk density, and sand content (Zhang et al., 2020). Additionally, soil characteristics can affect the WUD because they affect root growth (Weemstra et al., 2017) and because the water availability to plants depends on the capillary rise of the soil (Bertrand et al., 2014). Similarly, Bachofen et al. (2024) found that the WUD can vary with soil hydraulic conductivity. However, these correlations were not detected, which can be explained by the spatial resolution, the limited sample size, or the high spatial heterogeneity in the Studibach catchment.

It can be concluded that no difference in WUD between the elevation zones was observed due to the high model uncertainties and assumptions discussed in Chapter 6.2.2 and Chapter 6.2.3., combined with the relatively small range of soil moisture contents and the limited sample size. Additionally, the large number of variables affecting the WUD may result in no visible effects from individual variables in such a heterogeneous terrain with only three elevation zones.

6.3. Vegetation Water Isotopes

6.3.1. Positive lc-excess of Twig Water Isotopes

Twig water isotopes plot above the LMWL with a positive mean lc-excess of 10.3. Consequently, some twig water isotopes are outside of the range of the soil water isotopes. For example, twig water at L2 is more depleted in heavy isotopes than all potential water sources, causing a high uncertainty in the modelled WUDs. The offset is slightly more pronounced on the first and last sampling days and at L1 and L3 but with no significant correlation, and there is no relation to the sampling time of the day. Twig water isotopes were expected to plot along the LMWL within the 95% confidence band, as reported in several previous studies. In a mixed forest in Zurich, branch samples from spruce trees plotted along the LMWL with deviations of up to +/- 2.5% δ^{18} O (Floriancic et al., 2024), which is a smaller offset than measured in this study. In the mixed Swiss Lägeren forest, precipitation, soil, and xylem water isotopes plotted along the GMWL (Brinkmann

et al., 2019). Tree xylem water of Norway spruce trees that was significantly depleted in heavy isotopes compared to the LMWL has not been observed in previous studies.

Xylem water can be enriched in heavy isotopes due to various reasons. For example, in the dry Pfyn forest, the tree water isotopes had a more negative lc-excess than soil water isotopes (Bertrand et al., 2014), and in the Italian pre-Alps, mean beech sap water isotopes had a slightly negative lc-excess (Penna et al., 2013). Across Europe, mean stem xylem water isotopes were typically within the range of soil water isotopes, with most offsets enriched in heavy isotopes. These offsets were either explained by additional water sources, such as water stored in the organic surface layers, deeper soil water, or groundwater, or attributed to cryogenic water extraction biases (Lehmann et al., 2024). Tree xylem water can be enriched in heavy isotopes if trees use shallow soil water that is affected by evaporative fractionation (Sprenger et al., 2016). For example, in a mixed forest with clay loam soil in New Mexico, Grossiord et al. (2017) found that xylem water isotopes consistently had a negative lc-excess but plotted above the evaporation line, on which soil isotopes plotted. Another explanation for xylem water being enriched could be a deeper water uptake in autumn, which causes the isotopic composition of xylem water to be more similar to that of summer precipitation, which was enriched due to higher temperatures (Bertrand et al., 2014). Furthermore, water storage in the tree could lead to xylem water being more enriched in heavy isotopes than soil water, as observed in the Eastern Italian Alps (Brighenti et al., 2024).

It is unexpected that xylem water isotopes have a positive lc-excess. The most logical explanation is that an additional water source affects the isotopic composition of the twig xylem water. This could be deeper soil water, groundwater, water in the uppermost soil layer, or water vapour in the air. Deeper soil water as an additional water source would not explain the offset because soil water isotopes plot along the LMWL, and the isotopic composition of deep soil water remains relatively constant. Shallow groundwater and deep soil water are expected to have a similar isotopic composition (Bishop & Dambrine, 1995). The deep soil water isotopes are similar to shallow groundwater isotopes measured by Kiewiet et al. (2019), which suggests that a deeper water source would not explain the offset of the twig samples.

Additional water sources could include either dew or fog drip that is taken up by shallow roots near the soil surface or fog entering the twigs via foliar water uptake. The humidity remained relatively high throughout the entire study period, and fog was regularly observed in the mornings, especially on colder days in September and October. Bishop & Dambrine (1995) found that Norway spruce trees took up water from the shallowest soil and even the organic surface layer. Consequently, water could be taken up from the unsampled uppermost 5 cm of the soil and the organic surface layer.

Dew and fog drip can be more depleted in heavy isotopes than precipitation and have a positive lc-excess (Liu et al., 2006; Welp et al., 2008). At the same time, the isotopic composition of fog and dew varies with climate, water source, and the isotopic composition of atmospheric water vapour (Tian et al., 2023; Wen et al., 2012). Tian et al. (2023) found that dew plotted mainly above

the LMWL, with the lc-excess depending on the water source. The strongest offsets were measured for advective dew in Gobabeb and ocean-derived dew in Nice, while dew consisting of dew water or groundwater plotted partly above and partly along the LMWL. Additionally, the isotopic composition of dew varies over time. For example, Welp et al. (2008) measured a depletion of heavy isotopes in dew during dew formation but an enrichment during dew evaporation. Consequently, dew and fog drip could also be enriched in heavy isotopes compared to precipitation, atmospheric water vapour, and xylem water isotopes (Dawson, 1998; Wen et al., 2012). Nevertheless, lower δ^2H in the topsoil compared to deeper soil can be explained by condensation at night due to a large temperature gradient between day and night (Yang et al., 2016).

Several studies have identified dew or fog drip in the uppermost soil layer as a potential source of water for plants. Dawson (1998) studied fog inputs in heavily fog-inundated coastal redwood forests in northern California and found that all plants relied on fog as a water source. During dry summer months, the transpiration of redwood trees consisted of about 19% fog water on average. The importance of fog as a water source on an annual average is even higher due to the increased transpiration during summer. Furthermore, Muñoz-Villers et al. (2025) attributed a positive d-excess in plant water samples to dew water uptake from the uppermost soil layer. Dew was considered a relevant water source for the coffee plants during the dry season (Muñoz-Villers et al., 2025). In contrast, no effect of fog drip or dew water uptake was observed in other regions. For example, in a seasonally dry tropical montane cloud forest, plant xylem water isotopes plotted below the LWML along shallow soil water isotopes (Goldsmith et al., 2012). It can be concluded that dew or fog drip in the unsampled uppermost 5 cm of the soil and in the organic surface layer could have been an additional water source that was depleted in heavy isotopes. Future studies could analyse the isotopic composition and the temporal variability of fog in the Studibach catchment and compare the results to the lc-excess of twig water isotopes.

Another possibility is that foliar water uptake of fog affects the isotopic composition of the twigs. Norway spruce trees can take up water through the bark and needles (Katz et al., 1989), and branch water uptake has been found to be relevant for supporting hydraulic recovery in Norway spruce trees at the alpine tree line (Losso et al., 2021). Furthermore, trees can take up fog and dew via foliar water uptake (Wen et al., 2012). Eller et al. (2013) found that trees in a Brazilian cloud forest relied on foliar water uptake of fog to compensate for low soil water availability. Boucher et al. (1995) reported that the foliar absorption of artificial dew by Pinus strobus seedlings increased the shoot water potential, stomatal conductance, and root growth, especially for seedlings under water stress. Furthermore, Limm (2009) found that foliar water uptake of fog enhanced the hydration of trees in the fog-inundated redwood forest. However, research conducted on the foliar water uptake of fog by Norway spruce trees is insufficient to estimate its amount and effect on twig water isotopes.

Another cause for the offset in twig water isotopes is the choice of the measurement method for isotope analysis. δ^{18} O can be systematically enriched when measured with CRDS compared to

IRMS, while $\delta^2 H$ do not show significant differences (Herbstritt et al., 2024). However, a Micro-Combustion Module[™] (Picarro, Inc., 2015) was used to prevent spectral contamination from organic compounds. Nevertheless, future studies could include additional isotope measurements using IRMS for comparison, as IRMS is typically not affected by organic contamination (Herbstritt et al., 2024). Lastly, a bias of cryogenic vacuum extraction that causes the positive lc-excess of twig samples is excluded. In a study review, an average bias of -6.1 +/- 3.4‰ $\delta^2 H$ in xylem water was identified, while the bias in $\delta^{18} O$ was either negligible or not measurable (Allen & Kirchner, 2022). In contrast, twig water in this study is more depleted in $\delta^{18} O$ with a positive lc-excess of 13.7.

6.3.2. Difference Between Twig and Stem Core Water Isotopes

While twig water isotopes unexpectedly have a positive lc-excess, stem core water isotopes have a negative lc-excess. The range in $\delta^2 H$ is similar, but $\delta^{18} O$ in twig and stem core xylem water ranged from -13.4 to -8.7% and from -9.4 to -6.4%, respectively. Consequently, the model results from 03.10.2024 differed when using twig and core xylem water isotopes as mixture data. As discussed in Chapter 6.3.1, xylem water isotopes typically plot along or slightly below the LMWL, and a negative lc-excess can be explained by the uptake of enriched shallow soil water (Bertrand et al., 2014; Grossiord et al., 2017; Sprenger et al., 2016) or by enriched water stored in trees (Brighenti et al., 2024). However, the negative lc-excess cannot be explained by the uptake of enriched soil water, as all soil and lysimeter water isotopes plotted along the LMWL. Therefore, water storage within the tree is a potential explanation for the observed negative lc-excess as well as the difference between twig and core samples.

Differences between twig and stem water isotopes in spruce trees were analysed by Bernhard et al. (2024). As in this study, the stem xylem water plotted below the GMWL and the bulk soil water line. The stem xylem contained varying proportions of different water sources, with a within-tree standard deviation of 2.1‰ δ^2 H and 0.4‰ δ^{18} O. Stem water isotopes sampled closer to the ground were more similar to soil water isotopes, and stem water isotopes at the highest and lowest stem positions differed significantly. Furthermore, the branch xylem water isotopes had a mean offset to stem xylem water isotopes of about -10‰ δ^2 H and about -3‰ δ^{18} O (Bernhard et al., 2024).

Two processes can explain this variability in the isotopic composition within a tree. Firstly, the transportation of water through the tree, combined with changes in soil water isotopes, can lead to different isotope signals. This time lag increases as transpiration rates decrease (Bernhard et al., 2024). Conifers in a mixed forest in Washington had time lags between 2.5 and 21 days, with tree heights of 13.5 to 58 m (Meinzer et al., 2006). In a forest in Pennsylvania, trees measuring 12 to 20 m in height had time lags ranging from one day to one week (Gaines et al., 2016). However, all trees were actively transpiring in this thesis, and twigs were sampled from low branches, which further reduces uncertainty caused by a potential time lag (Bernhard et al., 2024). Consequently,

the time lag is expected to be less than the temporal variability in soil water isotopes and, therefore, negligible.

Secondly, the exchange of water stored in the stem xylem and phloem can explain differences in the isotopic composition at different positions within a tree (Bernhard et al., 2024). In spruce trees, phloem water can be enriched in heavy isotopes compared to xylem water (Bernhard et al., 2024). Furthermore, the observation of a cryogenic water extraction bias causing a δ^2 H offset between stem water and soil water could be explained by the exchange of stem organic hydrogen and xylem water (Chen et al., 2020). However, the difference between stem core water isotopes and twig water isotopes was more pronounced in δ^{18} O than in δ^2 H, so the sole effect of a cryogenic water extraction bias can be excluded. Nevertheless, the exchange of water stored in the trees could explain the difference between vegetation samples, especially since the exchange of water stored in heartwood and sapwood is not yet well understood (von Freyberg et al., 2020a).

Lastly, back-diffusion of water enriched by transpiration could cause tree xylem water to be enriched in heavy isotopes. In a meta-analysis, an average δ^{18} O enrichment of 2.21‰ in xylem water was found in comparison to groundwater. This enrichment results from isotope fractionation during transpiration and foliar water uptake, which could affect the isotopic composition of xylem water due to the back-diffusion of leaf water (Schreel et al., 2023). The effect of isotopically enriched water decreases with distance from the stem tip and is minimal about ten nodes away from the tip (Dawson & Ehleringer, 1993). However, water taken up through leaves may be transported via the xylem into the soil (Cassana et al., 2016), and reverse sap flow during fog events may transport fog taken up via foliar water uptake through the xylem down to the roots (Eller et al., 2013). This effect would be visible not only in stem core water isotopes but also in twig water isotopes, which had a positive lc-excess. Consequently, back-diffusion could explain the isotopic composition of stem core water but not that of twig water and would not explain the difference between stem core and twig samples.

6.4. Lysimeter and Soil Water Isotopes

Water extracted from bulk soil samples contains more strongly bound water, while water collected in suction lysimeters consists of more mobile water. The exchange between mobile and bulk soil water isotopes varies. Vargas et al. (2017) concluded from an experiment that 74 to 96% of mobile water exchanged isotopes with bulk soil water, varying with soil type. Contrarily, even in relatively wet soils, mobile and bulk soil water pools can have little exchange (Sprenger et al., 2019). In addition to the sampling method, the sampling periods for bulk soil and lysimeter water differ. The negative pressure was applied to the lysimeters about one week before the sampling day of the bulk soil samples, and the lysimeters collected water over several days. Furthermore, water in bulk soil samples was extracted using the cryogenic water extraction method and may potentially be affected by a bias (see Chapter 6.2.2), while lysimeter water was only filtered.

Bulk soil water is slightly enriched in heavy isotopes, while the isotopic composition of mobile soil water and precipitation is more similar, which is consistent with findings of previous studies (Floriancic et al., 2024; Sprenger et al., 2019). Bulk soil water is typically more affected by evaporation at the surface because mobile soil water infiltrates faster via preferential flow pathways (Goldsmith et al., 2012; Sprenger et al., 2018). Unexpectedly, shallow bulk soil water is more depleted in autumn, while mobile soil water isotopes are less affected by snowfall and lower temperatures. One explanation could be the earlier sampling period of lysimeter water, which started when temperatures were still higher. Additionally, lysimeters collected water during various weather conditions and, therefore, represent an averaged isotope signal.

Both bulk and mobile soil water isotopes remain relatively constant and show no temporal trend at depths below 40 cm, although deep bulk soil water isotopes tend to be more constant than deep mobile soil water isotopes. Sprenger et al. (2019) found that mobile soil water isotopes became more negative with depth, while bulk soil water isotopes were less negative near the surface but constant below a depth of 60 cm. Similarly, Floriancic et al. (2024) found that bulk soil water isotopes varied throughout the year down to a depth of 40 cm, while mobile soil water isotopes also varied at greater depths. The low temporal variability of deep soil water isotopes can be attributed to high soil moisture, the relatively short sampling period, and the relatively shallow sampling depths.

Model results based on lysimeter and soil water isotopes varied due to these differences. Lysimeter water isotopes typically indicate a lower WUD than soil water isotopes. However, the low reliability of model results based on lysimeter water isotopes must be considered, as there were little to no significant differences between shallow and deep lysimeter water isotopes per elevation zone and sampling day. This can be explained by the small sample size and the higher mobility of the water, which causes fluctuations throughout the soil profile. In addition to the low reliability of the model results when using lysimeter water isotopes, the model results based on soil water isotopes are more accurate because trees tend to rely on less mobile water sources, especially in the growing season (Kirchner et al., 2023).

7. Conclusions

This thesis investigated the spatial and temporal variability in the isotopic composition of bulk soil water, mobile soil water, twig xylem water, stem core xylem water, and precipitation in a headwater catchment in the Swiss pre-Alps.

There was a clear temporal trend in the isotopic composition of all sources, which were enriched during periods of higher temperatures and depleted due to snowfall and lower temperatures in autumn. The isotopic composition of precipitation fluctuated strongly, which indicates changes in moisture origin, air circulation, and rainout effects. The temporal variability in the isotopic composition of soil water was mainly related to changes in precipitation and was only slightly affected by evaporation at the soil surface. The isotopic composition of bulk soil water and mobile

soil (lysimeter) water varied significantly between study sites, as well as between elevation zones, indicating that various factors contribute to its spatial variability.

Unexpectedly, for the soil and lysimeter water samples, there was a positive correlation between $\delta^{18}O$ or $\delta^{2}H$ and altitude. This suggests that in small headwater catchments, elevation effects in the isotopic composition of precipitation are negligible compared to the effects of differences in storm tracks and other site characteristics. Although the isotopic composition of soil water did correlate with a few site characteristics on certain sampling days, there were no consistent correlations between the isotopic composition of xylem water and tree age, tree circumference, slope, aspect, TWI, and tree cover, likely due to the limited number of study plots and the numerous variables affecting spatial variability.

A mixing model was used to determine the relative fraction of shallow (5-20 cm) and deeper (25-50 cm) soil water in the xylem water in the twigs. The derived contributions of shallow and deeper soil water to xylem water (used as an indicator of the water uptake depth; WUD) suggested that xylem water was primarily a mixture of shallow and deep bulk soil water. However, there was no temporal trend in the modelled WUD, which could be explained by the consistently high soil moisture content, high model uncertainty, and the limited sample size.

Both the soil moisture content and the WUD varied between the elevation zones, but there was no effect of soil moisture on the modelled WUD. This could be due to the high model uncertainty and the limited number of study sites. Similarly, the WUD did not correlate with elevation or other study site characteristics, as already suggested by the lack of a consistent relation between the site characteristics and δ^{18} O or δ^{2} H.

The results of this thesis are constrained by the consistently high soil moisture content, especially at soil depths below 30 cm, and the relatively high humidity and precipitation. A key factor contributing to uncertainty was the similarity between shallow and deep water sources. The high moisture content throughout the soil profiles resulted in the absence of a visible evaporation effect in the shallow soil water, leading to the small differences between shallow and deep water sources. It would thus be useful to redo this study during a warm drought period. A more distinct soil profile could also be achieved by enhancing the differences between the water sources by applying labelled water. Furthermore, sampling deeper and shallower soil depths based on knowledge of the root distribution should be considered to ensure the inclusion of all potential water sources.

The unexpected positive lc-excess for the twig samples could indicate a neglected water source, which could be dew or fog drip that recharges the shallowest soil and is taken up by roots. Other potential explanations include foliar water uptake of fog or dew, or an artefact of the measurement method. Interestingly, stem core water isotopes had a negative lc-excess. A slightly negative lc-excess can be attributed to the uptake of enriched soil water, but the reason for the difference between twig and stem core samples remains unclear and requires further research. It would be interesting to study the temporal variability of fog and dew in the atmosphere, as well as in shallow

soil and in the organic surface layer. Furthermore, the within-tree variability of vegetation water isotopes could be analysed, and a potential time lag caused by the transport of water through the tree could be measured. Moreover, it is essential to gain a deeper understanding of the exchange between different water sources stored within the tree.

The findings of this study are relevant for future research on the tree water uptake in wet regions. Although the findings regarding the WUD and their representativity for the entire pre-Alpine region are limited, the discussion of variables affecting the WUD as well as the evaluation of the research design are helpful when planning future studies in wet areas. Further research on tree water uptake in the pre-Alps is required due to the increasing frequency of summer droughts, which could force trees to change their WUD to cope with water stress.

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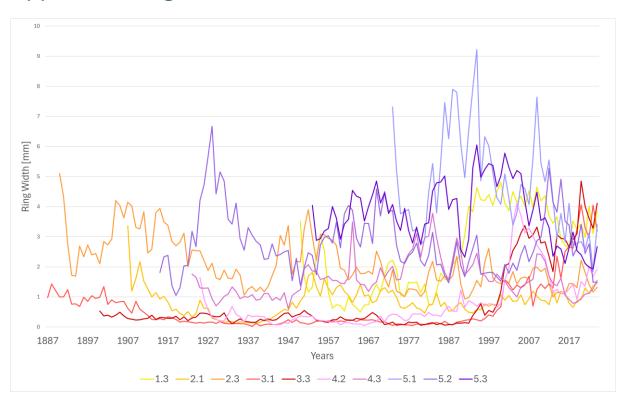
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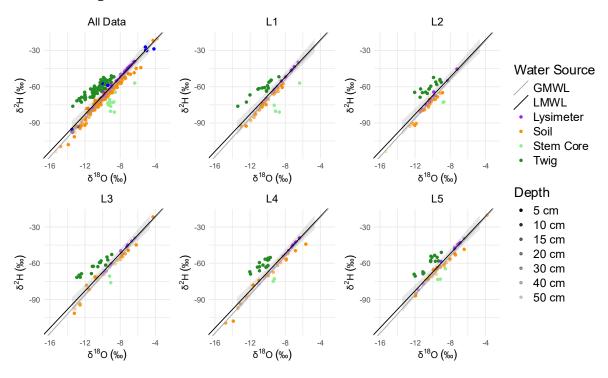
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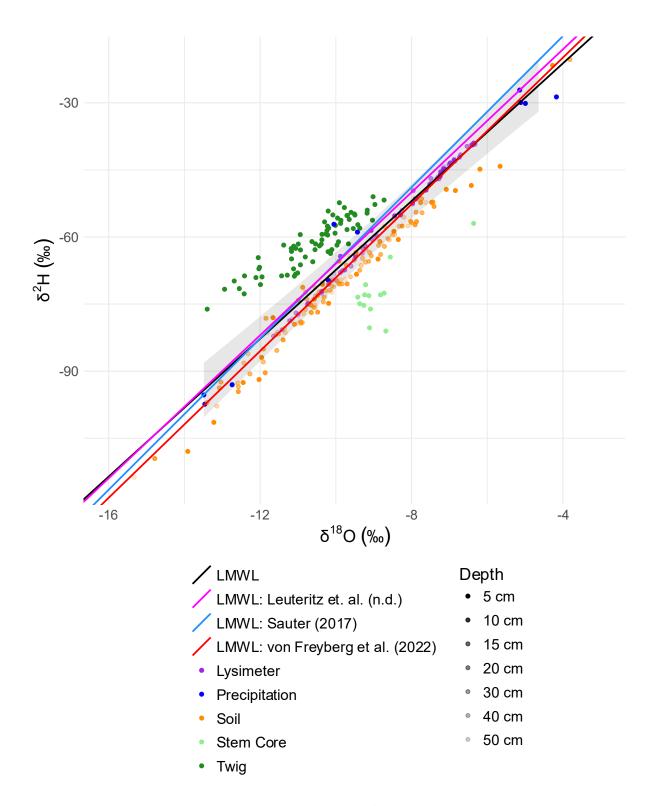
Appendix A: Figures



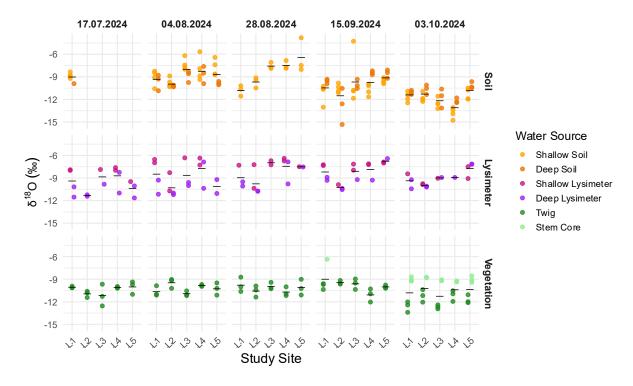
Appendix A.1: Tree ring widths of the three trees (.1, .2, .3) at the study sites L1 (1.) to L5 (5.) measured using the software CooRecorder.



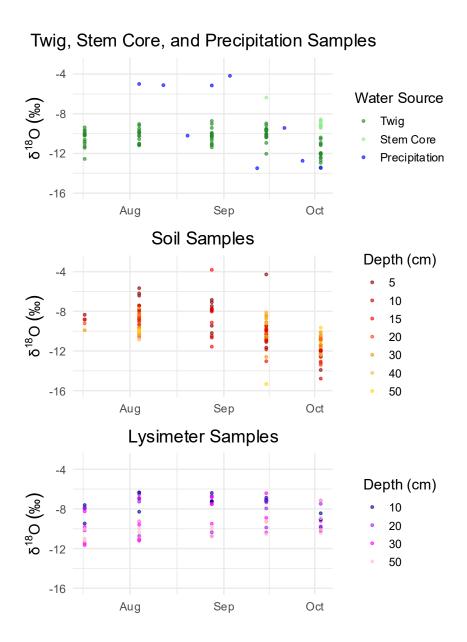
Appendix A.2: Dual isotope plots of all data and at each study site with the LMWL ($\delta^2 H = 7.70*\delta^{18}O + 9.61$) and its 95% confidence band. The opacity indicates the sampling depth.



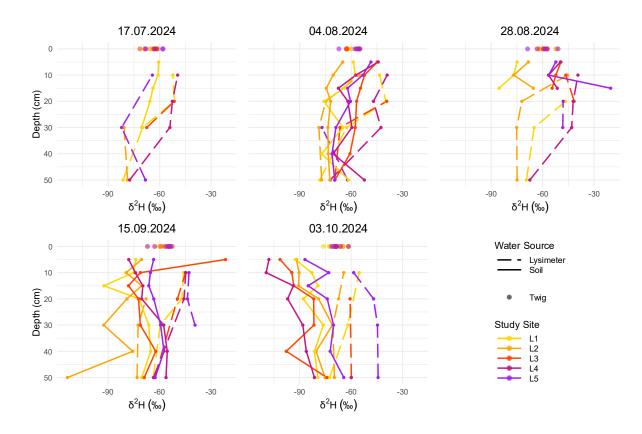
Appendix A.3: Comparison of the LMWL ($\delta^2H = 7.70^*\delta^{18}O + 9.61$) and its 95% confidence band with the GMWL and LMWLs calculated for headwater catchments in the Alptal. Sauter (2017) took precipitation samples in the Studibach catchment over the summer of 2016 to estimate the LMWL ($\delta^2H = 8.45 * \delta^{18}O + 18.7$). Leuteritz et al. (n.d.) collected 12 precipitation samples in the summer and autumn of 2021 and 2022 to estimate the LMWL ($\delta^2H = 7.99 * \delta^{18}O + 13.91$). Von Freyberg et al. (2022) used monthly, amount-weighted precipitation samples collected at the Alp and Erlenbach stations between 2015 and 2019 to estimate the LMWL ($\delta^2H = 12.9 + 8.2 \cdot \delta^{18}O$).



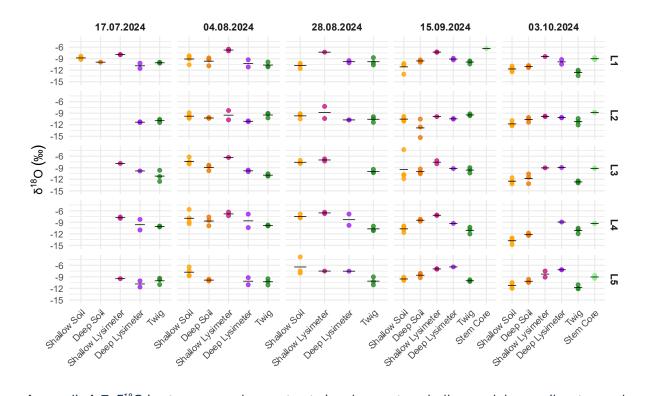
Appendix A.4: Difference in soil water, lysimeter water, and tree xylem water δ^{18} O between the study sites per sampling day. The horizontal black lines represent the mean values of each water source per day and study site.



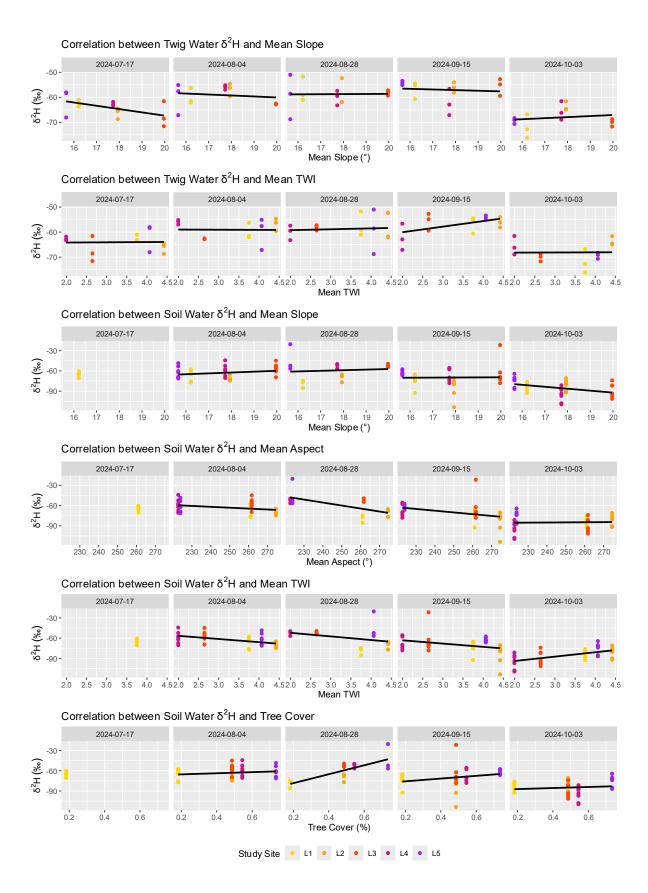
Appendix A.5: Temporal variability in δ^{18} O of different water sources and soil depths.



Appendix A.6: Depth profiles of $\delta^2 H$ in soil water and lysimeter water per sampling day and study site.



Appendix A.7: δ^{18} O in stem core xylem water, twig xylem water, shallow and deep soil water, and shallow and deep lysimeter water per sampling day and study site L1-L5



Appendix A.8: Correlations between δ^2H in twig xylem water or soil water and tree and site characteristics, with the study site highlighted.

Appendix B: Tables

Appendix B.1: Correlation (p-value) between $\delta^{18}O$ in soil water and study site characteristics for each sampling day and over the entire dataset. The sample sizes are 15 on 28.08.2024 and 35 on 04.08.2024, 15.09.2024, and 03.10.2024. *The correlation between the two variables if the correlation is significant.

	04.08.2024	28.08.2024	15.09.2024	03.10.2024	All data
Mean slope (°)	0.218	0.772	0.719	0.019	0.72
				(-0.394*)	
Mean aspect (°)	0.058	0.012	0.027	0.300	0.03
		(-0.629*)	(-0.374*)		(-0.20*)
Mean TWI	0.005	0.236	0.201	0.000	0.44
	(0.463*)			(0.666*)	
Tree cover (%)	0.248	0.001	0.158	0.765	0.03
		(0.771*)			(0.20*)

Appendix B.2: Correlation (p-value) between $\delta^{18}O$ in twig xylem water and tree and study site characteristics for each sampling day and over the entire dataset. The sample size is 15 on all sampling days. *The correlation between the two variables if the correlation is significant.

	17.07.2024	04.08.2024	28.08.2024	15.09.2024	03.10.2024	All
						data
Age	0.395	0.463	0.363	0.396	0.715	0.89
Circumference	0.169	0.427	0.736	0.363	0.487	0.39
Mean slope (°)	0.046	0.655	0.821	0.592	0.636	0.35
	(-0.522*)					
Mean aspect (°)	0.107	0.864	0.665	0.012	0.321	0.91
				(0.631*)		
Mean TWI	0.957	0.525	0.717	0.035	0.923	0.30
				(0.547*)		
Tree cover (%)	0.959	0.425	0.473	0.573	0.136	0.68

Appendix B.3: Kruskal-Wallis test of significance to test the differences between $\delta^2 H$ of the water sources on each sampling day.

Sampling Day	p-value
17.07.2024	1.27e- 3
04.08.2024	4.35e- 4
28.08.2024	1.51e- 2
15.09.2024	2.26e- 6
03.10.2024	1.36e-10
All sampling days	2.97e-16

Appendix C: Photos of Methods and Study Sites



Weather station in front of the forest stand at L1.



Groundwater well at L1.



Tree corer for sapwood sampling at L3.



Moisture Meter type HH2 with a ThetaProbe ML3 sensor.



Soil moisture, relative humidity, and air temperature logger at L1.



Core taken for sapwood sampling.



Study site L1.



Study site L3.



Study site L5.



Study site L2.



Study site L4.

Personal Declaration

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.

Meret Vogler

Wogler

Zurich, 16.07.2025