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by

Ginevra FABIANI

Born on 17 September 1993 in Massa (Italy)

TREE WATER USE ACROSS LANDSCAPE AND TIME

Dissertation defence committee
Prof. Dr. Julian KLAUS, dissertation supervisor
Professor, University of Bonn, Germany
Prof. Dr. Daniele PENNA, dissertation co-supervisor
Professor, University of Florence, Italy
Prof. Dr. Laurent PFISTER, Chairman
Professor, University of Luxembourg
Luxembourg Institute of Science and Technology, Esch-sur-Alzette, Luxembourg
Prof. Dr. Joachim HANSEN, Member
Professor, University of Luxembourg, Luxembourg
Prof. Dr Claudia COCOZZA, Member
Professor, University of Florence, Italy
Dr. Ilja VAN MEERVELD, Member
University of Zurich, Switzerland

To my grandma Silvana and Caterina

Abstract

The interaction between topography and climate has a crucial role in shaping forest composition and structure. The understating of how the ecohydrological processes across the landscape affect tree performance becomes especially important with the expected reduction in water availability and increase in water demand, which could enhance the thermal and hydrologic gradient along the slope. Incorporating soil moisture variation and groundwater gradient across the landscape has been found to improve the capacity to predict forest vulnerability and water fluxes in complex terrains. However, most of the information that can be retrieved by remote sensing technique cannot capture small scale-processes. Therefore, hillslope-catchment scale studies can shed light on ecosystem responses to spatially and temporally variable growing conditions.

In the present work, I investigated how hillslope position affects tree physiological response to environmental controls (i.e. soil moisture, vapor pressure deficit, groundwater proximity to the surface) and tree water use in two hillslope transects (Chapter 1 and 3). Sap velocity measurements and isotopic measurements have been applied along two hillslope transects, characterized by contrasting slopes angle, climate, and species composition. We found that the different hydrological processes occurring at the two sites lead to contrasting physiological responses and water uptake strategies. In the Weierbach catchment, the lack of shallow downslope water redistribution through interflow leads to no substantial differences in vadose zone water supply between hillslope positions and ultimately no spatial differences in the tree's physiological response to environmental drivers. Furthermore, beech and oak trees displayed different stomatal control resulting from their water uptake strategies and physiology. In the Lecciona catchment, the greater soil moisture content at the footslope, promoted by the steep slope, led to more suitable growing conditions and a longer growing season in the piedmont zone. These results emphasize the strong interconnection between vegetation, climate, and hydrological processes in complex terrains, and the need to consider them as a whole to better understand future ecosystem responses to changing climate.

Additionally, the present work sheds new light on the complex interaction between sapwood and heartwood. In Chapter 2, I provide experimental evidence about water isotopic exchange between the two compartments in four tree species

(*Fagus sylvatica*, *Quercus petraea*, *Pseudotsuga menziesii*, and *Picea abies*) characterized by different xylem anatomy, and timing of physiological activity. While the two functional parts display a consistent difference in isotopic composition in conifers, they are characterized by more similar values in broadleaved species in broadleaved species, suggesting a higher degree of water exchange. These results highlight the value of accounting for radial isotopic variation, which might potentially lead to uncertainties concerning the origin of the extracted water for water uptake studies.

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Introduction

The Critical Zone science as a bridging endeavor

The Critical Zone (CZ) science has started with the aim of achieving a better understanding of the complex interactions between the biotic and abiotic components of the Earth's surface by linking multiple research fields (e.g. hydrology, hydrochemistry, hydrogeology, ecohydrology, and plant physiology). The CZ extends from the top of the canopy, through the soil, down to the fresh bedrock, and the bottom of groundwater (Rempe and Dietrich, 2018). The thread that connects all these components is the water while interacting with bedrock, soil, vegetation, and atmosphere gets partitioned along the way into evaporation, transpiration, and recharge (Brantley et al., 2017). The small-scale variation in microclimate, lithology, biotic and abiotic disturbances shape the vegetation community assemblage (Hahm et al., 2019). The subsurface properties of the CZ (e.g., porosity, permeability, texture, regolith depth, layering, and weathered bedrock water storage capacity) play also a major role in controlling water availability through hydrological processes occurring in hillslopes and ultimately ecosystem composition (Brooks et al., 2015; Hahm et al., 2019; Klaus and Jackson, 2018; Penna et al., 2009). Trees, digging their roots in the subsurface and expanding their branches above-ground, connect and modulate water flux from the Earth's surface to the atmosphere by building and plumbing the critical zone (Brantley et al., 2017). From the soil-plant-atmosphere continuum perspective, water sources below the soil have long been neglected. Within the context of CZ, the role of the deep water resources (i.e. water stored across the weathered bedrock profile) have been emphasized and have improved plant ecophysiological research (Dawson et al., 2020).

Trees performance across landscapes and climatic conditions

A series of recurrent droughts and heatwaves in 2003, 2015, 2018, and 2019 has severely affected forest ecosystems in Europe (Obladen et al., 2021), and each one of them has set a new benchmark for future drought events. The latest is nowadays affecting several regions in Europe since late-spring 2022, where severe rainfall deficit, accompanied by increased temperature, is putting vegetation under pressure (Toreti et al., 2022). It is expected that environments north of 40°N will be the most affected by temperature increase (Serreze and Barry, 2011). In this context, the understanding of forest ecosystem response to atmospheric and soil dryness is key to predicting future species distribution and developing forest management practices suited for locations at risk. Thus, ecosystem science becomes an applied technique to describe the ecosystem processes occurring in time and space (Murphy et al., 2020).

Good et al. (2015) reported that globally plant transpiration is the majority of the evapotranspiration flux, and is estimated to account up to 74% of terrestrial evapotranspiration (Schlesinger and Jasechko, 2014). Transpiration is dependent on plant physiological activity, while evaporation depends on shallow soil water content and energy (Maxwell and Condon, 2016). The availability of water is one of the main constrain for forest productivity and health (Hogg et al., 2008; Michelot et al., 2012) and varies both over time and across landscapes (Kaiser et al., 2013). Trees can extract water from different layers of the soil, from the fractures of saprolite and weathered bedrock, and from the pore spaces of fresh bedrock (Dawson et al., 2020; Hahm et al., 2020). The accessibility to different water sources by trees is determined by the structure and scale of subsurface heterogeneity, and the transition from soil to saprolite and bedrock. Topography-driven processes modulate water and energy balance resulting in drier and wetter conditions at the higher and lower topographic positions, respectively (Song et al., 2022). Landscape surface topography promotes lateral water flow in the vadose and saturated zone, which ultimately drains at the valley bottoms (Fan, 2015). This hydrologic gradient directly translates in higher tree transpiration rates and productivity in the lower parts of the hillslope owing to the wetter condition (Kume et al., 2016; Méndez-Toribio et al., 2017; Pei et al., 2019; Pettit and Froend, 2018; Tromp-van Meerveld and McDonnell, 2006). Conversely, in hillslope positions where surface topography is seasonally or even permanently decoupled from the groundwater table and the evaporative demand is higher, tree transpiration is reduced (Méndez-Toribio et al., 2016). The accessibility of different water sources by trees is not only determined by water redistribution, subsurface structure, and the degree of subsurface heterogeneity, but also by root distribution and activity (Fan et al., 2017). Fan et al. (2017) have explained the effect of groundwater on root elongation as a pulling and pushing force. In high topographic positions, the groundwater table draws roots to tap the capillary rise or rock moisture (Rempe and Dietrich, 2018), whereas in concave areas it pushes roots in the shallow soil to avoid oxygen stress (Fan et al., 2017; Rossatto et al., 2014). Climate and seasonality can also affect root development. For example, Populus species grow shallow roots in sites with wetter climate, where soil moisture is not limited, whereas in dryer regions they root deeper to tap the capillary fringe above the groundwater table (Rood et al., 2011).

Several investigations have assessed the spatial origin of water uptake via isotopic tracing technique and showed the ability of trees to use a range of water pools such as soil, rock moisture, and groundwater (Bertrand et al., 2014; Barbeta and Peñuelas, 2017). However, it has been also highlighted that the proportional contributions of different sources may vary with season depending on their accessibility and availability (Bertrand et al., 2014; Brinkmann et al., 2019; Gessler et al., 2022).

Today, there is a pressing need for gaining a better understanding of how and when trees access different water pools and eventually influence water fluxes in the critical zone. Several works have highlighted the importance of incorporating soil moisture variation (Schwantes et al., 2018; Tai et al., 2017) and groundwater gradient (Maxwell and Condon, 2016) to land surface models to better predict forest vulnerability and water fluxes in complex terrains. However, most of the information that can be retrieved by remote sensing technique cannot capture small scale-processes (Murphy et al., 2020). Thus, hillslope-catchment scale studies can shed light on the spatial and temporal pattern of three water use and on ecosystem responses to spatially and temporally variable growing conditions.

Species-specific water relationship

To sustain the photosynthetic demand and cool the leaf surfaces, trees move large volumes of water from the belowground to the atmosphere through the xylem (Johnson et al., 2022). The water movement in the “belowground-plant-atmosphere continuum” follows a water potential gradient, expressed in pressure units (Pa). The evaporation of water from leaves is the driving force of water movement, which generates a continuous of water column from the leaves to the roots (Tyree and Zimmermann, 2002a). Water is a critical factor for tree physiological activity considering that more than 90% of water is lost via transpiration (Fonti et al., 2010). As long as soil water supply compensates for evapotranspiration loss, water flow from the belowground through the plant to the atmosphere is conservative, and no down-regulation occurs (Bréda et al., 2006). Water limitations do not necessarily translate into comparable stress levels among different tree species (Aranda et al., 2005; Mészáros et al., 2011). Each species has evolved ingenuous strategies to capitalize on resource acquisition while minimizing the risk of hydraulic disfunction (Grossiord et al., 2014; Pfautsch et al., 2015). Physiological, anatomical, and morphological adaptations from the root to the leaf levels concur to define species-specific hydraulic traits (Choat et al., 2018). Some of these responses act from a diurnal to a seasonal scale (e.g. stomatal control on vapor pressure deficit) (N. G. McDowell et al., 2008), while others require more than decades to take place such as acclimation strategies (e.g. growing deeper roots, xylem lumen diameter) (Zimmermann et al., 2021). In case of extreme drought events, branch sacrifice and leaf shedding may also occur in broadleaves species to reduce evaporative water loss and avert hydraulic failure (Salomón et al., 2022; Williams et al., 2022). However, at short-time scale, stomatal regulation is one of the primary strategies by which plants regulate water losses in response to rising vapor pressure deficit (VPD, i.e. the difference between actual and saturated air vapor pressures) and drought (Grossiord et al., 2020). When the water supply becomes limited and the VPD increases, trees can reduce water losses by stomata closure at the cost of carbon assimilation (Martínez-Vilalta and Garcia-Forner, 2017a). The leaf-level regulation is driven by the interaction between xylem architecture and stomatal sensitivity (N. McDowell et al., 2008). Thus, the regulation of water use differs among species that fall along the spectrum of isohydric to anisohydric behavior (Yi et al., 2017). While isohydric species close earlier the stomata to prevent hydraulic dysfunction in spite of reduced carbon allocation, anisohydric species maintain high stomatal conductance at risk of cavitation risk. Matheny et al. (2016) showed that rooting strategy, thus increasing accessibility to water with increasing rooting depth, concurs with leaf and xylem physiology to define the tree hydraulic strategy. Water stored in the subsurface is not the only water sustaining tree transpiration. Trees can also rely on the internal water storage, owing to the large biomass in stems per unit leaf area compared to other non-woody plants (Tyree and Yang, 1990). This translates in sap flow rates lagging behind leaf transpiration (Steppe et al., 2015) and time shifts between sap flow at the shoot tip to the stem base caused by the elastic transport system (Cermak et al., 2007). Water can be stored in the living cells (i.e. parenchyma, and rays) of the sapwood and in the xylem conduits and extracellular spaces (Meinzer et al., 2011). Recently, Barbeta et al. (2022) have highlighted through an isotopic approach that xylem conduits have a different isotopic composition than water stored in other xylem tissues. Thus, the combination of sap flow measurements with the isotope tracing technique can aid in understanding the relationship between water transport and storage. Water storage capacity varies considerably among plant species depending on wood density, sapwood area, and also on wood anatomy (Köcher et al., 2013; Phillips et al., 2003). The role of stem water reservoir does not only buffer transpiration demand during soil water shortage (Köcher et al., 2013) but also contributes to daily water use, accounting for 5-22% of the daily water use in broadleaved trees (Köcher et al., 2013) and for 20-25% in conifers (Cermak et al., 2007; Phillips et al., 2003). Although much is known about the key role of sapwood as a water source, we have still limited understanding about the relevance heartwood as water storage and the possible water exchange between the two functional parts.

Objectives of this thesis

This thesis aims to investigate the physiological response of trees growing in complex terrains and link it to species-specific and location-driven water use strategies. The general hypothesis driving the present work is that the seasonality and hillslope ecohydrological processes drive contrasting tree water use strategies and physiological responses in trees growing along different topographic positions.

By combining isotopic technique with sap flow measurements and environmental monitoring, the present work aims to provide a detailed comprehension of the seasonal interactions and feedbacks between vegetation and hydrological processes.

It is an important challenge for the scientific community to provide an insight into the ecohydrological functioning of temperate forests resulting from the complex interaction between topography, forestry, and hydrology in order to aid future management responses to local and climate change (Adams et al., 2014). In these habitats, the different plant functional groups and the stratification of soil water and root systems are key factors to ensure community composition and resilience of the whole ecosystem. European beech (*Fagus sylvatica*), sessile oak (*Quercus petraea*), Douglas fir (*Pseudotsuga menziesii*), and Norway spruce (*Picea abies*) are the species investigated in this thesis. The present work also aims to offer new insight on water movement and isotopic exchange within stems of trees characterized by different xylem anatomy and timing of physiological activity. The investigated species are abundant in many temperate European forest ecosystems, which could be strongly impacted by the predicted intensification of dry and wet spells (IPCC, 2013).

The following Chapters are based on manuscripts that have been published (Chapter 1 and Chapter 2) and in preparation (Chapter 3). Each chapter includes a detailed introduction, description of the methods, discussion, and conclusions, and focuses on specific objectives and research questions:

- Chapter 1: Transpiration patterns and water use strategies of beech and oak trees along a hillslope

This chapter combines the assessments of transpiration rates and water uptake depths along hillslope transects to better understand water use strategies and plasticity of beech and oak trees growing in the Weierbach catchment. The specific research questions are: (i) How does landscape position affect the spatial and temporal patterns of water use? (ii) How do two co-occurring species (beech and oak) characterized by different physiological and hydraulic traits respond to a variable water supply over the growing season?

Fig. 1. The mixed beech-oak forest in the Weierbach catchment (LU).

- Chapter 2: Sapwood and heartwood are not isolated compartments: consequences for isotope ecohydrology

By relying on the isotopic technique, this chapter aims to provide a better understanding the mixing processes between sapwood and heartwood among multiple species and functional groups. The specific research questions are: i) how does the isotopic composition of sapwood and heartwood change over the growing season? (ii) does xylem morphology (i.e. vessel- or tracheid-bearing species) drive different degrees of water compartmentalization between sapwood and heartwood?

Fig. 2. Pictures taken during the xylem sampling. The difference in wood core translucency given by the water content allowed us to separate sapwood from heartwood for spruce samples (left photo). The greater moisture content in the sapwood leads to a visible amount of condensation on the vials' walls (right photo).

- Chapter 3: Contrasting water use strategies of beech trees along hillslopes with different slope and climates

Chapter 3 aims to provide a better understanding of the ecohydrological functioning of different ecosystems by comparing the physiological response of beech trees growing on oceanic and gently sloping hillslope in Luxembourg and a Mediterranean and very steep slope in Italy. The specific research questions are: (i) does topography drive different water uptake strategies along two contrasting hillslopes? (ii) does hillslope topography increase tree sensitivity to drought?

Fig. 3. Photos from the two study sites. On the left, a beech tree on the steep slope of the Lecciona catchment (ITA) is equipped with a sap flow sensor. On the right, the casing of a groundwater well at the midslope location on the gentle slope of the Weierbach catchment (LU).

Chapter 1

Transpiration patterns and water use strategies of beech and oak trees along a hillslope

Abstract

The role of landscape topography in mediating subsurface water availability and ultimately tree transpiration is still poorly understood. To assess how hillslope position affects tree water use, we coupled sap velocity with xylem isotope measurements in a temperate beech-oak forest along a hillslope transect in Luxembourg. We generally observed greater sap velocities at the upslope locations in trees from average-sized trees, suggesting the presence of more suited growing conditions. We found a lower difference in sap velocity among hillslope positions for larger trees, likely due to the exploitation of deeper and more persistent water sources and the larger canopy light interception. Beech trees exploited a shallower and seasonally less persistent water source than oak trees, due to the shallower root system than oak trees. The different water exploitation strategy could also explain the stronger stomatal sensitivity of beech to vapor pressure deficit compared to oak trees. Xylem isotopic composition was seasonally variable at all locations, mainly reflecting the contribution of variable soil water sources and suggesting that groundwater did not contribute, or only marginally contributed, to tree transpiration. Overall, our results suggest that trees along the hillslope mainly rely on water stored in the unsaturated zone and that seasonally shallow groundwater table may not necessarily subsidize water uptake for species that do not tolerate anoxic conditions. Contrary to previous studies, at our site, we did not find higher sap velocity downslope as the subsurface hillslope structure promotes vertical water flux over lateral redistribution in the vadose zone.

This chapter is based on: *Fabiani, G., Schoppach, R., Penna, D., Klaus, J., 2021. Transpiration patterns and water use strategies of beech and oak trees along a hillslope. Ecohydrology 1–18. https://doi.org/10.1002/eco.2382.*

1.1. Introduction

Water availability in space and time is one of the key elements shaping forest ecosystems and their adaptive response to environmental stress. Landscape position is a dominant factor that controls the spatio-temporal variability of water available for tree transpiration (Looker et al., 2018). Several studies observed that a variation in water availability along a topographic gradient can result in different tree species distribution, growth rate, and transpiration fluxes (Elliott et al., 2015; Fan, 2015; Hawthorne and Miniat, 2018; Tromp-van Meerveld and McDonnell, 2006). Topography critically controls plant transpiration via the influence of slope and aspect on the amount of incoming solar radiation (Renner et al., 2016a) and on water availability through hillslope hydrological processes (Fan et al., 2017). The age distribution and composition of natural forests are typically adapted to the varying water availability in different parts of the ecosystem in order to meet species-specific growth requirements (Band et al., 1993; Lin et al., 2019). Trees covering the hilltop and ridgeline, which are often characterized by thin soils, are frequently less productive in biomass (Hoylman et al., 2018; Kume et al., 2016) but can adapt in certain cases to utilize water from weathered bedrock and rock moisture (Dawson et al., 2020; Klos et al., 2018; Rempe and Dietrich, 2018). On the contrary, trees located in topographically convergent locations may take advantage of shallower groundwater tables (Barbeta and Peñuelas, 2017; Brooks et al., 2015; Eamus et al., 2006; Pettit and Froend, 2018). Shallow groundwater replenishes soil water via upward capillary fluxes or temporary groundwater rise, modulating the water content in the unsaturated zone (Brooks et al., 2015). Groundwater can also directly sustain plant transpiration when the water table is within the root zone (Brooks et al., 2015; David et al., 2013; Miller et al., 2010). Trees at footslope locations can also benefit from water that is laterally redistributed from upslope areas (Band et al., 1993; Hwang et al., 2020; Lin et al., 2019). Nevertheless, some studies have observed high drought sensitivity, a small basal increment, and a reduced leaf area index in trees growing at downslope locations (Elliott et al., 2015; Hwang et al., 2020). This behavior has been associated with the combined effect of higher water consumption by upslope vegetation compared to downslope vegetation and consequent lower downslope water subsidy available for trees in convergent areas (Hawthorne and Miniat, 2018; Hwang et al., 2020). The lack of consistent findings on how hillslope position controls forest water use may originate from the interplay of the site-specific structure of the Critical Zone, which is the near-surface environment extending from the tree canopy through the soil up to the weathered bedrock (Brooks et al., 2015; Rempe and Dietrich, 2018). The subsurface properties of the Critical Zone (e.g., porosity, permeability, texture, regolith depth, layering, and weathered bedrock water storage capacity) control water availability through hydrological processes occurring in hillslopes (Brooks et al., 2015; Hahm et al., 2019; Klaus and Jackson, 2018; Penna et al., 2009).

Trees can utilize different below-ground water sources to sustain transpiration (Barbeta and Peñuelas, 2017). The accessibility of different water sources by trees is not only determined by water redistribution, subsurface structure, and the degree of subsurface heterogeneity, but also by root distribution and activity (Fan et al., 2017). Along a topographic gradient, rooting depth and biomass may vary depending on moisture availability in the subsurface, in order to meet the nutrient and water needs of trees (Tsuruta et al., 2020). Plants have developed different below-ground strategies to access nutrients and water and to respond to changes in their spatio-temporal availability (Bardgett et al., 2014; Fry et al., 2018). Tree water consumption is related to several species-specific features including architectural root traits (i.e., rooting depth and root length density) (Fry et al., 2018), xylem architecture (diffuse- vs. ring-porous species) (Wang et al., 1992), and stomatal regulation (isohydric vs. anisohydric species) (N. McDowell et al., 2008; Uhl et al., 2013). Since roots differ in their functional and physical roles (Fry et al., 2018), the mere presence of roots at a given depth is not a reliable indicator of their contribution to water use (Ehleringer and Dawson, 1992). Additionally, it has been shown that the correspondence between physically present and functionally active roots is species- and time-dependent (Volkmann et al., 2016).

Until today, a range of ecohydrological studies successfully employed stable isotopes (i.e., hydrogen (^{1}H/^{2}H) and oxygen (^{16}O/^{18}O)) in the water molecule in the soil-plant continuum to investigate tree water use (Penna et al., 2018). Xylem water isotopic composition is presumed to reflect the integrated isotopic composition of water sources accessed by the tree (Dawson et al., 2002). It was shown that trees can adapt their water source from shallow to deep soil water following water availability (Brinkmann et al., 2018; Lanning et al., 2020). This species-specific plasticity might be key for their survival and competitiveness under increasing water scarcity (Volkmann et al., 2016). The spatial and temporal water source partitioning between different species is still poorly tested and contrasting results have been found (Allen et al., 2019; Bello et al., 2019; Grossiord et al., 2014; Meißner et al., 2013; Volkmann et al., 2016). While Meinzer et al. (2001) highlighted the potential of niche complementarity as a competition avoidance strategy, Grossiord (2019) and Gillerot et al. (2020) suggested that tree diversity does not systematically increase the performance of forest communities. Indeed, it was only in drought-prone environments that forest resistance to drought was enhanced by higher diversity (Grossiord et al., 2014). Despite the body of previous work, we lack studies addressing plant water use along hillslope transects. Specifically, combined assessments of transpiration rates and water uptake depths along hillslope transects might help to better understand water use strategies and plasticity of different tree species. Such a combined assessment supports a more detailed comprehension of the seasonal interactions and feedbacks between vegetation and hydrological processes occurring at the hillslope scale.

To address this gap, we carried out an ecohydrological study along a hillslope transect populated by oak (*Quercus petraea* (Matt.) Lieb. x *robur*) and European beech trees (*Fagus sylvatica* L.) in Luxembourg. Beech and oak trees are coexisting species in Central Europe (Barbaroux and Bréda, 2002; Grossiord et al., 2014), however, they may become competitors during drought conditions, resulting in negative consequences on forest vitality and composition (Petritan et al., 2017). Although taxonomically related, the morphological and ecological differences between the two species can result in temporally and spatially different patterns of water utilization (Zapater et al., 2011). We monitored soil moisture, groundwater level, sap velocity, and hydro-meteorological variables for one growing season (April - October 2019). Additionally, we determined the isotopic composition of precipitation, soil water, groundwater, and xylem water in order to assess the influence of topography on water use. The combination of these measurements allows for discrimination between water sources used by tree species for assessing the physiological response of trees to water availability along a hillslope.

Our study was driven by the general conjecture that landscape position controls tree water use through subsurface water redistribution, including spatially-variable groundwater table depth along the hillslope. Furthermore, we conjectured that the characteristics of a species result in a different response between species at the same hillslope position. In particular, we addressed the following specific research questions: (i) How does landscape position affect the spatial and temporal patterns of water use? (ii) How do two co-occurring species (beech and oak) characterized by different physiological and hydraulic traits respond to a variable water supply over the growing season?

1.2. Materials and methods

1.2.1 Study area, ecohydrological monitoring, and sampling areas

The experiment was carried out between March and October 2019 at a transect along a south-facing forested hillslope ranging from 485 m to 515 m a.s.l. in the Weierbach catchment (45 ha) in Luxembourg (Fig. 1). The hillslope transect is 348 m long spanning from the stream bank up to a plateau area. The moderate, oceanic climate in the region is largely influenced by air masses from the Northern Atlantic Ocean (Hissler et al., 2021, Pfister et al., 2014). Long-term annual precipitation (2007-2018) is approximately 783 mm at the Roodt weather station (3.5 km from the study site). The mean annual air temperature is 8.3°C and 13.5°C during the growing season (01 April-31 October) from 2007 to 2018.

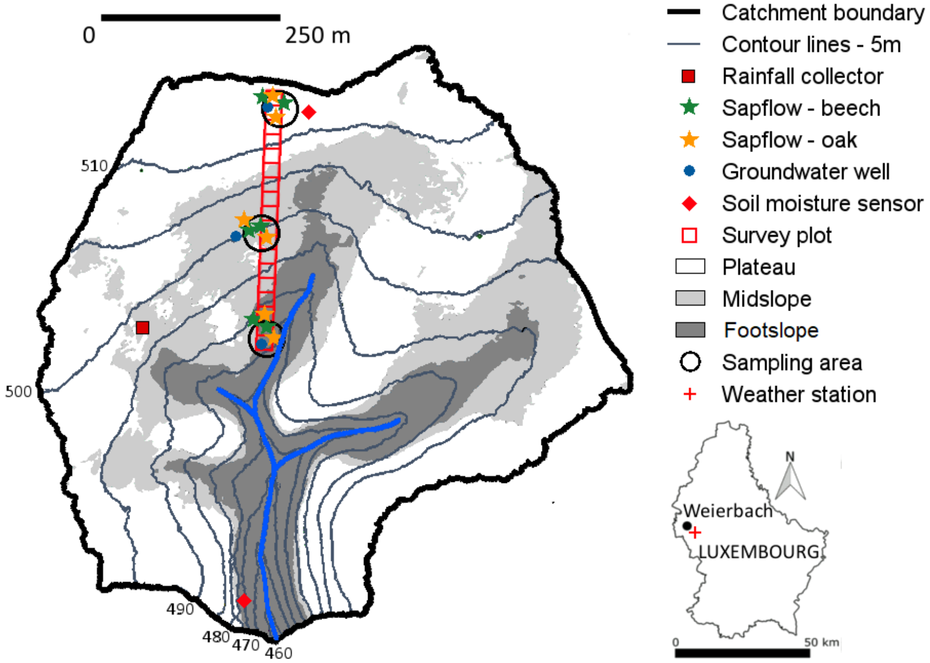

Figure 1. Site overview with the forest inventory plots (red squares). At each sampling area, a groundwater well (blue dot) was installed and two trees for each species (beech, oak) were equipped with sap flow sensors (green star for beech, yellow star for oak) with two different diameter classes.

The forest stand on the selected hillslope transect was characterized in a survey of 18 consecutive 20x20 m plots along the hillslope transect (Fig. 1) recording the number of trees, species, and diameter at breast height (DBH). The basal area for each tree was calculated from the DBH. Based on the digital elevation model (DEM), we derived the topographic position index (TPI, Hoylman et al., 2018; Weiss, 2001) to classify landscape position with respect to a defined neighborhood. The TPI was computed as follows:

$$TPI = z_i - \bar{z}_{i\_100} \qquad \text{(Eq. 1)}$$

where z is the elevation for the i-th pixel and $\bar{z}_{i\_100}$ is the average elevation in a 100 m radius around the i-th pixel. We defined the plateau area for TPI>0.5, the midslope for 0.5<TPI>-0.5, and the footslope for TPI<-0.5. The upper five plots fell into the category plateau, the subsequent nine plots into the midslope, and the lowest four plots were associated with the footslope. A fully stocked mixed forest with European beech trees (78% of the forest stand, 60% in basal area) and pedunculate and sessile oak hybrid trees (22% of the forest stand, 40% in basal area) populates the hillslope transect. The two oak species often form hybrids, which are phenologically difficult to differentiate. Hence, we did not differentiate between the species and refer to them as oak. Shrubs are absent and the understory mainly consists of blueberries (*Vaccinium myrtillus)*. Trees were divided into three diameter classes (Table 1). Given the relatively small diameter differences between individual trees in the plateau area (the upper part of the hillslope, see Fig. 1), we define the forest as even-aged. The forest becomes more heterogeneous downslope (Table 1).

Table 1. Number of trees (plant/ha) per tree diameter at breast height (DBH, cm) classes for beech and oak trees at the three locations along the hillslope. The total indicates the total number of trees per diameter class per hectare.

	Plateau		**Midslope**		**Footslope**		**Total**
DBH	beech	oak	beech	oak	beech	oak	
0-25	5	0	153	0	238	6	402
25-50	50	20	72	33	113	63	351
50-75	35	20	28	39	31	19	172

The plateau area is characterized by a lower basal area and lower forest density than the midslope and footslope (Fig. 2). The forest structure in the Weierbach catchment is the result of past and current management practices. Oak trees are evenly distributed across the whole area, while beech tree allometry strongly varies between the three areas with increasing density from the plateau to the footslope (Fig. S1). For each landscape position (plateau, midslope, footslope), we established one sampling area (Fig. 1).

Soils are shallow (<1 m) and the lithology consists mainly of a Pleistocene periglacial cover bed overlying Devonian slate from the Ardennes massif (Juilleret et al., 2011). The analysis of eight profiles showed that soil characteristics (e.g. structure, porosity, bulk density, particle density, texture) were similar across the catchment (Glaser et al., 2016). On the plateau, the subsolum is characterized by Regolithic Saprolite with gleyic properties, while the hillslope is characterized by a Regolithic Saprock substratum with dense vertical cracks (Juilleret et al., 2016). The solum is a stony loam soil with a mean thickness of 50 cm and an average porosity of 30%. In the subsolum, the size of schist/slate fragments strongly increases while the drainage porosity decreases. On average, the slate bedrock starts at a depth of 140 cm and is highly weathered (Gourdol et al., 2021) and permeable (Bonanno et al., 2021; Scaini et al., 2018). The subsurface structure leads to a dominance of vertical hydrological fluxes, while lateral flow occurs in the deeper hillslope (Glaser et al., 2019, 2016) and contributes to streamflow (Rodriguez et al., 2021; Rodriguez and Klaus, 2019).

Temperature (T), relative air humidity (RH), solar radiation, and precipitation (15 min logging intervals) data were available from the weather station. Using T and RH, we calculated the daily mean vapor pressure deficit (VPD) using Eq 2:

$$\mathrm{VPD} = 0.61375 \times \ \mathrm{e}^{(17.502 \times \mathrm{T}/(240.97+\mathrm{T}))} \times \left(1 - \frac{\mathrm{RH}}{100}\right) \qquad \text{(Eq. 2)}$$

Volumetric soil moisture was measured at the plateau and at a footslope at a different hillslope close to the catchment outlet with water content reflectometers (CS650, Campbell Scientific, UK). Each profile consisted of four probes installed horizontally at 10, 20, 40, and 60 cm depth recording at 30-min intervals (Fig. 1). At each location, one groundwater well was installed (10, 9, 3.5 m deep, and screened for 5, 4, 2.5 m from the bottom at the plateau, midslope and footslope, respectively) and was equipped with water pressure transducers (Orpheus Mini, OTT, Germany) recording data at 15 min logging intervals.

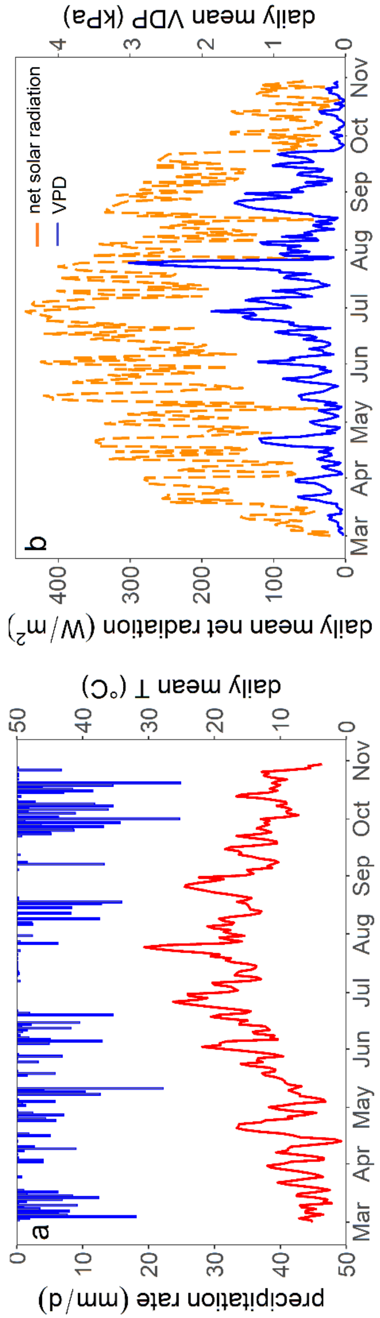

Figure 2. (a) Daily total precipitation amount (mm/d) and daily mean air temperature (°C) and (b) daily mean net radiation (W/m2) and daily mean vapor pressure deficit (VPD) (kPa) observed at the Roodt weather station.

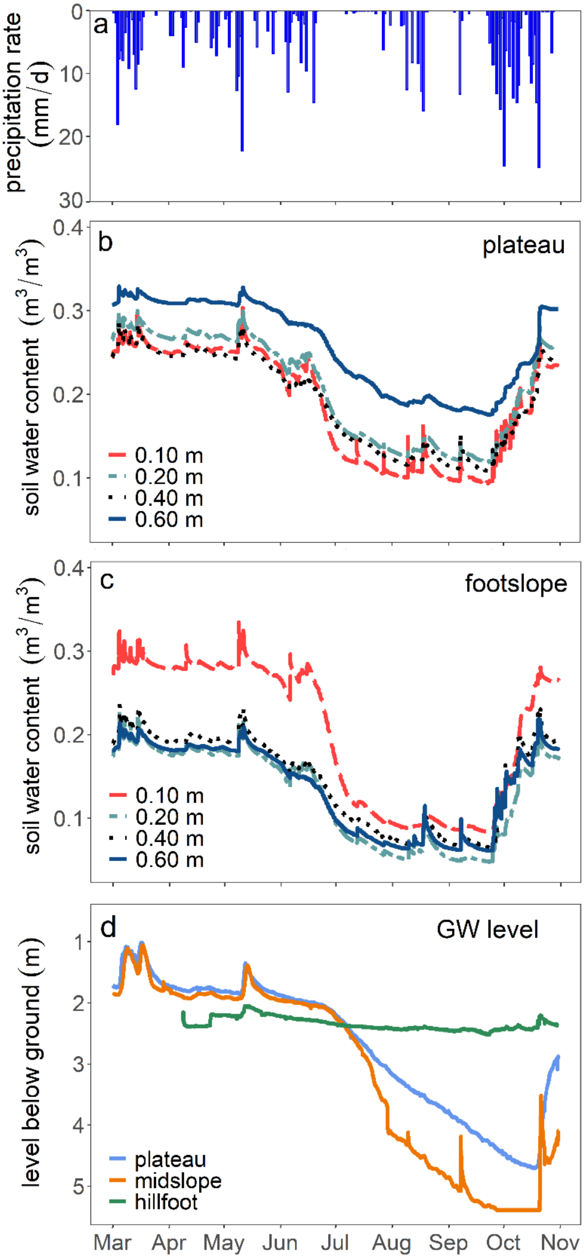

Figure 3. (a) Precipitation at the Roodt weather station, volumetric soil water content (SWC) recorded at 30 min intervals measured at the (b) plateau sampling area and at the (c) footslope location, and (d) groundwater level below ground surface (m) measured at the three sampling areas.

1.2.2 Measurement of sap velocity

At each of the three sampling areas (Fig. 1), we selected one tree from the most frequent diameter class (25-50 cm, referred to as average diameter class) and one tree from the diameter class 50-75 cm (referred to as large diameter class) of both species for sap velocity measurements (Table 1).
The selected trees were equipped with heat-pulse sap flow sensors (SFM1, ICT International Pty Ltd., Australia) (Table 2). We positioned the sap flow sensors at the north-east side of the trunk, 1.3 m above the ground and shielded them from direct sun exposure. The sensors consist of a central heating needle and two needles, each with two thermistors (located at 12.5 mm and 27.5 mm from the bark on a 35 mm long needle) recording the temperature upstream and downstream of the heater. Needles were installed one above the other with a vertical distance of 0.5 cm. The heat pulse velocity (V_h in cm h^{-1}) was calculated with Eq (3) (Burgess et al., 2001) at the inner and the outer thermistor:

$$V_h = \frac{k}{x} \ln\left(\frac{T1}{T2}\right) 3600 \qquad \text{(Eq. 3)}$$

where k is the thermal diffusivity (cm^2 s^{-1}) set to 0.0025 (Marshall, 1958), x is the distance between the heater and either temperature probe (0.5 cm), and T1 (°C) and T2 (°C) are the increases in temperature in the downstream and upstream thermistor, respectively.

Data were recorded from 7 April 2019 until 31 October 2019 at 30-min intervals covering the 2019 growing season. Sap velocity (V_{sap} in cm h^{-1}) for every monitored tree was calculated based on heat pulse velocity according to Eq. (4) (Barrett et al., 1995) (Fig. 4).

$$V_{sap} = V_h \mathrm{B} \frac{\rho_b(c_w + m_c \cdot c_s)}{\rho_s \cdot c_s} \qquad \text{(Eq. 4)}$$

where V_h is the calculated heat pulse velocity (cm h^{-1}), B is the wound correction factor set to 0.13 cm (Marshall, 1958), ρ_b the basic density of wood set to 0.5 g cm^{-3} (Burgess and Downey, 2014), c_w the specific heat capacity of the wood matrix (1200 J kh^{-1} °C^{-1} (Becker and Edwards, 1999)), Cs the specific heat capacity of sap (water, 4182 J kh^{-1} °C^{-1}, (Lide, 1992)), ρ_s the density of sap water (1 g cm^{-3}), and m_c the water content of sapwood (set to 0.5 g cm^{-3} (Burgess and Downey, 2014)).
Following Renner et al. (2016), sap velocities measured by the inner and outer thermistor were averaged to obtain the daily mean sap velocity, which is used as a

proxy of tree transpiration (Smith and Allen, 1996). For our analyses, we were interested in the response of sap velocity to environmental conditions (i.e., soil moisture and VPD) as an indicator for stomatal control, therefore we scaled daily mean sap velocities between 0 and 1 for further analysis, where 0 and 1 were the minimum and maximum daily mean velocities recorded by each tree over the entire growing season.

Table 2. Tree, location, tree DBH, sapwood area, and time period with available sap velocity data.

Tree	Location	DBH (cm)	Sapwood area (cm^2)	Available sap flow data
beech 1	plateau	32.8	544.8	07/04/2019- 31/10/2019
beech 2	plateau	58.9	1676.7	07/04/2019- 23/05/2019 – 09/07/2019 - 31/10/2019
beech 3	midslope	31.5	504.9	07/04/2019- 31/10/2019
beech 4	midslope	71.3	2420.6	07/04/2019- 31/10/2019
beech 5	footslope	30.9	485.2	07/04/2019- 31/10/2019
beech 6	footslope	57.9	1625.2	07/04/2019- 23/05/2019 - 09/07/2019 - 31/10/2019
oak 1	plateau	45.8	335.2	07/04/2019- 31/10/2019
oak 2	plateau	73.5	853.1	07/04/2019- 31/10/2019
oak 3	midslope	39.8	299.4	07/04/2019- 23/05/2019 - 09/07/2019 - 31/10/2019
oak 4	midslope	66.8	824.7	07/04/2019- 23/05/2019 - 09/07/2019 - 31/10/2019
oak 5	footslope	36.6	315.7	07/04/2019- 31/10/2019
oak 6	footslope	63.6	832.9	07/04/2019- 23/05/2019 - 09/07/2019 - 31/10/2019

1.2.3 Wood core, soil, and water sampling for isotopic analysis

We carried out 14 bi-weekly sampling campaigns over one entire growing season from 8 April 2019 (before leaf flush) until 21 October 2019 (when leaves were turning yellow). For each campaign, we randomly selected two beech and two oak trees from the dominant diameter class (25-50 cm DBH) at each of the sampling areas (Table 1). The maximum distance between trees equipped with sap flow sensors and trees that were sampled for xylem water was 25 m. We collected trunk cores encompassing only the sapwood with a Pressler borer, removed the bark, and transferred the remaining xylem wood into 30-ml glass vials sealed with caps and Parafilm®. Samples were stored in a freezer (-22°C) until water extraction (see section 2.4). During five sampling campaigns (8 April, 23 April, 3 June, 17 June, and 1 July), we also sampled soil cores to assess the soil water isotopic composition. At each sampling area, we extracted three soil cores from the top 60 cm divided into five depth classes (0-5, 5-10, 10-20, 20-40, 40-60 cm) with a soil auger. A 60 cm depth was the lower limit of sampling due to the interface between the soil and rock clasts of the basal layer (Juilleret et al., 2011). Each soil sample was stored in zip bags with as little air as possible until analysis (see section 2.4). We sampled groundwater at the sampling areas and rainfall bi-weekly with a rainfall collector (Palmex Ltd.) placed in a clearing (Fig. 1).

1.2.4 Water extraction from plant and soil material and isotopic analyses

We extracted xylem water from wood cores through a cryogenic vacuum distillation line (Orlowski et al., 2016) by submerging the sample in a 100°C oil bath for 3 h under a vacuum of 0.03 hPa. Evaporated water was collected in U-shaped tubes, which were submerged in liquid nitrogen (-197°C). The pressure was continuously recorded to assure that the lines remained leak-tight throughout the entire extraction. The average extraction efficiency of the cryogenic vacuum distillation line is 99.7%, which was assessed by oven drying 210 extracted samples at 105°C for 24h and weighing them afterwards. The isotopic composition of the extracted water (δ^2H and $\delta^{18}O$) was analyzed using a Picarro cavity ring down spectrometer (CRDS) (L2140-i, Picarro, USA) coupled with a Micro-Combustion Module^{TM} (MCM) in order to remove organic compounds (ethanol, methanol and/or other biogenic volatile compounds). To detect possible organic contamination, xylem samples were screened with ChemCorrectTM (Picarro Inc., Santa Clara, CA, United States) software, which attempts to identify contamination through fitting to a known library of spectral features. In order to minimize the potential memory effect (Penna et al., 2012), each sample was analyzed 10 times and only the last 5 measurements were used to calculate the average isotope signal. Our quality control lab standard water was 0.02‰ for $\delta^{18}O$ and 0.3‰ for $\delta^{2}H$. Precipitation and groundwater samples were analyzed on a LGR Liquid Water Isotope Analyzer (TIWA-45-EP, Los Gatos Research, Inc., Mountain View, CA, USA).

We analyzed soil samples for their water stable isotopic composition with direct liquid-water-vapor equilibration (Wassenaar et al., 2008).We prepared the sampling bags with a blot of silicone on the outside to later serve as a septum. After each sampling campaign, sampling bags were heat sealed upon arrival at the lab, pierced, and inflated with dry air with a needle, and left to equilibrate for 24 h to allow water liquid-vapor equilibration. In parallel, three bags were filled with 40 ml of water with known isotopic composition (ranging between-10.3 - -2.9 in $\delta^{18}O$ and -154.0 – -9.9 in $\delta^{2}H$) and dry air and left 24 h to equilibrate. Headspace vapor was sampled directly with a needle connected to the Picarro analyzer coupled with MCM for the samples and the three bags with known values. We used two known values as standards for calibration and one known value for validation. For this analysis, our quality control lab standard showed an average deviation from the real value of 0.07‰ for $\delta^{18}O$ and 1.7‰ for $\delta^{2}H$.

The local meteoric water line (LMWL) at the study site, based on biweekly precipitation samples collected from 2011 to 2018, is $\delta^{2}H = 7.4\ \delta^{18}O + 6.5$. This rather low slope is characteristic for the region as shown for the IAEA precipitation isotope station in Trier (Klaus et al., 2015; Stumpp et al., 2014), which is approximately 64 km from the study site. We calculated the *lc*-excess for xylem water following Landwehr and Coplen (2004):

$$lc\text{-}excess = \delta^{2}H - a \cdot \delta^{18}O - b \qquad \text{(Eq. 5)}$$

where a and b are the slope and intercept of the LMWL, respectively.

1.2.5 Data analysis

We analyzed differences in sap velocities and $\delta^{2}H$ and $\delta^{18}O$ in xylem, groundwater, and soil water between species and sampling areas using the software R (v.3.2.1; R Core Team, 2015). Comparisons of sap velocities between sampling areas, species, and diameter classes were analyzed with the Pairwise Wilcox test. The Pairwise

Wilcox test was also used to analyze differences in the isotopic composition of groundwater sampled at the three sampling areas. Comparisons of the xylem isotopic composition between different species, sampling areas, and sampling dates were analyzed via the non-parametric Mann-Whitney test.

1.3. Results

1.3.1 Environmental conditions over the 2019 growing season

The cumulative precipitation from 1 March 2019 to 31 October 2019 was 608.6 mm and the mean air temperature was 11.9 °C (Fig. 2a). The daily mean VPD and solar radiation increased during the growing season, reaching a peak in July (Fig. 2b). Soil moisture progressively decreased between May and September at all depths, but from September, it increased due to a decrease in evapotranspiration and recurrent precipitation. The topsoil moisture content (10 cm) mostly reflected the precipitation pattern; the soil moisture signal in the deeper soil was generally damped compared to the topsoil (Fig. 3 b, c). Deeper soil (60 cm) at the plateau displayed a consistently higher volumetric water content compared to shallower soil (Fig. 3b). At the footslope, soil moisture was higher in the topsoil and decreased over time reaching the same values of deeper layers from July onwards (Fig. 3c). The groundwater level at the plateau and midslope position showed a similar temporal trend. The groundwater level was highest in spring and decreased throughout the growing season, reaching 4.7 m and 5.7 m below the surface at the plateau and midslope positions, respectively (Fig. 3d). The groundwater table at the footslope location was quite steady over the study period with an average of 2.6 m below surface (Fig. 3d).

1.3.2 Sap velocity: Species, site, and size-specific differences

The daily mean sap velocity exhibited species, site, and size-specific variations. Due to instrument failure, not all sensors supplied a complete record over the growing season (Table 2). Beech trees showed earlier leaf emergence (approx. 18 April - 26 April) than oak trees (approx. 1 May - 7 May), consistent with increasing sap velocity during these periods (Fig. 4). We observed a strong seasonal pattern in sap velocity at the three sampling areas for both monitored diameter classes (Fig. 4). Sap velocity increased in spring, reaching the maximum at the end of June when atmospheric water demand and soil moisture were high. Concurrently with decreasing soil moisture and high evaporative demand, sap velocity decreased gradually and showed minimum values in mid-August. Short-term increases of sap velocity occurred during the summer following precipitation events (e.g., middle of August, cf. Fig. 2a). From the end of August, we observed a progressive decline in sap velocity, as soil moisture remained low and VPD decreased (Fig. 2, 3).

The daily mean sap velocities of beech trees from the average diameter class (25-50 cm DBH) were consistently higher at the midslope than at the other locations ($p<0.05$, Pairwise Wilcox test) (Fig. 4a). Beech trees at the footslope location experienced a delayed increase in sap velocity but reached the same velocities as plateau trees in summer. Sap velocity for beech trees from the large diameter class (50-75 cm DBH) (Fig. 4c) was not different between the sampling areas. Mean daily sap velocities in beech trees were statistically not different among the average and the large diameter class ($p>0.05$, Pairwise Wilcox test) (Fig. 4 a, c). Oak trees experienced significantly higher sap velocities at the plateau for both diameter classes than the other locations ($p<0.05$, Pairwise Wilcox test) (Fig. 4 b, d). For the average diameter class, sap velocity was statistically similar for oak trees at the footslope and midslope, while for oak trees of the large diameter class, the plateau tree displayed the highest, followed by the one midslope and footslope (Fig. 4d). For oak trees, sap velocity was approximately 2.5 times higher for the large diameter class than for trees from the average diameter class. We did not find a statistical difference between the two species in sap velocity for the large diameter class ($p>0.05$, Pairwise Wilcox test) when comparing dates where all sensor data was available (Fig. 4 c, d). Sap velocities were significantly higher for the average diameter class of beech trees than oak trees ($p<0.05$, Pairwise Wilcox test) (Fig. 4 a, b).

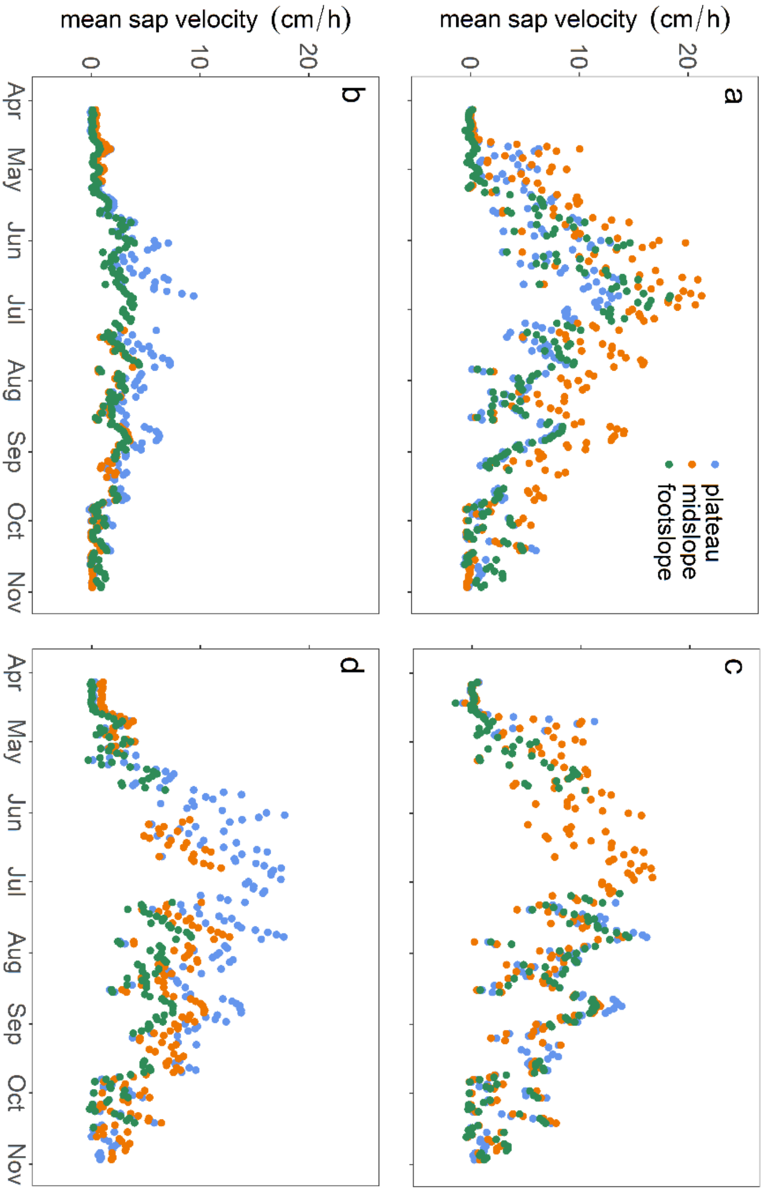

Figure 4. Sap velocity (cm h^{-1}) of beech and oak at three sampling areas; (a) beech from the average diameter class, (b) oak from the average diameter class, (c) beech from the large diameter class, and (d) oak from the large diameter class.

1.3.3 Relationship between sap velocity, VPD, and soil moisture

The relationship between normalized sap velocities from trees from the average diameter class and daily average VPD and the relationship between normalized sap velocity and daily average soil moisture across the four measured depths (from the plateau cluster) showed anticlockwise hysteresis for both species (Figs. 5, 6). However, the shape of the loop was different for the beech and oak trees (Figs. 5, 6) suggesting a species-specific response to the two environmental controls. In the early growing season, sap velocity progressively increased in all monitored plants concurrently with an increase in VPD (Fig. 5). For beech trees, maximum sap velocity occurred when VPD values ranged between 1.2-1.4 kPa (Fig. 5). However, at the end of July, when average VPD values where above 1.85 kPa, sap velocities in beech trees dropped by about 35-50% (Fig. 5). Contrary to beech, oak trees maintained maximum sap velocities (Fig. 5). When VPD ranged between 1.2-1.4 kPa again in August, beech trees did not meet the pre-stress sap velocities. (Fig. 56).

We also observed a marked temporal pattern in the relationship between sap velocity and soil moisture and different behaviors between the two species (Fig. 6). Sap velocity increased for both species at the beginning of the growing season when soil moisture was high. Sap velocity in beech trees experienced a marked decline compared to oak trees once soil water content approached 0.2 m^3m^{-3}. Oak trees maintained stable and high sap velocities and dropped only when the soil moisture content was lower than 0.15 m^3m^{-3}. We did not observe differences in the relation between VPD or soil moisture and normalized sap velocity between the different sampling areas (Fig. 5, 6). Sap flow data for trees from the large diameter class showed the same patterns (data not presented).

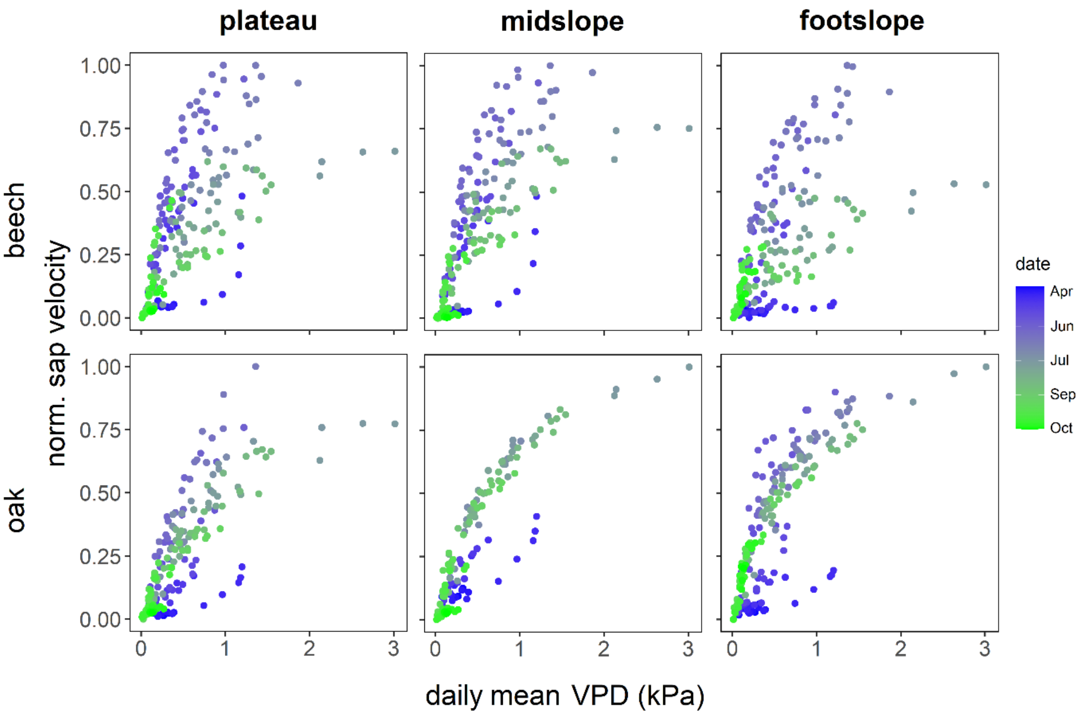

Figure 5. Relationship between normalized mean sap velocity and daily average vapor pressure deficit (VPD) (kPa) from beech and oak trees from the average diameter class at different hillslope positions with data from 5 April 2019 to 31 Oct 2019.

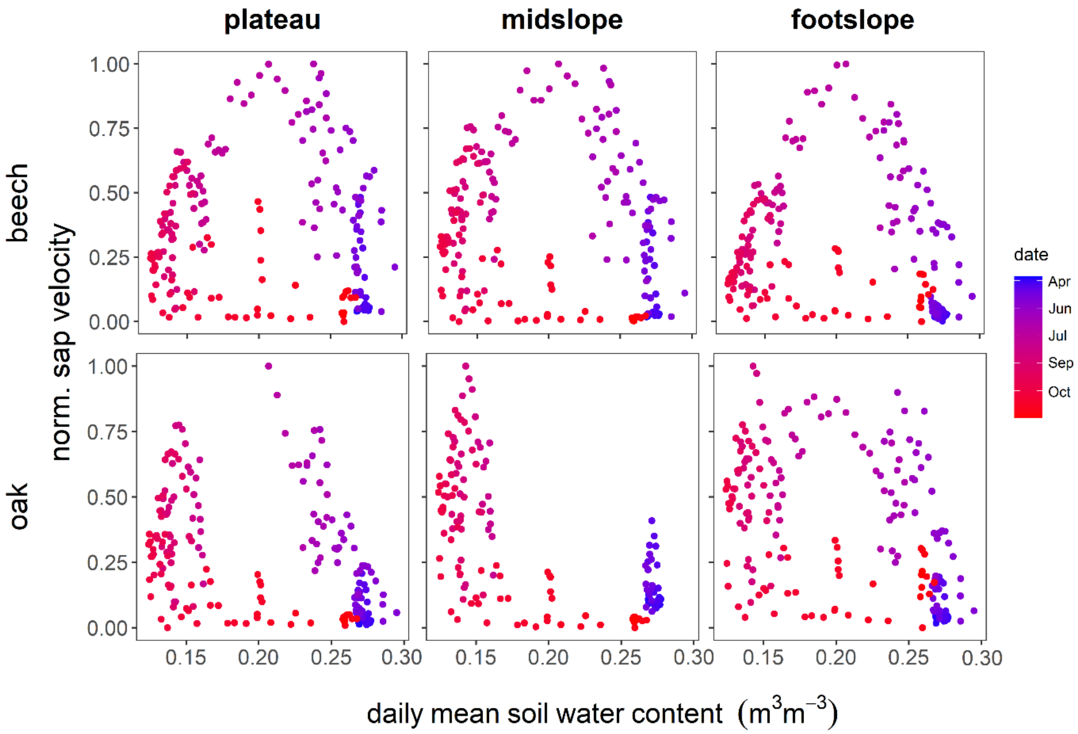

Figure 6. Relationship between normalized mean sap velocity of beech and oak trees from the average diameter class at different hillslope positions and daily mean soil water content (m^3m^{-3}) at the plateau site from 5 April 2019 to 31 Oct 2019

1.3.4 Isotopic composition of water, soil, and xylem samples

The isotopic composition of the bi-weekly precipitation samples collected between 1 March and 31 Oct 2019 ranged from -14.6‰ to -2.3‰ and from -97.6‰ to -17.2‰ in $\delta^{18}O$ and $\delta^{2}H$, respectively. The groundwater isotopic composition was quite stable over time but was statistically significantly different between the three sampling areas ($p<0.05$, Kruskall-Wallis test) displaying progressively lighter isotopic values moving from the plateau (mean -8.3‰ $\delta^{18}O$, -53.5‰ $\delta^{2}H$) to the midslope (mean -8.4‰ $\delta^{18}O$, -54.0‰ $\delta^{2}H$) and the footslope location (mean -8.5‰ $\delta^{18}O$, -55.1‰ $\delta^{2}H$). Soil water showed less negative isotopic values in shallow soil layers and more negative values in deeper soil layers (Fig. S2). No significant differences in soil water isotopic composition occurred between different sampling areas ($p>0.05$, Wilcoxon rank sum test). Deeper soil layers displayed a generally constant isotopic composition over time compared to the shallower layers, which tended to become isotopically heavier over time. In the dual isotope plot (Fig. 7), soil isotopic values plotted along the LMWL.

Xylem water from both species plotted below the LMWL in the dual-isotope space (Fig. 7). This was a consistent occurrence across all sampling campaigns and sampling areas (Fig. 7). In particular, the xylem samples from beech trees plotted farther away from the LMWL than those from oak trees (Fig. 7). The isotopic composition of xylem water was highly variable throughout the growing season (Fig. 8). Xylem water from the first sampling campaign (8 April 2019) before the leaf flush was significantly higher in δ-values for both isotopes (Wilcoxon rank sum test, $p<0.05$) compared to later dates (Fig. 8) and was situated in the upper right area of the dual-isotope plot (Fig. 7, SI Appendix Fig. S3). Concurrently with leaf flush, the δ-values became markedly lighter (Fig. 8 a, b) and xylem water fell in the lower left part of the dual-isotope space (Fig. 7, SI Appendix Fig. S3). Xylem water from both species became progressively heavier in both isotopes over the growing season, although two sampling campaigns that were carried out one day after rainfall led to a deviation from this general trend (cf. late July and September sampling, Fig. 8). For both isotopes, the interquartile range and variability over the sampling season were higher for beech trees than for oak trees (Fig. 8, 9). Xylem water from beech trees displayed a more pronounced variability at the sampling date and seasonal scale than xylem water from oak trees, which exhibited more consistent isotopic composition over time (Fig. 8). From the end of May to the end of the 2019 growing season, xylem water from beech trees showed heavier $\delta^{18}O$ values than in oak trees ($p<0.05$) but lighter in $\delta^{2}H$ ($p>0.05$). We found a statistical difference in xylem *lc*-excess between the two species (Fig. 9c) (Wilcoxon rank sum test, $p>0.05$) over the whole study period. We did not observe significant differences ($p>0.05$, Wilcoxon rank sum test) in beech tree xylem $\delta^{18}O$, $\delta^{2}H$, and *lc*-excess along the hillslope when xylem data were grouped together (Fig. 9). Additionally, no topographic effect was evident in *lc*-excess over time (Fig. 10). However, from June to September, beech trees at the plateau location exhibited more negative *lc*-excess than the other locations. For oak trees, we did not observe significant differences in xylem $\delta^{18}O$ and $\delta^{2}H$ ($p>0.05$, Wilcoxon rank sum test) between the three locations but *lc*-excess was significantly different between the plateau and the footslope ($p<0.05$, Wilcoxon rank sum test) (Fig. 9).

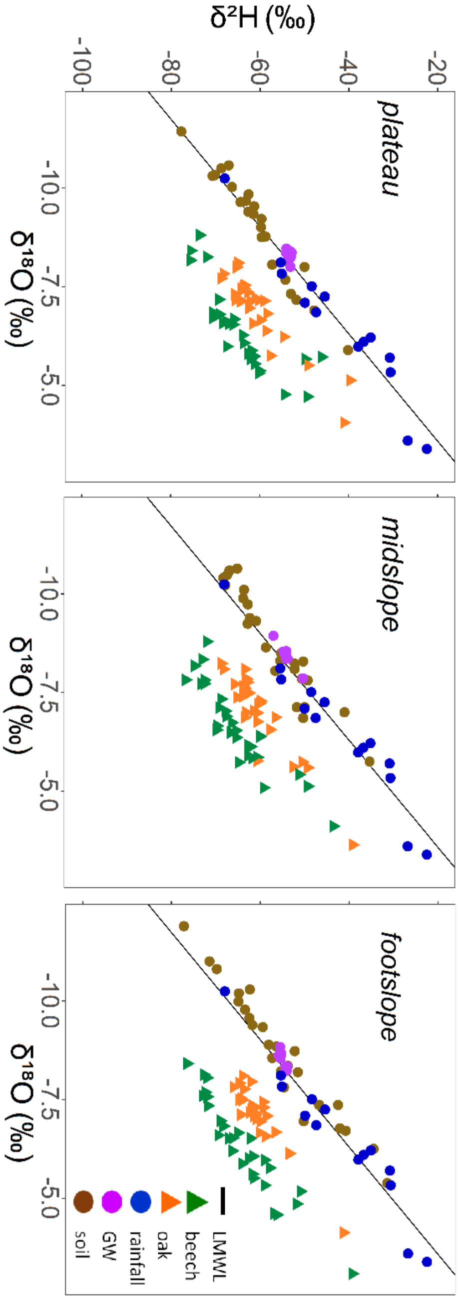

Figure 7. Dual-isotope (δ^2H and δ^{18}O) plot of xylem water from the two species studied and their potential sources (soil water at five depths, groundwater) for every sampling campaign conducted in 2019. Soil was sampled on 8, 23 April, 3, 17 June, and 1 July. The black line indicates the LMWL ($\delta^2H = 7.4\ \delta^{18}O + 6.5$).

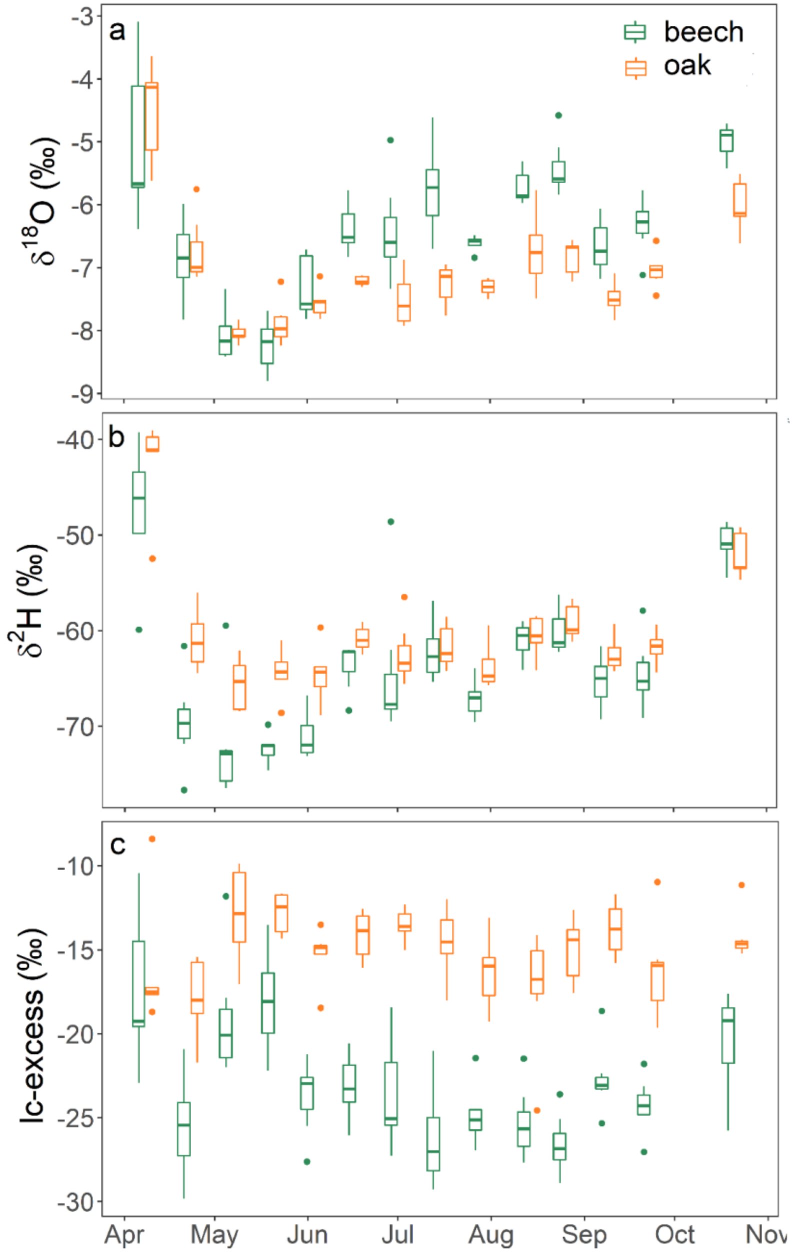

Figure 8. (a) δ^{18}O, (b) δ^{2}H and (c) lc-excess of xylem water for each sampling campaign across the growing season. The data from each species were averaged over the three sampling areas. The center line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartile, respectively. The whiskers indicate points within 1.5 times the interquartile range above or below the median.

1.4. Discussion

1.4.1 Spatial and temporal pattern in tree water use along the hillslope

We generally observed higher sap velocities in trees from the average diameter class growing at plateau and midslope locations compared to those at the footslope suggesting that growing conditions at upslope areas were more suitable. Here, trees may benefit from a lower degree of competition for water and greater access to light due to the lower forest density compared to the footslope location (Pretzsch and Forrester, 2017) (Fig. S1). We observed minor differences in sap velocity between locations for large diameter trees, because they likely have access to a deeper and more stable water reservoir (cf. Gaines et al., 2016; Goldsmith et al., 2012) and occupy dominant positions which ensure greater light interception than the average trees in the stand. From the beginning of the growing season until late June when leaves were completely unfolded, sap velocities increased along the hillslope due to the increasing VPD and sufficient soil water supply (Fig. 3, 4). From June onwards, xylem water isotopes progressively became heavier (Fig. 8) and the *lc*-excess became more negative indicating that trees relied on water sources that had been increasingly affected by isotopic enrichment, such as shallow soil water. From July onwards, soil moisture decreased, the groundwater table at plateau and midslope locations receded, and sap velocities decreased. The response of sap velocity to environmental controls (VPD and soil moisture) was species-specific but similar in all locations (Fig. 5, 6) indicating that the sampled trees mainly absorbed water from similar sources, irrespective of hillslope position. However, at the footslope, sap velocities were equal to or lower than at the midslope and plateau locations (Fig. 4) despite a seasonally high and stable groundwater table (Fig 3d). This may indicate that increased groundwater accessibility did not foster higher transpiration rates.

Xylem isotopic composition varied seasonally but differed between the sampling areas in only a few sampling campaigns (Fig. 7). This result suggests that at our study site trees might not directly rely on groundwater or used it to such a limited extent that no clear differentiation in isotopic composition between trees vegetating in higher positions in the landscape was detectable. In studies where trees were found to exploit groundwater, xylem water isotopic composition hardly varied over time because groundwater isotopic composition was rather stable compared to rain and soil water (Carrière et al., 2020; David et al., 2013). The observed high and seasonally stable groundwater table at the footslope may even restrict root development and confine roots to shallow soil and saprolite layers, since saturated environments can limit root expansion and plant productivity for species that do not tolerate permanent hypoxic or anoxic conditions (Fan et al., 2017; Hasenmueller et al., 2017; Rossatto et al., 2014; Roy et al., 2000) such as beech and oak trees (Schmull and Thomas, 2000). Additionally, the lack of daily groundwater table fluctuations, even when sap velocity was high (Fig. 3d), may indicate that trees did not rely, or only marginally relied, on groundwater (cf. Naumburg et al., 2005). Diurnal water table fluctuations have been mostly observed in riparian areas and used to directly assess groundwater consumption by plants (Lurtz et al., 2020; Martinetti et al., 2020; Soylu et al., 2012), but these studies involved wetland species that cope with anoxic conditions in the root zone . However, at our study site the potential tree water uptake from groundwater at the footslope might have been balanced by groundwater inflow from upslope areas (Rinderer et al., 2017). The progressively declining water table at upslope locations over the growing season may have left behind an aerated soil profile at field capacity, which became available for deeper root exploitation (Naumburg et al., 2005). Trees located in the upper portion of hillslopes are usually found to develop a dimorphic root system that allows them to access the weathered rock moisture during dry periods (Fan et al., 2017). Hahm et al. (2020) were able to demonstrate that the receding groundwater table progressively offers a large water reservoir held in the weathered bedrock, which is accessed by deeper roots, and that rock moisture can offer a significant contribution to transpiration in a variety of lithological settings (Rempe and Dietrich, 2018). However, the isotopic characterization of this potential water source is often neglected in isotope studies due to difficulties in accessing and measuring below-ground compartments (Rempe and Dietrich, 2018).

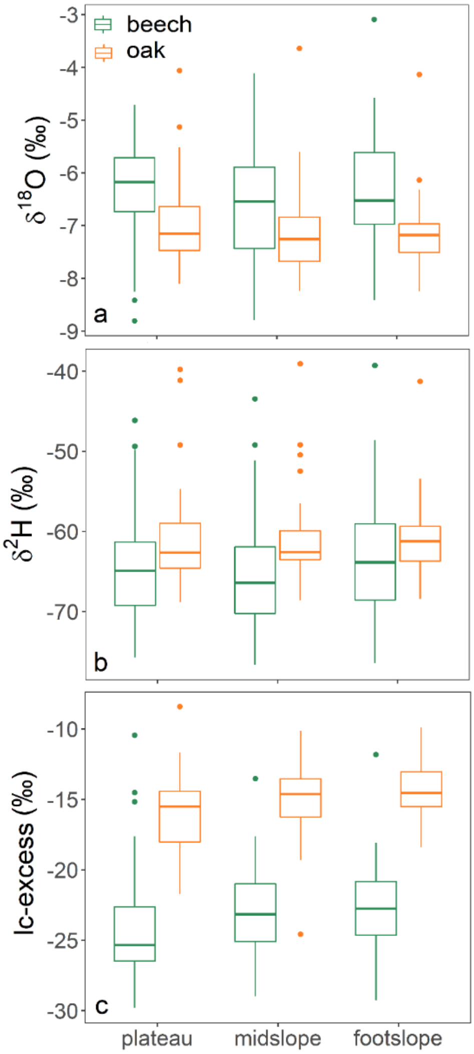

Figure 9. (a) $\delta^{18}O$, (b) $\delta^{2}H$ and (c) lc-excess of xylem water for each sampling campaign at the three sampling plots for all sampling campaigns conducted in 2019. The center line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartile, respectively. The whiskers indicate points within 1.5 times the interquartile range above or below the median.

Xylem water in our study displayed a hydrogen isotope ratio more depleted than any of the water sources considered, as shown in other studies (Barbeta et al., 2019; Oerter et al., 2019; Oerter and Bowen, 2017). Some possible explanations for this offset have been proposed in the literature, including isotopic separation between bound and mobile soil water (Brooks et al., 2010), water compartmentalization between flowing and stored water in the stem (von Freyberg et al., 2020; Zhao et al., 2016), and isotopic fractionation at the soil-root interface (Barbeta et al., 2020; Poca et al., 2019). Other studies have provided some indications that the isotopic offset could arise from $\delta^{18}O$ fractionation processes (Marshall et al., 2020; Vargas et al., 2017), challenging the argument of negligible ^{18}O fractionation during root water uptake (Rothfuss and Javaux, 2017). We do not have evidence that goes beyond the behaviors recently discussed (Beyer and Penna, 2021; von Freyberg et al., 2020), but we cannot exclude that unsampled saprolite and weathered bedrock waters could act as additional sources potentially explaining this offset.

Furthermore, the lack of higher sap velocity at the footslope location, which is contrary to observations in several other studies due to the lateral redistribution of soil moisture (Hawthorne and Miniat, 2018), deeper soil, and higher water holding capacity (Kumagai et al., 2008; Mitchell et al., 2012; Tromp-van Meerveld and McDonnell, 2006) provides some insight into the hydrological functioning of the Critical Zone at the Weierbach. Due to the high hydraulic conductivities in the Weierbach catchment (Glaser et al., 2016) and the lack of shallow impeding layers, the hillslope structure does not promote the lateral downslope redistribution of soil water via interflow (Klaus and Jackson, 2018). This results in reduced subsidies to soil moisture at footslope locations, contrary to what was observed in other studies (Hawthorne and Miniat, 2018; Lin et al., 2019). Due to the highly fractured bedrock (Juilleret et al., 2011; Martínez-Carreras et al., 2016), upslope patches are hydrologically connected only through the saturated zone with downslope areas allowing groundwater at the footslope location to maintain a constant and shallow water table over the investigated period (Rinderer et al., 2017). This behavior shows the critical importance of landscape characteristics determining water redistribution, water availability for vegetation, and ultimately growing conditions.

1.4.2 Species-specific response to variable water supply

Our study showed that beech and oak trees clearly have different water uses, representing a resource-driven niche partitioning. The systematically more negative and seasonally more variable xylem *lc*-excess (Fig. 8c) of beech trees compared to oak trees suggests the use of a water source more exposed to isotopic enrichment, likely near-surface soil water. Such a difference can be associated with species-specific root architecture. This explanation is consistent with *in situ* root measurements at other sites (Coners and Leuschner, 2005a; Leuschner et al., 2001). For instance, in a beech-oak forest stand, Leuschner et al. (2001) found that beech was a superior below-ground competitor of the topsoil where the maximum fine root densities occurred to colonize the nutrient-rich organic layer. In contrast, oak was considered a deep-rooted species able to access deeper subsurface water (Lanning et al., 2020). Through a metanalysis across several sites, Fan et al. (2017) found that the average rooting depth of the genus Quercus was 5.23 and only 0.8 m for Fagus.

The different timing of leaf emergence observed for the two species, as also shown by sap velocities (Fig. 4), indicates that water use partitioning does not only occur spatially in the subsurface driven by root distribution, but also temporally (Meinzer et al., 2001). This phenological variation has been linked to wood hydraulic conductivity (Wang et al., 1992). Over the dormant season, ring-porous species like oak with large vessels may experience a higher loss in hydraulic conductivity than diffuse-porous species like beech (Cruiziat et al., 2002). To overcome winter embolism and restore the water flow pathway, oak trees invest in the formation of early wood before leaf expansion (Bréda and Granier, 1996), while stem growth in beech trees starts after leaf flush (Barbaroux and Bréda, 2002). At the time when leaves flush out, the xylem water in our study displayed an extremely heavy isotopic composition. This is consistent with previous studies and results from evaporation through the bark during periods of limited sap flow (Barbeta et al., 2019; Martín-Gómez et al., 2017; Oerter et al., 2019; Phillips and Ehleringer, 1995).

Accessibility to different water sources could also explain the different hysteretic response of sap velocities to the environmental drivers (VPD and soil moisture). The increasing VPD and high soil moisture content in the first part of the growing season supported an increase in sap velocity in both species (Fig. 5, 6). However, the high VPD values and reduced water supply from late July onwards induced stomatal closure in beech trees in order to minimize water loss. Conversely, oak trees showed a lower sensitivity to these environmental forces with sap velocity reaching its maximum when VPD approached 3 kPa, despite reduced soil moisture content. The different degree of stomatal sensitivity to the environmental forcing coupled with different xylem isotopic composition suggests the use of a deeper and more stable water source for oak trees and a shallower and ephemeral water source in beech trees, supporting the findings of Bakker et al. (2008) and Grossiord et al. (2017).

In order to meet water requirements and regulate water status, trees have developed different adaptations, like stomatal response (isohydric vs anisohydric species), xylem architecture (diffusive vs. ring-porous), and rooting depth (Matheny et al., 2017a; N. McDowell et al., 2008). The interplay of these characteristics defines tree transpiration response to a variable water supply. The deep rooting strategy of oak trees enables them to overcome the hydraulic failure risk given their anisohydric stomatal regulation (Matheny et al., 2017a) and ring-porous xylem (Cruiziat et al., 2002) by exploiting deeper water sources. Anisohydric species have a weaker stomatal sensitivity than isohydric species such as beech trees, which reduce water consumption via stomata closure to avoid water stress damage (Magh et al., 2020).

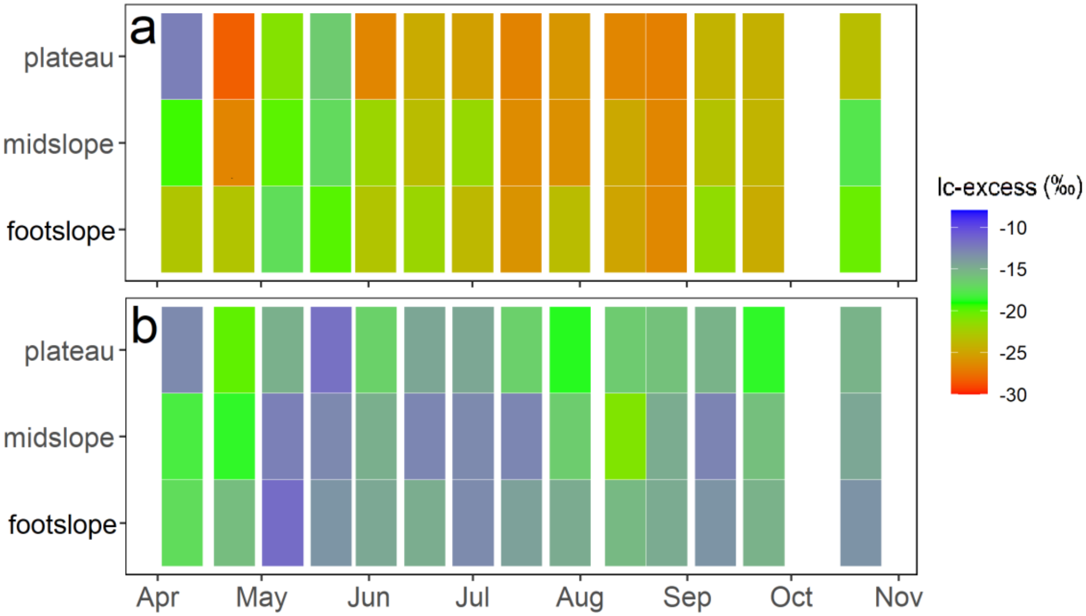

Figure 10. *lc*-excess of xylem water from (a) beech and (b) oak trees at the three sampling areas over the growing season.

1.5. Conclusions

In this work, we examined the role of landscape topography (hillslope position) on the spatial and temporal patterns of water use in a mixed forest of beech and oak trees through sap velocity and stable water isotope measurements. We showed different patterns of sap velocity in different hillslope positions, with trees generally displaying higher sap velocity in upper locations than in downslope areas where the groundwater table was shallower and temporally less variable. These results, coupled with seasonally variable xylem isotopic composition, suggest that the trees studied relied on vadose zone water storage irrespective of the hillslope position and the corresponding groundwater table depth. Contrary to many previous studies that showed the influence of the shallow groundwater table on transpiration rates, the shallower and more stable groundwater table at the footslope did not foster higher sap velocities at our study site compared to upper locations. Additionally, our study site does not experience shallow downslope water redistribution through interflow which results in no substantial differences in vadose zone water supply between hillslope positions and ultimately no higher sap velocity in downslope areas. Thus, we reject our first conjecture that hillslope position controls tree water use though subsurface water redistribution. In our case, high and stable groundwater table at the footslope location might even reduce root expansion in species that do not tolerate saturated environments.

Furthermore, our results confirm that beech and oak trees have different ecohydrological niches driven by their species-specific water exploitation strategies and hydraulic traits, which are crucial to determining a tree ability to recover from water shortage periods. Beech trees, although more drought-sensitive than oak trees, are a superior above-ground competitor. This characteristic, combined with a marked shade tolerance, allowed them to regenerate extensively at our study site, while oak seedlings and saplings were absent. This confirms our second conjecture that species-specific characteristics result in a different response in different species at the same hillslope position.

Overall, our findings highlight that the link between forest community and the Critical Zone structure is highly dynamic due to species-specific interaction with water availability and subsurface flow patterns. Future management practices should operate to create optimal conditions for forest resilience, accounting for subsurface structure to promote more drought-tolerant species in order to ensure ecosystem functioning in face of future climate change.

Chapter 2

Sapwood and heartwood are not isolated compartments: consequences for isotope ecohydrology

Abstract

In most tree species, xylem consists of two different functional parts: sapwood and heartwood. While sapwood, as the flowpath for sap, has received more attention in isotope studies assessing water sources accessed by trees (e.g., soil water from different depths, groundwater, stream water or a mixture of these), much remains unknown about heartwood and the possible water exchange between the two functional parts. We investigated four tree species (*Fagus sylvatica*, *Quercus petraea*, *Pseudotsuga menziesii*, and *Picea abies*) characterized by different xylem anatomy, and timing of physiological activity to evaluate the degree of differentiation in isotopic composition of water between sapwood and heartwood on a biweekly time scale. We found that the sapwood and heartwood of all species displayed a concurrent variation in their isotopic composition throughout the growing season and on a day-night scale suggesting that the two are not isolated compartments. While the two functional parts display a consistent difference in isotopic composition in conifers, they are characterized by more similar values in broadleaved species in broadleaved species, suggesting a higher degree of water exchange. Furthermore, we have also observed a progressive change in the isotopic composition in broadleaved species with sampling depth rather than functional parts of xylem. Our study highlights the value of accounting for radial isotopic variation, which might potentially lead to uncertainties concerning the origin of the extracted water for water uptake studies.

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2.1 Introduction

A widely applied method to investigate tree water uptake is the use of stable isotopes of hydrogen ($^{2}H/^{1}H$) and oxygen ($^{18}O/^{16}O$) in the water molecule. Xylem water isotopic composition is deemed to reflect the combined isotopic composition of water sources accessed by the tree, with the assumption that no isotopic fractionation occurs during root water uptake and water flow in plant xylem (Flanagan and Ehleringer, 1991; White et al., 1985). However, this assumption was challenged when multiple studies observed that the isotopic composition of xylem water does not always fall within the range of the isotopic composition of the source waters (Penna et al., 2018). Some studies hypothesized that physiological mechanisms at the soil-root interface (Poca et al., 2019), leaf-atmosphere scale (Cernusak et al., 2016), and along the flow of the sap within the xylem might cause a depletion in ^{2}H xylem water and consequently, a deviation from the uptake sources (de la Casa et al., 2021). Additionally, there is increasing evidence that water compartmentalization between flowing and stored water can occur within the stem (Barbeta et al., 2022; Zhao et al., 2016) and that the daily withdrawal of water from storage cells might affect the xylem isotopic composition (Dubbert and Werner, 2018). Zhao et al. (2016) found that bulk stem water in poplar trees displayed a different isotopic composition compared to sap water. Relying on a new water extraction technique for tree stems able to selectively extract sap water from xylem conduits, Barbeta et al. (2022) provided experimental evidences that a certain degree of compartmentalization exists between water in non-conducting woody cells and sap, with the latter being a better proxy of source water than bulk stem water. Water compartmentalization in plant tissue implies the occurrence of redistricted "communication" between water compartments (Yakir, 1992). This makes the identification of tree water sources challenging, since isotopic heterogeneity in the wood matrix may imply that bulk xylem water does not accurately reflect its source water but is rather an approximation of it (Barbeta et al., 2022; Beyer and Penna, 2021; von Freyberg et al., 2020).

In most tree species, xylem consists of two physiologically different parts: sapwood (SW) and heartwood (HW). SW is characterized by the presence of living cells, which act as water and nutrient storage, and non-living cells that function as the route of sap flow (Spicer, 2005). As the tree ages, old SW is compartmentalized in the center of the stem as HW (Spicer, 2005) and becomes disconnected from the root system, which also develops HW close to the stem base (Hillis, 1987). HW xylem provides the mechanical support to the tree in spite of not participating in the vital activities of the tree as result from the loss to conduct water due to pit aspiration, incrustation, and vessel tyloses (Nakada et al., 2019; Siau, 1984). Thus, no flow occurs in the HW (Cermak et al., 1992). The volumetric ratio of HW to SW is not only species-specific (Hillis, 1987) but also varies depending on tree age, climatic and soil conditions, and the height at which the analyzed stem cross-section is located (Nawrot et al., 2008). The SW of conifers contains a larger amount of water than HW (Cermak et al., 1992) where moisture content is almost equal to the fiber saturation point (Nakada et al., 2019), whereas the HW of some broadleaved species contains more moisture than the SW (Gartner, 1995; Hillis, 1987; Peck, 1953; Treydte et al., 2021). The large majority of the water transport in the SW xylem occurs in the axial direction through a dense network of dead cells constituting water conduits; these are tracheids in conifers and vessels in broadleaves. Functional connectivity is also enabled by the axial and ray parenchyma, the only living cells present in the xylem that act both as a storage of water and nutrient (Morris et al., 2016a). However, water does not flow exclusively as a bulk flow via water conduits, because water transport also occurs via diffusion as transfer of water vapor through the air in cell lumen (Siau, 1984).

Water is also present in SW living cells as symplastic water, between cells as capillary water and within cells wall as fiber water. Multiple studies have reported the relevance of the internal storage of SW as a water reserve for transpiration in several species, depending on wood density, SW area, and also on wood anatomy (Köcher et al., 2013; Phillips et al., 2003). The stem water reservoir does not only buffer transpiration demand during soil water shortage (Köcher et al., 2013) but also contributes to daily water use, accounting for 5-22% of the daily water use in broadleaved trees (Köcher et al., 2013) and for 20-25% in conifers (Cermak et al., 2007; Phillips et al., 2003). By measuring water potential variations in the trunk, Betsch et al. (2011) proved that water is exchanged between the elastic tissues and the vertical transpiration flow within the tree. By testing different degrees of storage scenarios, Knighton et al. (2020) shed light on the importance of considering internal mixing in relation to potential water sources when interpreting xylem isotopic composition. Despite there being evidence of a potential exchange between mobile and less mobile water within the tree stem, there is still little knowledge available about the magnitude of this isotopic exchange and how this uncertainty affects the identification of sources of tree water uptake (Berry et al., 2017).

Several attempts to investigate the variability of isotopic composition at the tree scale, the canopy scale (Goldsmith et al., 2019), and within leaf tissues (Cernusak et al., 2016; Goldsmith et al., 2019) were recently carried out but few studies have focused on the isotopic composition of the wood matrix and wood functional parts (Treydte et al., 2021; White et al., 1985). Stems are difficult to study and in most cases require destructive sampling that can compromise the integrity of the plant (Holbrook, 1995). Several studies sampled the xylem water by coring the tree trunk (Goldsmith et al., 2012; Knighton et al., 2020; Matheny et al., 2017a; Meißner et al., 2014, 2012; Muñoz-Villers et al., 2018). However, few studies specified whether the sampling encompassed only the SW (Gessler et al., 2021; Kahmen et al., 2021; Kühnhammer et al., 2021; Tetzlaff et al., 2021), which occupies the outer sheath of the stem that is the cross-sectional area deployed for water flow. The structural heterogeneity of stems has been often overlooked, leaving a crucial component of the regolith-soil-plant-atmosphere continuum almost neglected. Additionally, current knowledge on the isotopic difference and rate of water exchange between HW and SW is still scarce, which potentially leads to uncertainties as to the estimated origin of the extracted water. To date, a few studies addressed this topic by applying isotopically-enriched water (James et al., 2003; Treydte et al., 2021) and found the occurrence of radial water transport. Despite the early warning by White et al. (1985) on the need to better understand the mixing processes between SW and HW, to our knowledge, no study has yet documented the temporal evolution of SW and HW isotopic composition among multiple species and functional groups. In order to address this gap, we sampled SW and HW from two broadleaved species, European beech (*Fagus sylvatica*) and sessile oak (*Quercus petraea*) and two evergreen conifers, Douglas fir (*Pseudotsuga menziesii*) and spruce (*Picea abies*) in a temperate forest in Luxembourg. The sampling covered one growing season (March - October) and took place on a biweekly basis in order to address the isotopic composition changes over time and relate this pattern to species-specific xylem properties. We included a night-sampling campaign to test whether flow conditions are linked to a change in xylem isotopic composition. The four forest species addressed are characterized by different xylem anatomy and timing of physiological activity, aspects that may regulate the water exchange between SW and HW.

More specifically, we propose the following hypotheses: (i) the isotopic composition of SW is more seasonally-variable than that of HW, because the latter is generally presumed to be disconnected from water sources, and (ii) conifers have a higher degree of water compartmentalization between SW and HW compared to broadleaved species due to the lower water storage capacity of their HW.

2.2 Materials and Methods

2.2.1 Sampling strategy

We conducted the study during the 2020 growing season (16 March – 19 October 2020) in a species-rich temperate forest in the Weierbach experimental catchment in Luxembourg (Hissler et al., 2021; Rodriguez and Klaus, 2019; Schoppach et al., 2021), where *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Pseudotsuga menziesii* (Mirb.) Franco, and *Picea abies* (L.) Karst. are present. A mixed beech-oak-forest is covering the vast majority of the catchment, with some minor cover by spruce and Douglas fir in pure artificial stands. We selected healthy mature trees (diameter ranged between 35 and 55 cm) in order to exclude tree-size related effects. Biweekly wood cores were taken at breast height (ca. 1.3 m above ground) with a 5-mm-diameter increment borer from the same three individual trees for each species. Tree cores were collected around the stem circumference avoiding sampling above or below previous cores to minimize possible disturbances due to the wounding of trees. Based on the wood core translucency given by the water content, we separated SW from HW (Kravka et al., 1999; Wang et al., 2019) in conifers and beech trees. Light is transmitted through the water-filled conduits lumen but reflects and scatters at the surface of embolized conduits, resulting in contrast in wood color (Taneda et al., 2021) (Fig. S1). For oak, the SW–HW boundary was determined visually based on the distinct color change between the two (Fig. S1). The sampled SW length for oak, spruce, and Douglas fir was on average 3.28, 5.44, and 3.79 cm, respectively. Beech tends to develop HW only after decades (Gebauer et al., 2008; Zweifel and Sterck, 2018), thus we sampled cores for beech with an average length of 7 cm which was split in two, representative for outer and inner SW. SW and HW cores were directly placed in vials after removing bark and phloem. All vials were directly capped and sealed with Parafilm® and kept frozen until extraction. In preliminary experiments we did not observe a strong statistical difference between SW and HW in broadleaved species, therefore, we decided to test whether sampling depth would result in a stronger differentiation between the sampled xylem portions. To do this, we sampled deeper cores in broadleaved trees (beech and oak) during five sampling campaigns (June18, June 30, August 16, and September 4). We sampled deeper core in beech trees in order to reach the actual HW during these sampling dates. To verify if the degree of compartmentalization between SW and HW decreased during low flow conditions (predawn), we repeated the SW and HW sampling during the night between September 4 and 5 between 1 and 2 a.m. We collected a total of 78 samples for each coniferous species and 91 samples for each broadleaved species.

2.2.2 Laboratory analysis

We extracted xylem water through a cryogenic vacuum distillation line (Orlowski et al., 2016). After de-capping the sampling vials, we immediately inserted them into larger extraction vials which were submerged in a 100°C oil bath for 3 h and connected to a vacuum extraction line. The pressure was maintained at 0.03 hPa and was continuously monitored. We collected the evaporated water from U-shaped tubes, which were submerged in liquid nitrogen (-197°C). We continuously recorded the pressure to ensure that the lines remained leak-tight throughout the entire extraction.

We assessed the gravimetric water content and extraction yields for each xylem sample by weighting them before and after cryogenic water extraction. We monitored the extraction efficiency for 271 out of 338 samples (covid-lockdown prevented us to have access to labs and to monitor this parameter from the very beginning of the experiment) by oven drying the samples at 105°C for 24h and weighting them afterwards. The extraction efficiency, defined as the amount of water extracted divided by the total water of each sample, was above 99.7%, value which has been argued to guarantee a negligible effect of residual water content on the isotopic ratios of stem samples (Zhao et al., 2022). For each sample, we assessed the wood moisture content defined as the ratio of the weight of extracted water (liquid mass) to the weight of dry wood (Berry and Roderick, 2005; Peck, 1953; Siau, 1984; Steppe et al., 2010; Steward, 1967) after cryogenic water extraction (Eq. 1):

$$\text{moisture content } (\%) = \frac{(fresh\ weight - dry\ weight)}{dry\ weight} \times 100 \qquad \text{Eq.1}$$

Oven - dry density (g cm^{-3}) was also assessed on a subset of 253 samples as the ratio of the mass of dry weight wood to volume of dry wood (Eq. 2):

$$dry\ density = \frac{mass\ of\ dry\ wood}{volume\ of\ dry\ wood} \qquad \text{Eq.2}$$

The isotopic composition of the extracted water (δ^2H and $\delta^{18}O$) was analyzed using a Picarro cavity ring-down spectrometer (CRDS) (L2140-i, Picarro, USA) coupled with a Micro-Combustion Module^{TM} (MCM) in order to remove organic compounds (ethanol, methanol and/or other biogenic volatile compounds). For each sample, aliquots of 1.8 µl were injected and analyzed in ten replications. The first five injections were always discarded, and the remaining five injections were averaged in order to account for the memory effect (Penna et al., 2012). Every 12 samples, two standards were used for calibration. Another sample with a known value was analyzed in triplicate throughout the sequence as a quality control. To detect possible organic contamination, xylem samples were screened with ChemCorrectTM (Picarro Inc., Santa Clara, CA, United States) software, which attempts to identify contamination through fitting to a known library of spectral features. No organic contamination was found in any sample. For the sake of convenience, when mentioning SW and HW isotopic composition, we refer to the isotopic composition of the water extracted from these two functional parts.

2.2.3 Data analysis

We calculated the line-conditioned excess (lc-excess) as a function of the slope (a = x) and the intercept (b = x ‰) of the Local Meteoric Water Line (LMWL) (Eq. 3) (Landwehr and Coplen, 2004) for each sample:

$$lc - excess\ (‰) = \delta^{2}H - a \cdot \delta^{18}O - b \qquad \text{Eq.3}$$

The lc-excess describes the deviation of the sample’s δ^2H value from the LMWL in the dual-isotope space (Landwehr et al., 2014), which indicates non-equilibrium kinetic fractionation processes due to evaporation after precipitation. The LMWL at the study site was based on biweekly precipitation samples collected from 2011 to 2020 and is $\delta^2H= 7.5\ \delta^{18}O + 7.4$ and consistent with the long term regional precipitation behavior at Trier (Klaus et al., 2015; Stumpp et al., 2014).

We analyzed differences in xylem water isotopic composition (δ^2H and δ^{18}O, and lc-excess) with the software R, version v. 4.0.5 (R Core Team, 2021). Statistical analysis between species, SW and HW were assessed with the non-parametric Mann-Whitney U test, which examines the differences in medians for two independent groups. Statistical differences between the two groups derived from the Mann-Whitney U test are indicated by an asterisk (p-values ≤ 0.05) (Fig. 1, 5, S3). We used a = 0.05 to determine statistical significance for all statistical tests. We calculated the difference in tissue water (Δmoisture %), δ^2H (Δδ^2H), and δ^{18}O (Δ δ^{18}O) between SW and HW. We applied a linear regression analysis to test for statistic relationship between Δmoisture and Δ in isotopic composition.

Table 1. Values of mean, standard deviation, and interquartile range (IQR) for $\delta^{18}O$ and $\delta^{2}H$ per species and then grouped according to their functional group.

	Sapwood				*Heartwood*			
	$\delta^{18}O$	IQR $\delta^{18}O$	$\delta^{2}H$	IQR $\delta^{2}H$	$\delta^{18}O$	IQR $\delta^{18}O$	$\delta^{2}H$	IQR $\delta^{2}H$
beech	-6.5±0.6	0.68	-64.4±4.7	5.17	-6.1±0.5	0.83	-64.7±4.1	4.62
oak	-7.1±0.7	0.97	-61.3±4.9	5.78	-6.7±0.6	1.1	-57.3±3.3	6.39
Douglas fir	-6.5±1.0	0.89	-60.0±4.7	7.71	-5.8±0.9	0.81	-73.8±5.6	6.07
spruce	-6.6±1.0	1.02	-56.8±4.7	7.18	-5.6±1.0	1.26	-66.5±5.9	7.97
broadleaves	-6.7±0.7	1.00	-63.0±4.9	6.47	-6.4±0.6	1.01	-60.3±5.3	9.24
conifers	-6.5±1.0	0.97	-58.0±5.0	6.96	-5.7±0.9	1.16	-69.5±6.3	8.52

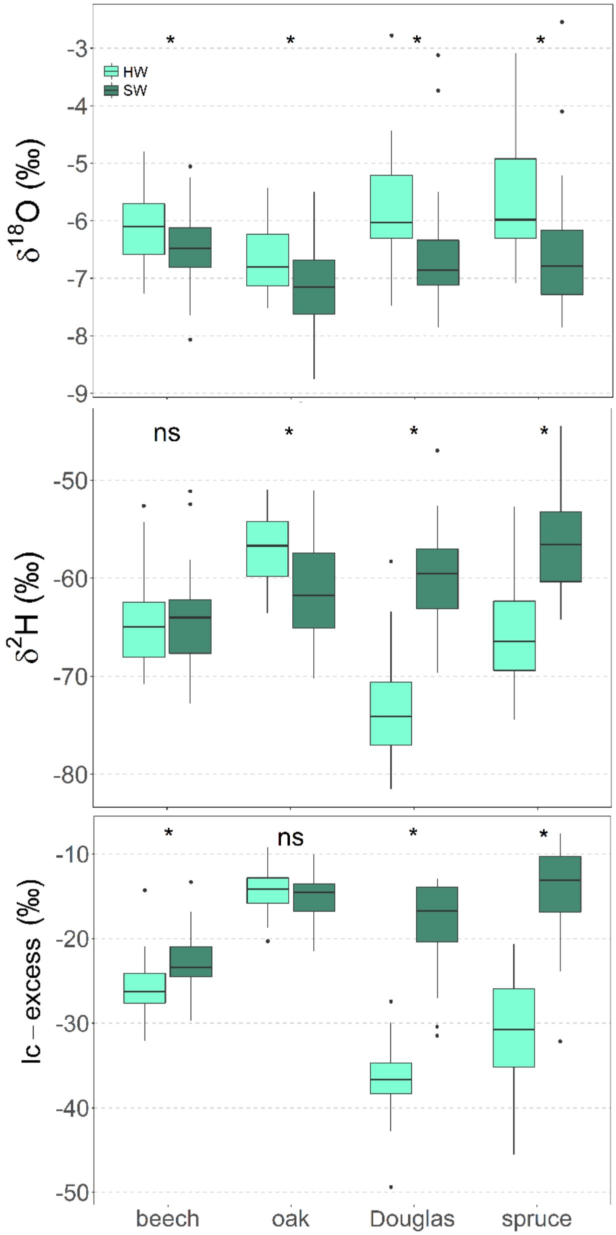


Fig. 1. (up) $\delta^{18}O$, (mid) $\delta^{2}H$ and (bottom) lc-excess of xylem water for all sampling campaigns conducted in 2020. The center line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartile, respectively. The whiskers indicate points within 1.5 times of the interquartile range above or below the median. Above each species, asterisks (symbols "*", "ns" correspond to p-values ≤ 0.05 or > 0.05, respectively) denote significant statistical differences between HW and SW (Mann-Whitney U test)

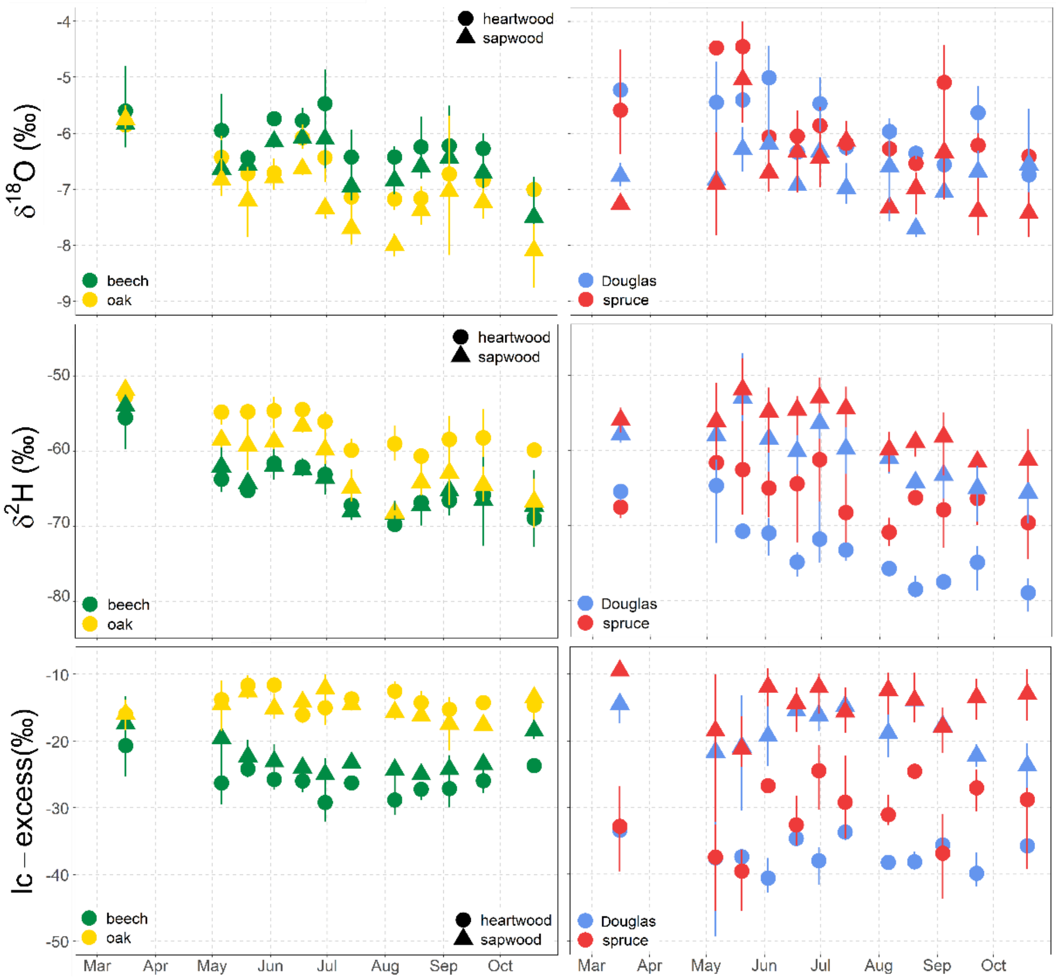


Fig. 2. Isotopic composition (upper and mid panels) and lc-excess (bottom panel) of xylem water for each sampling campaign across the growing season. Data of each species were averaged. Solid dots represent the mean, the whiskers indicate the confidence interval (95%). For beech trees, SW and HW correspond to inner and outer SW.

2.3. Results

2.3.1 SW and HW isotopic composition in coniferous and broadleaved species

SW isotopic composition was significantly lighter in $\delta^{18}O$ ($p < 0.01$, Mann-Whitney U test) than HW for the four investigated species (Fig. 1). On the contrary, $\delta^{2}H$ values were significantly heavier in SW than in HW in conifers, but not in beech trees ($p > 0.05$, Mann-Whitney U test) (Fig. 1). In oak trees, $\delta^{2}H$ values in SW were instead significantly lighter than HW. The studied conifers displayed a prominent difference between SW and HW in both isotopes compared to broadleaved species (Table 1). For both isotopes, the interquartile range and variability over the whole sampling season were highest for spruce followed by oak, Douglas fir, and beech trees (Table 1, Fig. 1). Before leaf-flush in beech and oak trees were, SW and HW displayed a similar isotopic composition in both isotopes but maintained a constant difference at later stages of the growing season (Fig. 2). We observed a consistent difference in $\delta^{2}H$ throughout the study period in conifers (Fig. 2). While we also observed a consistent difference between SW and HW in $\delta^{18}O$ for Douglas fir, the SW and HW of spruce tended to have similar values from June until the end of July (Fig. 2). Relationships between the delta values of SW and HW for ^{18}O and ^{2}H were statistically significant ($p<0.05$, shown in Fig. S3) for the four investigated species. R^2 values were lower for conifers (0.25 and 0.33 for spruce and Douglas fir respectively) than broadleaves (0.71 and 0.69 for beech and oak).

2.3.2 Temporal variability of the isotopic composition of water from SW and HW

Xylem water from all four species consistently plotted below the LMWL in the dual-isotope space across all sampling campaigns (Fig. 3, S2). SW samples from beech trees plotted further away from the LMWL than SW samples from the other three species (Fig. 3, 1). SW samples of spruce plotted closer to the LMWL than any other species. The isotopic signature in SW and HW was highly variable throughout the growing season for the four species (Fig. 2, 3). Xylem water from the first sampling campaign (16 March) before leaves were flushed out from beech and oak, was significantly higher in δ-values for both isotopes (Wilcoxon rank sum test, $p < 0.05$) compared to later sampling dates (Fig. 2) and plotted in the upper right area in the dual-isotope space (Fig. 3). In broadleaved species, both isotopes displayed a progressively lighter isotopic composition over time (Fig. 3). The isotopic composition of conifers displayed a different and less clear pattern than broadleaved species (Fig. 3).

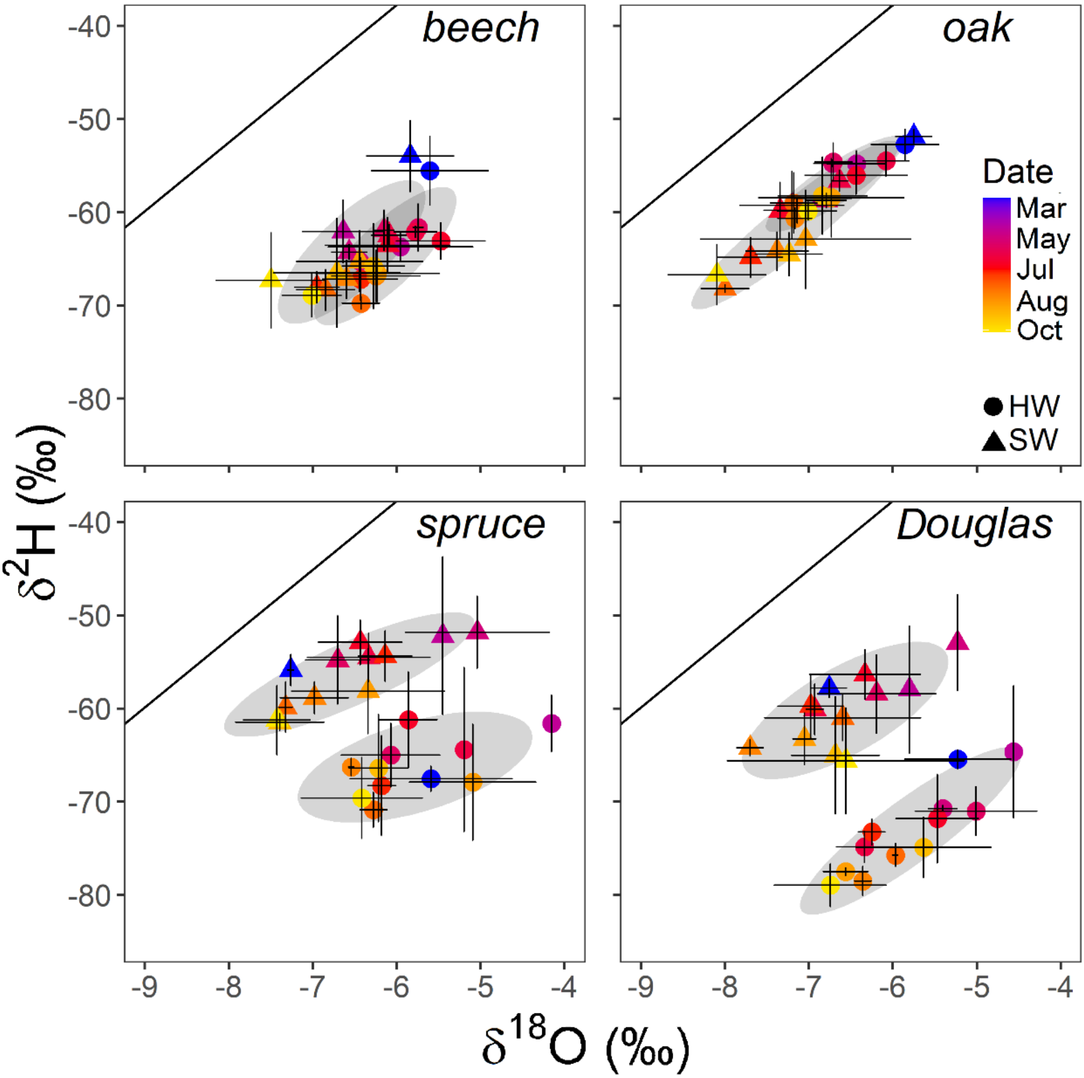


Fig. 3. Dual-isotope plot of xylem water per species for all sampling campaigns conducted in 2020. Each point represents the average isotopic composition and whiskers indicate standard deviation. The black line indicates the LMWL. Ellipses are drawn on the basis of 80% confidence intervals. For beech trees, SW and HW correspond to inner and outer SW.

2.3.3 Relationship between moisture content and xylem isotopic composition

Moisture content (%) (Fig. S4) was significantly higher ($p < 0.05$) in SW than in HW for beech, Douglas fir, and spruce but not for oak trees. We also observed a stronger temporal variability of moisture content in SW than in HW for all species

(Fig. S5). Correlations between Δmoisture and $\Delta\delta^{18}O$ were highly significant ($p < 0.001$) (Fig. 4) in general but not for each species individually ($p > 0.05$). The relationship between Δmoisture and $\Delta\delta^{18}O$ was statistically significant, yet the R^2 value was low (Fig. 4) showing that Δmoisture is not able to explain the variation in $\Delta\delta^{18}O$ alone. Conversely, correlations between Δmoisture and $\Delta\delta^{2}H$ were highly significant ($p < 0.001$) (Fig. 4), not only when all species were considered, but also for the individual species ($p < 0.05$). Conifers scored higher Δ-values for moisture content, $\delta^{18}O$, and $\delta^{2}H$ compared to broadleaved species (Table S1). Xylem density was higher in broadleaved species (0.60 and 0.57 g cm^{-3} in beech and oak, respectively) than in conifers (0.49 and 0.38 g cm^{-3} in Douglas fir and spruce trees, respectively).

2.3.4 Day-night variability of isotopic composition and moisture content in SW and HW

Due to the limited number of samples (three samples per functional part per species) we could not perform any statistical tests, but we reported the data as mean and standard deviation of $\delta^{18}O$, $\delta^{2}H$, and moisture content in Table 2. HW isotopic composition displayed generally a lighter isotopic composition at night than during the day for all species, while a less consistent pattern was observed in SW. The moisture content (%) of SW tended to increase over night for Douglas fir, oak, and beech trees, but it decreased in spruce trees. The moisture content of HW tended to increase overnight only for beech trees and remained rather constant for the three other species. We did observe a strong differentiation but there were small changes in the SW-HW isotopic composition and moisture content between day- and night-time (Table 2).

2.3.5 Xylem depth-related variability of isotopic composition in broadleaved species

For beech trees, xylem $\delta^{18}O$ and $\delta^{2}H$ values were not significantly different between the different sampling depths (i.e., outer, mid, inner) and functional parts (i.e., SW and HW) (Fig. 5). Despite the lack of statistical difference, $\delta^{18}O$ values tended to become progressively heavier with depth. In oak trees, both $\delta^{18}O$ and $\delta^{2}H$ values became progressively heavier with depth and the outer and inner portion of the cores were significantly different (Wilcoxon test, $p < 0.05$) (Fig. 5). While moisture content remained unvaried in beech, oak moisture content was significantly higher in the inner part than in the outer part (Fig S6).

Table 2. Mean and standard deviation for δ^{18}O and δ^{2}H and moisture (%) per species (n=3). In orange, the day-time sampling values, in blue the night-time sampling values.

	Sapwood				*Heartwood*			
	δ^{18}O day	δ^{18}O night	δ^{2}H day	δ^{2}H night	δ^{18}O day	δ^{18}O night	δ^{2}H day	δ^{2}H night
beech	-6.4±0.7	-6.4±0.6	-65.2±3.3	-64.2±4.3	-6.2±0.7	-6.1±0.3	-66.6±3.0	-64.9±1.7
oak	-7.0±1.2	-7.5±0.4	-62.9±5.3	-64.8±0.6	-6.7±0.9	-7.0±0.4	-58.4±4.2	-60.6±1.9
Douglas fir	-7.0±0.1	-5.9±1.3	-63.0±2.8	-61.0±3.6	-6.5±0.3	-6.8±0.4	-77.5±0.4	-81.8±0.6
spruce	-6.3±0.9	-7.1±0.5	-58.8±4.5	-62.1±2.2	-5.1 ±0.7	-6.5 ±0.5	-67.9±6.2	-72.8±9.9

	Sapwood		*Heartwood*	
	Moisture (%) day	Moisture (%) night	Moisture (%) day	Moisture (%) night
beech	59.6 ±3.6	61.7±3.1	55.3 ±5.7	60.6±4.8
oak	74.9±13.7	78.6±21.0	88.8±9.6	86.1±14.1
Douglas fir	81.5±14.3	90.8±3.9	33.3±0.6	33.4±1.1
spruce	123.3±15.6	112.1±4.2	33.7±1.9	31.7±0.0

2.4. Discussion

2.4.1 SW and HW do isotopically exchange

We found that both SW and HW displayed a similar temporal variability in their isotopic composition (Figs. 2, 3 and S3) suggesting that the two functional parts were interconnected. If an exchange between SW and HW had not occurred, HW isotopic composition would have displayed a rather constant pattern, if we assume that the isotopically dynamic sap flow is likely the only process able to underlie such isotopic variations. A certain degree of isotopic exchange between the two functional parts would require the radial transport of water in both directions, and this has been observed in previous studies (Gessler, 2021; James et al., 2003; Kitin et al., 2009). Contrary to our results, White et al. (1985) observed that δ^2H of HW showed rather uniform values over time but the authors did not provide a clear interpretation due to the short measurement period in their study. In addition to the bi-weekly variation, we also observed that in some species, both SW and HW isotopic composition changed even on the day-night scale (Table 2), indicating that SW and HW water exchange can occur at the sub-daily time scale.

We observed that SW and HW in broadleaved species displayed similar isotopic composition only when sap flow fluxes were very low or negligible for extended periods, i.e. before bud break (Fig. 2, 3). We did not observe the same isotopic difference during night when transpiration ceases (Table 2), which suggests that the isotopic equilibrium between SW and HW would require more than ca. 6 hours (time between sunset and sampling time) to be reached. We can envisage that the two functional parts tend to reach an isotopic equilibrium over the dormant season due to the inactivity of the SW and the consequential lack of temporal change in the SW isotopic composition by water uptake. Additionally, during the dormant season δ^{18}O and δ^2H showed the heaviest values of the studied period (Fig. 2, 3). The occurrence of the heavy isotopic composition in xylem water during leafless periods was previously reported in other studies (Martín-Gómez et al., 2017; Oerter et al., 2019; Phillips and Ehleringer, 1995) and also observed in the study area in 2019 (Fabiani et al., 2021). This observation has been explained by the evaporation through the bark that occurs over the dormant season when sap flow is ceased (Martín-Gómez et al., 2017; Oerter et al., 2019; Phillips and Ehleringer, 1995). Therefore, the heavy isotopic composition of xylem water observed before leaf flush both in SW and in HW is another indication that SW and HW are not isolated compartments in broadleaved species. At the onset of the growing season, the xylem water of the broadleaved species became progressively lighter in both isotopes and at both functional parts (Fig. 2, 3), likely caused by the input of fresh, non-enriched soil water. However, as the growing season progressed, SW and HW of broadleaves began to display different isotopic composition, as the equilibration process between SW and HW seems to be slower than the rate of change in the isotopic composition in the SW (from root uptake).

In conifers, we observed a consistent difference over time between SW and HW even at the beginning of the growing season (Fig. 2, 3). This is likely due the lack of a complete cessation of transpiration in the dormant season for evergreen species (Chan and Bowling, 2017), which reduces the effect of fractionation through the bark. Overall, the differences between the isotopic composition of water in functional parts of conifers and broadleaved trees (Table 1) might not be exclusively linked to stem hydraulic processes (Martín-Gómez et al., 2017) and xylem anatomy (i.e. water storage capacity, parenchyma fraction, Gessler, 2021; Treydte et al., 2021), but could also be related to the phenology of the species.

Although water does not flow readily between growth rings and water movement is mainly promoted within a growth ring (Gartner, 1995), our observed seasonal variability in isotopic composition in SW and HW (Fig. 2, 3) suggests that isotopic exchange between the functional parts does occur to a certain degree. Consistently with our findings, James et al. (2003) showed through a labelling experiment in tropical species that substantial diffusion of deuterated water occurs from SW to HW through the rays and axial parenchyma. Treydte et al. (2021) injected deuterated water into *Eucalyptus* trees and identified the presence of this tracer even in the HW of the opposite side of the injection point. This was interpreted as evidence for the circumferential water movement as bulk flow through the SW and the subsequent equilibration with HW. Bulk flow might be allowed by the radial connectivity, which is granted by the intercellular spaces of the xylem participating in gas exchange and water conduction (Kitin et al., 2009; Zhang et al., 2004) and by parenchymatic rays (Pfautsch, 2016; Pfautsch et al., 2015). Parenchyma provides storage for water during water shortages and grants functional connectivity to water transport both in an axial direction by axial parenchyma (Chen et al., 2020) and radial direction between the inner and outer xylem by ray parenchyma (Steward, 1967). The axial and ray parenchyma run perpendicularly to each other, originating a living network interspaced among fibers and water conduits (Morris et al., 2016b). The proportionality of different parenchymatic rays and xylem elements varies among coniferous (3-12%) (Panshin and De Zeeuw, 1980) and broadleaved species (5-33%) (Siau, 1984, Pfautsch et al., 2015) and their water contribution to the total transpiration flux might be related to the tree water status and how easily water is withdrawn from storage (Tyree and Yang, 1990). Conifers are also characterized by the presence of heterogeneous rays, where parenchymatic cells and tracheids run radially (Siau, 1984) and facilitate water transport in a radial direction. Heterogeneous rays are lacking in broadleaved species, but the connectivity among adjacent xylem vessels is granted by inter-vessel pit pairs that allow water to spread from one vessel to the next at points of vessel to vessel contact (Tyree and Zimmermann, 2002b). Inter-vessel pits size and density varies among species and determine their effectiveness in decreasing resistance to lateral flow and the tangential spread of water (Orians et al., 2004). Although HW is presumed to be characterized by tyloses formation and the complete dieback of living cells (parenchymatic cells), some of the cells may actually survive and pits (which allow water to pass between xylem conduits) may maintain their functionality (Frey-Wyssling and Bosshard, 1959). An additional driving force for the internal radial movement from SW to HW is the water potential gradient between the two (Nakada et al., 2019). Nakada et al. (2019) argued that water moves radially in the vapor phase rather than in the liquid phase. This argument is also consistent with the lighter isotopic composition observed in δ^2H of the HW (Fig. 1), as the light isotopes are more likely to be transported in higher fractions through the vapor phase. However, we observed the opposite for $\delta^{18}O$ (Fig. 1) leaving the transport path subject to further research.

Our hypothesis of a different degree of compartmentalization (i.e., isotopic exchange) between broadleaved species and conifers is also supported by the linear relationship observed between $\Delta\delta^{18}O$ and $\Delta\delta^2H$ with Δmoisture between the two functional parts (Fig. 4, Table S1). When the Δmoisture was low (Fig. 4), the two functional parts displayed a closer isotopic composition compared to high Δmoisture. This indicates a higher degree of exchange. Seasonal variations in moisture content in wood are confined largely to SW (Fig. 4), confirming that species-specific moisture content of wood in living trees is governed by their anatomy (Steward, 1967). The wood of conifers, which is characterized by a lower density than broadleaved species (see 3.3 Results), is able to absorb more water than broadleaved species (Fig. S4). Despite this, HW has limited storage capacity (Fig. S4) due to the physiological changes that xylem has encountered during SW-HW conversion (Tyree and Zimmermann, 2002b). The seasonal variations in moisture content are less pronounced in the SW-HW of coniferous species than broadleaved species, probably reflecting the hydraulic separation between the conducting portion of the stem and the HW (Holbrook, 1995). An exceptional case among broadleaved species is that of oak (Tomczak et al., 2018), where HW moisture content is comparable and sometimes even exceeds SW moisture content (Fig. 4, S4, Table 2). However, the contribution of HW water to the transpiration for this species remains uncertain. It may be that the day-night scale fluctuations of moisture content in the HW of broadleaves indicates the occurrence of water logging and withdrawal of water on the day-time scale. In conifers, we observed a change in the isotopic composition of HW independent of a variation in moisture content, potentially indicating that HW likely does not contribute significantly to tree transpiration despite the occurrence of an isotopic exchange.

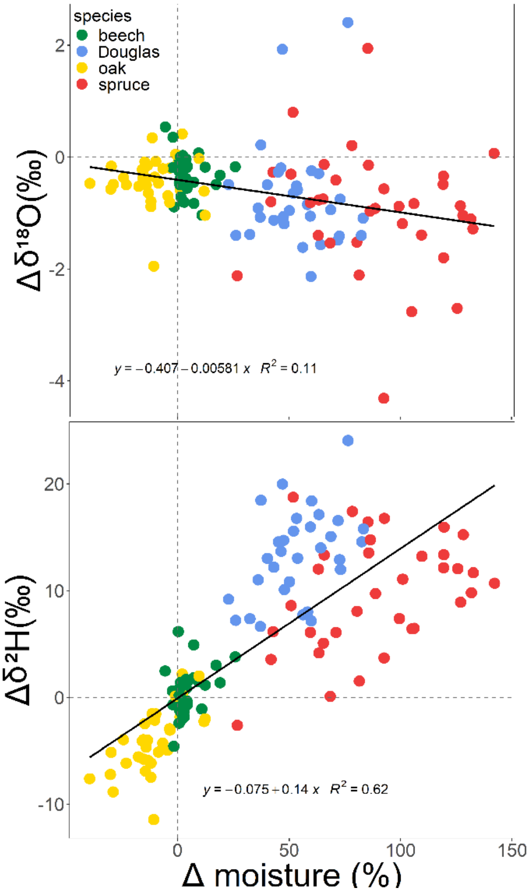


Fig. 4. Relationship between Δmoisture and $\Delta\delta^{18}O$ (upper plot) and Δmoisture and $\Delta\delta^{2}H$ (bottom plot) for all species. Δ is the difference in isotopic composition (top panel) and moisture (bottom panel) between SW and HW of the same tree.

From a methodological point of view, we must bear in mind that the cryogenic water extraction does not only extract water flowing through xylem conduits, but rather a mixture of symplastic water, capillary water, and fiber water (Barbeta et al., 2022). Thus, we acknowledge that our study incorporates multiple degrees of isotopic heterogeneity occurring not only between different functional parts (i.e., SW and HW) but also at a finer scale between different xylem elements within the same portion of functional xylem. Xylem is composed of conducting elements (i.e. vessels in broadleaved species and tracheids in conifers), parenchyma, and fibers (Chen et al., 2020), which contribute to different extent to the total hydraulic functioning of the tree. Water in the intercellular space and xylem conduits are isotopically in-equilibrium (Barbeta et al., 2022), while water in parenchymatic cells and within cell walls has been assumed to be more depleted in hydrogen (Barbeta et al., 2022; Zhao et al., 2016). The most recent literature has focused on whether xylem depletion in hydrogen originates from the isotopic heterogeneity between xylem elements (Barbeta et al., 2022; Zhao et al., 2016) or rather whether it results from the technological limitations of the extraction technique (Chen et al., 2021). It has been argued that cryogenic water extraction has systematically biased δ^2H estimates due to the hydrogen exchange of water with organic compounds during distillation (Chen et al., 2021). However, the work of Barbeta et al. (2022) showed that an extraction artifact alone cannot quantitatively explain the depletion in deuterium, and thus other mechanisms would be behind the relative depletion of water in non-conducting woody cells. Among possible mechanisms, chemical bonding and capillary (i.e. surface) forces could have an effect on the hydrogen (Berry and Roderick, 2005) (and not oxygen) especially when moisture content is low, such as in conifer HW (Fig. S4). More research is clearly needed in order to quantify the influence of organics and capillary forces on deuterium exchange during the xylem extraction process and define the implications for tree water uptake studies. Still, both studies agree on the reliability of δ^{18}O as a tracer (Barbeta et al., 2022; Chen et al., 2021), pointing to the need to build interpretations on both isotopes.

2.4.2 The radial variability in isotopic signal

We observed a progressive transition in isotopic composition with increasing radial depth toward the stem center suggesting the occurrence of an isotopic gradient or the presence of a transition zone between SW and HW (Fig. 5). This might be an indication of high xylem connectivity, granted by inter-vessel pitting that allows water movement between adjacent vessels (Orians et al., 2004). The increasingly heavier isotopic composition with sampling depth observed in oak trees (Fig. 5) may result from the radial variability in xylem sap flux as SW does not conduct water uniformly with depth. The importance of SW as source water for leaf transpiration increases with depth (Cermak et al., 2007) and most of the water flow is largely confined in the outer SW (Steward, 1967). In ring-porous species such as oak trees, the axial flow is restricted to either the last or last few annual rings (Cermak et al., 1992; Granier et al., 1994; Kozlowski and Winget, 2015; Matheny et al., 2017a) where the width of the late-wood vessel creates sufficient transport capacity to supply the entire crown (Gartner, 1995). In oak species, it has been estimated that 80% of flow occurred in the outer portion of the SW, while only some xylem transport occurs in the inner rings. Diffuse porous species such as beech trees rely on multiple growth rings to sustain transpiration (Matheny et al., 2017a), which could potentially explain the lack of statistical difference observed in isotopic composition between different sampling depths in the xylem of beech (Fig. 5).

The progressive decrease in water flow with depth is explained by two co-occurring phenomena: (i) leaf petioles attached to the xylem that withdraw water from the outer ring (Gartner and Meinzer, 2005) and (ii) the higher flow resistance experienced by the water flow in the inner part of the SW (Ford et al., 2004). As the light isotopes are more likely to be transported in higher fractions through the vapor phase and leave trough the bark, we speculate that water stored in older SW might have developed heavier isotopic composition over time. Furthermore, the outer sheath of xylem may be considered the more faithful proxy of the water taken up as it contains a higher percentage of the water which flows to the leaves. As outer xylem sustains the upper and outer crown and inner xylem sustains lower and older branches (Pfautsch, 2016), we expect that different vegetative parts will be supplied by water with a different isotopic composition that matches that of the corresponding xylem-depth supplier.

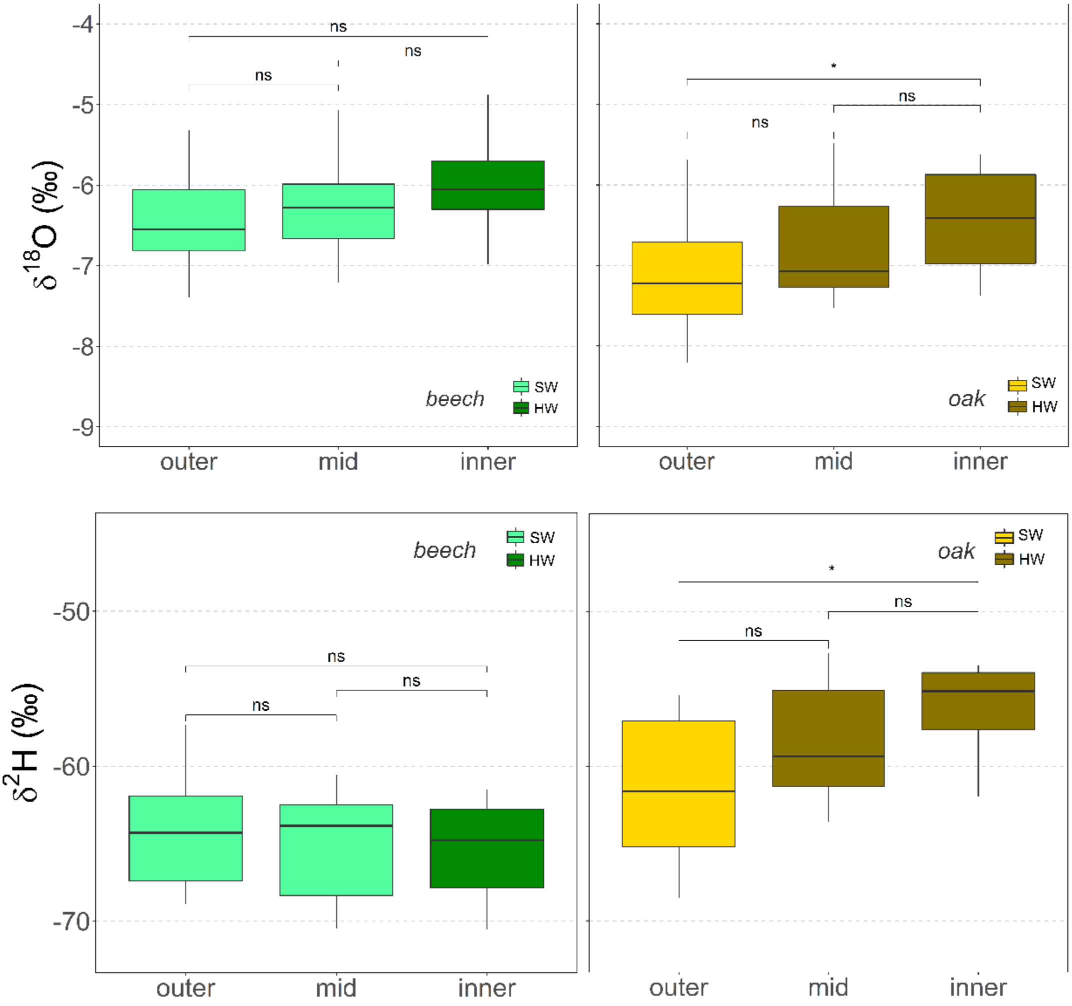


Fig. 5. $\delta^{18}O$ (top panels), $\delta^{2}H$ (bottom panels) isotopic composition of beech (left plots) and oak (right plots) trees for the different sampling depths and functional parts (SW and HW). The center line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartile, respectively. The whiskers indicate points within 1.5 times of the interquartile range above or below the median. Above each boxplot, asterisks (symbols “*”, "ns” correspond to p-values ≤ 0.05 or > 0.05, respectively) denote significant statistical differences between the different sampling depth (Wilcoxon test).

2.4.3 Implications of isotope patterns in xylem for ecohydrological studies

Our results add to the recent literature showing how the xylem structure (Barbeta et al., 2022, Zhao et al., 2016) and tree physiology (Martín-gómez et al., 2016) affects xylem water isotopic composition. Although the anatomical structure of xylem provides slow rates of radial movement for water in comparison to the axial transport (Kitin et al., 2009), we showed that this exchange might take place even on the day-time scale for some species. In light of this, we can think of different xylem compartments as potential end-members that complicate the identification of tree water use. This is in agreement with Seeger and Weiler (2021) who highlighted that xylem water incorporates the transient or intermediate sources such as roots, trunks, branches and leaves. It is still unclear to what extent non-uniform isotope signatures might also impact measurements with gas-permeable in-situ probes (e.g. Volkmann et al., 2016), since a mixture of recent water uptake flowing in vessels and water stored in fibers, parenchymatic cells, and intercellular spaces, possibly affecting the interpretation of the measurements. Furthermore, Seeger and Weiler (2021) raised concerns that the measurements with in-situ probes might be influenced by the isotopic signature of immobile water stored in the HW impairing the characterization tree water uptake. We suggest that the analysis of the tracer breakthrough curve with in-situ probes (Kahmen et al., 2021; Seeger and Weiler, 2021) could provide valuable information about the xylem hydraulic capacitance driving the exchange of water between storage compartments and the transpiration stream. As hydraulic capacitance depends on wood density (Köcher et al., 2013), we argue that the breakthrough curve arrival time would be species-specific. Future studies should aim to discriminate between the total bulk of xylem water and conduits water in order to understand how the xylem anatomy might affect the isotopic measurements and ultimately, to better understand tree hydraulic functioning.

2.5. Summary and conclusions

Our results provide previously missing evidence of different degrees of water exchange between SW and HW for multiple forest species and functional groups. We found that:

- Both SW and HW display a variable isotopic composition throughout the growing season and on a day-night scale, but conifers display a higher isotopic compartmentalization reflected by the consistent isotopic difference between SW and HW compared to broadleaved species as a result of the difference in xylem anatomy and phenology.

- In broadleaved species, the radial variation in isotopic composition is driven by sampling depth rather than xylem functional parts

With the present work, we hope to draw attention to the need for ecohydrological studies to be consider the multi-directionality of water transport in wood and the heterogeneity of the isotopic composition in xylem. Future work should seek to link species-specific xylem hydraulic capacitance with water transport processes to better understand the processes underling the isotopic exchange between storage compartments and the transpiration stream.

Chapter 3

Contrasting water use strategies of beech trees along hillslopes with different slope and climates

Abstract

The understanding of the interaction between topography and vegetation across different sites is important to assess how ecohydrological processes affect tree performance. This becomes especially important with the expected reduction in water availability and increase in water demand driven by climate change, which could enhance the thermal and hydrologic gradient along the slope. Here, we set up a comparative study on oceanic and gentle slope hillslope in Luxembourg and a Mediterranean and very steep slope in Italy. We aimed at testing if different climatic and hydrological conditions affect the physiological response to environmental variables of beech trees (*Fagus sylvatica* L.) along a toposequence. To do so, we combined sap velocity measurements with isotopic measurements of soil, freshwater, and xylem over 2019 and 2020 in Luxembourg and 2021 in Italy. We found that in the Weierbach catchment, trees response to environmental variables (i.e. vapor pressure deficit and relative extractable water in the soil) was similar among hillslope positions and between the two monitored years resulting from homogeneous growing conditions along the toposequence. We also did not find any statistical difference in isotopic composition of xylem water among positions suggesting that beech trees relied on similar water sources across the landscape. In the Lecciona catchment, we observed lower sap velocities and shorter growing season in trees growing in the upper portions of the slope, presumably due to drier soil and atmosphere. Xylem isotopic composition was significantly lighter at the footslope location throughout the growing season than at upslope locations, suggesting the use of a less fractionated water source. These results emphasize how differing hydrometeorological processes occurring at the hillslope scale can lead to contrasting tree performances.

This chapter is based on: *Fabiani, G., Klaus, J., Keim, R., Penna, D., 2022. Contrasting water use strategies of beech trees along hillslopes with different slope and climates*. In preparation, intended for Hydrology and Earth System Sciences

3.1. Introduction

Climate change is expected to strongly affect forest ecosystems by exacerbating environmental extremes, such as droughts and heatwaves (Allen et al., 2010; Choat et al., 2012). The general increase of the atmospheric evaporative demand concurrently with reduced water availability exposes trees to the risk of water deficit (Salomón et al., 2022), which impairs normal tree functioning (Giménez et al., 2013). Additionally, droughts coupled with warmer temperatures amplify forest vulnerability to other biotic and abiotic disturbances (Jaime et al., 2022; Jung, 2009), which may result in tree mortality and irreversible changes in forest ecosystem functioning and productivity (Bréda et al., 2006). The severity of drought is variable in space, time, and among domains of the hydrological cycle (Tijdeman et al., 2021), and is expected to affect tree performance in species ranges both at the limits and centers (Parmesan, 2006).

Multiple works investigating forest vulnerability to drought have often neglected the landscape heterogeneity at fine spatial resolutions, even though soil conditions, slope, and aspect are important abiotic factors for forest health (Schwantes et al., 2018). The interaction of these factors results in thermal and water gradients across the landscape and ultimately shapes species composition and distribution (Méndez-Toribio et al., 2017). The hydrologic gradient along a hillslope transect determines surface and subsurface water flow patterns (Dunne et al., 1975; Jencso et al., 2009) resulting in different water availabilities in the subsurface, one of the main drivers of tree productivity (Hogg et al., 2008). Topographically convergent areas are generally wetter in the subsurface and atmosphere due to the lateral redistribution of water flow (Western et al., 1999) and the reduced solar radiation, respectively (Hoylman et al., 2018). Deeper soils with higher storage capacity and organic matter typically occur at the footslope and are generally associated with greater stand primary productivity compared to hilltop locations (Elliott et al., 2015; Hawthorne and Miniat, 2018; Tromp-van Meerveld and McDonnell, 2006), where trees experience more water stress (Oberhuber and Kofler, 2000). The effects of the physiological drought, which occurs when water is not sufficient to sustain plant needs, are dampened where soil water storage capacity is greater. (Meusburger et al., 2022). Indeed, spatially variable soil characteristics and depths can lead to different levels of stress for the same forest species (Tai et al., 2017). With the expected increase of drought events, topography-related changes in soil moisture availability (Hawthorne and Miniat, 2018) combined with the land-surface energy balance (Dobrowski, 2011) may lead to a gradient of drought risk on vegetation increasing from lower to higher parts of the hillslope catena.

However, greater water accessibility in concave areas does not always translate into more tree growth because very wet places restrict rooting expansion to oxygenated soil above the water table (Fan et al., 2017). Bosch et al. (2014) found that landscape position, and subsequently access to groundwater, did not appear to strongly influence sap flow rates. Instead, they were mainly influenced by water content in the vadose zone (Bosch et al., 2014). Similarly, in one of our previous studies, we found that high and steady groundwater table does not necessarily enhance sap velocity in two co-occurring species (Fabiani et al., 2021). Low oxygen levels in saturated soil or regolith can hamper root functioning and development in trees sensitive to anaerobic conditions and force them to rely on shallow soil water and use little groundwater (Thorburn et al., 1993). European beech (*Fagus sylvatica* L.) is among those species that do not grow roots in the saturated zone, and it mainly relies on water stored in the top soil (Gessler et al., 2021; Kreuzwieser and Rennenberg, 2014).

European beech is a key component of the European mesic forests, with a distribution range covering wide climatic and geological settings (Durrant et al., 2016). In the last decade, its physiological activity (i.e. leaf phenology and cambial activity) has been mildly to severely restricted by forest droughts in 2003, 2015, and 2018 (Cavin and Jump, 2017; Obladen et al., 2021; Salomón et al., 2022; Walthert et al., 2021) and is expected to face high mortality rates with the future predicted heatwaves. In Germany, drought decreased beech productivity in lowland regions compared more than in montane areas, despite being in the center of the species' distribution range (Dulamsuren et al., 2017). Also in Italy, at the southern limit of beech geographical range, beech growth was found to be limited especially at low elevations (Piovesan et al., 2008). The reduced increments might be explained by the conservative strategy of this species for high vapor pressure deficit (VPD), which leads to strong stomatal control and reduced cambial activity (Köcher et al., 2013).

Inter-annual meteorological variability represents another potentially important control on the patterns of plant physiological activity (Murphy et al., 2020). Vegetation can deploy physiological (i.e. stomatal conductance) and structural (i.e. leaf area, crown architecture, rooting depth) adaptations to acclimate to changing hydrometeorological conditions at annual to decadal timescales (Grossiord et al., 2017a; N. G. McDowell et al., 2008). Although the hydraulic system of beech trees has shown pronounced modification as a response to drought (Zimmermann et al., 2021), it is still unclear how beech trees will cope with a future increase in climate aridity, when rainfall deficits are associated with high atmospheric dryness.

So far, site-specific case studies with contrasting results have offered a limited understanding of how hydrological processes occurring at the hillslope scale affect tree water use of the forest communities in complex terrain. This information cannot be retrieved from larger scale approaches (Murphy et al., 2020). Therefore, hillslope-scale studies, are needed to identify areas where and when tree physiological activity is more constrained by dryness and develop management practices suited for locations at risk. Through a cross-site comparison, we aim to advance the understanding of the ecohydrological functioning of different ecosystems (Brooks et al., 2015) and allow deeper comprehension of forest dynamics and survival. To do so, we set up a comparative study on contrasting hillslopes in Luxembourg (the Weierbach catchment) and in Italy (the Lecciona catchment). These sites differed in steepness, climate, geology, and soil characteristics, but beech is the dominant species in both. We combined sap velocity and isotope measures with environmental monitoring to capture beech trees water response to environmental variables along hillslope transects. We hypothesized that (i) trees located at the hilltop in a steeper slope experience a greater decoupling from water sources than in footslope location, (ii) the physiological response of beech trees varies following topography related changes in water availability, (iii) in the Mediterranean site, the physiological activity of beech trees would be more constrained compared to the central European population.

We address these hypotheses by posing the following research questions: (i) does topography drive different water uptake strategy along two contrasting hillslopes?, (ii) does hillslope topography increase tree sensitivity to drought?

3.2. Materials and methods

3.2.1 Study sites

The Weierbach catchment: study area, environmental monitoring

The experiment was carried out during two consecutive growing seasons (March-October 2019 and 2020), at a transect along a south-facing forested hillslope ranging from 485 m to 515 m a.s.l. in the Weierbach catchment in Luxembourg (Fig.

1). The site has been described in detail previously by Fabiani et al. (2021) and only the key details are repeated here (Table 1). The hillslope transect is 348 m long and spans from the stream bank up to a plateau area with a slope of 10 degrees. The forest consists of European beech trees (78% of trees, 60% in basal area) and pedunculate and sessile oak hybrid trees (22% of trees, 40% in basal area). The moderate, oceanic climate in the region is largely influenced by air masses from the Northern Atlantic Ocean (Hissler et al., 2021, Pfister et al., 2014). Long-term annual precipitation (2007-2018) is approximately 783 mm at the Roodt weather station (3.5 km from the study site) (Fig.1). The mean annual air temperature is 8.3°C and 13.5°C during the growing season (01 April-31 October) from 2007 to 2018. Volumetric soil moisture was measured at the plateau at 10, 20, 40, and 60 cm depth with water content reflectometers (CS650, Campbell Scientific, UK). Groundwater wells were installed along the hillslope transect in the plateau, midslope, and hillfoot locations. Groundwater table depth was monitored with water pressure transducers (Orpheus Mini, OTT, Germany) recording data at 15 min logging intervals.

The Lecciona catchment: study area, environmental monitoring

Lecciona (Fig. 1) is a subcatchment of the Re della Pietra experimental catchment (2.05 km^2) located in the Tuscan-Emilian Apennines, in Tuscany, Italy. We selected a north-facing hillslope transect ranging from 940 to 970 m a.s.l. with a slope of 23 degrees (Table 1). The hillslope is covered by a monospecific stand of beech trees. Understory and tree regeneration is absent, and a thick litter layer of leaves washed off the upper portion of the slope accumulates downslope. At the ridgeline, trees are replaced by Scotch broom bushes, a species that typically grows in dry and sunny sites. The climate is typical of Mediterranean mountain environments, with an average annual temperature of 10.5°C and annual precipitation of 1200 mm (2007-2016) spread along wet (winter, spring, and fall) and dry (summer) periods. The mean air temperature during the growing season is 15.1°C (01 April-31 October). The lithology consists of “Macigno” sandstone, a turbidite unit with pelitic dominant lithofacies and a reduced thickness. Soils have sandy loam to sandy clay loam textures with low water storage capacity. Volumetric soil moisture was measured at the hilltop, midslope, and footslope at 15 and 45 cm depth with water content reflectometers (TEROS 10, Meter Group). Groundwater wells were drilled in 2019 at the footslope and in the riparian area. Wells were equipped with water pressure transducers (Orpheus Mini, OTT, Germany) recording data at 15 min logging intervals. Meteorological data (temperature, relative humidity, wind speed, and precipitation) were monitored at 5 min resolution in an open area at the top of the hillslope.

Relative extractable water (REW) and vapor pressure deficit (VPD)

Water retention curves are not available to compare soil water supply to plants across study sites. Therefore, in order to account for inter-site differences in soil properties and facilitate the inter-site comparison we estimated the daily relative extractable water (REW, unitless) (Granier et al., 1999) over all monitored depth classes at each site, calculated as (Eq. 1):

$$REW = \frac{SM - SM_{min}}{SM_{95th} - SM_{min}} \qquad \text{Eq. 1}$$

where SM is daily soil moisture, and SM_{95th} and SM_{min} are the site-specific soil moisture approaching field capacity and minimum values, respectively. REW varies between 0 (i.e. permanent wilting point) and 1 (i.e. field capacity) and represents the ratio between available soil water and maximum extractable water. REW was calculated for the Weierbach catchment based on soil moisture records from 2012 to 2020 and for the Lecciona catchment from 2020 to 2021. Following Salomon et al. (2022), we chose the 95th percentile, and not the maximum value, to exclude heavy rain events that may result in soil water saturation. REW is generally quantified to characterize soil drought intensity (Bréda et al., 2006; Grossiord et al., 2017b).

For each study site, we assessed the daily mean vapor pressure deficit (VPD), calculated as (Eq. 2):

$$VPD = 0.61375 \cdot e^{\left(17.502 \cdot \frac{T}{240.97+T}\right)} \cdot \left(1 - \frac{RH}{100}\right) \qquad \text{Eq. 2}$$

where T and RH are temperature and relative air humidity recorded from the weather station at each study site (Fig. 1).

Table 1. Summary of the characteristics of the instrumented hillslopes

Site	Altitude (m a.s.l)	Slope (degrees)	Aspect	Soil	Geology	Stand
Weierbach	485-515	10	south	loam	slate	Beech-oak mixture
Lecciona	910-990	23	north	sandy clay	sandstone	Beech pure

Table 2. Average precipitation (mm), temperature (°C), VPD (kPa), radiation (W/m^2), soil moisture (m^3/m^3), and REW (relative extractable water) of growing season (1 April -31 October) in the Weierbach and the Lecciona catchment.

	Site	Year	Precipitation (mm)	T (°C)	VPD (kPa)	Radiation (W/m^2)	Soil moist (m^3/m^3)	REW
Growing season	Weierbach	2019	506	13.8 ± 4.3	0.53 ± 0.28	231 ± 84	0.202	0.491
		2020	423	14.4 ± 3.6	0.63 ± 0.28	197 ± 74	0.185	0.398
	Lecciona	2021	319	15.1 ± 5.8	1.88 ± 0.66	190.7 ± 58	0.199	0.541

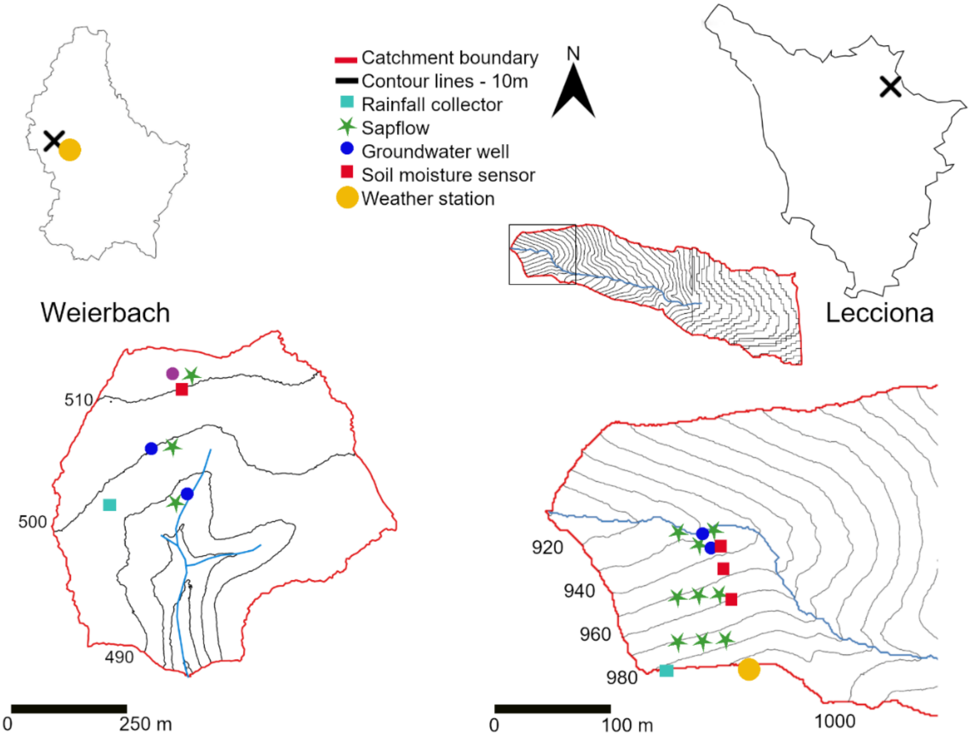


Fig. 1. Sites overview with sensors in place: on the left the Weierbach catchment and on the right the Lecciona catchment. In the Weierbach, we monitored groundwater level (blue dot) and sap velocity (green star) in one beech tree at each hillslope position, and soil moisture (red square) at the plateau. In the Lecciona catchment, we measured sap velocity (green star) in three trees, and soil moisture content (red square) at each hillslope location, and groundwater level (blue dot) was recorded at the footslope.

3.2.2 Measurement of sap velocity

At each sampling location, we selected one tree and three trees in the Weierbach and the Lecciona catchment, respectively (Fig. 1). We selected mature trees with the same diameters to exclude tree-size related effects (between 30 and 32 cm in both sites). The selected trees were equipped with heat pulse sap flow sensors (SFM1, ICT International Pty Ltd., Australia). The sensors were installed before leaves flushed and remained in place until leaves had fallen. In 2020 the installation in the Weierbach catchment was delayed due to the covid-lockdown that prevented access to the study site. In the Weierbach catchment, we positioned the sap flow sensors at the north-east side of the trunk at 1.3 m heigh. In the Lecciona catchment, the sensors were installed on the west side to avoid installing tension wood, which is grown by hardwood species growing on steep slopes. The heat pulse technique uses heat as a tracer to determine sap velocity. Briefly, a heater and two temperature sensors are inserted into holes drilled horizontally into the sapwood, with the sensors at fixed distance above and below the heater (5 mm). More details on the method can be found in Burgess et al. (2001). The mechanical damage of sensors installation induces xylem wounding interrupting the water flowpath (Barrett et al., 1995) and an increase in the proportion of non-conducting tissue. We applied a wound correction factor (Green et al., 2003) to account for this effect. We filled the data gaps due to power malfunctioning via linear interpolation with sensors at the same location (Table S1).

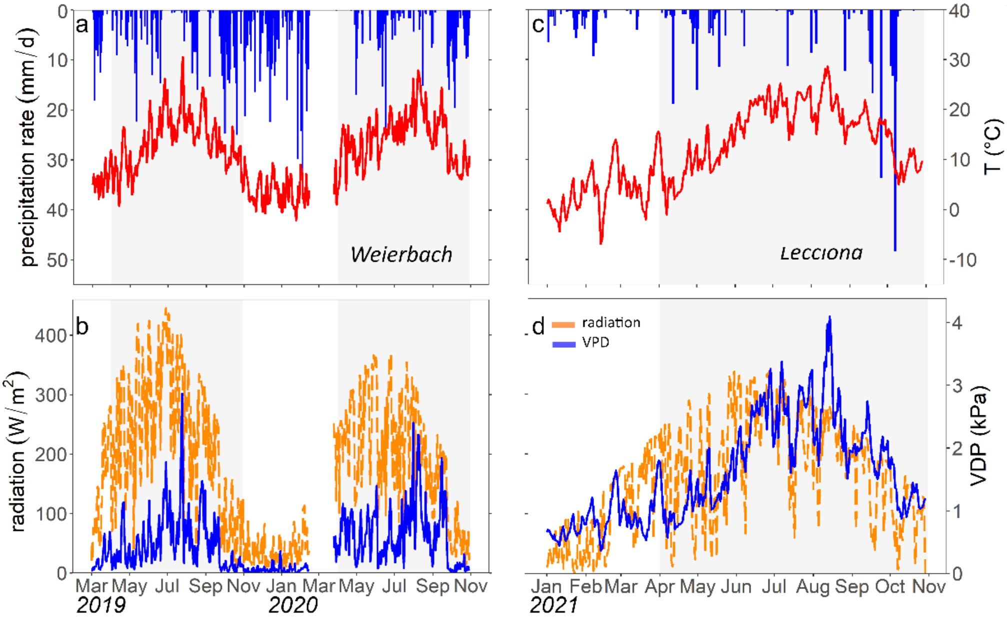


Fig. 2. Daily total precipitation amount (mm/d) and daily mean air temperature (°C) and daily mean radiation (W/m^2) and daily mean vapor pressure deficit (VPD) (kPa) observed at the Roodt (left plots, a and b) weather station and the Lecciona catchment (right plots, c, and d).

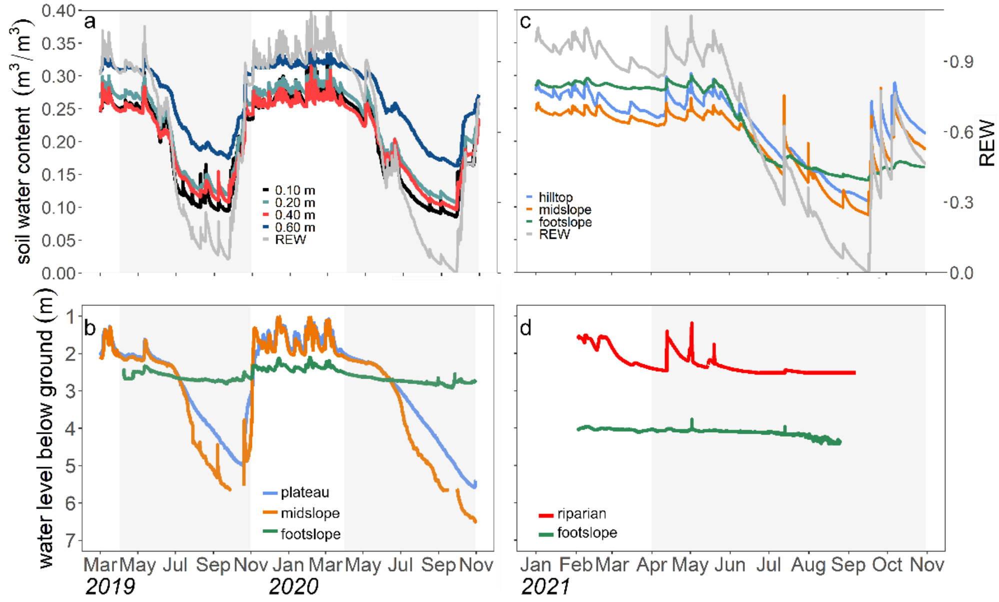


Fig. 3. Volumetric soil water content (m^3/m^3) and REW (relative extractable water) in the Weierbach (a) and the Lecciona catchment (c). Soil moisture in the Weierbach catchment

was measured at four depths in the plateau location. In the Lecciona catchment, soil moisture was recorded in three locations at two depths, which are averaged in the present plot. Groundwater level below ground surface (m) in the Weierbach (b) and the Lecciona catchment (d).

3.2.3 Wood core, soil, and water sampling

We sampled tree xylem water for isotopic composition biweekly over the 2019 and 2020 growing seasons in the Weierbach (26 sampling campaigns) and 2021 in the Lecciona catchment (11 sampling campaigns). The trees selected for xylem sampling were in the same diameter range as the trees equipped with sap flow sensors and in close proximity (within 25 m). Tree cores encompassing only the sapwood were collected around the stem circumference with a Pressler borer avoiding sampling above or below previous cores to minimize possible disturbances in sampled trees due to prior wounding. Xylem samples were quickly placed in glass vials, capped, and sealed with Parafilm©. We simultaneously sampled soil cores to assess the soil water isotopic composition. At each sampling area, we extracted three soil cores from the uppermost 60 cm divided into five depth classes (0–5, 5–10, 10–20, 20–40, and 40–60 cm) with a 60 cm long soil auger. Soil samples were collected in laminated aluminum bags, which have proven to be save against evaporation even over several days (40 days) (Gralher et al., 2021), and heat-sealed all bags upon arrival at the lab. For Weierbach soil samples, we stored them as explained in more detail in Fabiani et al. (2021). Lecciona soil samples were shipped at the end of the growing season 2021 to the Luxembourg Institute of Science and Technology (LIST) and kept frozen until analysis. This cooling effect prior to analysis likely reduces microbial activity and the associated buildup of CO_2 (Gralher et al., 2021). We sampled groundwater at the sampling areas in the Weierbach catchment only, and rainfall bi-weekly with a rainfall collector (Palmex Ltd.) placed in a clearing at both sites. In the Lecciona catchment, we did not sample groundwater due to the peristaltic pump malfunctioning.

3.2.4 Water isotopic analyses

Xylem water was extracted via cryogenic water extraction (detailed procedure in Fabiani et al. 2022) at LIST. We assessed the gravimetric water content and extraction yields for each xylem sample by weighting them before and after cryogenic water extraction. We assessed the wood moisture content defined as the ratio of the weight of extracted water to the weight of dry wood (Berry and

Roderick, 2005; Peck, 1953; Siau, 1984; Steppe et al., 2010; Steward, 1967) after cryogenic water extraction. The isotopic composition of the extracted water (δ^2H and δ^{18}O) was analyzed using a Picarro cavity ring-down spectrometer (CRDS) (L2140-i, Picarro, USA) coupled with a Micro-Combustion ModuleTM (MCM) to remove organic compounds (ethanol, methanol and/or other biogenic volatile compounds). To detect possible organic contamination, xylem samples were screened with ChemCorrectTM (Picarro Inc., Santa Clara, CA, United States) software, which attempts to identify contamination by fitting to a known library of spectral features. No organic contamination was found in any sample. We analyzed the water stable isotopic composition of soil samples with the direct liquid-water-vapor equilibration method (Wassenaar et al., 2008). Samples while being allowed to thaw for 2 days were equipped with silicon blots, which was the time sufficient to let them harden and avoid the risk of outgassing volatile organic compounds from freshly applied silicone (Gralher et al., 2021). Bags were inflated with dry air and allowed to equilibrate for 48h at a constant temperature for isothermal equilibration. Headspace vapor was sampled directly with a needle connected to the Picarro analyzer coupled with MCM for the samples and the five bags with known values (ranging between -16.2 - -2.9 in δ^{18}O and -123.7 - -9.9 in δ^2H). Water vapor concentrations were always above 28000 ppm where the concentration dependent deviation becomes low and thus measurement precision is not compromised. The local meteoric water line (LMWL) at the Weierbach catchment is δ2H = 7.4 δ18O + 7.4 and is based on biweekly precipitation samples collected from 2011 to 2020. This rather low slope is characteristic of the region (Klaus et al., 2015). The LMWL at the Lecciona catchment is δ2H = 6.2 δ18O + 7.0 and is based on biweekly to monthly samples collected from 2018 to 2021.

3.2.5 Data analysis

For our analyses, we were interested in the response of sap velocity to environmental conditions (i.e., REW and VPD) as an indicator for stomatal control, therefore we normalized daily mean sap velocities between 0 and 1 for further analysis, where 0 and 1 were the minimum and maximum daily mean velocities recorded by each tree over the entire growing season. This procedure facilitates the comparison between the two study sites and has been applied in previous studies (Gimenez et al., 2019). We analyzed differences in sap velocity and xylem water isotopic composition (δ^2H and δ^{18}O) among locations with the software R, version v. 4.2.0 (R Core Team, 2022). Comparisons of sap velocities between locations were analyzed with the Pairwise Wilcox test. The Pairwise Wilcox test was also used to analyze differences in the isotopic composition of groundwater sampled at the three sampling areas. Comparisons of the xylem isotopic composition between locations were analyzed via the non-parametric Mann–Whitney test. The significance level was set to $\alpha \leq 0.05$ to determine which year or zone was statistically different from each other.

3.3. Results

3.3.1 Environmental conditions across years and sites

The Weierbach catchment. In the Weierbach catchment, 2020 was characterized by drier conditions compared to 2019. Despite the lower radiation in 2020 than in 2019 (Table 2, Fig. 2b), the 2020 growing season was characterized by higher evaporative demand concurrently with reduced precipitation input (Table 2, Fig. 2a) and lower soil moisture (Table 2, Fig. 3a). Over the past 8 years, the minimum REW was reached in 2020 in the second half of September (data not shown). However, the daily maximum VPD was higher in 2019 with 3 kPa compared to 2020 when VPD reached 2.5 kPa. In the hydrometeorological space, each month of the 2020 growing season was characterized by lower REW but higher VPD than in 2019, except from June and July when VPD was higher in 2019 (Fig. 4). This general dry-out of the system was also observed in the groundwater table which progressively depleted (Fig. 3b) up to 5.58 and 6.50 m in depth in 2020 compared to 4.98 and 5.65 m in 2019 in the plateau and midslope, respectively. The groundwater table maximum and minimum remained unvaried between the two years at the footslope location (Fig. 3b). The drying of soil was accompanied by progressive dehydration of xylem (S3a) leading to a significant relationship between soil moisture and wood moisture content (Fig. 9). In the Weierbach catchment, there was no statistical differences in wood water content between the 2019 and 2020 growing seasons, or among months values were compared (Kruskal-Wallis, $p>0.05$). However, wood moisture was significantly lower in trees located at the plateau (Kruskal-Wallis, $p<0.05$) compared to the other locations.

The Lecciona catchment. The Lecciona catchment was characterized by almost 100 mm less of precipitation and higher temperature compared to the Weierbach catchment (Table 2), but soil moisture content was on average similar between the two sites (Table 2). In the Lecciona catchment, soil moisture pattern from June until

September. Soil moisture at the hilltop and midslope desiccated simultaneously, while soil moisture at the footslope dried out to a lesser extent and remained higher and steadier (Fig. 3). The groundwater table at the footslope location was rather constant with an average depth of 4.1 m, and responded only after major rainfall events (Fig. 3). By comparing the hydrometeorological space between the two study sites, we found slightly higher REW in the Lecciona catchment, and up to 3.9-fold higher VPD compared to the Weierbach catchment (Fig. 4). The progressive decrease in water input over the growing season did not only affect the soil but also the wood water content (Fig. S3). There was a significant relationship between soil moisture and wood moisture content at all locations (Fig. 9) and a marked temporal variability (Fig. S3). There was no statistical difference in wood moisture content among locations in the Lecciona catchment (Kruskal-Wallis, p>0.05).

According to hydrometeorological space (Fig. 4), defined by the relationship between REW and VPD (Salomon et al. 2022), we selected the months with more contrasting environmental conditions in terms of water availability in proportion to the maximum extractable water (REW) and evaporative demand (VPD). To investigate tree physiological response from the site-specific environmental stress and compare between locations, we regressed the normalized sap velocity against site daily VPD and REW.

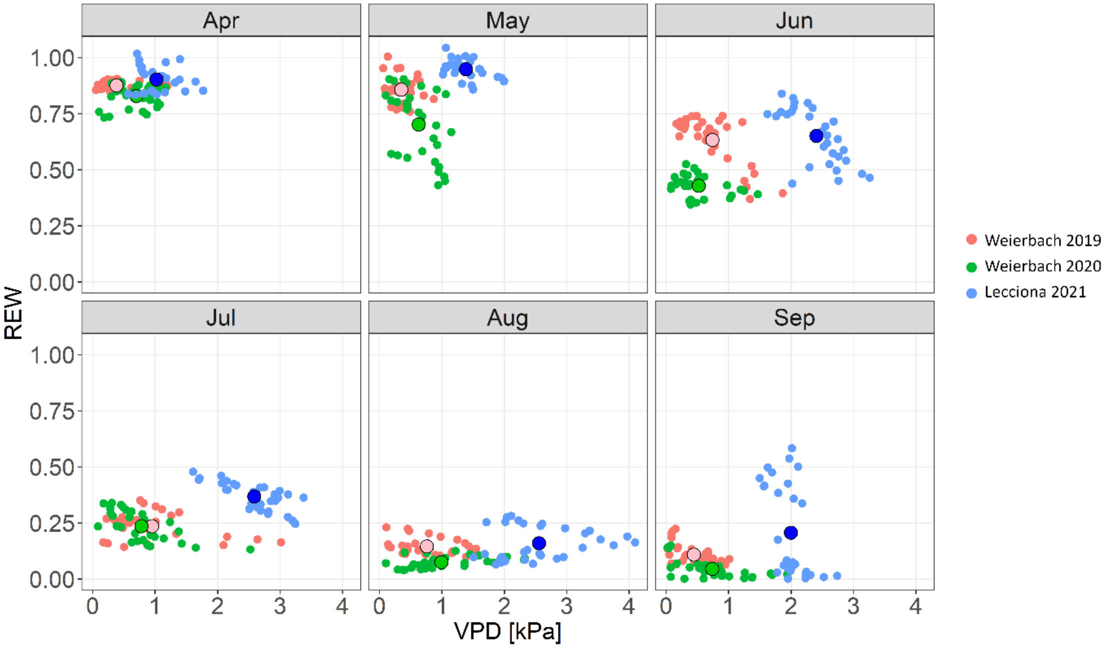


Fig. 4. Relationship between VPD (kPa) and REW over the growing season (2019-2020) in the Weierbach catchment and (2021) in the Lecciona catchment. Full dots with black outlines represent monthly means.

3.3.2 Sap velocity

In the Weierbach catchment, there was a consistent pattern in sap velocity between the consecutive monitoring years with monthly sap velocities higher in June compared to other months (data not shown), but daily sap velocity peaking in July (Fig. 5b). Sap velocity was significantly higher (Wilcoxon test, $p<0.05$) at the midslope location than other locations followed by plateau and footslope when both years were considered (Fig. 5). In 2020 sap velocities were statistically higher than 2019 in May and September at all locations. Despite the early yellowing and shedding of leaves observed in August 2020, which are symptoms of partial dysfunction of the hydraulic system, sap velocities were not lower than in 2019. In the Lecciona catchment, there were no statistical differences in overall mean velocity among locations (Wilcoxon test, $p>0.05$) (Fig. 5). However, there was a location-specific behavior by month. All trees shared a common pattern for the first part of the growing season: after leaf-out (19-26 April), sap velocity increased for all trees and locations and peaked toward the end of June (Fig. 5). From August onwards, sap velocity markedly dropped in plants at the hilltop (Fig. 5). In contrast, at the footslope sap velocity remained high and unvaried between July and August. Trees at the midslope location displayed a behavior in between the other locations. In September, the sap velocity of trees located at the footslope was significantly higher than at other locations (Kruskal-Wallis, $p<0.05$) and 2- and 4-fold higher compared to midslope and hilltop, respectively.

Growing season (leaf-out) started later in the Lecciona catchment compared to the Weierbach catchment; data are not available from the late season (October onwards) to compare the duration of the growing season between the two sites.

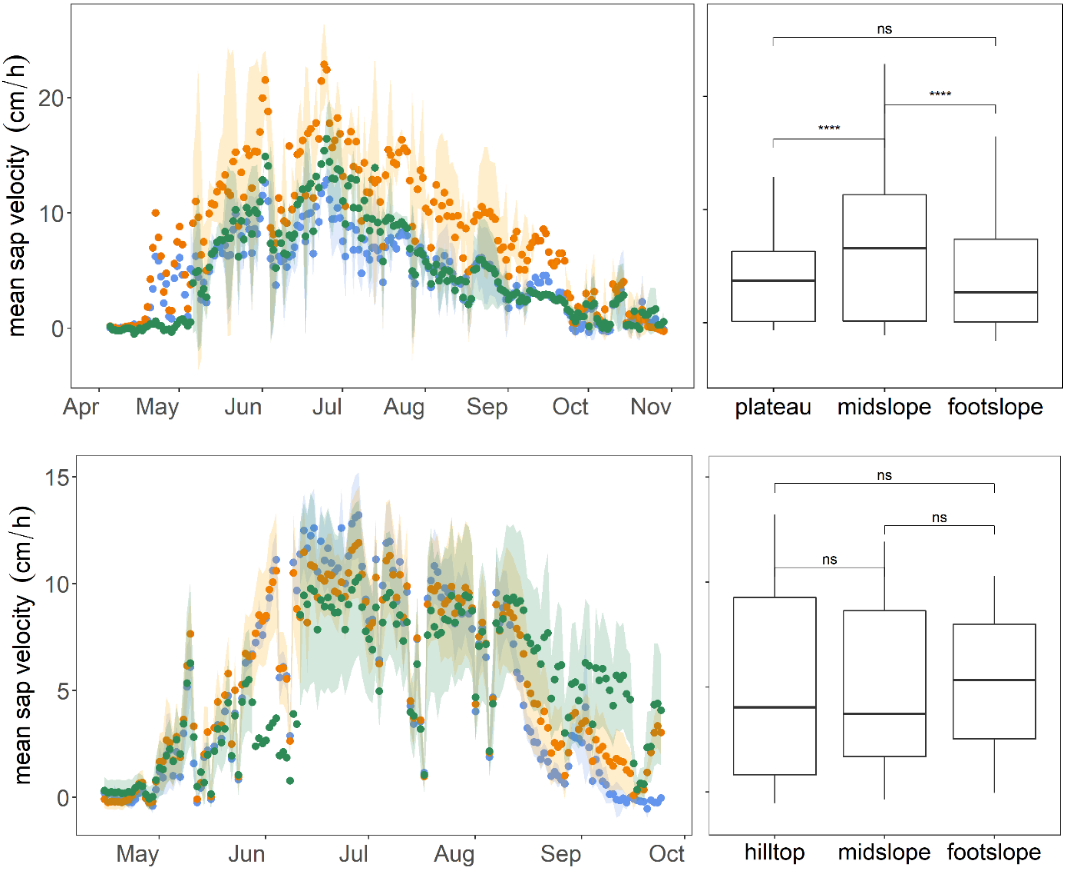


Fig. 5. Daily mean sap velocity (cm h-1) of Weierbach (upper plots) and Lecciona (lower plots) trees. Data from the Weierbach catchment cover the 2019 and 2020 growing seasons (April‐October), while data from the Lecciona catchment cover the 2021 growing season (mid-April – September). Each color corresponds to location (green= footslope, orange= midslope, blue= hilltop). The area inside the colored lines is the mean average deviation between trees at the same location. Boxplots on the right side show the average sap velocity by location. The whiskers indicate points within 1.5 times the interquartile range above or below the median. Above each boxplot, asterisks (symbols "*" and "ns" correspond to p-values ≤ 0.05 or > 0.05, respectively) denote significant statistical differences between locations (Wilcoxon test).

3.3.3 Sap velocity response to VPD and REW

Normalized sap velocity response to VPD in the growing season did not differ between locations and years in the Weierbach catchment (Fig. 6). However, for the same VPD values, sap velocity was lower in August and September at the footslope compared to the other locations indicating a stronger stomatal control. There was a progressive decline of sap velocity throughout the growing season despite similar monthly VPD (Fig. 4). In both years maximum sap velocities were reached when VPD ranged between 1.0 and 1.4 kPa (data not shown). In the Lecciona catchment, there was a strong differentiation of sap velocity response to VPD across the toposequence (Fig. 4). For the first months of the growing season, sap velocity response to VPD was consistent among hillslope positions. In August, trees at the footslope maintained sustained sap velocity even when daily mean VPD values were 4.1 kPa, the highest value of the season (Fig. 2d). On the other hand, sap velocity of trees at the hilltop and midslope dropped progressively in August and ceased in September, while remaining high at the footslope.

There was no difference in the relationship between normalized sap velocity and REW among hillslope positions and over the years in the Weierbach catchment (Fig. 7). Following the reduction in REW over the months, sap velocity was progressively reduced at all locations (Fig. 7). In the Lecciona catchment although REW reduction influenced sap velocity, its effect on sap velocity varied between locations likely resulting from the different soil moisture content between locations. In June, the high REW did not cause any differentiation in sap velocity response among locations, but in August with reduced REW, sap velocity at the hilltop and midslope decreased but not at the footslope. In September, despite recharge of soil moisture (Fig. 2c) by precipitation, sap velocity remained high only at the footslope and approached minimum values at the hilltop.

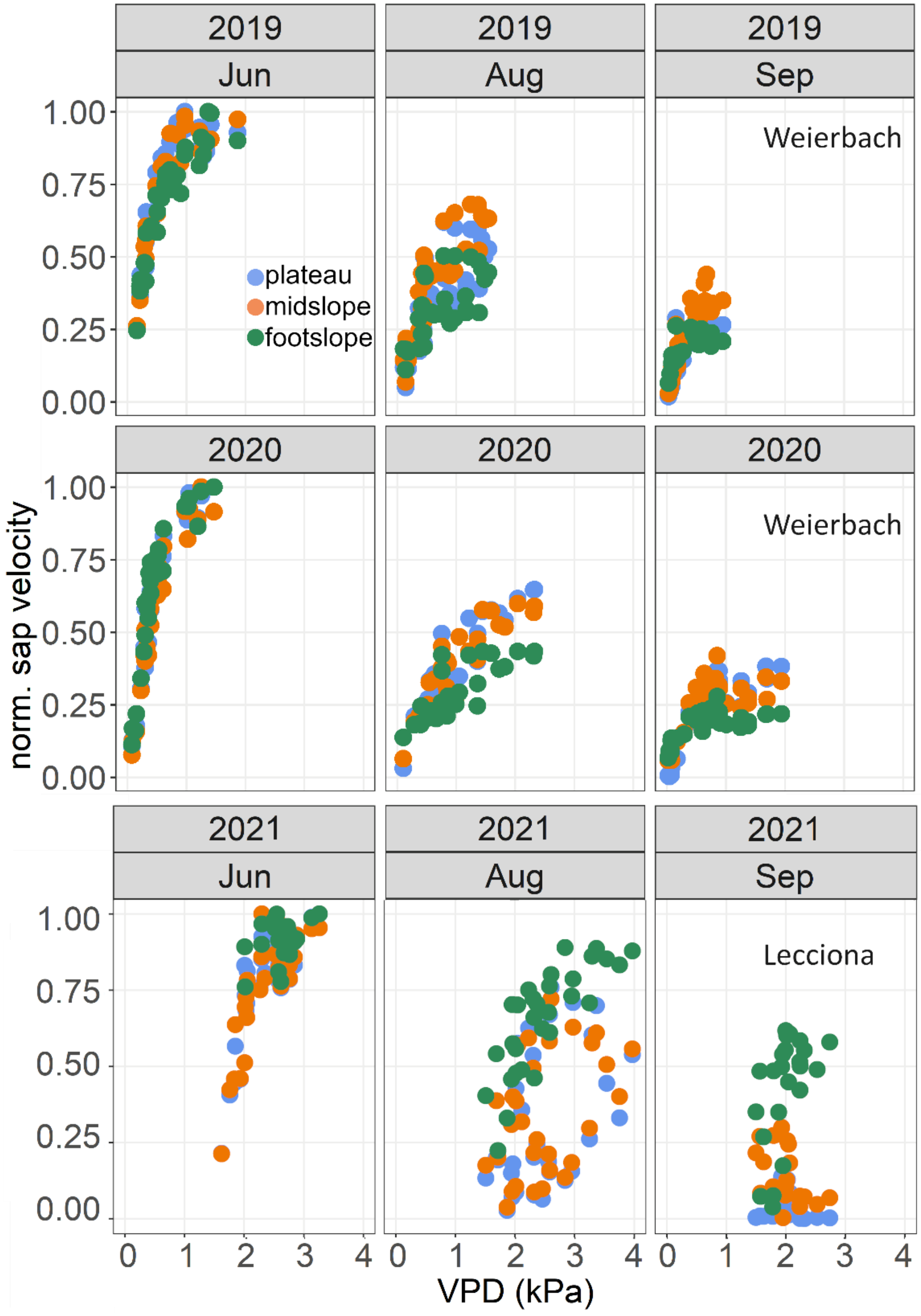


Fig. 6. Relationship between normalized mean sap velocity and daily vapor pressure deficit (VPD) (kPa) at different hillslope positions over three months of 2019 and 2020 in the Weierbach catchment and 2021 in the Lecciona catchment.

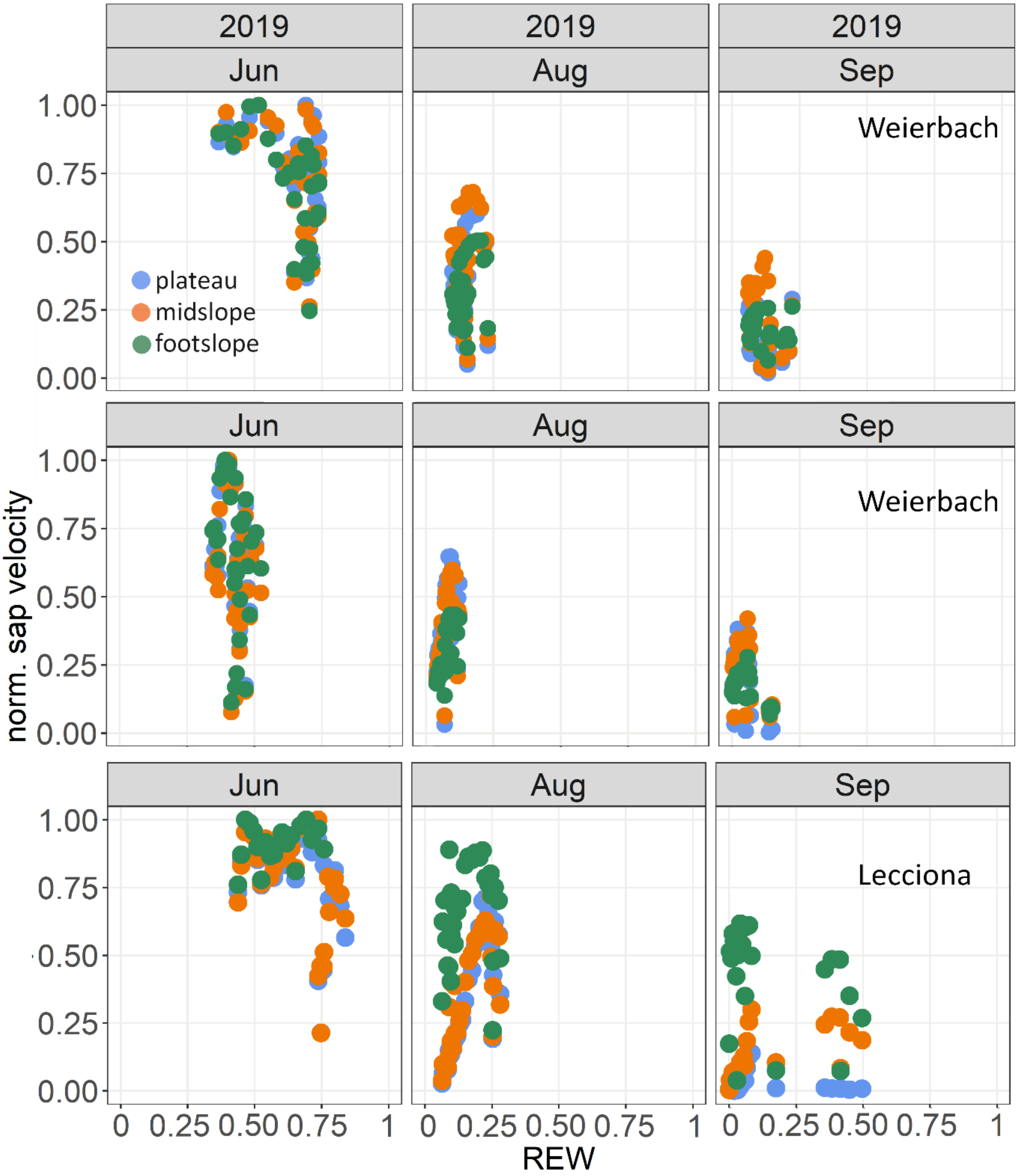


Fig. 7. Relationship between normalized mean sap velocity and relative extractable water (REW) at different hillslope positions over three months of 2019 and 2020 in the Weierbach catchment and 2021 in the Lecciona catchment.

3.3.4 Isotopic composition of xylem, soil, groundwater, and precipitation

In the Weierbach catchment, xylem isotopic composition (157 samples, see Table S2) ranged between -8.8 and -3.1‰ in $\delta^{18}O$ and -76.6 and -39.3‰ in $\delta^{2}H$. The mean isotopic composition was -6.4 ± 1.0‰ and -63.7 ± 7.1‰ in $\delta^{18}O$ and $\delta^{2}H$, respectively. There was a marked temporal variability at all locations with heavier isotopic composition during the dormant season values (Fig. S5). We did not find any statistical difference in isotopic composition between sampling locations ($p>0.05$, Wilcoxon rank sum test) (Fig. 8). Soil water $\delta^{18}O$ and $\delta^{2}H$ showed the typical depth-gradient with heavier isotopic composition in the shallow layers (mean -5.5 ± 1.6 ‰ $\delta^{18}O$, -38.7 ± 9.6 ‰ $\delta^{2}H$) and lighter values in the deeper one (-8.5 ± 1.4 ‰ $\delta^{18}O$, -55.7 ± 8.9 ‰ $\delta^{2}H$). The groundwater isotopic composition was stable over time and was statistically significantly lighter at the footslope (mean -8.5 ± 0.1 ‰ $\delta^{18}O$, -54.3 ± 1.1 ‰ $\delta^{2}H$) compared to the midslope (mean -8.3 ± 0.3 ‰ $\delta^{18}O$, -56.9 ± 1.8 ‰ $\delta^{2}H$) and plateau (mean -8.5 ± 0.3 ‰ $\delta^{18}O$, -52.6 ± 2.3 ‰ $\delta^{2}H$) ($p<0.05$, Kruskall-Wallis test).

In the Lecciona catchment, xylem water (96 samples, see Table S2) showed values between -8.0 and -3.5 ‰ in $\delta^{18}O$ and -65.9 and -42.3 ‰ in $\delta^{2}H$. During the growing season, xylem water displayed a strong temporal pattern (Fig. S5). From May onwards, xylem water became heavier in both isotopes over time. After reaching the heaviest values on June 11, it progressively got lighter (Fig. S6). Xylem water of trees located at the footslope (mean -6.5 ‰ $\delta^{18}O$ and -57.6 ‰ in $\delta^{2}H$) was significantly lighter in both isotopes compared to trees at the midslope (mean -5.9 ‰ $\delta^{18}O$ and -53.9 ‰ in $\delta^{2}H$) and hilltop (mean -5.4 ‰ $\delta^{18}O$ and -51.6 ‰ in $\delta^{2}H$) (Fig. 8). Soil water in Lecciona was heavier compared to the Weierbach catchment (upper portion of the GMWL, Fig. 8) and showed a marked depth gradient. While topsoil (0-5 cm) isotopic composition displayed an average isotopic composition of -3.6 ± 1.9 ‰ in $\delta^{18}O$ and -16.8 ± 13.9‰ in $\delta^{2}H$, the deepest soil depth sampled (40-60 cm) had an average isotopic composition of -6.5 ± 1.8 ‰ in $\delta^{18}O$ and -31.9 ± 14.1 ‰ in $\delta^{2}H$. As in the Weierbach catchment, we did not find significant differences in soil water isotopic composition between sampling locations ($p>0.05$, Wilcoxon rank sum test). At both sites, xylem water did not overlap with the potential water sources sampled (Fig. 8), but we observed a stronger deviation between xylem water and soil water in the Lecciona catchment (Fig. 8)

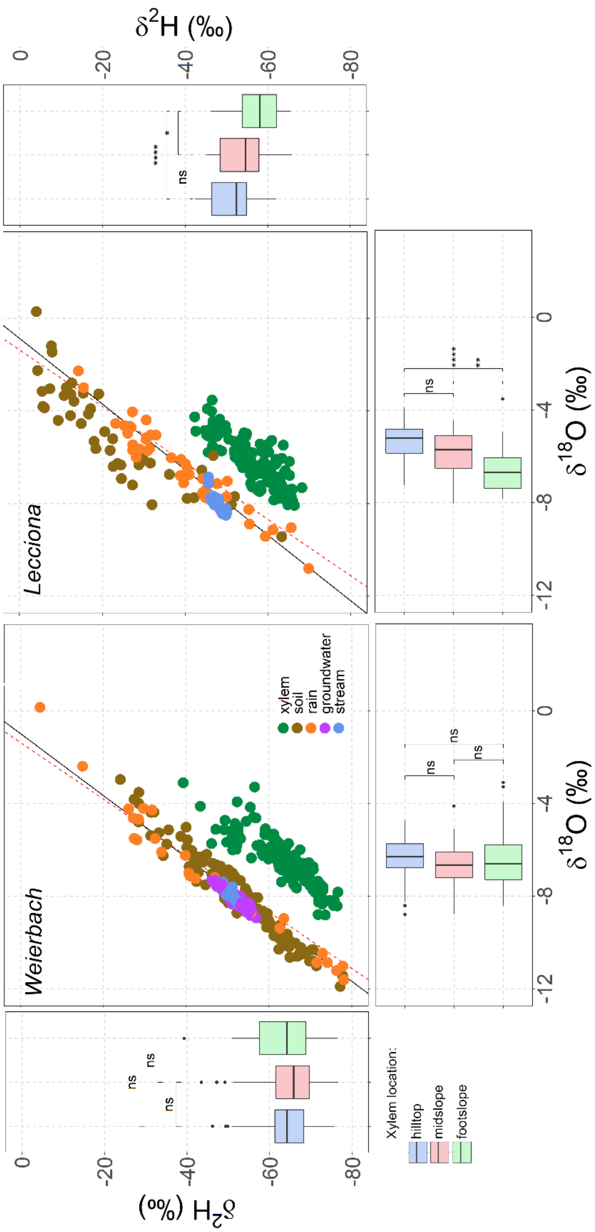


Fig. 8. Dual isotope plots showing soil water, rain, groundwater, stream, and xylem water isotopic composition sampled in the Weierbach catchment (left plot) and the Lecciona catchment (right plot). The boxplots show the median (black line in the box), the interquartile range (extent of the box), the range (whiskers), and outliers (points) for xylem isotopic composition for the three sampling locations. The dashed and solid lines represent the global meteoric water line (GMWL) and local meteoric water line (LMWL) for each site.

3.4. Discussions

3.4.1 Dry conditions did not lead to stronger sensitivity of beech trees to topography

According to our results, lower water availability in 2020 growing season in the Weierbach catchment (which was also the lowest recorded in the last eight years at our site) did not lead to a stronger sensitivity of tree water use to topography. The 2020 growing season was dryer and hotter compared to 2019 (Table 2, Fig. 4), which is in line with the general drying and warming trend observed over the last decade at the same meteorological station (Schoppach et al., 2021). Despite this, sap velocity did not decrease in 2020 compared to 2019, and sap velocities were even greater in May and September at all locations. Similarly, Gutierrez et al. (2021) found that drier conditions did not affect transpiration compared to the previous year because small precipitation events were able to reset drought spells. The gradual decrease in soil moisture content concurrently with an increase in VPD caused a reduction in normalized sap velocity, especially at the footslope location in both years (Fig. 6, 7). We found that maximum sap velocities were reached for the same VPD range (1.0-1.5 kPa). However, the number of days with VPD higher than 1.5 kPa doubled in 2020, which, coupled with the depletion in soil water reservoir, might be the cause for the early leaf yellowing and defoliation observed from mid-August at our site. According to Walthert et al. (2021), early defoliation in beech can be used as a predictor for crown dieback in the following year. In fact, the effects of water shortage cannot be confined to the year under review alone because the carry-over effects from current conditions will be expressed over the next seasons.

In agreement with what we observed in 2019 (Fabiani et al., 2022), the high vertical hydraulic conductivity in the Weierbach catchment (Glaser et al., 2016) does not promote shallow lateral subsurface from the plateau to the footslope (Fig. 10). Instead, the footslope is hydrologically connected to the plateau through saturated zone which leads to shallow and stable groundwater table at the footslope (Fabiani et al., 2021). However, groundwater proximity to the soil surface was not predictive of tree physiological response even during drier conditions in 2020 (Fig. 3). Previous minimum groundwater table depths at the plateau and midslope locations were exceeded in 2020, reaching a level typically below the rooting depth of *F. sylvatica* (0.83±0.46) (Fan et al., 2017). A close proximity of the groundwater table to the soil surface leads to anoxic conditions that limit tree root development (Phillips et al., 2016). Taken together, this evidence, combined with the lack of statistical difference in xylem isotopic composition among locations (Fig. 8), supports the conclusion that there is a negligible role of groundwater as a water source for beech trees in the Weierbach catchment. In agreement with this conclusion, Weemstra et al. (2013) did not observe any impact of interannual variation in groundwater depth on beech growth rates. Although plants can grow roots deeper in the soil profile as a response to water limitation (Bréda et al., 2006), beech trees have shown little plasticity and rely on shallow soil storage regardless of the drought condition (Gessler et al., 2022; Volkmann et al., 2016). This result emphasizes the importance of occasional rainfall to wet the topsoil, where most of the fine roots of *F. sylvatica* are located (Coners and Leuschner, 2005b; Meier and Leuschner, 2008).

Another potential water source to buffer water shortage is stem water (Tyree and Yang, 1990). The progressive dehydration of the xylem from winter to summertime suggests that the stem internal water storage acted as an additional water source for tree transpiration (Fig. 9, S3a). Seasonal changes in stem water content have already been observed in other studies with progressive water depletion over summer and refill after leaf fall (Tyree and Zimmermann, 2002a). Stem-stored water has been found to play a crucial role in the water balance of beech trees, improving their ability to cope with water stresses (Betsch et al., 2011; Köcher et al., 2013; Matheny et al., 2017a). Although we did not observe a statistical difference in wood moisture among locations and years (Fig. 9, S3), we acknowledge that our measurements provided only snap-shots of xylem water status and cannot capture fine-scale differences that could exist across the landscape. A clearer understanding of the role of stem water reserves on tree hydraulic integrity can be better addressed with the deployment of band dendrometers concurrently with point dendrometers (e.g. Dietrich et al., 2018; Salomón et al., 2022).

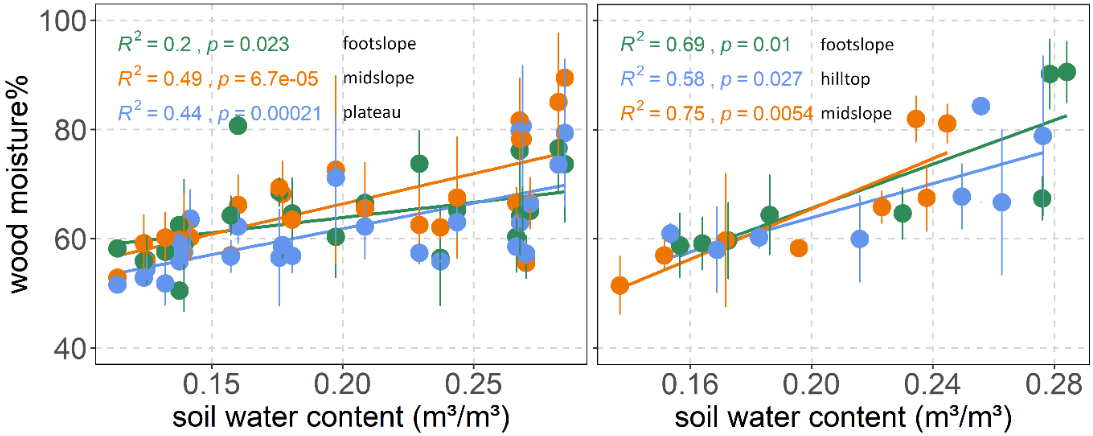


Fig. 9. Relationship between wood moisture content (%) variation and volumetric soil moisture content of the xylem sampling date in the Weierbach catchment (left side) and the Lecciona catchment (right side). Solid dots represent the mean of xylem moisture, the whiskers indicate the confidence interval (95%). In the Weierbach catchment, we used the average soil moisture content over the four depths, while in the Lecciona catchment, we used the average soil moisture over the two monitored depths recorded at each sampling location.

3.4.2 Topography drives different physiological response in the Lecciona catchment

Contrary to the Weierbach catchment, there was a significant effect of topography on sap velocity in the Lecciona catchment, supporting our hypothesis that steeper topography enhances the variability in energy and water inputs (Fig. 10). The steep slope in the Lecciona site facilitates the drainage of water away from the ridge leading to wetter soil conditions at the footslope than at the upslope locations (Fig. 3). Additionally, the thick litter pack accumulated downslope and lacking along the slope might have act as a barrier reducing soil evaporation.

We found that topography had a direct influence on tree physiological response to VPD and REW (Fig. 6, 7) through the regulation of soil moisture availability. Until July, the relationship between sap velocity and VPD was similar among hillslope locations suggesting that the soil water supply was sufficient to compensate for evapotranspiration loss (Bréda and Granier, 1996). However, in August the reduced water availability at the hilltop and midslope lead to a down-regulation of sap velocity (Fig. 6, 7). When more favorable conditions were restored owing to shallow soil wetting from rainfall events in September (Fig. 3), sap velocities at the hilltop and midslope locations did not recover the pre-drought transpiration (Fig. 6, 7). This result confirms the anticipated effects of landscape characteristics on tree transpiration, leading to more conservative hydraulic behavior in higher topographic positions due to the xeric conditions (Kumagai et al., 2008; Song et al., 2022). We have also observed that the decrease in soil water content at the upslope location led to a progressively shorter vegetative season moving from the footslope to the hilltop location (Fig. 5). This result might also be interpreted as evidence for physiological acclimation of beech trees located at the hilltop and midslope locations. The drier and more evaporative-demanding conditions at the midslope and hilltop areas, as evidenced by the presence of broom bush at the ridge, could cause trees in higher topographic positions to experience shorter growing season to withstand water limitations (Fig. 5). Similarly, Méndez-Toribio et al. (2017) observed shorter growth time-window in the upper part of slopes as a strategy to evade drought via leaf shedding or by the use of the internal water storage. The statistical difference in xylem isotopic composition between locations observed in the Lecciona and not in the Weierbach catchment (Fig. 8) might support the hypothesis that along the steep slope, trees are adapted to capitalize water sources with different isotopic compositions. We found significantly heavier xylem isotopic composition at the hilltop and midslope compared to the footslope location in the Lecciona catchment (Fig. S5), which suggests that trees at upper hillslope positions rely on a more fractionated water source compared to the footslope throughout the entire growing season. However, from the soil isotopic composition (Fig. 8), we cannot confirm this interpretation due to the large offset between xylem and soil water isotopic composition, as has been commonly observed in literature (Barbeta et al., 2019; de la Casa et al., 2021; Poca et al., 2019). We speculate that the trees located at the footslope might tap deeper water retained in the soil and in the weathered bedrock, which generally displays lighter isotopic composition compared to soil water.

We observed that transpiration responses to VPD differed between the two study sites despite soil moisture being in the same range. While maximum sap velocities were markedly reduced for VPD above 1.5 kPa in the Weierbach catchment (Fig. 6), in the Lecciona catchment only VPD values above 3 kPa were associated with a decrease in sap velocity (Fig. 3, 4). This suggests that trees in the Lecciona catchment were not water limited and likely had access to water stored in deep soil or saprolite. When compared to other species, *F. sylvatica* is generally considered to have an isohydric behavior (Fabiani et al., 2021; Gessler et al., 2021; Magh et al., 2020; Martínez-Vilalta and Garcia-Forner, 2017b), which means that with increasing desiccating atmosphere (i.e. VPD), beech trees tend to close their stomata to minimize water loss (Grossiord et al., 2017b). However, the different sap velocity response to VPD observed at our sites (Fig. 6, 7) suggests that the hydraulic behavior of this species could also change from site to site as an adaptation to hydrometeorological condition and topographic features. As already pointed out by Hochberg et al. (2018) when comparing data from multiple sites we do not always have the capability to separate environmental and genotypic variability.

The wide distribution range occupied by *F. sylvatica* in Europe results from the high plasticity of this species and the phenotypic variation including those relating to drought resistance (Stojnić et al., 2018). Previous literature has already emphasized that trees' hydraulic strategy should not be considered strictly as a dichotomy between iso- and anisohydric behavior, but rather a continuum between the two (Martínez-Vilalta et al., 2014). The high adaptation potential of beech trees to changing environmental conditions has also been speculated in a study that revealed the high plasticity capacity of the stem hydraulic system in *F. Sylvatica* over decades (Zimmermann et al., 2021). However, it remains unclear how a long-term increase in soil and air dryness may impact this species, vulnerable to drought (Walthert et al., 2021), across its wide distribution range.

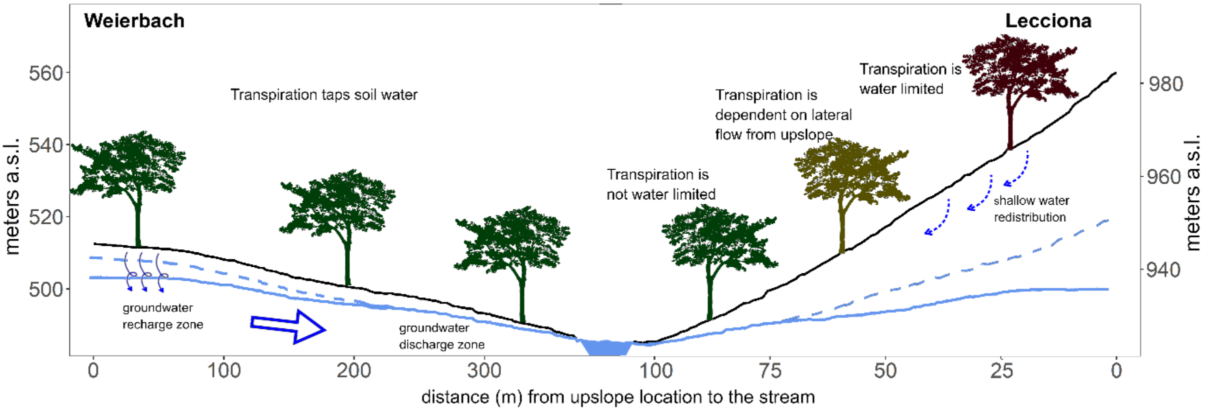


Fig. 10. Conceptual graphs showing the individual monitoring locations at each catchment. Terrain profiles were extracted from the digital terrain model with Qgis.

3.5. Conclusions

In the present study, we found that topographically driven hydrologic variability results in varying sap velocity responses to environmental controls (VPD and REW) but is not consistent across landscapes. In the Lecciona catchment, the shallow water redistribution along the slope leads to longer growing seasons for trees located at the footslope. On the contrary, at upslope locations trees showed a strong stomatal control and heavier isotopic composition due to a higher atmospheric desiccating effect. The xeric and evaporative demanding conditions upslope suggest that these topographic locations might be more prone to drought and overheat. Conversely, in the Weierbach catchment, the gentle slope did not lead to a strong hydraulic gradient, which translates into a lack of spatially different water use strategies and responses to vapor pressure deficit and relative extractable water (Fig. 10). Drier conditions in 2020 growing season did not increase the sensitivity of beech trees to topography. We have also shown that beech trees at our study sites exhibited a spectrum of hydraulic behaviors suggesting that this species may be fairly plastic.

Synthesis

Conclusions

So far, there has been a lack of consistent findings on how hillslope position controls forest water use may originate from the interplay of the site-specific structure of the Critical Zone (Brooks et al., 2015; Rempe and Dietrich, 2018). The subsurface properties of the Critical Zone (e.g., porosity, permeability, texture, regolith depth, layering, and weathered bedrock water storage capacity) control water availability through hydrological processes occurring in hillslopes (Brooks et al., 2015; Hahm et al., 2019; Klaus and Jackson, 2018; Penna et al., 2009) and ultimately tree transpiration.

This thesis aimed to improve the understanding of how water is partitioned in the critical zone and how vegetation water use patterns change seasonally and spatially in water-limited systems. To be able to address this, we adopted an interdisciplinary approach combining catchment hydrology, ecohydrology, and plant physiology. In the thesis, environmental conditions (temperature, humidity, rainfall, etc.), soil moisture, groundwater table level, and sap velocity have been monitored at high-resolution in combination with isotopic measurements to gain continue observations of different compartments of the Critical Zone. Additionally, the present work provides experimental evidence of water isotopic exchange between sapwood and heartwood in multiple species and functional groups. The three studies presented in the Chapters of this thesis contribute (i) to the advancement of our comprehension of how hydrological processes at the hillslope scale affect tree physiological activity (Chapter 1 and 3) and (ii) to the short- and long-term dynamics of water movement within tree stems (Chapter 2).

The main scientific findings are summarized as follows:

- Beech and oak trees have different ecohydrological niches driven by their species-specific water exploitation strategies and hydraulic traits, which are crucial to determining tree's ability to recover from water shortage periods. The interplay of these characteristics defines tree transpiration response to a variable water supply. Beech trees, although more drought-sensitive than oak trees, are a superior above-ground competitor and regenerate extensively in the Weierbach catchment (Chapter 1).
- Groundwater table proximity to the soil surface does not determine the magnitude of its contribution to transpiration for species not tolerating

- anoxic conditions in the rooting zone. Beech and oak trees in the Weierbach catchment mainly relied on water stored in the vadose zone (Chapter 1).
- Cross-site comparisons offer a great opportunity to increase our understanding of hillslope ecohydrological processes. Hillslope position does not everywhere control tree water use. The different hydrological processes characterizing the two investigated sites lead to contrasting tree physiological response. In sites where the hydraulic and climate gradient is stronger, tree physiological response among locations is spatially variable (Chapter 3).
- The occurrence of isotopic exchange between sapwood and heartwood improves the understanding of water movement within tree stems. Radial movement of water is not restricted to sapwood only, but also affects heartwood despite the loss to conduct water. Xylem structure (tracheid- and vessels-bearing tree species) and tree physiology (growing season duration) affect xylem water isotopic composition (Chapter 2).
- Xylem isotopic composition changes over radial depth. The progressive transition in isotopic composition with increasing radial depth toward the stem center indicates the occurrence of an isotopic gradient or the presence of a transition zone between SW and HW (Chapter 2).

Future outlook

In the context of the present work, I take the opportunity to emphasize the importance of long-term monitoring to estimate drought impact on forest ecosystems in complex landscapes. Carry-over effects of water limitation can be expressed over multiple years in forest ecosystems and affect different forest species according to their xylem, root, and leaf phenology.

While trying to answer the main research questions of the present Ph.D. project, several others came along. Most of them remained open due to time, expertise, or methodological limitations, but here they are discussed as propositions for the future.

Chapter 3 took up the challenge of making a cross-site comparison with the goal of advancing the generalized understanding of the interactions and feedback between topography and vegetation. The Chapter highlights that topographically driven hydrologic variability results in varying sap velocity responses to environmental controls but is not consistent across landscapes. However, replicating a consistent characterization of the CZ properties at both sites is a daunting task, and some aspects might have remained unaccounted, but can be addressed in future works. As an example, in the present study, our capacity to monitor below-ground water storage was limited to the upper soil layers (40 and 60 cm in the Lecciona and Weierbach catchment, respectively) and groundwater table depth (at one and three locations in the Lecciona and Weierbach catchment, respectively). Yet, water available to plants is also retained in deeper soil layers, saprolite, and between rock cracks, and their contribution to tree transpiration becomes relevant when the soil dries out. The use of a neutron probe has offered the possibility of monitoring the water content in depths generally uncovered by soil moisture sensors (Bréda et al., 1995; Rempe and Dietrich, 2018). Another aspect that has not been covered when evaluating the physiological activity of beech trees in the Lecciona and Weierbach catchment is the effect of genotypic variability. Populations growing in different areas of the distribution range are generally subject to genetic differentiation which includes drought resistance traits. Therefore, the data produced here contain both environmental and genotypic variability, but separating one from another is a difficult task (Hochberg et al., 2018). Future studies may investigate and consider the genotypic variability when combining tree physiological data from multiple field sites.

In Chapters 1 and 3, we deployed sap flow sensors to monitor species- and location-specific stomatal control and water use based on sap velocity response to vapor pressure deficit and soil moisture content in two study sites. However, sap velocity measurements provide only point measurements that do necessarily reflect the overall complexity and heterogeneity of the forest stand. When selecting trees for continuous measurements, we aimed at trees of similar diameter classes and with a symmetrical crown. To get a better understanding of how the forest stand organizes in response to spatially variable energy and water supply along a topography gradient a detailed characterization of the forest stand (i.e. stand structure, crown classes, density, basal area, crown architecture, but also the history of the past treatments) are needed. An interesting opportunity to characterize aboveground biomass is offered by airborne light detection and ranging (LiDAR) and photogrammetry technologies. Future studies in the Lecciona and Weierbach catchment may consider this technology to advance the understanding of the forest ecosystem functioning in complex topography.

Chapters 1 and 3 rely on techniques that can only provide insights of the monitored growing season. However, retrieving information about how a given species has adapted in the past to spatially and temporally variable water availability could aid to understand better its current behavior and predicting its future response to changing environmental conditions. Water limitations induce a reduction in cambial activity and cells enlargement, which translates in vessels with smaller lumen and reduced hydraulic conductivity (Hacke et al., 2005). Trees can respond to current climate conditions with xylem traits adaptation, and beech is known for its plasticity to growth conditions (Noyer et al., 2017). Therefore, a follow-up of the studies presented here (Chapter 1 and 3) may analyze whether the increase in climate aridity has exacerbated the landscape effects on tree xylogenesis and productivity along the topographic gradient.

Future studies may also combine isotopic technique with sap flow and dendrometer measurements to test if tree reliance of internal water storage as a potential strategy can aid trees to cope with topographic-related water limitations. Chapter 2 has demonstrated the occurrence of isotopic exchange between sapwood and heartwood, but the approach used here is unable to quantify the contribution of heartwood to sapwood and ultimately to the transpiration stream. Due to the low moisture content of heartwood in most tree species, the heartwood is presumed to not contribute to tree transpiration (Holbrook, 1995). Instead, the gas filling the voids has been argued to have a mechanical role in increasing the structural rigidity of a standing tree (Gartner et al., 2004). In some species, heartwood can develop a condition called “wetwood”, which leads to even higher moisture content in the heartwood than sapwood. An exceptional case among broadleaved species is that of oak (Tomczak et al., 2018), where heartwood moisture content is comparable and sometimes even exceeds sapwood moisture content in healthy trees (Chapter 2). Capacitance sensors have been successfully applied in tree stems to monitor the water content, but generally, the needle length was adjusted to cover the sapwood only (Matheny et al., 2017b). In this case, capacitance sensors with longer needles could be used in this species to test the occurrence of temporal changes in moisture content in the heartwood. This information coupled with the isotopic composition of the xylem could offer new insights on the mechanical or physiological role of this water reservoir.

Appendix

Appendix Chapter 1

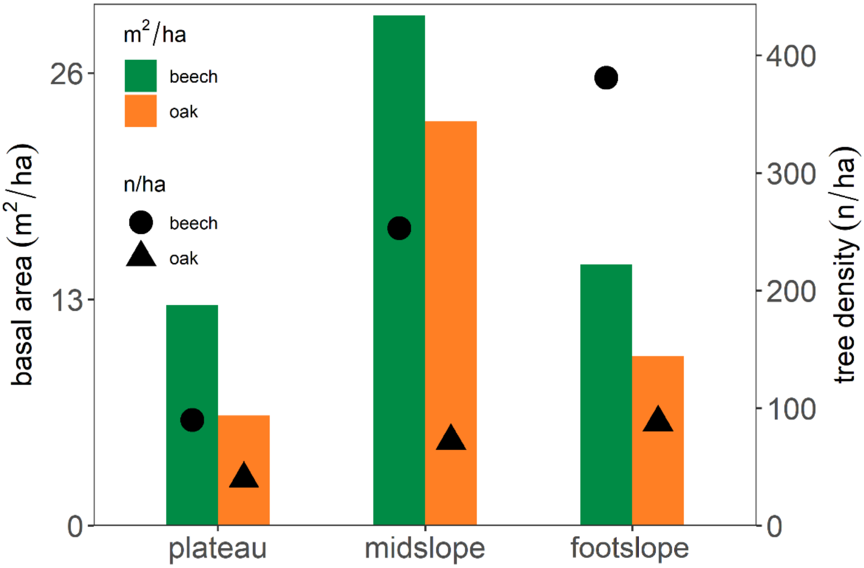
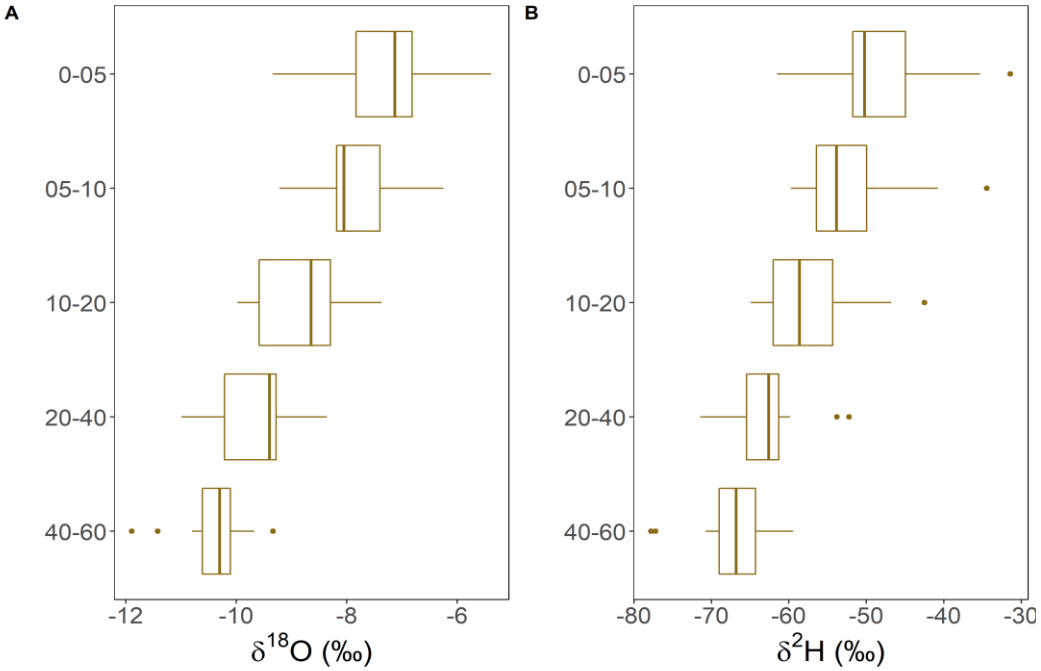


Figure S1. Basal area (m^2/ha) and tree density (n/ha) for beech and oak trees along a hillslope transect at the plateau, midslope, and footslope areas.

Figure S2 (next page). $\delta^{18}O$, $\delta^{2}H$ of soil water for the three sampling areas for all sampling campaigns conducted in 2019 (8 April, 23 April, 3 June, 17 June, and 1 July). The box size represents the interquartile range, the whiskers indicate the variability outside the upper and lower quartiles and the individual points are outliers.



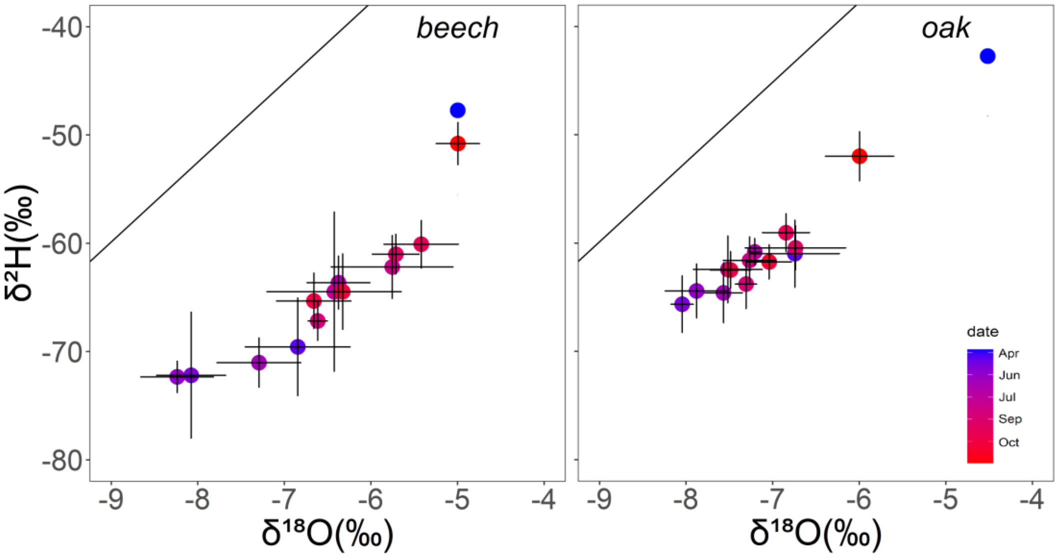


Figure. S3. Dual-isotope (δ^2H and $\delta^{18}O$) plot of xylem water for each sampling date averaged over the three sampling areas. Each point represents the average isotopic composition and standard deviation. The black line indicates the LMWL.

Appendix Chapter 2

Fig. S1. Pictures taken during the xylem sampling. The difference in wood core translucency given by the water content allowed us to separate SW from HW for spruce samples (left photo). For oak, the SW–HW boundary was determined visually based on the color change: SW displays a yellowish color, while HW has a dark-brown shade.

Table S1. Δ variation in moisture content (%), $\delta^{18}O$, and $\Delta\delta^{2}H$ between SW and HW for the investigated species

	Sapwood- Heartwood		
	$\Delta\delta^{18}O$	$\Delta\delta^{2}H$	Δmoist
beech	-0.3±0.3	0.45±2.0	4.1±6.3
oak	-0.4±0.4	-3.98±2.9	-11.0±11.7
Douglas fir	-0.7±0.9	13.0±4.5	52.5±17.4
spruce	-0.9±1.0	9.6±5.1	88.7±29.4

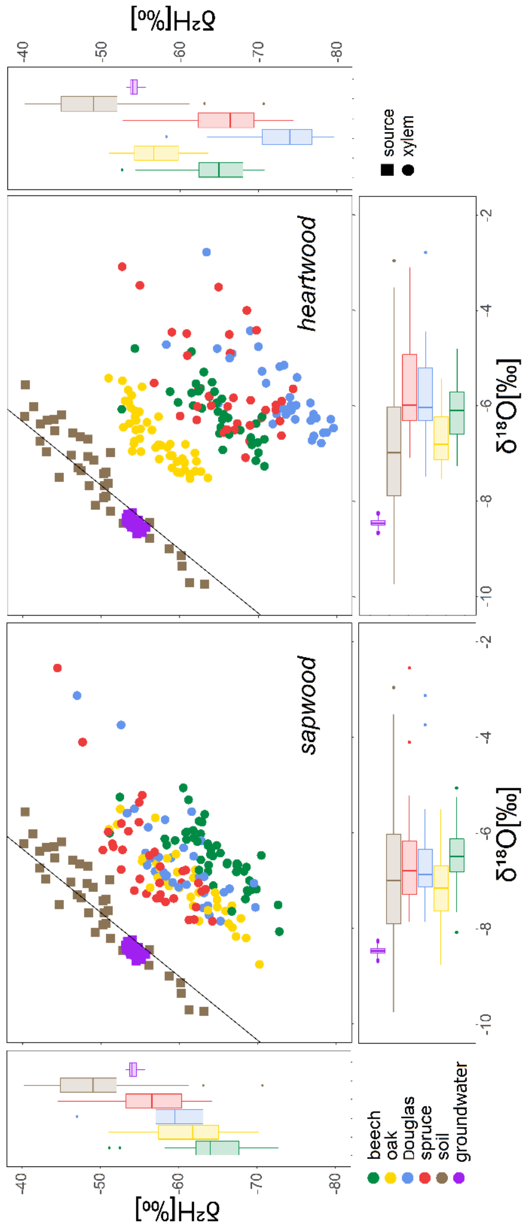


Fig. S2. Dual isotope plots and bar plots showing all soil water, groundwater, and xylem water samples for the different species. Boxplots show the median (black line in box), the interquartile range (extent of the box), the range (whiskers), and outliers (points).

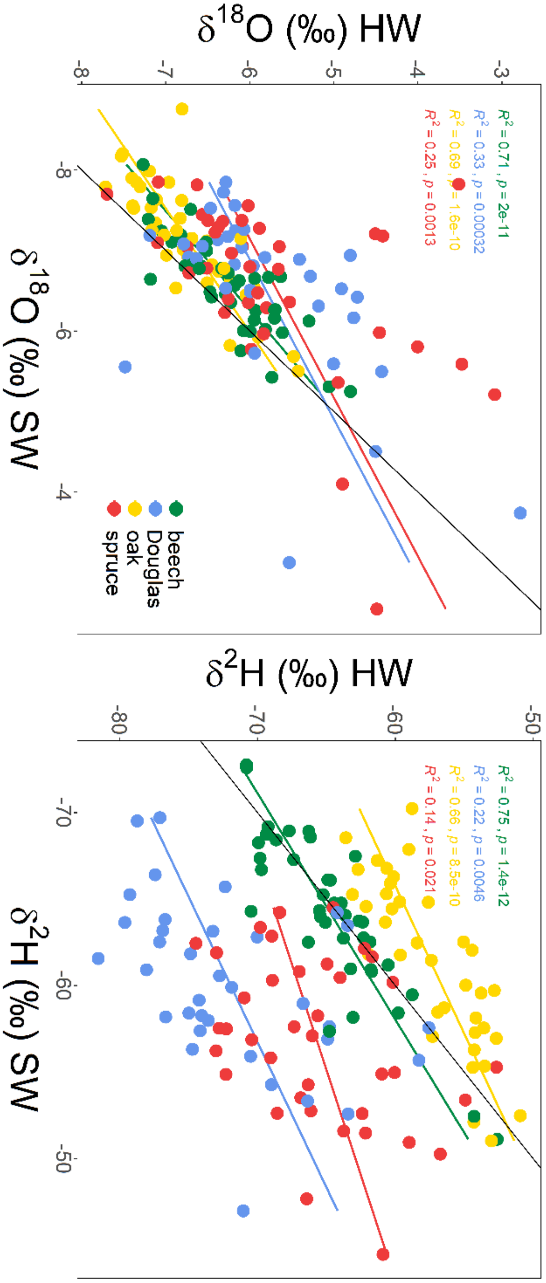


Fig. S3. Relationship between SW and HW $\delta^{18}O$ values (left panel) and $\delta^{2}H$ values (right panel) for all species.

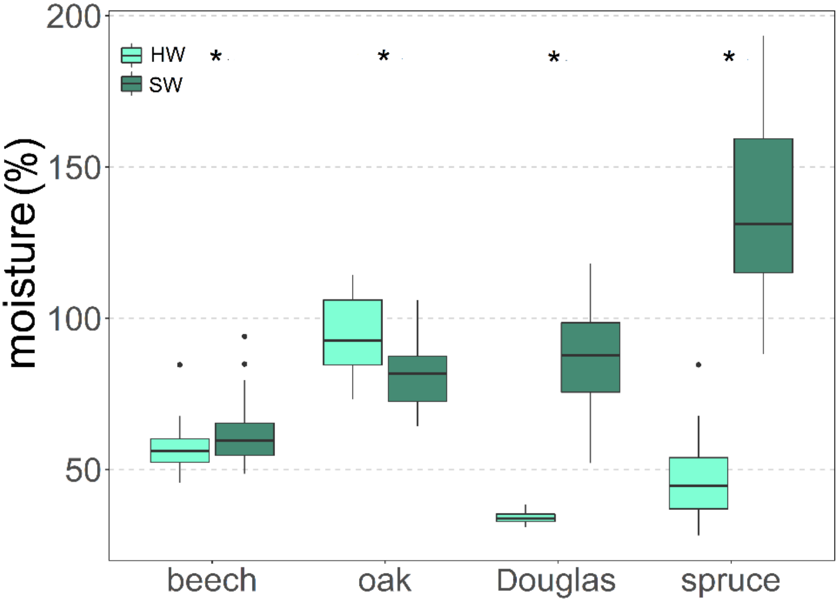


Fig. S4. Moisture content of xylem water for all sampling campaigns conducted in 2020. The center line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartile, respectively. The whiskers indicate points within 1.5 times of the interquartile range above or below the median. Above each species, asterisks (symbols “*”, "ns” correspond to p-values ≤ 0.05 or > 0.05, respectively) denote significant statistical differences between HW and SW (Mann-Whitney U test). For beech trees, SW and HW correspond to inner and outer SW.

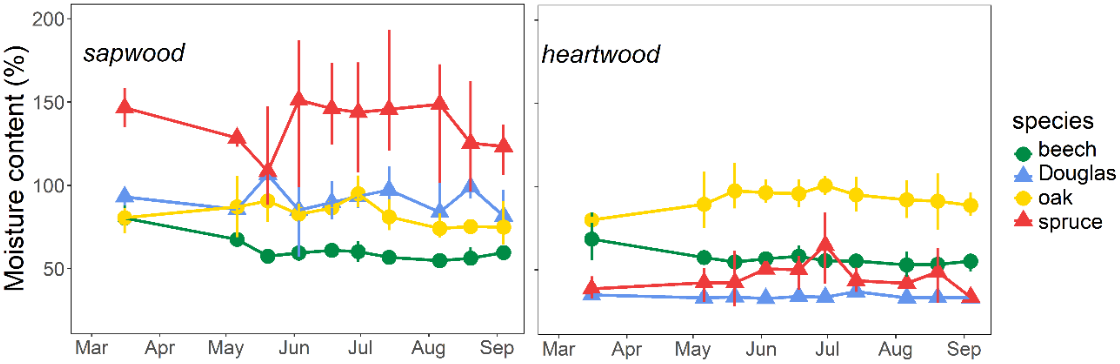


Fig. S5. Moisture content variation of sapwood and heartwood over time for the four investigated species. Solid dots represent the mean, the whiskers indicate the confidence interval (95%). For beech trees, SW and HW correspond to inner and outer SW.

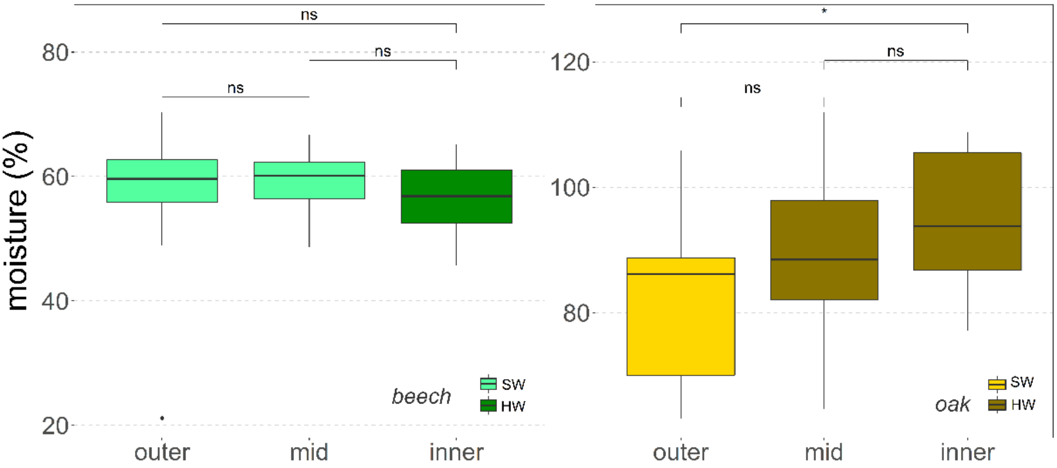


Fig. S6 Moisture content of beech (left plot) and oak (right plot) trees for the different sampling depths and functional parts (SW and HW). The center line in the boxplot indicates the median, and the lower and upper extremes indicate the first and third quartile, respectively. The whiskers indicate points within 1.5 times of the interquartile range above or below the median. Above each boxplot, asterisks (symbols “*”, "ns” correspond to p-values ≤ 0.05 or > 0.05, respectively) denote significant statistical differences between the different sampling depth (Wilcoxon test).

Appendix Chapter 3

Table S1. Data gaps filled. Color corresponds to the color code of Fig. S2.

Sensor	Color	Gap filled	R^2
Hilltop_3	pink	2021-04-21 – 2021-05-13	0.93
Midslope_2	blue	2021-05-15 – 2021-06-10	0.88
Midslope_3	pink	2021-06-14 – 2021-06-22, 2021-09-21 – 2021-10-03	0.92
Midslope_1	black	2021-04-15 – 2021-04-22, 2021-05-29 – 2021-06-10	0.92
Footslope_3	pink	2021-04-15 – 2020-04-29, 2021-06-11 – 2021-07-20, 2021-09-22 – 2021-10-04	0.93
Footslope_2	blue	2021-05-15 – 2021-05-28	0.93
Footslope_1	black	2021-05-15 – 2021-05-27	0.96

Table S2. Number of total samples collected at each site.

Site	Xylem samples	0-5 cm	5-10 cm	10-20 cm	20-40 cm	40-60 cm
Weierbach	157	95	95	95	95	95
Lecciona	96	27	27	27	27	27

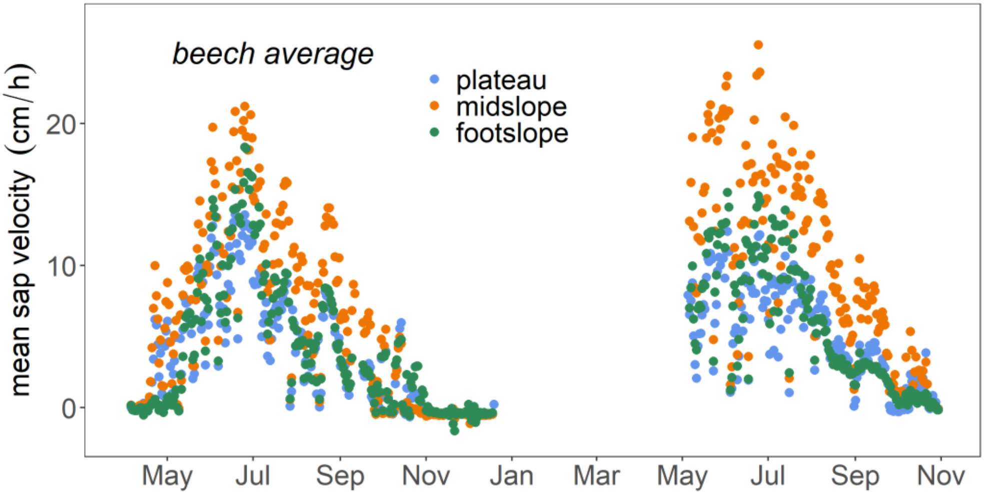


Fig. S1. Sap velocity (cm h-1) of beech at three sampling areas (plateau, midslope, footslope) in the Weierbach catchment over 2019 and 2020 growing season.

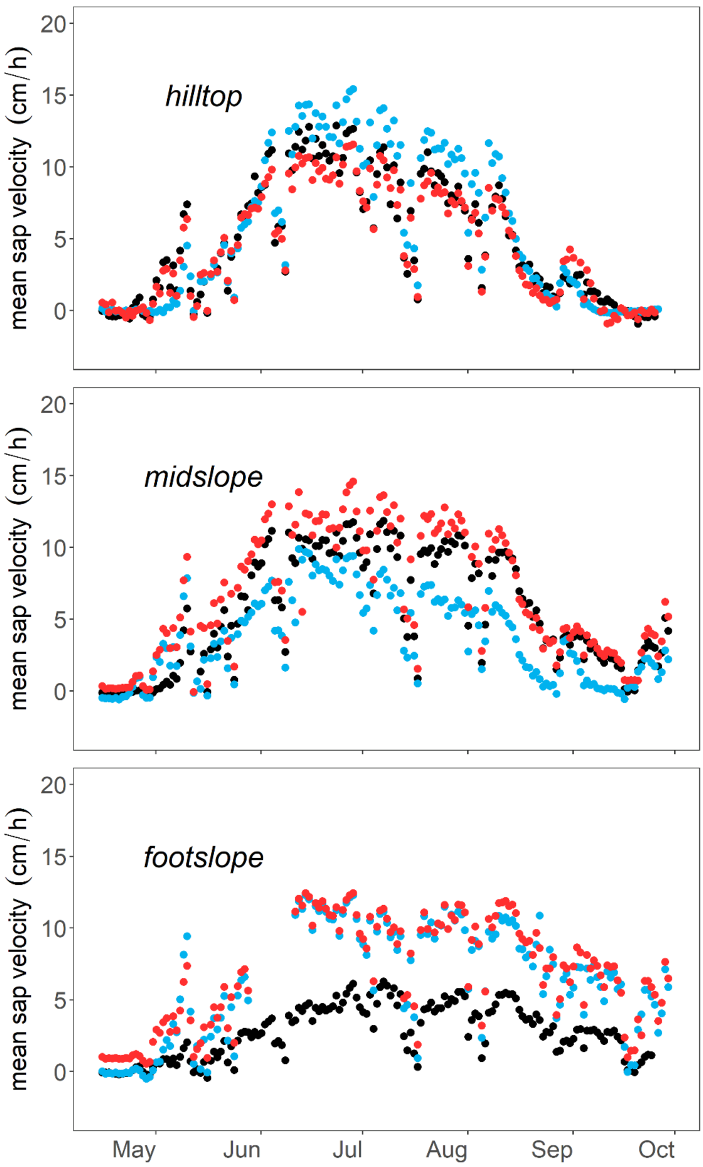


Fig. S2. Sap velocity (cm h-1) of beech at three sampling areas (hilltop, midslope, footslope) in the Lecciona catchment; each colour corresponds to one equipped tree. Missing data are the result of power interruption. When possible, data gaps were filled by interpolation, details are reported in Table S1.

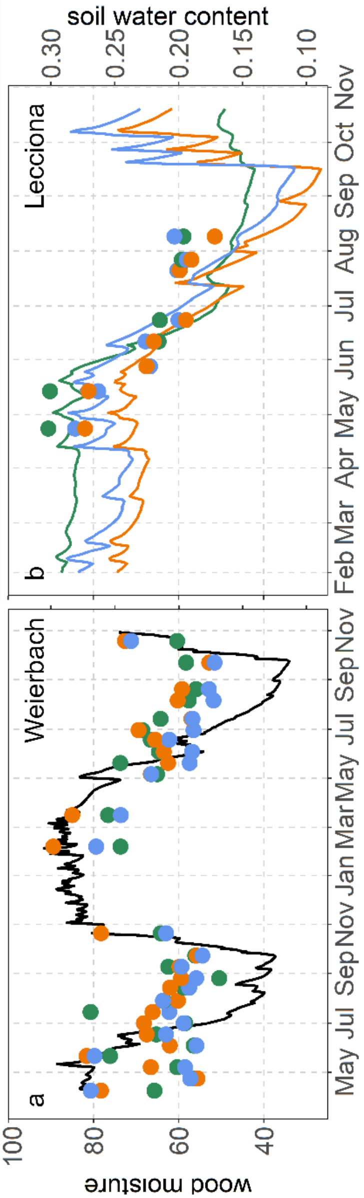


Fig. S3. Temporal pattern of wood moisture content (%) and volumetric soil moisture content in the Weierbach catchment (a) and in the Lecciona catchment (b). Solid dots represent the mean, each color correspond to sampling locations (blue= plateau/hilltop, orange= midslope, green= footslope). In the Weierbach catchment we used the average soil moisture content over the four depths, while in the Lecciona catchment we used the average soil moisture over the two monitored depths recorded at each sampling location.

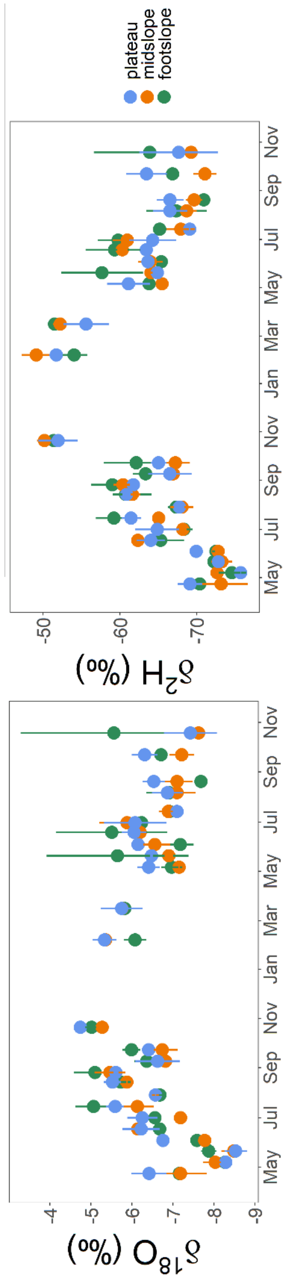


Fig. S4. Isotopic composition of xylem water for each sampling campaign across 2019 and 2020 growing season in the Weierbach catchment. Data were averaged by location. Solid dots represent the mean, the whiskers indicate the confidence interval (95%).

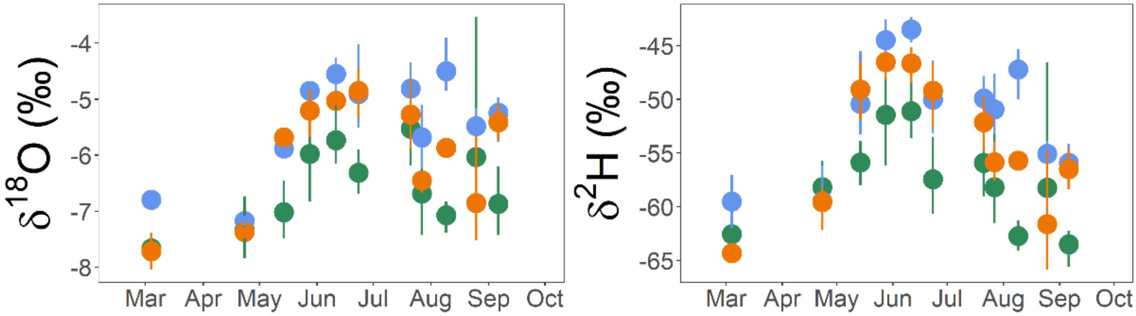


Fig. S5. Isotopic composition of xylem water for each sampling campaign across the growing season in the Lecciona catchment. Data were averaged by location. Solid dots represent the mean, the whiskers indicate the confidence interval (95%).

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List of publications

First author manuscripts in peer-reviewed journals:

Fabiani, G., Schoppach, R., Penna, D., Klaus, J., 2021. Transpiration patterns and water use strategies of beech and oak trees along a hillslope. Ecohydrology 1–18. https://doi.org/10.1002/eco.2382.

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Fabiani, G, Klaus, J. Penna, D, 2021. Contrasting water use strategies of beech trees along different hillslopes. In preparation, intended for: Trees

Co-authored manuscripts in peer-reviewed journals:

Schoppach, R., Chun, K.P., He, Q., Fabiani, G., Klaus, J., 2021. Species-specific control of DBH and landscape characteristics on tree-to-tree variability of sap velocity. Agric. For. Meteorol. 307, 108533. https://doi.org/10.1016/j.agrformet.2021.108533

First name contributions to international conferences:

Fabiani, G., Penna, D., Klaus, J. (2019): How does tree water uptake change over time along a hillslope?, MID-TERM AIIA: Biosystems Engineering for sustainable agriculture, forestry, and food production, Matera, Italy, oral presentation.

Fabiani, G., Penna, D., Klaus, J. (2019). Temporal dynamics of tree water uptake along a hillslope transect. EGU-Leonardo: Global change, landscape ageing and the pulse of catchments - EGU Leonardo Topical Conference Series, Belval, Luxembourg – 16-18 October 2019, poster presentation.

Fabiani, G., Penna, D., Klaus, J. (12 November 2019). Where do trees take up water from? An isotope-base study along a hillslope, Catchment Hydrology Day, University of Florence, DAGRI Department, oral presentation.

Fabiani, G., Penna, D., Klaus, J. (May 2020). How do meteorological variables and topography control species-specific water uptake strategy along a forested hillslope?, EGU European Geoscience Union, Vienna, poster presentation.

Fabiani, G., Penna, D., Barbeta A., Klaus, J. (19-30 April 2021). Sapwood-Heartwood isotopic composition in four forest species: implications for isotopes studies. EGU European Geoscience Union, Vienna.

Fabiani, G., Schoppach, R., Penna, D., Klaus, J. (11-12 October 2021). Transpiration patterns and water use strategies of beech and oak trees along a hillslope. Workshop 2021 - Water and nutrient fluxes in ecosystems under a changing climate - a tracer-based perspective, University of Freiburg.

Fabiani, G., Klaus, J., Schoppach, R., Penna, D., (26 May 2022). Contrasting tree water use strategies along hillslopes in forested catchments in Luxembourg and Italy. EGU European Geoscience Union, Vienna.

Fabiani, G., Klaus, J., Schoppach, R., Penna, D., (7-10 June 2022). Contrasting tree water use strategies along hillslopes in forested catchments in Luxembourg and Italy. Poster presentation at ERB conference Euromediterranean Network of Experimental and Representative Basins, Isola d’Elba, Italy.

Fabiani, G., Penna, D., Barbeta, A., Klus, J., (26 July 2022). Experimental evidence of isotopic exchange between sapwood and heartwood for different tree species. Workshop on isotope-based studies of water partitioning and plant-soil interactions, Florence, Italy

GINEVRA FABIANI

CURRICULUM VITAE

Born in Massa, Italy on 17 September 1993
ginevra.fabiani@gmail.com

ABOUT ME

I am a forest hydrologist, entusiast of field and lab work,
My research interests are ecohydrology, forestry, plant phisiology and tree functional ecology.

Mother tongue 🇮🇹

Fluent (C2) 🇬🇧

Intermediate (B1) 🇩🇪

EDUCATION

PhD in Docteur en Sciences de l'Ingenieur and Doctor of Suistainable Management of Agricultural Forestry and Food Resources
University of Luxembourg (LU) and University Florence (ITA)
September 2018 - December 2022

Master degree (110 cum laude) in Science and technology of forestry systems, curriculum Forestry management
University of Florence (ITA)
January 2016 - April 2018

Bachelor degree (110 cum laude) in Forestry and Environmental Science
University of Florence (ITA)
September 2012 - December 2015

WORK AND RESEARCH EXPERIENCE

Research as Phd Candidate
Luxembourg Institute of Science and Technology (LIST) and University of Florence
September 2018 - December 2022

Internship in Microbiology
Microbiology department - University Innsbruck (AT)
July 2017 - December 2017

Internship in Pedology
Swiss Federal Institute for Forest Snow and Landscape Research (WSL), Birmensdorf (CH)
September 2014 - December 2014

SKILLS

Softwares:
- R: statistics, data analysis, data visualization;
- QGIS;
- Hydras
- Sap flow tool

Lab instruments:
- Laser spectrometer - Picarro and Los Gatos LGR;
- Micro Combustion Module - Picarro;
- Cryogenic extraction ramp;
- Gel electrophoresis of nucleic acids;
- Real-time PCR cyclers.

Field instruments:
- Sap flow sensor ICT International (SFM1);
- Conductivity, Temperature, Depth sensor OTT;
- Soil moisture sensor Theros;
- Dendrometers ICT international;
- Pluviometer HOBO;
- Rainfall collector Palmex;
- Forestry tools: GPS, clinometer, relascope, increment borer.