Ecohydrology

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Ecohydrological Dynamics and Temporal Water Origin in a European Mediterranean Vineyard

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ABSTRACT

Viticulture is an essential sector in agriculture as wine production plays a vital role in the socio-economic life of many countries, especially in the Mediterranean area. Grapevines are a valuable, long-lived species able to grow in hot and dry regions. We currently do not know whether rain-fed grapevines entirely rely on deep soil water or make substantial use of shallow water from summer precipitation events. Without knowing this, we poorly understand what fraction of summer precipitation inputs contributes to grapevine transpiration. This has implications for how we quantify grapevine-relevant precipitation budgets and for predicting the impacts of climate change on grape and wine production. We investigated grapevine water use in a vineyard in the Chianti region, central Italy. During the growing season of 2021, we monitored precipitation and soil moisture at 30- and 60-cm depth. We collected over 250 samples for stable isotope analysis from rainfall, soil, and plants. Since traditional plant water sampling is problematic for grapevines, we collected samples from shoots, leaves, and condensed leaf transpiration after sealed plastic bags were wrapped around a shoot. We use these alternative plant samples to reconstruct the isotopic signal in the xylem water and infer the plant's seasonal water origin throughout the growing season. The analysis of the seasonal origin of water revealed that, throughout the growing season, soil water and plant water received disproportional contributions by rain that had fallen in the winter, even when compensating for the Mediterranean climate of the area. Only in late summer did the grapevines use substantial amounts of summer rainfall, whose contribution occasionally became dominant. These results provide a better understanding of ecohydrological interactions and uptake dynamics in valuable socio-economic agroecosystems such as vineyards.

1 | Introduction

Gaining a thorough understanding of crop water use strategies and water flux exchanges between crops, soil, and the atmosphere has always been a priority to define crop water requirement, assess crop response to water scarcity, and efficiently and sustainably manage water resources (Sivapalan et al. 2014; Rodríguez-Iturbe and Porporato 2005). Crop-water interactions and ecohydrological dynamics in agricultural settings are becoming more and more important under the predicted increase in the frequency of extreme

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meteorological events, such as drought episodes that have the potential to severely damage crops with noticeable socio-economic impacts (Leng and Hall 2019). The ecohydrology of plant-stressed environments has emerged as a discipline after seminal work by Rodríguez-Iturbe and Porporato (2005) and highlighted how different plants have specific water-use strategies to respond to the erratic changes in soil water availability and environmental forcing (Rodriguez-Iturbe 2000; Rodriguez-Iturbe et al. 2001; Laio et al. 2001).

Complementary to the problem of how plants respond to hydrological variability, a growing branch of ecohydrology focuses on identifying plant water sources through isotope tracing methods (Penna et al. 2018). Despite some intrinsic uncertainty (Beyer and Penna 2021; Rothfuss and Javaux 2017), water tracing techniques and mixing models based on the stable isotopes of hydrogen and oxygen (^{2}H and ^{18}O) have great potential to track sources of plant root water uptake from different soil depths and to understand plant water use strategies (Sprenger et al. 2019; Penna et al. 2018). In temperate climates with marked seasonality, assessing whether plants can extract soil water stored during rainy winter periods allows for assessing their resilience to drought episodes. For this reason, isotope methods are commonly used both in forested and agricultural ecosystems (Orlowski et al. 2023; Penna et al. 2020). Recent examples of isotope-based ecohydrological investigations aimed at detecting water use strategies in agroecosystems include studies on a large variety of crops, encompassing apple trees (Shi, Gai, and Li 2023; Yang et al. 2022; Penna et al. 2021; Liu, Jia, and Yu 2020; Aguzzoni et al. 2022; Giuliani et al. 2023), cherry trees (Li et al. 2020), walnut trees (Liu et al. 2019), shrub or herbaceous species, such as winter wheat (Liu et al. 2021, 2023), coffee (Muñoz-Villers et al. 2020), cotton (Goebel and Lascano 2019), rape and barley (Wu et al. 2018) and maize (Wu et al. 2016). The basic concept underlying isotope methods in plant apportioning studies is that the outflow, that is, xylem water, integrates all waters accessed by the plants from different spatial (e.g., depth) or temporal (e.g., winter vs. summer) sources. Therefore, the isotopic composition of xylem water reflects the proportions of water taken up by the root system having different spatial and temporal origins and, assumingly, a different isotopic composition. Being able to sample and determine the isotopic composition of each potential water source used by a given plant as well as its xylem water allows researchers to identify and quantify the fraction of each source taken up by the plant. The application of this approach is challenged by the possible isotopic fractionation occurring during the water transport mechanisms from the root system to the leaves, which can alter the original isotopic composition of the water tapped by the plant and mask the estimate of the actual contribution of different water sources. Isotopic fractionation occurring within the plant has sometimes been observed in potted experiments under natural conditions (e.g., Uugulu, Wanke, and Koeniger 2024; Poca et al. 2019; Zhao et al. 2016), but other studies reported no or negligible fractionation of xylem water during the transport process through the stem (Barbeta et al. 2022; Amin et al. 2021) or showed contrasting results according to different species (e.g., Sánchez-Murillo et al. 2023).

Viticulture is one of the most economically valuable agricultural sectors, especially in Europe, which hosts 3.2 million hectares of vineyards and includes the three world's top wine-producing countries, that is, France, Italy, and Spain (Fraga 2019). Viticulture is particularly sensitive to climate change-related alterations in temperatures and water availability as premium wine production occurs within very narrow climate ranges (Sodini et al. 2023; Shtai et al. 2024). Grapevines are generally well adapted to hot and dry environments, such as those in Mediterranean regions but prolonged or intense droughts cause negative effects such as early shoot growth cessation, reduced photosynthesis, and limited yield (Gambetta et al. 2020; van Leeuwen et al. 2019; Hannah et al. 2013). Moreover, in some of the most renowned wine-producing districts, such as Tuscany in central Italy, irrigation is not allowed except in the case of emergency conditions. Therefore, grapevines rely almost exclusively on naturally occurring water availability, even during dry spells.

The grapevine's ability to withstand periods of drought largely depends on the type of rootstock used. Rootstocks were introduced in European grapevine cultivation during the XIX century as a measure to counteract phylloxera, a pest that inflicts severe damage to the root system of *Vitis vinifera sativa* (Keller 2020). The rootstocks employed today are typically hybrids derived from the crossbreeding of various American grapevine species (*V. riparia, V. rupestris, V. berlandieri*), all known for their resistance to phylloxera. These hybrids exhibit varying capacities to adapt to soil properties, including water availability (Keller, Mills, and Harbertson 2012). Notably, drought-resistant rootstocks are typically characterized by roots capable of growing deep into the soil, ensuring a water supply to the grapevine even in situations where the superficial layers of soil are dry (Swanepoel and Southey 1989). Irrespective of the available rootstock, soil water limitations can negatively impact grapevine growth and physiological performance in different ways, depending on the seasonal phenological stages (Romero, Navarro, and Ordaz 2022; Wenter et al. 2018). During certain growth phases, such as blooming and/or berry set, grapevines are particularly susceptible to water limitations, leading to significant yield reduction and a loss of berry quality. In contrast, in other growth stages (from veraison until harvest), grapevines exhibit relative drought tolerance. In addition to using irrigation to prevent water stress during susceptible phenological stages, traditional grapevine cultivation relies heavily on appropriate soil management techniques in the vineyard. These practices include soil tillage beneath the grapevines or the mowing of grasses in the inter-row space, with the goal of reducing competition for water from other plants (Medrano et al. 2015), especially during the most susceptible phases.

A few isotope applications are reported in vineyards. Savi et al. (2018) monitored grapevine water status and assessed the spatial origin of water used by grapevines growing on a karstic plateau between North-Eastern Italy and Slovenia. They found that even during harsh conditions that occurred during a dry summer, plant water status showed only a moderate water deficit never reaching critical levels, and that this was related to deep soil water sources accessed by the grapevines (Savi et al. 2018). Meng et al. (2023) sampled grapevine tissues and organs (root, stem, leaf, and fruit) in grapevines of a wine-producing region in China to assess the effect of different irrigation strategies on the isotopic fractionation of grape juice from different varieties. Their results revealed a strong fractionation effect

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during the transport of water to different grape organs and that isotopic fractionation in grapes occurred more frequently under drought conditions (Meng et al. 2023). Kovač et al. (2023) relied on the isotopic composition of precipitation and soil water to understand water circulation patterns and mixing in a hillslope hosting a vineyard in Croatia, but without sampling and focusing on water used by grapevines. Gómez-Alonso and García-Romero (2010) analysed both oxygen and carbon isotopes in a vineyard in Spain, showing that the variability in the isotopic composition of water and sugar in berries was mostly controlled by irrigation, and it was less related to the vegetative cycle of each grapevine variety (Gómez-Alonso and García-Romero 2010).

Isotope techniques have mainly been applied to the wine-production market (e.g., Tardaguila et al. 1997), and there is a substantial under-use of stable isotopes of hydrogen and oxygen for identifying and quantifying the spatial and temporal origin of water used by grapevines. This lack of studies hampers a clear identification and quantification of the spatio-temporal variability of water sources used by grapevines, preventing a robust conceptualization of water flux exchange in vineyards and a solid assessment of grapevines' resilience to drought, that is, their capability to access different water pools during periods of water scarcity. Moreover, the isotope technique requires sampling and determining the isotopic composition of the potential water sources (i.e., soil water at different depths) and of xylem water. Sampling xylem water in grapevines is challenging as the stem is too small for taking wood cores or for installing *in situ* probes (Beyer, Kühnhammer, and Dubbert 2020). While shoots can be sampled easily without causing permanent damage to the grapevine, repeated sampling from the same plant is limited by the number of secondary shoots that do not bear fruits. These drawbacks can severely limit the application of isotope techniques in vineyards, hampering our understanding of the plasticity of different cultivars and rootstocks to access different water depths and sources, preventing a clear assessment of the capability of grapevines to use water stored during winter or summer periods. Therefore, developing alternative sampling methods of grapevine xylem water is critical to take full advantage of the tracing capability of isotopes to assess vineyards' resilience to drought.

We contribute to filling these knowledge gaps by conducting isotope-based experimental work in a Mediterranean vineyard in the Chianti region, a hilly area in Central Italy, one of the most famous wine-producing regions in the world. We measured weather data, soil moisture at different depths and locations, and we sampled precipitation, soil water at different depths and locations, and plant water from grapevines grafted on two different rootstocks (characterized by different root depths) across multiple campaigns during a year. We collected plant samples from shoots and leaves and also tested a recently proposed approach to collect transpired water in the field (the so-called 'bag water method'), exploring its possible usefulness for source water estimation and quantification. Based on these experimental data, we addressed the following research questions:

- What are the relative contributions of winter and summer precipitation in soil water at different depths?
- How does the temporal origin of water used by grapevines change over the growing season?
- How do alternative plant samples, such as the shoot water or bag water, allow inferring water sources for grapevines?

While addressing these research questions, we also explore whether vines grafted on different rootstocks show significant differences in their main water sources.

2 | Methods

2.1 | The Experimental Vineyard

The experimental activities were carried out between April 2021 and March 2022 in a vineyard at the Villa Montepaldi farm, a productive winery owned by the University of Florence, and

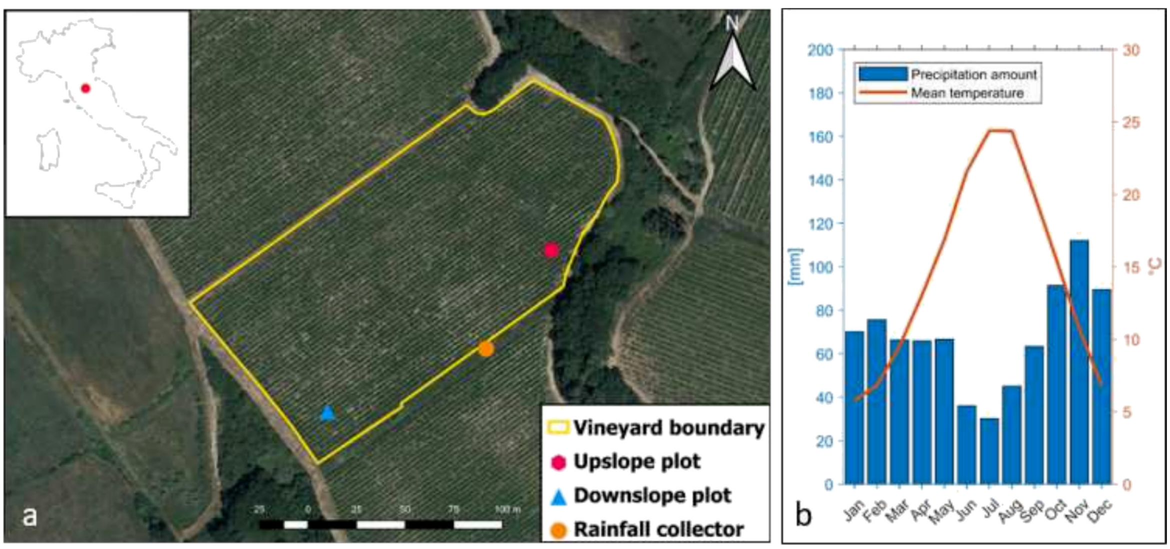

FIGURE 1 | (a) Location of the experimental vineyard (43° 40′14.47′ N, 11° 08′20.46′ E, WGS84), highlighting the position of the rainfall collector and the upslope and downslope sampling plots; (b) mean monthly temperature and precipitation amounts measured between 2004 and 2022 at a regional weather station located 4.5 km from the field site.

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located on the hills of the famous wine region Chianti, Tuscany, central Italy. The climate at the site is temperate and warm and falls in class Csa according to the Köppen-Geiger classification (Peel, Finlayson, and Mcmahon 2007). The long-term (2004–2022) mean annual precipitation in the study area measured by a weather station operated by the Regional Hydrological Service and located at Montespertoli (roughly 4.5 km straight from the study site) is 822 mm, and the mean annual temperature is 13.7°C (Figure 1b). Precipitation is unevenly distributed over the year, with dry summers and wet winters. The experimental vineyard, named 'Nincio 3' (43° 40′14.47″ N, 11° 08′ 20.46″ E, WGS84), has a size of 2.3 ha and is located on a slope degrading towards a small river (Figure 1a). Elevation ranges between 109 and 146 m a.s.l., and the aspect is predominantly south-west. The soil has a dominant clay texture; it is rich in skeleton made by calcareous rocks and has a relatively low content in nitrogen and organic matter. The vineyard is cultivated with cv. Sangiovese (*V. vinifera*) grapevines grafted either on 1103 Paulsen or 420A rootstocks, which are typically characterised by a deeper and shallower rooting system, respectively. The grapevines are trained as permanent cordon, with planting distances of 1.5 m and inter-rows of 2.3 m. The inter-row space is covered with grass, which is mowed twice over the growing season. At the time of our field campaign, grapevines were 12 years old.

We installed Frequency Domain Reflectometry volumetric soil moisture probes at 30- and 60-cm depth, with replicas, at both the top and bottom hillslope locations (ten probes overall). The probes measured at 5-min temporal resolution between June 2021 and May 2022, and values were aggregated at 15-min resolution. We used the calibration equation for mineral soils provided by the manufacturer. An evaporation-free rainfall collector for isotopic analysis of rainfall (Gröning et al. 2012) was placed on the vineyard boundary in May 2021. All the hydrologic data collected at the site are available in Benettin et al. (2024).

2.2 | Water Sampling for Isotope Analysis

Samples for isotopic analysis (Figure 2) were collected approximately twice a month between April and early October 2021, with additional sampling in December 2021, January 2022, and March 2022 (a total of 11 campaigns). On each occasion, we collected water from the precipitation sampler, which cumulated all the rainfall since the previous sampling date. Through a soil auger, we collected two bulk soil samples at 30- and 60-cm depth at both the top and bottom hillslope locations (a total of eight samples on each occasion). The collected bulk soil material was placed in Exetainer vials and stored at 4°C for subsequent water extraction. We selected two adjacent grapevines for each rootstock and hillslope location (a total of eight plants each time), and from each, we removed one shoot and one leaf sample. The shoot sample consisted of two internodes (still green, taken from the centre of a shoot) from which the buds were removed. From the same shoot portion, we collected a leaf sample after removing the petiole. As for the soil samples, plant material was placed in Exetainer vials and stored at 4°C. We also tested an alternative type of plant water sample, extracted in the field using transparent plastic bags (Kulmatiski and Forero 2021). We sealed 6-L plastic bags around one shoot of each selected grapevine, including approximately eight-ten leaves each. The bags were kept in place for approximately 2 h, during which the temperature inside the bag could be up to 10°C higher than the air temperature. While the bags were on, the leaves continued to transpire and the accumulated water vapour condensed on the bag walls. After manually shaking the bags, water accumulated at the bottom was collected by piercing the bag with a syringe needle. The volume that accumulated within the bag was variable and ranged between 0.5 and 5 mL, with typically smaller volumes during drier days. The collected water was placed in 300-μL vials and stored at 4°C for subsequent isotopic analysis. All the isotope data collected at the site are available in Benettin et al. (2024).

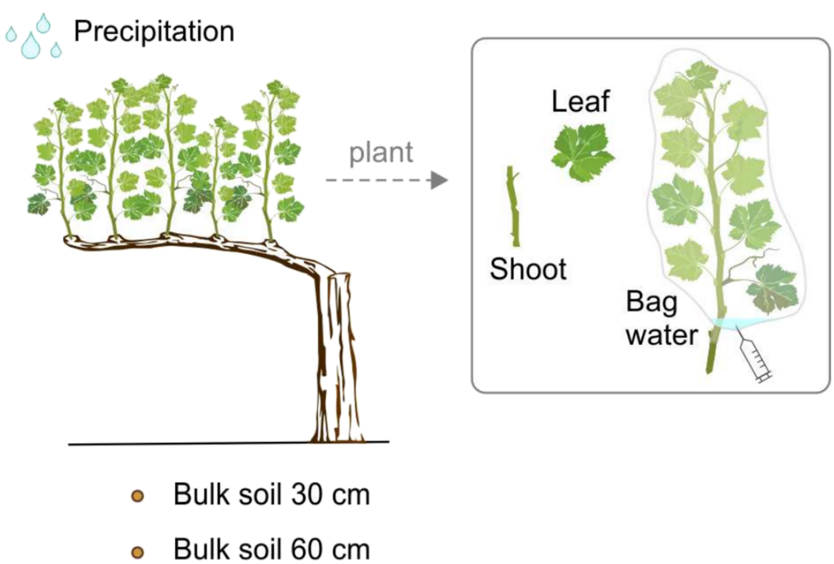

FIGURE 2 | Illustration of the water sample types used in this study: precipitation, bulk soil at 30- and 60-cm depth, leaves, shoot and water extracted from transpiring leaves using a plastic bag in the field.

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2.3 | Laboratory Analysis

Water from soil, leaves, and shoot samples was extracted through cryogenic vacuum distillation at the Isotope Laboratory of the Faculty of Agricultural, Environmental and Food Sciences of the Free University of Bozen-Bolzano. The recovery rate of the cryogenic line is > 99%. Water samples were analysed for their isotopic composition through cavity ring-down spectroscopy (Picarro Inc. L-2130i) at the Faculty of Agricultural, Environmental and Food Sciences of the Free University of Bozen-Bolzano, following the procedure by Penna et al. (2012) to minimize the memory effect. Xylem water samples that were flagged as contaminated by organic compounds were analysed by a Gas Bench coupled with an isotope-ratio mass spectrometer (IRMS Delta V, Thermo Fisher Scientific). The instrumental precision is 1‰ for δ^2H and 0.25‰ for δ^{18}O for laser spectroscopy and 5‰ and 0.2‰ for δ^2H and δ^{18}O, respectively, for the IRMS. The consistency between the laser spectroscopy and the IRMS measurements was assessed as in Penna et al. (2021). Due to a technical issue with the IRMS catalysers, some runs containing soil and xylem water samples were corrupted, potentially affecting the measurements. As a quality check, all the samples included in those runs were re-analysed at least twice, and we removed from the dataset those re-analysed samples that returned inconsistent values (i.e., differences among repeated analyses that were significantly larger than the instrument precision). Overall, we discarded 23 of the 254 collected samples. Stable isotope values are reported throughout the paper using the delta notation (δ) in per mil (‰) against the international reference standard Vienna Standard Mean Ocean Water. The line-conditioned excess (LC-excess) was calculated for each water sample following Landwehr and Coplen (2006):

$$\text{LC-excess}(‰) = \delta^2\text{H} - a \times \delta^{18}\text{O} - b \quad (1)$$

where a and b represent the slope and intercept of the local meteoric water line (LMWL). Water samples that experienced fractionation by evaporation have negative LC-excess values and plot below the LMWL in a dual-isotope plot (Landwehr, Coplen, and Stewart 2014).

2.4 | Computation of the Seasonal Water Origin

We used the Seasonal Origin Index (SOI) introduced by Allen et al. (2019) to evaluate whether a water sample was mainly comprised of winter versus summer precipitation. The SOI was developed specifically for the evaluation of the temporal origin of xylem and soil water at locations where 'typical' winter and summer values are sufficiently distinct and may be estimated through regional data interpolation (Bowen et al. 2019) without high-resolution isotope records. The SOI quantifies the isotopic deviation of a sample from the annual average precipitation and then scales that deviation by the amplitude of the seasonal isotope cycle, as follows:

$$\text{SOI} = \begin{cases} \dfrac{\delta_x - \delta_{ann_P}}{\delta_{sum_P} - \delta_{ann_P}}, & \text{if } \delta_x > \delta_{ann_P} \\ \dfrac{\delta_x - \delta_{ann_P}}{\delta_{ann_P} - \delta_{win_P}}, & \text{if } \delta_x < \delta_{ann_P} \end{cases}$$

where δ_x represents the fractionation-compensated δ^{18}O or δ^2H signature of the sample and δ_{win_P}, δ_{sum_P}, and δ_{ann_P} represent the isotopic signatures of winter, summer, and volume-weighted annual precipitation. The SOI will be near −1 for water samples derived entirely from winter precipitation and near 1 for samples derived entirely from summer precipitation. Samples with SOI values near zero approximate the annual average precipitation and can potentially represent many possible mixtures of waters from spring, summer, autumn, and winter (Allen et al. 2019). Importantly, the effects of evaporative fractionation, which may alter the sample composition, need to be compensated before computing the SOI. These procedures are summarized in Figure 3, which illustrates the SOI and the evaporative fractionation effects in the dual-isotope space.

We defined the typical winter and summer precipitation values by fitting a sine wave to the isotope composition of precipitation. We combined local measurements available over the period May 2021–April 2022 with reconstructed monthly compositions based on the Piso.AI tool (Nelson, Basler, and Kahmen 2021) over the period January 2020–December 2020. We computed the optimal sine wave parameters and their uncertainty using the DREAM algorithm (ter Braak and Vrugt 2008; Vrugt et al. 2009), which computes probability density functions (posterior distributions) for each parameter. We defined the summer and winter source as in Allen et al. (2019) and characterized the uncertainty as plus or minus the standard deviation of the amplitude parameter. Since the climate is Mediterranean, the mean annual composition was computed by weighting the isotope composition by precipitation amounts. For the mean annual precipitation, the uncertainty was characterized as plus or minus the standard deviation of the offset parameter. Because of the lower rainfall amounts in the summer season, the mean annual composition is closer to the winter composition.

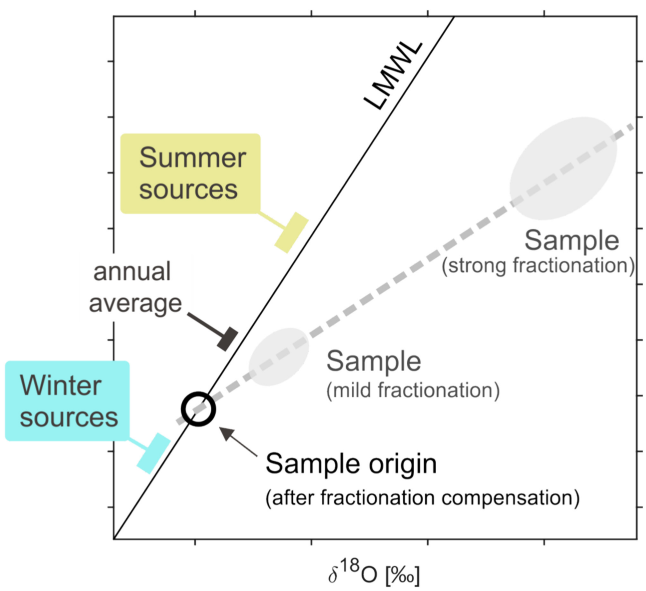

FIGURE 3 | Illustration of the concept of the Seasonal Origin Index (SOI, Allen et al. 2019) in the presence of fractionated samples. The SOI approach is based on comparing the sample origin with the typical isotope values of winter and summer sources. However, when isotope values show evidence of isotope fractionation, their origin is not readily available and needs to be estimated by compensating for isotope fractionation.

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To compensate the samples for isotope fractionation, we followed the probabilistic approach by Bowen et al. (2018) as implemented in Benettin et al. (2021). In this approach, the samples' estimated origin is defined by a probability distribution, which is typically broader (i.e., more uncertain) for samples that are more fractionated. In the paper, we summarize this distribution by reporting its mean and ± 1 standard deviation. Note that, after fractionation has been compensated for, the use of both ^{18}O and ^{2}H is redundant because their values are almost perfectly correlated. Thus, the SOI analyses are reported for ^{18}O only.

3 | Results

3.1 | Hydrometeorologic Data

Hydrometeorological and soil moisture dynamics over the monitoring period were consistent with the Mediterranean character of the region. The summer period June–August 2021 received less than 20 mm of rainfall, with temperatures often over 30°C and relative humidity lower than 50% during the day. The volumetric water content (VWC) was insensitive to summer rainfall events and kept on decreasing until the arrival of fall precipitation in October and November (Figure 4). VWC at 30 cm started to increase between October and November 2021, while VWC at 60 cm increased mainly in November and showed a clear delay of 2 weeks between the top and the bottom of the hillslope.

3.2 | Isotope Data

All the isotope data collected in the field are shown in Figure 5. Precipitation samples aligned well along the Local Meteoric Water Line (LMWL) $\delta^2\text{H} = 7.14 \times \delta^{18}\text{O} + 5.82$, determined from historical data (Natali et al. 2021) collected at the stations of Florence and Siena (15 and 40 km away from the field site, respectively). The sampled rainfall isotope composition ranged between −7.5‰, −1.3‰ in δ^{18}O and −50‰, −4‰ in δ^2H. Soil samples at 30-cm and 60-cm depth showed evidence of evaporative fractionation as they consistently plotted below the LMWL

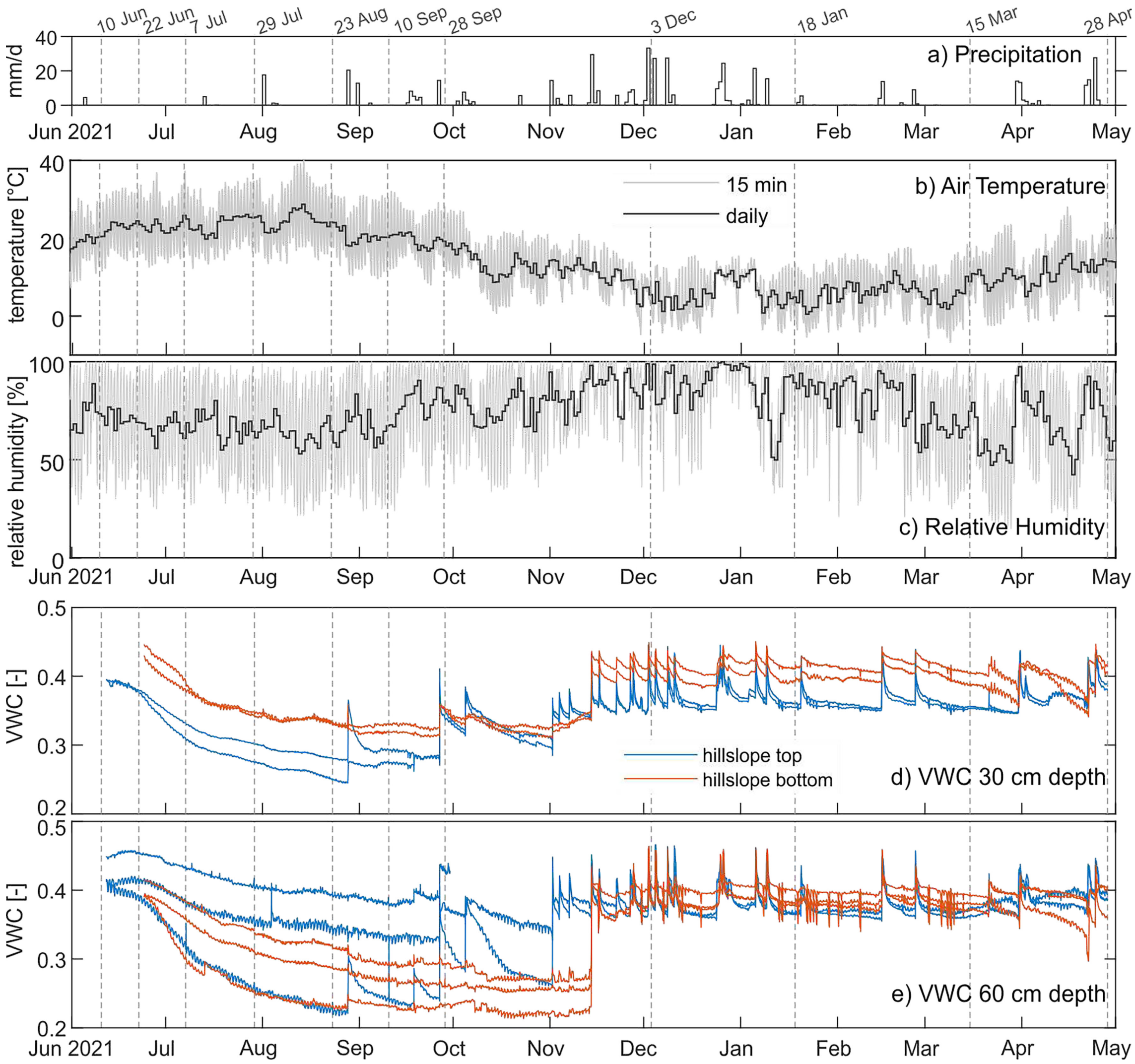

FIGURE 4 | Hydrometeorological conditions during the monitoring period, including data from the regional weather station in Montespertoli (panels a–c) and volumetric water content (VWC) measured at the top and the bottom of the experimental vineyard (panels d–e). The vertical dashed lines mark the 11 isotope sampling campaigns.

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with lc-excess values (Figure 5C) in the range (−12‰, −4‰). Plant samples (shoot, bag water, leaves) occupied distinct portions of the dual-isotope space, reflecting their different degrees of isotope fractionation. Shoot samples tended to overlap with soil samples and to have similar isotope values and lc-excess values. Leaf samples, as expected, plotted far away from the LMWL, with lc-excess values in the range (−70‰, −40‰) and an average isotope difference compared to the shoot samples of 20‰ in δ^{18}O and 50‰ in δ^2H. Bag water samples were in between shoot and leaf samples, in terms of both isotope values and lc-excess.

While Figure 5 shows all isotope data collected over the entire field campaign, Figure 6 groups the soil and shoot isotope data by sampling period, to assess the temporal variability in their isotope composition. All grouping periods (early summer, midsummer, early fall, and winter) were characterized by marked variability across samples, reflecting the spatial heterogeneity in soil and shoot isotope composition. The mean summer, winter, and annual isotope compositions (see also Figure 7) are provided as a reference. Despite the variability across samples, there was a clear shift in the direction of summer rain sources during the summer until early fall and a shift back to winter rain sources in the winter. However, these trends must be taken qualitatively because most samples were fractionated and the seasonal origin cannot be evaluated without compensating for fractionation.

Statistical tests were performed to assess the possible difference or similarity between the isotopic composition of soil samples taken at different depths and xylem water samples extracted from grapevines with a different rootstock. For the comparison, samples taken at the same time were selected, data from the two vineyard locations (upper and lower plot) were pooled together, and the test was performed for δ^2H, δ^{18}O, and lc-excess values. For both soil and xylem samples, the normality of the sample distribution was assessed by a Shapiro-Wilk test. In the case of normally distributed samples, we performed the parametric *t*-test to compare the means, while for non-normally distributed samples, we performed a non-parametric Mann-Whitney test to compare the medians. The isotopic composition of the soil samples at the two investigated depths was always significantly different. On the contrary, the isotopic composition of shoot water from the 1103 and 420A rootstocks was always not significantly different (Table 1).

3.3 | Seasonal Water Origin

The typical summer, winter, and mean annual isotope values (δ^{18}O) in precipitation estimated for the Montepaldi site are illustrated in Figure 7, along with measured and reconstructed precipitation values and the fitted sine wave. The highest and lowest seasonal values occurred in July and January,

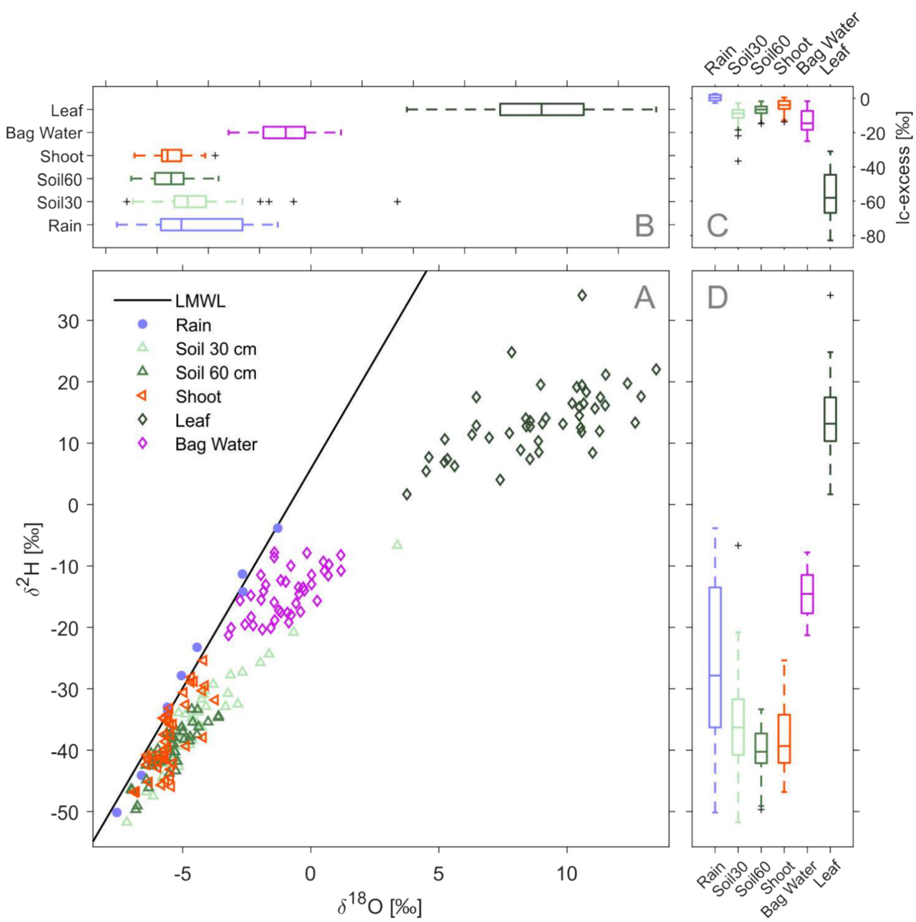

FIGURE 5 | All isotope data collected during the sampling season 2021–2022 at the Montepaldi farm. (A) Isotope values in dual-isotope plot; (B, C) boxplots of single isotope values; (C) boxplot of lc-excess values, indicating the degree of evaporative fractionation.

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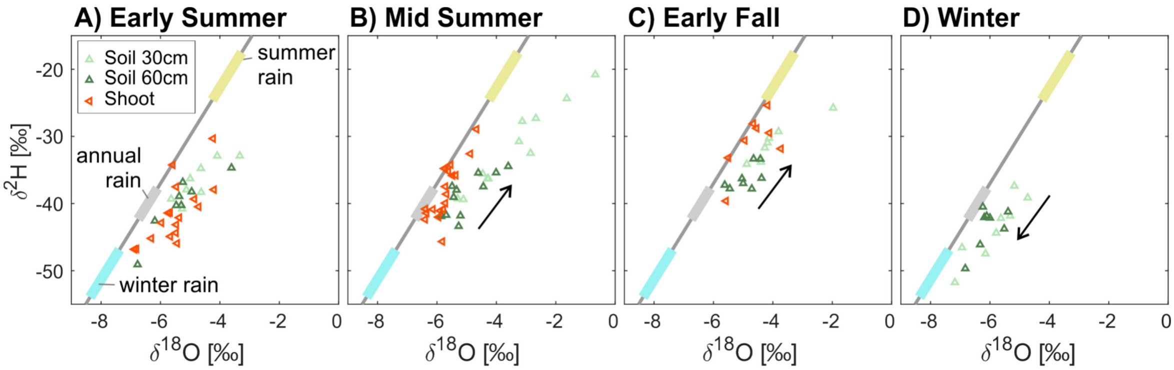

FIGURE 6 | Soil and shoot isotope data grouped by sampling period. Despite the marked variability across samples, the cloud of points tends to move consistently towards the summer sources during and right after the summer and back towards winter sources during the winter. Shoot samples are not available after the early fall when the vines are dormant. The periods are as follows: Early summer, with the three campaigns between June 10 and July 7; Mid summer with the three campaigns until September 10; Early fall with the two campaigns until December 3; Winter with the three campaigns in January, March, and April 2022.

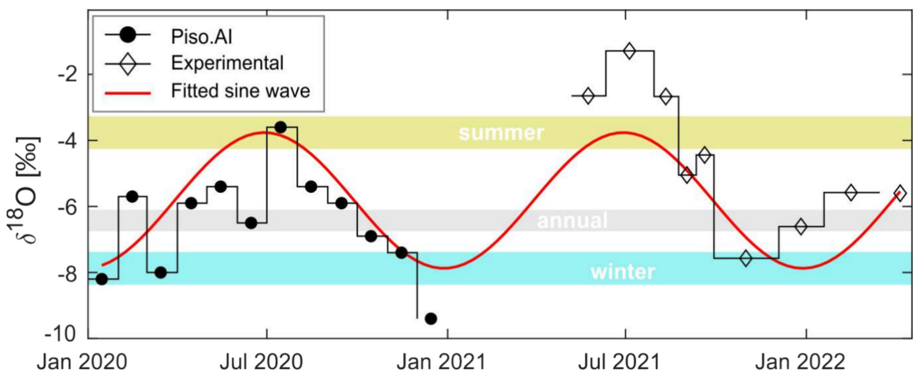

FIGURE 7 | Typical summer, winter, and mean (precipitation-weighted) annual δ^{18}O values in precipitation at the Montepaldi site. Values are based on fitting a sine wave over a 30-month period where isotope data are available either from field measurement or from Piso.AI reconstructed values. Since there is uncertainty in the sine wave parameters, summer/winter/annual values are shown as bands that represent the mean plus or minus one standard deviation.

TABLE 1 | *p*-values deriving from the *t*-test or the Mann-Whitney test to compare the isotopic composition and the lc-excess values of either soil samples taken at the 30- and 60-cm depth, or of shoot samples taken from Sangiovese vines grafted on rootstocks 1103 and 420A.

	n	δ^2H	δ^{18}O	lc-excess
Soil 30 cm vs. 60 cm	42	< 0.01	< 0.01	< 0.01
Shoot rootstock 1103 vs. 420A	20	0.219	0.125	0.839

Note: Significant values at the 99% confidence level ($p < 0.01$) are reported for soil water; that is, the difference in the median or mean values between soil water at 30 and 60 cm is greater than would be expected by chance. On the contrary, non-significant values are reported for shoot water; that is, the difference in the median or mean values of shoot water from rootstocks 1103 and 420A might be due to random sampling variability.

respectively. Because of the Mediterranean climate, the mean annual precipitation-weighted values were closer to winter than summer sources. Summer values were − 3.76 ± 0.50‰ (the maximum is reached in July), winter values were − 7.88 ± 0.50‰ and the mean flow-weighted annual values were − 6.43 ± 0.32‰. The distance of about 4‰ in δ^{18}O between summer and winter sources is much larger than their uncertainty and allows for the application of SOI.

Figure 8 shows the SOI of all soil and shoot samples, grouped by sampling date. Each individual value in the plot represents a sample's estimated origin (triangular marker in the plot) with an error band indicating its uncertainty (see Section 2.4). Higher uncertainties corresponded to samples that were more fractionated. Because of the large variability across samples collected during the same day and to help better see trends, we reported the mean SOI across all samples collected on the same day (larger dots in the plot). The estimated SOI values revealed a general dominance of winter rainfall sources (values closer to −1 than to +1) across our samples, as well as a seasonal dynamic with water origins moving closer to summer values late in the growing season. The winter water dominance was not an artefact of the Mediterranean climate, because the rainfall amounts have already been accounted for in the determination of the SOI. The sources of shallow soil water were more dynamic than those of deeper soil water and the sources of shoot water appeared to be even more dynamic, with both the highest and lowest SOI values during the growing season.

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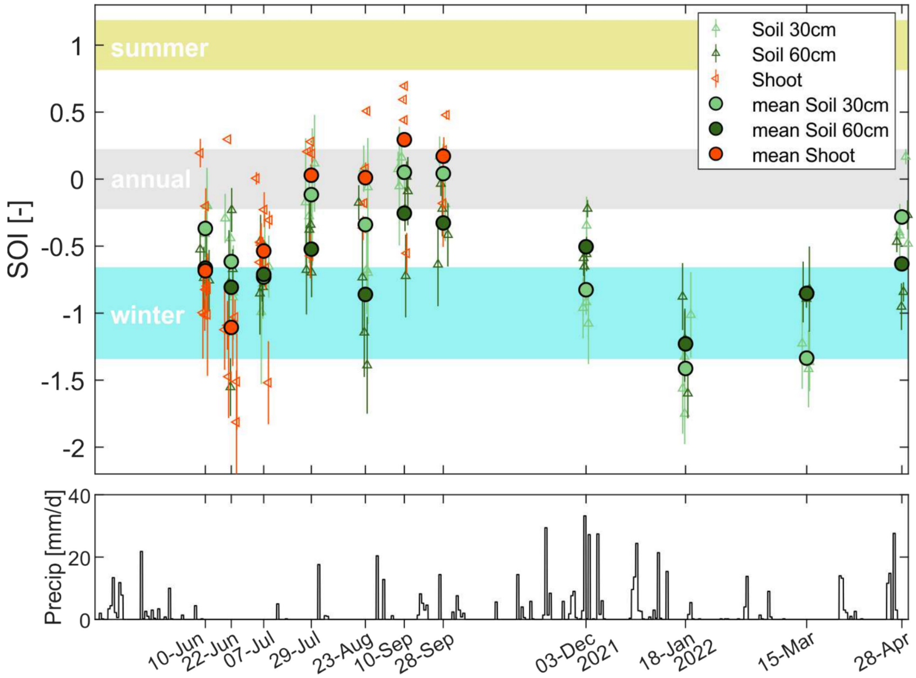

FIGURE 8 | Evolution of the seasonal water origin during the sampling campaign. The SOI is reported for each sample of shoot, shallow, and deep soil along with its uncertainty. The dots indicate the mean SOI value during a sampling day. The bands in the background indicate the typical summer/annual/winter rainfall SOI values, with their uncertainty.

TABLE 2 | *p*-values deriving from the *t*-test applied to compare the isotopic composition of the source of soil water at 30 and 60 cm and the source of shoot water from rootstocks 1103 and 420A.

	n	δ^2H	δ^{18}O
Soil water source 30 cm vs. 60 cm	42	0.116	0.108
Shoot water source rootstock 1103 vs. 420A	19	0.409	0.408

Note: All values are not significant at the 99% confidence level ($p > 0.01$); that is, the difference in the mean values between sources of soil water at 30 and 60 cm and sources of shoot water from rootstocks 1103 and 420A might be due to random sampling variability.

The *t*-test was applied to assess the possible difference in the mean isotopic composition of the sources of soil and shoot samples (i.e., de-fractionated samples). In contrast to what was observed for the original soil samples (Section 3.2, Table 1), the isotopic composition of the sources of soil water was not statistically different (Table 2), indicating that sample compositions were different because they experienced different rates of evaporation, and not because they came from different sources. Similarly, the difference in the mean values between sources of shoot water from the two rootstocks was not significant (Table 2).

In addition, when the statistical tests were performed separately for the dry (June 10–September 28) and wet (December 3–April 28) periods, we observed significant differences in soil water sources at 30 and 60 cm during the dry period only (Table 3).

4 | Discussion

4.1 | Soil and Plant Seasonal Water Origin

This research was driven by the necessity to better understand how grapevines change their temporal water sources (i.e., the relative contribution of winter vs. summer precipitation) over the growing period. The aim was to advance our knowledge on how different plants adopt specific water-use strategies to respond to changes in meteorological forcing (Rodriguez-Iturbe 2000) and to provide still missing practical information, useful to farmers, on grapevines' resilience to periods of water scarcity. At the same time, we adopted an alternative and minimally invasive method (the so-called 'bag method') to sample plant water from grapevines to test its potential to identify and quantify plant water sources and reduce the uncertainty in plant water source apportioning studies (Penna et al. 2018; Beyer and Penna 2021). As such, this is the first isotope-based study that investigates soil and plant water sources in a vineyard environment.

Our first two research questions (Section 1) focused on (i) the assessment of the relative contribution of winter and summer precipitation in recharging soil water in the study vineyard and (ii) the identification of the possible change in the temporal water origin accessed by grapevines over the monitored season. Based on the knowledge of the characteristic seasonality of the Mediterranean climate and the plasticity of the root system of grapevines, at the beginning of this research, we hypothesized that (i) the contribution of winter precipitation to soil water would be dominant, reflecting the larger precipitation amounts

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TABLE 3 | *p*-values deriving from the *t*-test applied to compare the isotopic composition of the source of soil water at 30 and 60 cm separately for samples collected in the wet and in the dry period of the monitoring year.

	n	δ^2H	δ^{18}O
Soil water source 30 cm vs. 60 cm in the dry period	27	< 0.01	< 0.01
Soil water source 30 cm vs. 60 cm in the wet period	15	0.575	0.578

Note: Significantly different values at the 99% confidence level ($p < 0.01$) are reported during the dry period, whereas the difference in the isotopic composition of samples collected during the wet period is not significant.

in winter compared to summer, and that (ii) water sources for grapevines changed over the growing season as a function of soil water availability, becoming deeper and older during particularly dry periods.

The marked seasonality in meteorological forcing typical of Mediterranean climates was reflected in soil moisture dynamics, with a clear shift between dry conditions during late summer and early fall and wet conditions during the remaining months (Figure 4). Interestingly, VWC at 30 cm was overall consistently higher at the bottom of the hillslope compared to the top plot for the entire monitoring year, but VWC at 60 cm was generally lower at the hillslope bottom compared to the hillslope top during the dry period, whereas similar VWC was observed in the two plots during the wet period (Figure 4). This suggests similar infiltration and redistribution dynamics over the deeper soil profile, irrespective of the hillslope position. This finding is in agreement with observations from another agricultural hillslope in Colorado, USA, where soil properties were relatively similar across the hillslope (Green and Erskine 2011), but disagrees with the results of a recent study conducted in a cropped hillslope in China reporting different infiltration properties at different hillslope positions as a function of soil structure (Zhu et al. 2023). These comparative observations suggest that soil properties are more important controls on infiltration and redistribution dynamics than topographic properties (e.g., slope) in agricultural sites.

Isotope data in the dual-isotope space revealed a large variability across samples from different waters (Figure 5). As expected, rain samples plotted over the LMWL, and overall, soil samples slightly deviated from it, with a larger degree of deviation and more negative lc-excess for shallow soil water samples, clearly indicating evaporative fractionation that influenced more strongly the surface soil layer, as observed at other agricultural sites (Penna et al. 2021; Liu et al. 2021; Liu, Jia, and Yu 2020; Wu et al. 2018). The isotope evaporative fractionation caused statistically different isotope values between soil samples at 30- and 60-cm depth (Table 1). Indeed, when the effect of evaporative fractionation was removed, the composition of both isotopes at the two depths was not statistically different anymore(Table 2). However, it is interesting to note that when analysing the isotopic composition of the sources of soil water at 30 and 60 cm separately for the dry and wet periods, the difference in the isotope signature at the two depths was statistically significant only during the dry period (Table 3). This suggests that seasonality plays an important role in the soil water recharging processes at different depths in this Mediterranean agricultural site, as observed in other contexts with Mediterranean climates (Del Toro-Guerrero et al. 2018; Ries et al. 2015; Viola et al. 2008).

On the contrary, the relative vicinity of shoot water samples to the LMWL and the lack of statistical difference in their isotopic composition between 1103 and 420A rootstocks (Table 1), even when considering the compensation for evaporative fractionation (Table 2), indicate that rootstock was not a strong controlling factor on the soil depth of root water uptake by the two grapevines, at least in this vineyard, despite the potential capability of these two rootstocks to access water from different soil depths (Swanepoel and Southey 1989). This lack of distinct behaviour might suggest that, despite the dry summer climate, enough water was generally available within the soil profile without the necessity for the 1103 rootstock (which is capable of rooting deeper) to explore/use deeper water.

Overall, the isotopic variability of shoot samples was comprised within the variability of soil water samples (see boxplots in Figure 5) indicating that soil water in the 30- to 60-cm depth profile was the main water source for grapevines during the monitoring period. However, it must be noted that not all shoot samples perfectly fell in the mixing space defined by the samples of soil water at 30 and 60 cm (Figure 6). This behaviour suggests that our experimental design, which did not sample the entire soil profile, might have simply missed capturing the whole range of possible soil water sources and that additional water sources (e.g., soil water shallower than 30 cm, between 30 and 60 cm, or deeper than 60 cm) could be accessed by grapevines.

Despite the marked variability among samples from different water types and in space, our data revealed quite clear temporal trends as the isotopic composition of soil and shoot samples moved consistently across the sampling campaigns, shifting towards the signal of summer rain first and winter rain later in the season (Figures 6 and 9). This behaviour was confirmed and quantified by calculating the SOI that was characterized by a marked variability over the monitoring period but that revealed a dominant winter source contribution for shoot water and soil water at both depths, although the contribution of summer sources to plant water was occasionally high in late summer (Figure 8). The most negative SOI values in soil samples (indicating the dominance of winter sources) were observed during the winter field campaigns when the VWC was highest reflecting higher precipitation depths (Figure 4). Interestingly, the mean SOI in the soil at 30 cm was consistently higher than that of soil at 60 cm during the summer but this trend reversed during the sampling campaigns in December, January, and March (Figure 8). Since the SOI was weighed by precipitation amounts, it was not biased by the seasonal differences in precipitation depths typical of Mediterranean climates. Therefore, SOI in soil samples suggests that soil water was disproportionately composed

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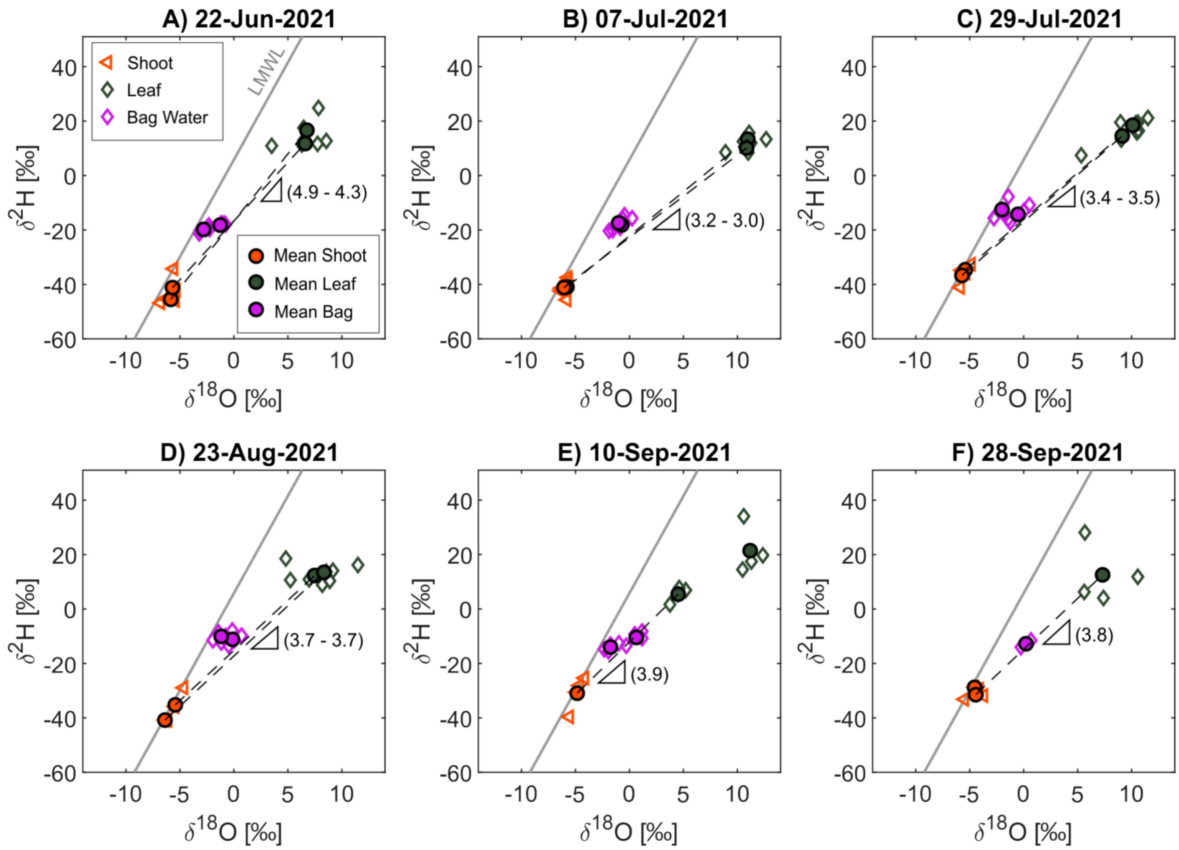

FIGURE 9 | Isotope composition of plant samples organized by sampling day. The dots indicate the average value across different plants at the top or at the bottom of the hillslope. The trajectory that connects the mean shoot and leaf samples can be interpreted as the leaves' empirical evaporation lines (the slopes are reported in the plot and indicated by triangles). Plots E) and F) only feature one evaporation line because either the shoot or the leaf isotope analysis failed at one of the locations.

of water that fell during the winter and that remained in the soil for months, validating our first hypothesis. This finding is in contrast with observations conducted in an experimental site in central-southern China, characterized by soil with a not-so-different texture compared to the Montepaldi soil (silt-dominated vs. clay-dominated, respectively) but different climate (humid subtropical monsoon climate with higher precipitation in the Chinese site) where soil water down to 130 cm mainly originated from summer precipitation, and soil moisture was mostly replenished during summer but only in the shallow layers (Luo et al. 2023). Our results are, however, in greater agreement with those from Allen et al. (2019) who showed a dominance of winter sources, especially below 30 cm, in forest soils across Switzerland.

The SOI pattern shown by xylem water was not entirely consistent with that of soil water (Figure 8). Indeed, except for the first two sampling occasions when xylem water likely reflected a 'memory' of winter sources (Allen et al. 2019; Brinkmann et al. 2018; Fabiani, Klaus, and Penna 2024), overall, the SOI of shoot samples was always higher than that of soil samples, suggesting that grapevines took up water deriving from comparable proportions of winter and summer sources. These patterns, in agreement with the little fractionation observed in shoot samples (Figure 5), suggest that grapevines in the study area were able to benefit from recent rainfall events that occurred during the summer, although such events only slightly increased soil moisture at 30-cm depth. This behaviour was recently documented for forest trees (Bailey et al. 2023; Berkelhammer et al. 2020), characterized by different root depths and water use strategies compared to grapevines. Although we do not have a sufficiently large dataset to make conclusive statements, these observations highlight the capability of these grapevines to transpire shallow water from recent rain events, when available. Therefore, this finding only partially agrees with our second hypothesis about the seasonal switch from shallow to deeper and older water sources during dry periods, as grapevines were shown to be able to rely on shallower and more recent soil water provided by occasional summer rainfall events.

4.2 | Evaluation of 'Alternative' Plant Water Samples

Our plant sampling campaign featured the collection of three types of 'alternative' and minimally invasive sample types: shoots, leaves, and bag water (Figures 2 and 5). Shoot water samples, although coming from fresh and still green tissue, seemed to be a good estimator of xylem water because they were not more fractionated than soil water samples. The collection of shoot samples is partly invasive but to avoid damage to the

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grapes, samples can be collected from secondary shoots that do not bear fruits (there are often 4–5 per plant). Leaf samples, as expected, were extremely fractionated because evaporative fractionation occurs within leaf stomata (Farquhar, Cernusak, and Barnes 2007; Cernusak et al. 2022; Liu et al. 2023; Zhan et al. 2019). Work by Benettin et al. (2021) based on isotope data from a willow tree showed that leaf isotope composition can, in some circumstances, be used to reconstruct the seasonal origin of plant water. To do so, one needs to dispose of additional meteorologic data like air temperature and humidity, as well as an estimate of the isotope composition of the atmosphere during the sampling day. We could not reliably estimate the isotope composition of the atmosphere at our site and, therefore, we cannot evaluate whether leaf water samples alone could be used to retrieve the grapevine's water origin. Yet, these samples are useful to investigate isotopic enrichment dynamics from xylem to leaves in Mediterranean grapevines and to interpret bag water isotope composition.

The isotope composition of bag water was in between the compositions of shoot and leaf water (Figure 5). The bag water approach was proposed by Kulmatiski and Forero (2021) and tested within the framework of a tracer experiment with highly deuterated water. The authors showed that for the labelled water there was a good correlation between the isotope composition of bag water and xylem water, while at natural abundance there was no correlation. Moreover, and contrary to our results, bag water samples were not more enriched than the xylem samples. The only other study that investigated the use of bag water at natural abundance is an unpublished preprint (Menchaca et al. 2007). Therein, the authors reported that bag water samples collected over periods of increasing length progressively lost the fractionation signal to become closer to the expected source water. While these previous studies provide the basis to apply the bag method, they do not offer a theoretical framework to interpret the isotope composition of bag water and which processes it should reflect.

Our starting hypothesis is that the bag water represents mixing between (fractionated) leaf water included within the leaf at the time of bagging and (unfractionated) xylem water flowing towards the leaf after the bag has been closed. Indeed, once the bag is sealed, it forms a closed system where equilibrium conditions are expected to form quickly, especially in the case of a highly transpiring plant like grapevine. We must note that some ambient vapour was inevitably trapped inside the bag at the time of bagging, but such a volume is small compared to the amount of extracted bag water. Indeed, considering that 1 m^3 of atmosphere at 25°C temperature and at relative humidity as high as 80% only contains 16 g of water (see, e.g., a psychrometric chart), our 6-L bags could not include more than 0.1 mL of atmospheric vapor, which is a very small volume (3%–5%) compared to the 2–3 mL of water that were usually sampled out of the bags. According to our closed-system framework, the first water to transpire and condense inside the bag is the initial (fractionated) leaf water, which is progressively replaced by the (unfractionated) xylem water that transpires under equilibrium conditions. Therefore, bag water should lie between these two end members, and longer bag application times should result in bag water values closer to xylem values.

To explore whether the bag water can be interpreted as the simple mixing between xylem and leaf water, we show the isotope composition of our plant samples organized by sampling day (Figure 9). We also computed the average shoot and leaf isotope composition across the four different plants at the top or the bottom of the hillslope. The trajectory that connects such average values can be interpreted as the empirical evaporation line (Flanagan and Ehleringer 1991) from shoot to leaf water on a given sampling day. In all our sampling days, the evaporation lines were very similar between the top and the bottom of the hillslope, with slopes higher than 4 in the first sampling day and otherwise within the range 3–3.9.

After displaying the data by sampling day and comparing the bag water composition against the empirical evaporation line, we realized that our initial hypothesis was partly unattended: while bag water composition lay almost mid-way between shoot and leaf compositions, it was also systematically higher than the evaporation lines. This implies that the line connecting leaf water and bag water was not an evaporation line (it was flatter), and using it to infer the xylem source would result in a biased estimate (overestimation of summer rainfall contribution).

The reason why the bag water isotope composition did not lie on the evaporation line is currently unexplained and will require further investigation. A possible explanation could be mixing with an additional end-member that ‘pulls’ the bag water composition towards the LMWL. Potential factors could be the atmospheric vapour trapped within the bag and dew that was possibly attached to the leaves at the time of bagging. Both these members would move the bag water composition in the right direction since they both lay on the LMWL around the typical summer rainfall composition. However, such additional end member would need to contribute at least 20% in volume to explain the departure of the bag water from the evaporation line (such a value is obtained from an elementary isotopic mass balance between leaf water, xylem water, and the hypothetical third end member). Instead, the trapped atmospheric vapour cannot account for more than 5% of the sample volume (see computations above), and it is unlikely that enough dew had formed systematically at every sampling campaign, especially considering the dry weather during a Mediterranean summer. Alternative explanations for the observed bag water offset may be sought in non-equilibrium and non-isothermal fractionation effects that may occur inside the bag right after its application. However, exploring this option would require additional experiments that monitor temperature and humidity inside the bag and the development of an appropriate theoretical framework, which are beyond the scope of this work and are left for future research.

As noted by Kulmatiski and Forero (2021), the bag water approach is not comparable to more rigorous approaches that involve leaf chambers (Kübert et al. 2022), yet it offers a simple and especially affordable way to extract a sample of about 2 mL (at least in our case) directly in the field. We believe it is worthwhile to continue exploring the use of bag water samples from transpiring grapevines because a better understanding of their isotope signature could be used to reconstruct the plant water sources or at least their seasonal origin.

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4.3 | Limitations and Ways Forward

A better understanding of water flux exchanges, temporal variability of water sources, and crop water use strategies in agricultural systems is becoming more and more critical under the current pressing drought conditions, in Europe and worldwide. In this context, this work represents the first attempt to analyse the seasonal origin of water taken up by grapevines and assess its temporal evolution. Overall, our results confirm that the stable isotope technique is an ubiquitous tool that provides useful insights in vegetated ecosystems and agroecosystems. However, we point out some limitations of this research that should be addressed in future isotope-based studies in vineyards.

Although we carried out isotope sampling regularly throughout the year, and with a higher frequency during the growing season, a more systematic and high-resolution sampling in time (e.g., every week) and space (e.g., in different locations of the field and across different soil depths) would be ideal to better capture finer temporal dynamics in water uptake and potential spatial differences in the isotopic composition of water. Particularly important is soil sampling at depths greater than 60 cm to assess the possible water uptake by grapevines from deeper soil water. Closely related to this point is the advocated benefit of acquiring information on root density and root architecture as it can provide supporting evidence for changing depths and spatial coverage of water uptake by the grapevines (Delrot et al. 2010) to be coupled with isotope-based observations. Furthermore, we see the availability of the isotopic composition of precipitation in the months before the field experiment as important ancillary data to characterize the input isotope signature of the soil. In this study, we were not able to perform preliminary precipitation sampling, but the isotope composition reconstruction we carried out based on existing local databases was reasonable and acceptable. Moreover, our results did not prove different behaviours in water uptake by grapevines with different rootstock perhaps also due to the relatively limited spatio-temporal sampling of plant water. Future isotope-based studies more specifically focused on comparative behaviours are needed to assess the plasticity and adaptation capabilities of different rootstocks to variable soil moisture availability.

Here, we evaluated source water dynamics by computing the SOI (Allen et al. 2019), which is a simple and data-based approach. Our results show that the SOI of soil and shoot samples was dampened and shifted with respect to the seasonality of precipitation (the peak in precipitation isotope signal was in July, while the peak in SOI was in September). This transformation of the input isotope signal was caused by transport and mixing processes occurring within the soil. The application of numerical models to assess water transport and age dynamics in soils (e.g., Smith et al. 2020; Asadollahi et al. 2020; Maxwell et al. 2018; Schwemmle and Weiler 2024) could be the next step to interpret the time lags between precipitation and both soil and shoot isotope compositions we observed in our study, and to provide a more quantitative evaluation of water residence times in the subsurface.

5 | Conclusions

In this work, we applied the stable isotope technique in a Mediterranean vineyard to trace, for the first time, the temporal origin of water sources accessed by rain-fed grapevines. We sampled precipitation, soil water at two depths, shoot water, leaf water, and transpired water through the bag method. In summary, our study led to the following conclusions:

- As expected for a Mediterranean climate, there was a marked seasonality in the meteorological forcing that reflected in large seasonal variability in VWC in the monitoring plots.
- The isotopic composition of fractionation-corrected soil water samples collected at 30- and 60-cm depth was statistically significant only during the dry period, stressing the role of seasonality in recharging soil at different depths.
- On the contrary, there was no difference in the isotopic composition of shoot water taken from the two different rootstocks, suggesting that enough water was available during the growing period and the rooting systems did not need to access different (e.g., deeper) water sources.
- The SOI in soil water markedly varied over the monitoring period but was dominantly characterized by winter source contributions. The SOI of shoot samples was always higher than that of soil samples, suggesting that grapevines were able to also take up shallow soil water derived at least partly from very recent summer rainfall events. Further studies are required to better understand the extent to which such erratic summer rain events help the grapevines to cope with increasingly frequent summer heat waves.
- The isotopic composition of bag water was intermediate between that of shoot and leaf water but it did not seem to be representative of simple mixing between leaf water and xylem water. Our preliminary results need to be validated by other experiments and theoretical frameworks aimed at explaining the enrichment patterns observed here.

The findings from this work represent the first isotope- and field-based evidence on the plasticity of grapevines to take up water from different temporal sources in Mediterranean climates. These results can be helpful for the practical evaluation of the resilient behaviour of grapevines in accessing different water sources, also considering different rootstocks. At the same time, our work offers new insight into the capability of isotopes in ecohydrological studies to trace the seasonal origin of plant water in economically valuable agroecosystems, and it paves the way for future analysis of alternative, non-invasive plant water sampling methods.

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information. The results of this study were discussed within the COST Action: 'WATer isotopeS in the critical zONe: from groundwater recharge to plant transpiration - WATSON' CA19120. Open access publishing facilitated by Universita degli Studi di Firenze, as part of the Wiley - CRUI-CARE agreement.

Data Availability Statement

The data associated with this paper can be found in https://doi.org/10.5281/zenodo.12708488 under Creative Commons Attribution 4.0 International Public License. The meteorological data from the Montespertoli weather station are available from the portal of the Tuscany hydrologic and geologic service (Settore Idrologico e Geologico Regionale Toscana) under the section 'Dati/Archivio storico'.

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