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RESEARCH ARTICLE

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Water uptake of apple trees in the Alps: Where does irrigation water go?

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Abstract

Understanding root water uptake sources in agricultural systems is becoming increasingly important in the sustainable management of water resources under changing climatic conditions. In this work, a stable isotope approach was adopted to investigate water sources accessed by apple trees in two orchards growing in two different locations in the upper Etsch/Adige valley (Eastern Italian Alps). We tested the general hypothesis that soil water, composed of a mixture of rain and irrigation water, was the main source for tree transpiration in both fields, but trees could also access groundwater according to the different proximity to the groundwater table of the two orchards. Our results revealed that apple trees during the 2015 and 2016 growing seasons relied mostly on soil water present in the upper 20–40 cm of soils, with an apparently negligible contribution of groundwater, irrespective of the field location in the valley bottom. The isotopic composition of xylem water did not reflect irrigation water composition (or that of groundwater) but rather of rainfall and throughfall, and soil water. We related this behaviour to the intense rate of soil evaporation during the growing period that modified the original isotopic signature of irrigation water in the shallower layers, masking its actual contribution. This work contributes to improving the understanding of water uptake strategies in Alpine apple orchards and paves the way for further analysis on the proportion of irrigation and rainwater used by apple trees in mountain agroecosystems.

KEYWORDS
apple trees, irrigation, isotopes, root water uptake, soil water evaporation

1 | INTRODUCTION

Agriculture is globally characterised by having the highest rates of water consumption (FAO, 2011). Current and future changes in climate forcing, in terms of alterations to precipitation inputs and temperature regimes, pose increasing pressure on water management in agricultural systems, requiring more sustainable strategies of water use. Obtaining a detailed understanding on the future of precipitation and irrigation water in agroecosystems, and on the water sources exploited by crops is a key factor in implementing efficient and sustainable water resource management strategies while simultaneously optimising crop yield and quality.

The stable isotope ratios of hydrogen and oxygen ($^{2}H/^{1}H$ and $^{18}O/^{16}O$) in the water molecule can be used as ecohydrological tracers and are among the most powerful tools available to researchers for investigating water source dynamics in vegetated systems (Beyer et al., 2020; Kübert et al., 2020; Penna et al., 2018; Sprenger et al., 2016). Isotope-based studies are typically applied in forested environments but are being increasingly used in agricultural systems to estimate the proportion of water from different sources (e.g., soil water from different depths, groundwater) accessed and transpired by crops (Penna et al., 2020). The stable isotope approach has been especially utilised in maize and wheat (e.g., Liu et al., 2021; Ma & Song, 2016, 2019; Wu et al., 2018). Studies that focus on water sources exploited by trees in orchards are much rarer, and mostly limited to cherry trees (e.g., Cao et al., 2018; Li et al., 2020; Li, Tan, et al., 2019), walnut trees (e.g., Lauteri et al., 2005; Liu et al., 2019; Sun et al., 2011), and apple trees. For the latter, we are aware of five published studies, all conducted in China's Loess Plateau. Wang et al. (2018) applied an isotope-based mixing model to 10-, 15-, and 22-year-old apple trees in the hilly Loess Plateau to investigate the contribution and the seasonal variation of different water sources for plant growth. They found a large variability in the shallow, medium, and deep soil water sources exploited by the trees of different ages, during different stages of the growing period, with a tendency of older trees to mainly use shallow and middle-depth soil water (Wang et al., 2018). In a recent follow-up study in the same region, Wang et al. (2020) applied the stable isotope technique combined with a modelling approach to compare water uptake patterns of different aged apple trees in semi-arid and semi-humid zones in the study area. Their results showed that the soil water content decreased with increasing tree age due to increased absorption of deep water (400–500 cm) by old trees compared to young ones, particularly in the semi-arid zone. They conclude by suggesting that old, deep rooted apple trees could excessively consume deep soil water, severely affecting the sustainability of apple production in the study region (Wang et al., 2020). Similarly, Zheng et al. (2018) quantified root water uptake of 5-year-old apple trees using isotope-based mixing models and a numerical model with the aim of improving water use efficiency under limited irrigation conditions. Their analysis revealed contrasting results compared to those of Wang et al. (2020, 2018). They showed that the principal depth of root water uptake was in the 0–60 cm range, with the main contribution being within 0–40 cm, giving an indication towards reducing surface irrigation depth in order to improve water use efficiency (Zheng et al., 2018). Building on these results, the same research group estimated the soil layer where apple tree roots absorbed most of the soil water: 0–40 cm when trees were irrigated by traditional surface irrigation and 20–100 cm when the water storage pit irrigation system was deployed (Zheng et al., 2019). Finally, Liu et al. (2020) applied the isotope method to assess water sources of apple trees intercropped with maize in an agroforestry ecosystem. They showed that the layer with a depth of 60–100 cm was the main water source of the apple trees, but that they also absorbed water from depths of 40–60 cm in the early stage of the growing period, whereas maize exclusively relied on water extracted from 20 to 60 cm depths, indicating competition for water use at 40–80 cm between apple trees and maize (Liu et al., 2020).

From this brief review of previous studies, a knowledge gap is evident as to the water sources exploited by apple trees in regions outside of China's Loess Plateau, where different climatic and soil conditions, rootstocks, and cultivars can lead to different ecohydrological dynamics and water use strategies. Moreover,the understanding of the role of surface waters (e.g., rivers) in rechargingshallow groundwater potentially available to apple trees is lacking. In fact,shallow groundwater tables are typical in mountain valley bottoms (i.e., onfloodplains), and apple orchards growing on such fluvial landforms might benefit from the river–floodplain hydrological connectivity. To contribute to bridging this knowledge gap, we selected two small, irrigated orchards in the driest mountain valley of South Tyrol (Northern Italy), where apple cultivation is extensive and fundamental for the local economy. In this valley, the continental climate is characterised by scarce precipitation during the growing season and thus requires careful water resource management by Land Reclamation and Irrigation districts (in Italy, public bodies responsible for water management in agricultural areas).

We tested the general hypothesis that apple trees in the two selected orchards predominantly used soil water derived from a mixture of irrigation and rainwater but could also access groundwater (likely connected to river water) according to the different topographical positions of the fields, that is, proximity to the groundwater table. Particularly, we aimed at addressing the following three specific questions:

i. Which water sources do apple trees predominantly use in dry Alpine valleys?
ii. Do trees growing at different locations in a valley bottom have access to different water sources?
iii. Can isotopes support a conceptual model of water uptake strategies and ecohydrological dynamics in Alpine apple orchards?

2 | MATERIAL AND METHODS

2.1 | Study site

Experimental activities were carried out close to the village of Laas/Lasa (German and Italian name, respectively), in the Vinschgau/Venosta Valley, a west–east oriented Alpine valley in South Tyrol, Northern Italy. The Vinschgau Valley corresponds to the upper course of the Etsch/Adige River, one of the most important rivers of the Alps. Due to its inner location and continental climate, the valley receives very little precipitation: the average annual precipitation recorded for the years 1989–2012 at the Laas weather station (874 m above sea level [a.s.l.], operated by the Hydrographic Office of the Autonomous

Province of Bozen-Bolzano) was approximately 480 mm. Minimum average temperatures fall below 0°C in December, January and February, while maximum average temperatures can reach 24°C and 23°C in July and August, respectively. Around 300 sunny days per year are usually recorded in the area. The valley bottom and the large alluvial and debris flow fans created by the lateral tributaries to the Etsch River are dominated by extended cultivations of apple trees that cover more than 5000 ha between 500 and 1100 m a.s.l. and produce around 314,000 t of apples per year. This makes apple growing the most important element of the local economy, followed by tourism. Due to the dry conditions, irrigation plays a major role in apple cultivation. Water for irrigation in Laas is sourced at approximately 1600 m a.s.l. from a snowmelt- and glaciermelt-fed stream flowing in a lateral valley, and is distributed to the orchards in the main valley by the local Land Reclamation and Irrigation district through an extended pipe system.

Two plots of approximately 400 m^2 located on the Etsch river floodplain at the same elevation (860 m a.s.l.) were selected for the experimental analyses. Hereafter they will be referred to as the right field (RF) and left field (LF) based on their orographic position with respect to the river. The most obvious difference between the two fields is indeed their position: RF is roughly 50 m from the river whereas LF is roughly 450 m from the river (Figure 1, left panel). Both orchards were planted in 2005 with *Malus domestica* (var. Pinova) grafted on M9 dwarfing rootstock, spaced every 80 cm in north–south rows with 3 m between rows (Figure 2, upper picture). The intensive planting density combined with the use of dwarfing rootstock is common in South Tyrol, typically resulting in higher root density in the upper 40 cm of soil and relatively close to the tree trunk (Scandellari et al., 2015; Tomè et al., 2016). The apples are normally harvested at the end of August. Soil samples were taken at 0–20 and 20–40 cm depth in both fields on 30 July 2015 for soil texture analysis. Soil texture was determined using the hydrometer method (Bouyoucos, 1962) and the United States Department of Agriculture (USDA) soil classification chart and can be classified as silty loam for both fields at both depths, with a greater fraction of sand in the RF compared to the LF (Table 1).

FIGURE 2 Upper photo: Apple tree rows in the right field in April 2015. Lower photo: Tension lysimeters (at 25 cm on the left hand, at 50 cm on the right hand) and wires connected to the buried soil moisture probes

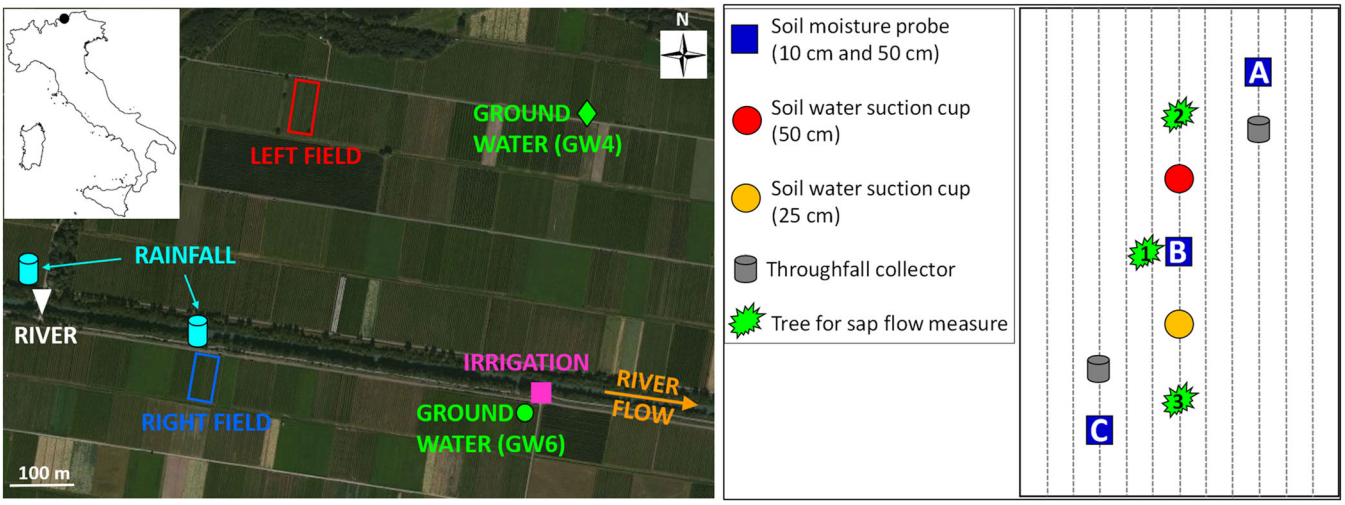

FIGURE 1 Left panel: Study area with sampling locations. Isotopic data from samples taken in the two rainfall collectors were averaged due to their vicinity in space. Right panel: Position of the sensors in each field. The letters A, B, and C indicate the position of the three couples of soil moisture probes. The vertical dashed lines indicate the apple tree rows

TABLE 1 Soil texture for the left field and right field determined from soil samples collected on 30 July 2015

Location	Depth (cm)	Sand (%)	Silt (%)	Clay (%)
Left field	0–20	43	55	2
	20–40	39	59	2
Right field	0–20	33	55	13
	20–40	24	66	10

2.2 | Field measurements and isotope sampling

2.2.1 | Collection of hydrometeorological and ecohydrological data

Experimental activities were carried out from April 2015 to October 2016, and a subsequent soil sampling campaign was performed in July 2019. Each field was equipped with sensors for the measurement of different hydrological and physiological variables and for isotope sampling (Figure 1, right panel). Time Domain Transmissivity soil moisture probes (TMS-4, TOMST s.r.o., Czech Republic) were installed at three different locations (A, B, C) at 10 and 50 cm depth, so that each field had three shallow probes and three deep probes (Figure 2, lower picture). Raw soil moisture values at 10-min temporal resolution were converted to volumetric soil moisture values (%) using the calibration equation for silty loam soils provided by the manufacturer. Soil moisture data were only available in 2015 due to instrumental failure in 2016. Thermal dissipation probes (Granier, 1985) were installed on three trees at different positions within the field. On each stem, of roughly 10 cm in diameter, two pairs of thermal dissipation probes (2 mm of diameter, entering 22 mm in the xylem below the bark, with a distance of 10 cm from each other along the trunk) were installed in opposite positions to capture the variability of flow around the stem circumference with minimum alteration of the conductive area (Pasqualotto et al., 2019). Raw data at a 15-min resolution were converted to sap flow density ($L/dm^2/h$) using Granier's calibration equation (Granier, 1985). It is well documented that the parameters of this calibration equation might underestimate sap flow and are therefore not suitable for reliable transpiration estimates (e.g., Bush et al., 2010; Peters et al., 2018). However, in our case, the standard calibration is appropriate because we used sap flow data for comparing time series patterns between the two fields, and not to derive a water balance. Sap flow sensors worked irregularly during 2015 in the RF and were not available in 2016. Air temperature and rainfall were provided by a weather station located approximately 2 km from both fields.

2.2.2 | Collection of isotopic data

Water samples for isotope analysis were collected from different sources, namely, precipitation, throughfall, irrigation, river, soil, groundwater, and xylem water (Figure 1). Sampling frequency was typically monthly but occasionally, during summer, bi-weekly sampling campaigns were conducted. Precipitation was collected by two rainfall collectors specifically designed to minimise evaporation of the sampled water and subsequent isotopic fractionation (Gröning et al., 2012) and located at two positions along the river, in open areas (Figure 1, left panel). Throughfall was sampled from two collectors per field, placed on the ground, made by inserting a funnel of 20 cm in diameter into a 5-L plastic bottle, filled with around 2 cm of mineral oil to avoid water evaporation. Irrigation was provided by an overhead sprinkler system so that water homogeneously reached all trees. As a consequence, the collectors captured not only throughfall (precipitation not intercepted by the tree crowns) but also irrigation water, as there was no way to separate the two components. Throughout the manuscript we refer to throughfall as the combination of these two waters. Irrigation water was sampled from a pipe connected to the irrigation system, and only available from April to October (Figure 1, left panel). River water samples were taken from a bridge a few hundred metres from the two fields (Figure 1, left panel) using a bucket connected to a rope to sample the well-mixed centre of the flow avoiding possible stagnation areas on the riverbanks. Soil water, that is, water from the unsaturated zone, was extracted by means of a syringe connected to a 50-cm-long plastic tube from tension lysimeters (suction cups) installed at 25 and 50 cm depths in the vicinity of the middle soil moisture probes and the middle tree equipped with sap flow sensors for each field (Figure 1, left panel and Figure 2, lower picture). Moreover, on 19 August 2015, two 60-cm-long soil cores (one for each field) were retrieved with an auger and separated in 10-cm layers and soil water was extracted through the cryogenic vacuum distillation method (Koeniger et al., 2011) in the laboratories of the Helmholtz Zentrum in Munich (Germany). Throughout the paper, we define tension lysimeters-extracted soil water as gravity-drained soil water, and cryogenic-extracted soil water as matrix soil water, according to Brantley et al. (2017).

A second soil water sampling was performed in July 2019 in the RF in a plot of approximately four trees in consecutive rows. A 130-cm-deep piezometric well was installed (not provided with a level logger). In order to prevent the infiltration of rain and irrigation water and create a moderate level of soil water deficit for a subsequent labelling experiment (Aguzzoni et al., 2020), an impermeable plastic sheet was placed on the plot. The last irrigation cycle before covering the soil occurred on 5 July (20 mm) and the last rainfall event occurred on 6 July (3 mm) 2019. Soil samples over different depths between 0 and 80 cm were collected on 23, 24, 25, and 30 July (defined as Time 1 to Time 4). Two groundwater samples from the well were also collected on 23 and 30 July. Soil water was extracted through the cryogenic vacuum distillation method (Koeniger et al., 2011) at the laboratories of the Faculty of Sciences and Technology of the Free University of Bozen-Bolzano.

Groundwater in the 2015–2016 field campaigns was sampled with a syringe connected to a 1.5 m long plastic tube from two wells installed by the Hydrographic Office of the Autonomous Province of Bozen/Bolzano and located approximately at the same distance downstream from each field. The well named GW6 was assumed representative for the groundwater isotopic composition of the RF, and

the well GW4 for the groundwater isotopic composition of the LF (Figure 1, left panel). Finally, xylem water was sampled at night (during stomata closure) using a portable Scholander-type pressure chamber (Scholander et al., 1965). The Scholander-type pressure chamber uses an external pressure to retrieve the water column present in the xylem conduit to determine the leaf water potential, but it can also be used to collect xylem water for isotopic analyses (Geiβler et al., 2019; Penna et al., 2013). Compared to cryogenic vacuum distillation, which extracts the entire volume of water from the plant tissues, possibly including water stored in dead and living cells for months or years, the pressure chamber has the advantage to extract only xylem water that is being transported at the time of sampling (Zuecco et al., 2020). Two or three twigs of roughly 5 mm in diameter from three trees randomly selected within the orchards at each sampling campaign were cut, inserted into the chamber, and pressurised until xylem water flowed from the cut end of the branch, at 300–800 kPa. Xylem water was removed from the pressure chamber using a glass pipette and transferred to a 2-ml gas chromatography vial with a 300 µl vial insert. Except for xylem water, which was directly placed in vials, all other water samples were placed in 50-ml, double-capped, high density plastic bottles. All samples were stored at 4°C until isotopic analysis.

2.2.3 | Collection of root data

Tree roots were sampled on 31 May 2018. Five cores were collected in each field, one in the middle of the field and one at each corner at a rough distance of 5 m from the border. A split tube sampler with an internal diameter of 53 mm and length of 40 cm was positioned halfway between the tree trunk and the grassed alley down to a depth of 80 cm. Undisturbed cores were wrapped in a plastic sheet and stored in an insulated container for transport to the laboratory where they were unwrapped and divided in 20-cm-deep pieces from which fine (diameter < 2 mm) and coarse roots (diameter > 2 mm) were separated under a magnifying lens using tweezers. Root samples were dried in a ventilated oven at 65°C for 72 h and then weighed. Root density was calculated by dividing the dry weight by the volume of soil cores assuming a perfectly cylindrical shape of 10 cm height and 5.3 cm diameter.

2.3 | Isotopic analysis

Water samples were analysed for their isotopic composition through cavity ring-down spectroscopy (Picarro Inc. L-2130i) at the Faculty of Sciences and Technology of the Free University of Bozen-Bolzano, following the procedure by Penna et al. (2012) to minimise 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 5‰ for δ^2H and 0.25‰ for $\delta^{18}O$ for laser spectroscopy, and 0.2‰ for both δ^2H and $\delta^{18}O$ for the IRMS. Throughout the paper, data of $\delta^{18}O$ are preferably shown compared to those of δ^2H. The consistency between the laser spectroscopy and the IRMS measurements was assessed by running a subset of 18 samples from different organically uncontaminated waters in the range of −41.76‰ and +5.92‰ in $\delta^{18}O$. The comparison showed a high correlation ($R^2 = 0.999$), a mean absolute error of 0.64‰, and a root mean squared error of 1.11‰. Particularly, when considering the range between −4.00‰ and −9.50‰ in $\delta^{18}O$ ($n = 8$), that encompasses more than 95% of all xylem water samples, the correlation was still high ($R^2 = 0.992$) and the differences between the measurements by the two machines decreased. Particularly the differences were roughly in the range of the instrumental precision (mean absolute error = 0.21‰, and a root mean squared error of 0.25‰) and were therefore considered negligible. Stable isotope values are reported using the delta notation (δ) in per mil (‰) against the international reference standard Vienna Standard Mean Ocean Water.

For each water sample, the line-conditioned excess (LC-excess) was calculated according to Landwehr and Coplen (2006) as follows:

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

where *a* and *b* represent the slope and intercept of the local meteoric water line (LMWL), that is, the regression line in a $\delta^{18}O$-δ^2H (dual-isotope) plot of monthly precipitation samples collected in the study area during 2015 and 2016 (Dansgaard, 1964). Water samples that experienced fractionation by evaporation have negative LC-excess values and plot below the LMWL in a dual-isotope plot (Landwehr et al., 2014).

3 | RESULTS

3.1 | Hydrological variability

For a preliminary evaluation of the hydrometeorological variability in the study area spanning the two study years, we focussed on the period from May to October that includes the growing season for apple trees (Figure 3). The period May–October in 2015 and 2016 in the study area was characterised by overall similar values of cumulative precipitation (411 and 433 mm, respectively) but having a slightly higher average temperature in 2015 compared to 2016 (15.1°C and 14.1°C, respectively, Figure 3, upper panel). Field-average soil moisture, that is, the average of the three soil moisture probes for each depth, was higher at 50 cm than at 10 cm in both fields and was markedly higher in the RF compared to the LF at both depths (Figure 3, middle panel). Soil moisture reacted quickly to rainfall and irrigation inputs (the latter were inferred by increases in soil moisture during no-rain periods) at 10 cm in both fields. The soil moisture response at 50 cm was more damped compared to that in the shallower layer in both fields but lower variability was observed in the RF due to the higher values and therefore a smaller soil water deficit (Figure 3, middle panel).

The depth to water table measured in the two wells revealed a similar behaviour to soil moisture, with consistently higher (i.e., closer to the ground surface) water table levels in the RF compared to the

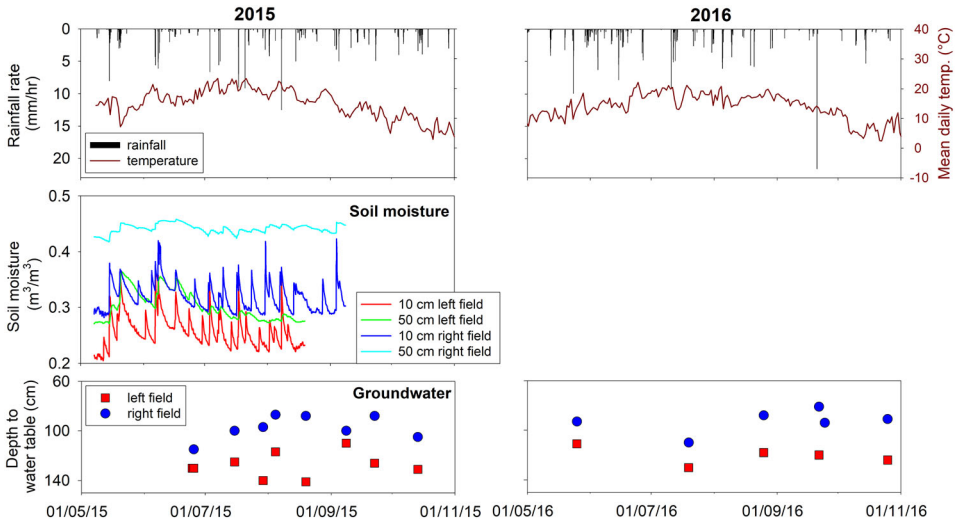

FIGURE 3 Rainfall rate, mean daily temperature, depth to water table and soil moisture at two depths (average of three probes for each depth and field) in the two fields for the two monitoring years 2015 and 2016 (May–October). Groundwater in the left field refers to well GW4 and in the right field to well GW6 (Figure 1). Irrigation inputs (approximately 20 mm each week between May and August) are not shown. Soil moisture in 2016 was not available due to instrumental failure

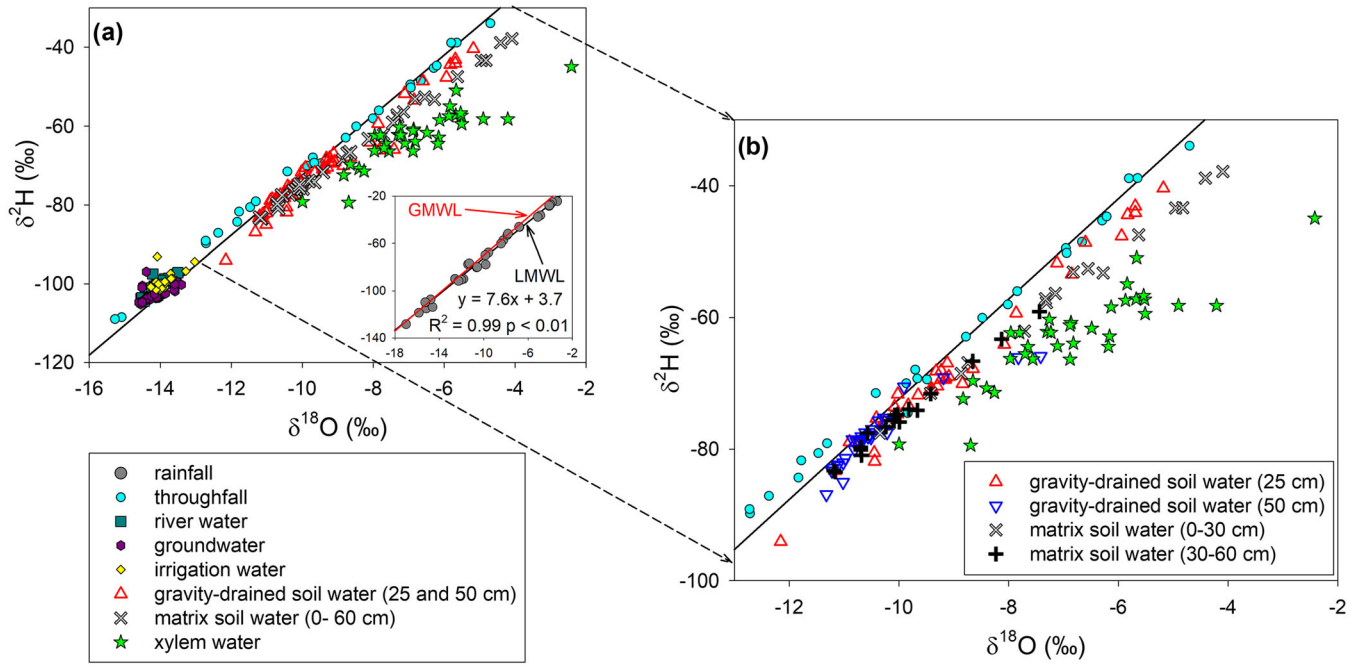

FIGURE 4 (a) Dual-isotope plot of all samples collected in this study. In the inset: Rainfall samples, global meteoric water line (GMWL) and local meteoric water line for Laas (LMWL). (b) Zoom of panel (a) focused on soil water samples, colour-coded by sampling depth. Gravity-drained soil water refers to water extracted by tension lysimeters, and matrix soil water refers to cryogenically extracted soil water (see Brantley et al., 2017). Matrix soil water samples were collected on 19 August 2015 in the two fields from 60-cm-long soil cores at 10-cm increments. These samples were colour-coded in two depths (0–30 and 30–60 cm) for comparison with the gravity-drained soil water collected by tension lysimeters at 25 and 50 cm

LF, and up to roughly 70 cm depth (Figure 3, lower panel). Groundwater levels therefore corroborate the observation of increased wetness in the RF than in the LF.

When measured, sap flow showed, as expected, daily variations with higher peaks during warm and hot days and lower peaks during rainy days (Supporting Information Figure S1). Negligible differences in sap flow values were recorded among the three sampled trees, although slightly higher values were reached for Tree 2 in the LF and Tree 3 in the RF, and sap flow intra-field dynamics were comparable. Due to the limited sap flow data in the RF, it is difficult to compare transpiration patterns between fields; however, the available data suggests, on average, similar sap flow values and the same sap flow dynamics in the two fields (Figure S1).

3.2 | Isotopic characterisation of the different waters

The LMWL was close to the global meteoric water line (Craig, 1961) but with a slightly lower slope (7.6‰ vs. 8‰) and lower intercept (deuterium excess, 3.7‰ vs. 10‰) (Figure 4a, inset).

Throughfall samples fell well aligned to the LMWL and spanned a wide isotopic range mainly reflecting the temporal variability of precipitation (Figure 4). Irrigation water, groundwater, and river water samples plotted along the LMWL but formed a cluster characterised by noticeably more depleted values in heavy isotopes compared to soil water and xylem water. The median $\delta^{18}O$ values of irrigation water in the two wells (−14.25‰ and −14.20‰), groundwater (−13.97‰), and river water (−14.07‰) samples taken on the same days were very close to each other although statistically slightly different (Kruskal–Wallis test, $n = 18$, $p = 0.017$). However, the isotopic composition of groundwater in the two sampling wells was not statistically different (Mann–Whitney rank test on $\delta^{18}O$ data, $n = 18$ taken on common dates, $p = 0.275$, not shown in Figure 4).

Gravity-drained and matrix soil water samples spanned a large range in the dual-isotope space, roughly similar to that of throughfall (Figure 4). A closer inspection of gravity-drained and matrix soil water reveals that, in both cases, the depleted samples—lying on the LMWL—were extracted from the deepest depths while the enriched samples—deviating from the LMWL—were extracted from shallowest depths (Figure 4b). Xylem water samples always plotted below the LMWL only partially overlapping with soil samples (Figure 4).

These differences among the sampled water sources observed in the dual-isotope plot were highlighted by the distribution of $\delta^{18}O$ and LC-excess for the period between June and September (assumed to be the period with most active tree transpiration) (Figure 5). River water, groundwater, and irrigation water showed very low variability in $\delta^{18}O$ and featured median values very similar among them and highly different (more negative) from all the other water sources (Figure 5, upper panel). At the same time, river water, groundwater, and irrigation water showed the highest (and positive) values of LC-excess (Figure 5, lower panel; Figure S2). As expected, rainfall was characterised by a large variability in the isotopic composition deriving from its seasonal variability and temperature dependence (Araguás-Araguás et al., 2000). Most of the isotopic variability of throughfall was included in the variability of rainfall but the median value of throughfall was lower (more negative) than that of rainfall due to the influence of the much more depleted irrigation water that contributed to throughfall (Figure 5, upper panel). As previously observed, differences in the isotopic composition of soil water could be identified according to depth: soil water from the shallow layer was isotopically more enriched and variable and had more negative LC-excess values compared to soil water from the deep layer (50 cm gravity-drained water and 30–60 cm matrix water) (Figures 6 and S3). This behaviour, combined with the observed deviation from the LMWL (Figure 4), is typically related to isotopic kinetic fractionation due to evaporation (Benettin et al., 2018). The $\delta^{18}O$ composition of xylem water was overall consistent with that of water in the shallow soil layer (Figure 5, upper panel) but the highly negative LC-excess reveals that xylem water samples were noticeably more isotopically fractionated than soil water, even the sample extracted from the shallow depth (Figure 5, lower panel).

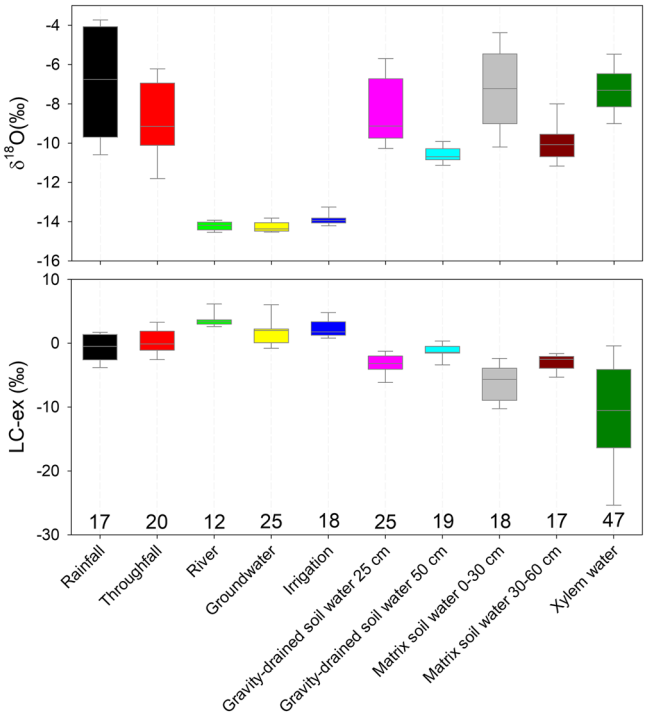

FIGURE 5 Isotopic composition ($\delta^{18}O$, upper panel) and LC-excess (lower panel) of samples collected from the different water sources from June to September (the more active period of the growing season) of both monitoring years. Samples from the two fields were grouped together. The boxes indicate the 25th and 75th percentiles, the whiskers indicate the 10th and 90th percentiles and the horizontal line within the box marks the median. The numbers at the bottom of the lower panel indicate the sample size

3.3 | Isotopic composition of soil and xylem water in the two fields

The isotopic composition of the gravity-drained soil water samples simultaneously collected in the two fields during the monitoring periods confirms the general pattern observed in Figure 5, with more enriched and variable values at 25 cm and more depleted and less variable values at 50 cm (Table 2). Particularly, despite the isotopic composition of soil water at both depths in the LF was slightly more enriched than in the RF at the corresponding depths, no statistical difference existed between the two fields (Mann–Whitney rank sum test, $p > 0.1$ both at 25 and at 50 cm between the LF and the RF).

The distribution of the isotopic composition of gravity-drained soil water at 25 and 50 cm and of xylem water in the two fields for the June–September period is reported in Figure 6. Soil water at 50 cm was more depleted in heavy isotopes in both fields compared to soil water at 25 cm and xylem water, but soil water at 25 cm in the LF was more enriched compared to that in the RF (Figure 6). Indeed, soil water at 25 cm in the LF was statistically similar to xylem water, whereas soil water at 50 cm in the LF, and soil water at both depths in the RF were statistically different from xylem water (Table 3). However, the median isotopic composition in $\delta^{18}O$ in xylem water

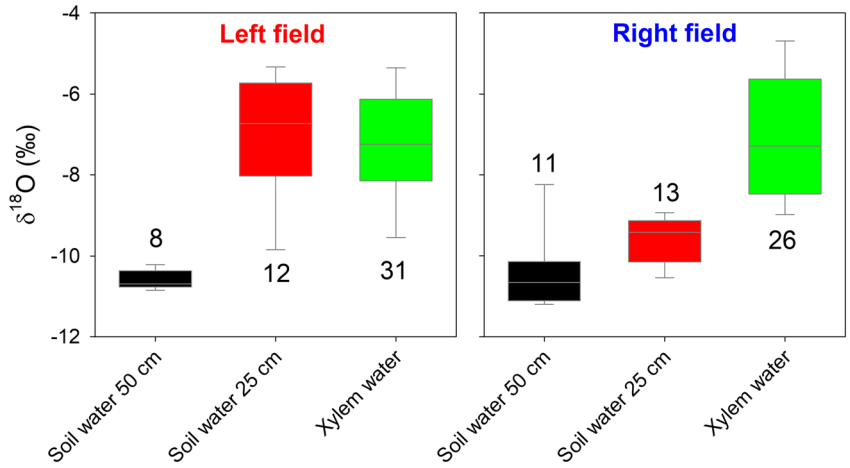

FIGURE 6 Isotopic composition ($\delta^{18}O$) of gravity-drained soil water and xylem water samples collected from June to September of both monitoring years distinguished by field. The boxes indicate the 25th and 75th percentiles, the whiskers indicate the 10th and 90th percentiles and the horizontal line within the box marks the median. Numbers above or below the boxes indicate the sample size (please note that there is no temporal consistency among samples)

		Left field		Right field	
	N	Mean	Standard deviation	Mean	Standard deviation
Soil water 25 cm	14	−8.47	2.41	−9.75	0.59
Soil water 50 cm	11	−10.15	1.01	−10.69	0.36

TABLE 2 Basic statistics of the isotopic composition ($\delta^{18}O$ ‰) for gravity-drained soil water concurrently collected at the two depths in the two fields in 2015 and 2016

	Variables	Statistical difference	p value
Left field	Soil water 50 cm vs. xylem water	Yes	<0.001
	Soil water 25 cm vs. xylem water	No	0.490
Right field	Soil water 50 cm vs. xylem water	Yes	<0.001
	Soil water 25 cm vs. xylem water	Yes	<0.001

TABLE 3 Results of the non-parametric Mann–Whitney test performed to compare soil water and xylem water samples shown in Figure 6 for each field

between the LF and RF was not statistically different (Mann–Whitney rank sum test, $p > 0.1$).

The isotopic composition of matrix soil water samples extracted from soil cores on a summer day in both fields reveals a marked decrease of the $\delta^{18}O$ signal with depth, that is, soil water was more depleted in heavy isotopes in the deeper layers and gradually became more enriched towards the soil surface (Figure 7 upper panel and Figure S3). Furthermore, more enriched values were clearly observed in the LF compared to the RF (Figure 7, upper panel and Figure S3). Consistently, the LC-excess of matrix soil water increased as a function of soil depth, that is, less negative values were observed in the deeper layers (Figure 7, lower panel). An analogous behaviour was observed for the soil water data collected during the 1-week monitoring period conducted in July 2019. There was a clear isotopic gradient along the soil profile, with deeper soil water isotopically similar to groundwater (−12.9‰ ± 0.3‰ in $\delta^{18}O$), and then becoming more enriched with decreasing soil depth over the four sampling days (Figure 8, upper panel). The LC-excess noticeably varied as well, from positive or slightly negative values in the deeper layers to markedly negative values in the upper soil layers (Figure 8, lower panel). Moreover, the isotopic composition of the soil samples generally became more depleted in heavy isotopes over time (from Time 1 to Time 4).

3.4 | Temporal variability of water isotopic composition

The isotopic composition of rainwater, as expected, was temporally variable due to its seasonal meteorological controls (Figure 9, upper panel). The isotopic composition of throughfall was temporally variable as well although to a lesser degree, and the seasonal pattern was comparable in the two fields, especially during the 2016 monitoring period (Figure 9 middle and lower panels). Conversely, irrigation water, river water, and groundwater at the two fields were much less variable over time and also well correlated to each other. Additionally, groundwater showed a very low degree of spatial variability between the two wells. River water values observed during the summer months were only slightly more depleted likely due to the influence of meltwater in the upper part of the Etsch River catchment (snow and glacier, typically isotopically depleted; see Schmieder et al., 2018; Penna et al., 2017, 2014; Engel et al., 2016, 2019, for examples in this area, and Ceperley et al., 2020, for the general effect of meltwater on streamflow isotopic composition) (Figure 9). However, no seasonal pattern was evident in groundwater. On the contrary, gravity-drained soil water at two depths and xylem water were characterised by a large temporal variability (Figure 9, middle and lower panel). An irregular temporal pattern was observed for soil water during the two

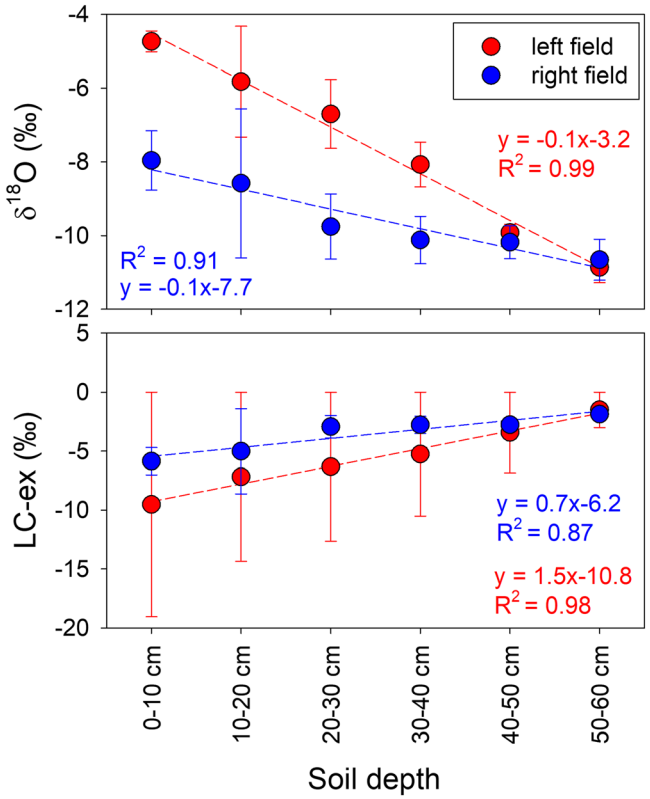

FIGURE 7 $\delta^{18}O$ (upper panel) and LC-excess (lower panel) of matrix soil moisture extracted from soil cores at six depths collected on 19 August 2015. Error bars represent the standard deviation

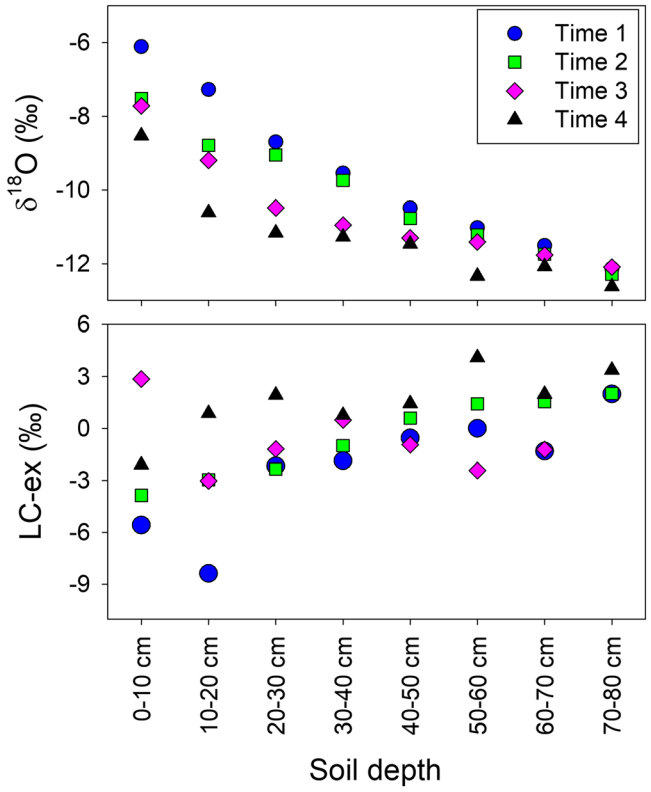

FIGURE 8 $\delta^{18}O$ (upper panel) and LC-excess (lower panel) of matrix soil moisture extracted in the right field from eight depths at four sampling times in July 2019. Error bars were not included to improve readability

monitoring periods, not consistent between the two fields. The $\delta^{18}O$ signal at 50 cm in the LF seemed to slightly increase in 2015 but appeared decoupled from the decrease at 25 cm, and also in 2016 contrasting patterns emerged (Figure 9, middle panel). Temporal soil water isotope patterns at the two depths appeared more consistent in both years in the RF (Figure 9, lower panel). The $\delta^{18}O$ signature of xylem water was very scattered in both years and in both fields (Figure 9, middle and lower panel) and only partially reflected the isotopic signature of rainwater. A clearly similar temporal pattern of the isotopic composition of xylem water and soil water at the two depths was not observed. Additionally, in 2015, the isotopic composition of soil water at 25 cm in the RF was more depleted than that of xylem water, while in the LF their values were much closer. A certain similarity, although less evident, could be observed in 2016 in both fields (Figure 9, middle and lower panel). No similarity existed between the isotopic composition of xylem water and irrigation water, river water, and groundwater.

The $\delta^{18}O$ values of groundwater, river water, gravity-drained soil water at 25 and 50 cm, and xylem water are reported for selected sampling dates when most samples were available (Figures 10 and S4). During this time, a more similar soil–xylem water temporal pattern emerged compared to what was observed in Figure 9. In fact, in both fields the isotope values of xylem water were similar to those of soil water at 25 cm and, to a much lesser extent, at 50 cm. As observed in Figures 4 and 5, the isotope signal of river water, groundwater (Figure 10), and irrigation water (Figure S4) plotted consistently far off that of xylem water in both fields. These observations indicate that the $\delta^{18}O$ composition of xylem water more clearly reflected the signal of soil water at 25 cm and, to a lesser extent, at 50 cm, and did not reflect the isotopic signature of irrigation water, groundwater, and river water.

3.5 | Root density patterns

Root density data revealed that the majority of coarse roots were concentrated in the upper 40 cm of soil, especially in the RF, and a very limited density of coarse roots was observed below 60 cm (Figure 11). Fine roots, which are responsible for most of the water uptake, were progressively less concentrated in the RF moving from the most superficial to the deepest layers. In the LF root density was relatively high (although very variable) also in the 40–60 cm layer (Figure 11).

4 | DISCUSSION

4.1 | Water sources used by apple trees

The isotopic composition of xylem water reflected more clearly the isotopic composition of gravity-drained soil water at 25 cm and

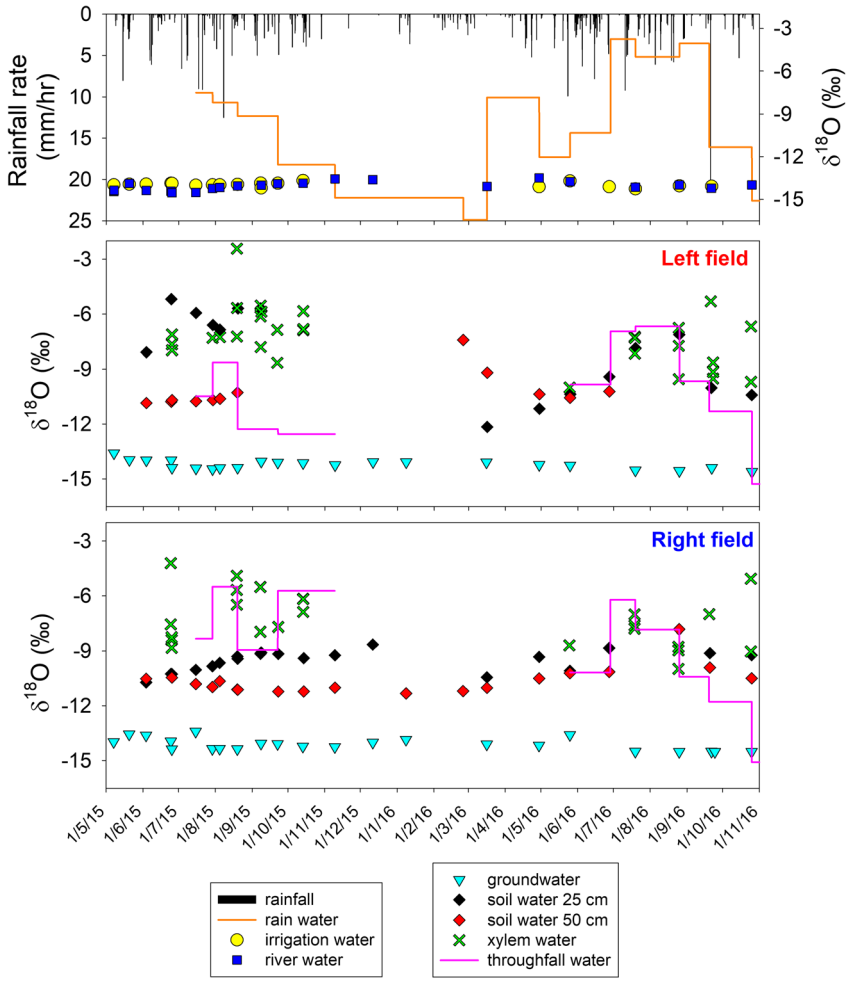

FIGURE 9 Time series of rainfall and the isotopic composition of rainwater, throughfall, river water, irrigation water, groundwater, gravity-drained soil water at the two sampling depths and xylem water distinguished by field. Groundwater in the left field and the right field refers to GW4 and GW6, respectively (see Figure 1)

secondarily at 50 cm (Figures 6, 9, and 10), suggesting that the main source for apple tree transpiration in the study area was water extracted from the upper 40 cm of the soil. Fine roots were mostly concentrated in the upper soil layer (0–40 cm) although they were also present at higher depths, especially in the LF (Figure 11). Moreover, despite the lack of soil water isotope data in the upper 10–20 cm, the negative LC values of xylem water and matrix soil water in the 0–30 cm layer (Figure 5) and the relatively high values of fine root density also in the topmost layer (Figure 11) suggest that soil water in the upper 10 or 20 cm might contribute to tree transpiration. These observations are in full agreement with the studies by Zheng et al. (2018) and Zheng et al. (2019) under surface irrigation conditions where the reported main contribution to root water uptake for *Malus domestica* and *Malus robusta* trees came from the 0–40 cm layer. Our results are different from other studies that showed that apple trees (species and cultivar not reported) preferred deeper soil water sources (60–100 cm, Liu et al., 2020) even up to 500 cm for older trees (Wang et al., 2020), revealing a large variability in water uptake strategies and large physiological flexibility and adaptation to local conditions. However, differently from our case, the Loess Plateau area where the studies previously mentioned were conducted is characterised by thick soils and deep groundwater tables. Additionally, it must be noted that trees in our experimental field were grafted on a dwarf rootstock, suitable for high density planting, whereas in the study by Wang et al. (2020) apple trees were likely grafted on vigorous genotypes, based on their stem and crown size, typical of low plantation density.

Furthermore, our results suggest a negligible contribution of groundwater to apple tree transpiration, as there was a marked difference in the isotopic composition between groundwater and xylem water (Figures 4, 5, 9 and 10). Such noticeable differences in δ values of xylem water compared to groundwater (and river water) made it not possible (and meaningless) to run isotope-based mixing models to quantify the contribution of groundwater because mixing models rely on a comparison between the isotopic composition of xylem water and its potential water sources (Amin et al., 2020; Rothfuss & Javaux, 2017). Despite the scarce evidence regarding groundwater contributions to apple tree transpiration, there might be a groundwater control on soil water recharge through capillary rise. Particularly in the RF, capillary rise might sustain soil moisture, especially in the deeper layers, as the water content in the RF was constantly higher than that in the LF, and the 50 cm layer in the RF was comparatively particularly wet (Figure 3). This possibility is also supported by the observed depleted isotopic composition in the deeper soil layers,

more similar to the isotopic signal of groundwater compared to the shallow layers (Figure 8). However, despite soil water being higher in the RF than in the LF (Figure 3) the isotopic composition of gravity-drained soil water was not statistically different between the two fields for the same sampling depth (Table 2), thus not supporting the hypothesis that groundwater substantially recharges the vadose zone. Unfortunately, our data did not allow for a robust estimation of the magnitude of the upward flow associated with capillary rise. However, published data for soil texture similar to our case reported, for groundwater depths between 80 and 100 cm, possible upward flow rates up to 5–6 mm/day but only up to 60 cm depth (Doorenbos & Pruitt, 1977).

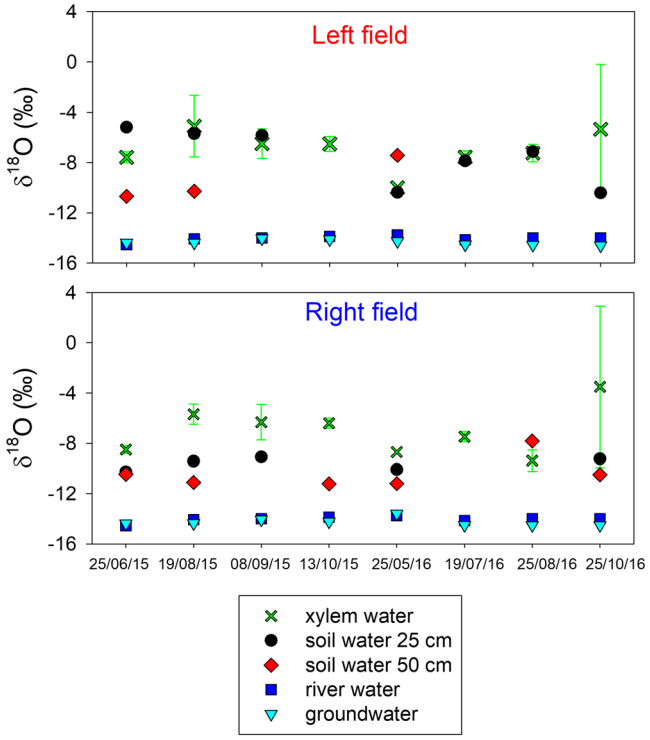

FIGURE 10 Groundwater, gravity-drained soil water at 25 and 50 cm, xylem water and river water isotopic composition ($\delta^{18}O$) for the right and left field for sampling dates when most samples were available. Xylem data are the average of samples collected at different times of the day, and error bars represent the standard deviation

4.2 | Different fields, same water sources but from different depths

The inferred negligible contributions of groundwater and river water to tree uptake in the two different fields at different distances from the river (50 vs. 450 m for RF and LF, respectively) does not support our general hypothesis that apple trees in the study area could access different water sources such as groundwater based on their potentially different hydrological connectivity to such sources. Based on the plasticity of apple trees to develop roots and access water at different depths (e.g., Hughes & Gandar, 1993; Wang et al., 2020) and be potentially several decades old (Li, Si, et al., 2019; Zhang et al., 2017), we can speculate that trees growing closer to surface water bodies (e.g., rivers, lakes) are well connected to shallow groundwater and might access this additional water source for transpiration during dry periods. This is likely not the case of our study area where frequent irrigation events (approximately 20 mm once per week for the entire growing season) keep soil moisture consistently relatively high thus favouring the absorption from shallow water sources, irrespective of the availability of deeper sources. This should lead to relatively low values of suction heads in soils and thereafter to small degrees of capillary rise, limiting the contribution of groundwater to soil recharge and subsequent tree water uptake. Our analysis revealed that the main water sources for tree transpiration in both fields was soil water in the shallow layers. However, we found some differences in the isotopic signatures between the LF and RF. In the LF, xylem water isotopically resembled gravity-drained soil water especially at 25 cm and

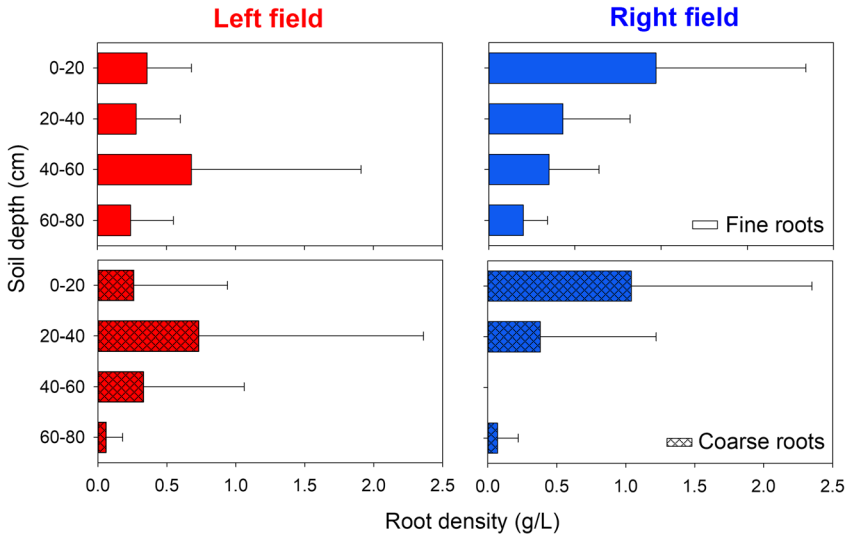

FIGURE 11 Root density (expressed in g/L) for coarse and fine roots at different depths for both fields. The standard deviation is reported as error bars

showed a lower degree of overlapping with soil water at 50 cm whereas in the RF xylem water was more enriched and statistically different from gravity-drained soil water both at 25 and 50 cm (Figures 6, 9, and 10). We recognise that the overlap between the isotopic composition of xylem water and soil water at a specific depth does not necessarily mean that trees exploit water from that soil depth. However, in this case, the striking difference between deep and shallow soil water, and the high degree of similarity between the isotopic signature of xylem water and shallow soil water, especially in the LF (Figure 6), leads to reasonably expect that the studied trees took up water mainly from the upper centimetres of the soil. Considering the isotopic gradient in the soil that showed an enrichment trend from the deeper to the shallower soil layers (Figures 7 and 8), we argue that the main sources for tree water uptake in the RF might be even shallower than 25 cm. This is consistent with the observed high soil moisture at 10 cm in the RF that may provide most of the water needed for tree uptake, and the higher root density at 0–20 cm depth in the RF compared to the LF (Figure 11). However, these observations are based only on a relatively small number of samples (though distributed over two growing periods), and further analyses and more frequent samplings are necessary to corroborate and validate these results.

4.3 | Where does irrigation water go? A conceptual model

What is strikingly noticeable in our results is the extremely different isotopic composition between xylem water in both fields and irrigation water. On the contrary, δ values of irrigation water were more negative and statistically identical to those of groundwater in both wells (see Figures 4 and 9). This is reasonable as irrigation water is abstracted from a tributary stream featuring a nivo-glacial hydrological regime, and meltwaters are typically characterised by more negative δ values (see Araguás-Araguás et al., 2000 for general explanations and Penna et al., 2017 for an example close to the study area). However, this similarity did not allow us to apply mixing models to assess the possible contributions of these water sources to tree transpiration as it violates the main assumption regarding the distinct tracer signature of the end-members (Rothfuss & Javaux, 2017; von Freyberg et al., 2020).

Rainwater and throughfall showed, as expected, a large range in δ values as a result of the seasonal variability of air temperature that directly controls the isotopic composition of precipitation (Gat, 1996). The variability in the isotope signature of rain and, to a certain extent, of throughfall was overall larger than that of soil water and xylem water, suggesting that rainfall and throughfall were the main hydrological inputs recharging the soil and therefore feeding trees. However, the variability in the isotope signature of rain and throughfall was not larger than that of groundwater, irrigation, and river water that were more depleted and looked like separate water pools (Figure 4 and more evidently in Figure 5). This indicates that the isotopic composition of rainfall sampled in the valley bottom was not entirely representative for the more depleted isotopic composition of the water sources that feed groundwater and irrigation water, and for the Etsch River water that originates from the mountain ranges of the upper Vinschgau Valley.

The apparent negligible role of irrigation water for tree use, indicated by the marked difference between the isotopic composition of irrigation water and xylem water, could be explained by an isotope-based conceptual model on water movement in these apple orchards (Figure 12). This conceptualisation relies on a subsequent mixing of evaporated soil water with non-fractionated precipitation and irrigation water. Such a process is similar to that described by Sprenger et al. (2016) (see their fig. 7) and discussed by Beyer et al. (2020). The strong solar radiation (related to the continental climate) during the growing periods led to the evaporation of soil water in the shallow layers and to a subsequent isotopic fractionation that was especially apparent in the topsoil, decreasing in the deeper layers. This was revealed by the strong gradient in isotopic composition and LC-excess in the soil profile, becoming more depleted and less negative or more positive with depth, respectively (Figures 7 and 8), as also observed in natural, not irrigated environments (Dudley et al., 2018; Sprenger et al., 2017). Fractionation must also have occurred in the water transport process from the root zone to the canopy, as xylem water samples plotted below the LMWL and were characterised by LC-excess values generally more negative than those of soil water (Figures 4 and 5). These observations are in agreement with recent research that showed that fractionation might occur during the root water uptake process at least in some species and/or under specific conditions (Poca et al., 2019; Vargas et al., 2017). Additionally, other possible interconnected effects, mainly related to the structural heterogeneity of plants and soils, might have contributed to the xylem–soil water offset (Barbeta et al. (2020); see also the discussions in Beyer and Penna (2021), and von Freyberg et al. (2020) on the role of heterogeneity in isotope-based ecohydrological studies, and the different factors affecting xylem water isotopic composition). Overall, incoming precipitation (throughfall) and irrigation waters were not isotopically fractionated as samples laid on the LMWL and LC-excess values were mostly positive (Figures 4 and 5). This unfractionated water infiltrated in the topsoil and mixed with already fractionated soil water modifying its isotopic composition. During hot summer days, following irrigation or rain events, infiltrated water recharging the shallower soil layers became fractionated and more enriched in heavy isotopes whereas the soil kept its more depleted signature in the deeper layers. Particularly, irrigation water was always isotopically very depleted. In contrast, a more enriched composition was attained in the shallower layers, due to mixing with enriched and fractionated water from previous irrigation and/or rain events. Roots took up a mixture of irrigation and rainwater from different, but mainly shallow (0–40 cm) soil depths, and the resulting xylem water showed an average isotopic composition of all the water sources. The contribution of rain and especially irrigation water was somehow masked by the high evaporation rate and successive fractionation, and therefore the original isotopic composition of rain and irrigation water did not reflect that of the xylem water.

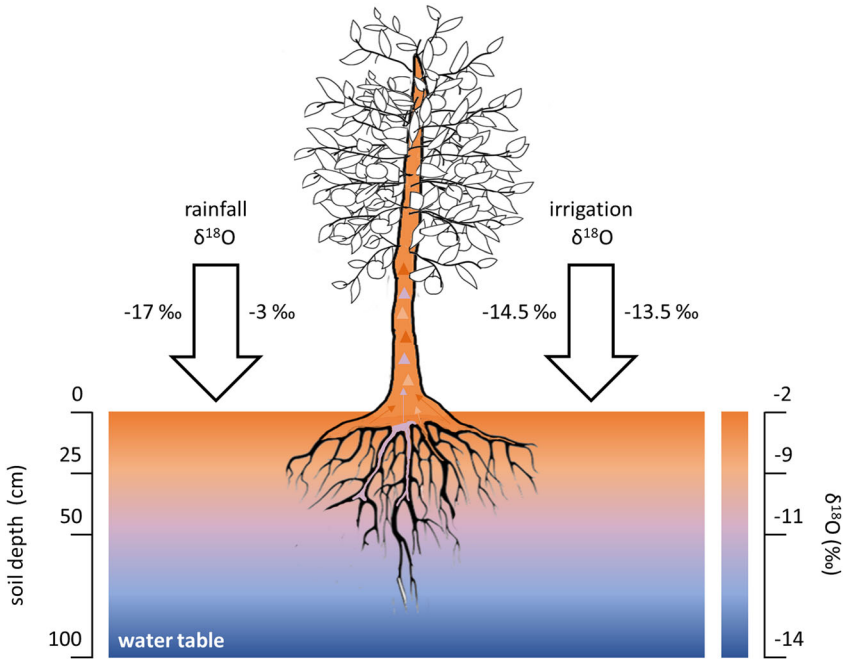

FIGURE 12 Conceptual isotope-based model of water movements in the study apple orchards. Vertical downward arrows indicate the two main sources of soil recharge, that is, rainfall and irrigation. The δ values indicate the approximated range in the isotopic composition in rainfall and irrigation water observed in this study (see Figure 4). The reported values stress the difference between the largely variable isotopic composition of rainfall that reflects the seasonal variations in air temperature and the constantly depleted signature of irrigation water originating from high elevations (Figures 4, 5 and 9). Both rainwater and irrigation water do not show evidence of isotopic fractionation (Figures 4 and 5). The colour gradient in the soil indicates the isotopic gradient from more depleted values of groundwater to more enriched and fractionated values in the shallow soil layers (Figures 4, 5, 7 and 8), as a main result of the evaporation process. Rain and irrigation water mix in the soil, and apple trees preferentially take up water approximately from the upper 20–40 cm in the soil, coherently with their root depth and coarse and fine root density (Figure 11). The xylem water isotopic composition reflects this mixed signature

5 | CONCLUDING REMARKS

This research was motivated by the need to better assess water uptake strategies by apple orchards in an Alpine valley where apple cultivation is of paramount importance for the local economy. Isotopic data revealed that apple trees relied mostly on soil water from the shallow layers (0–40 cm), where most of the roots are concentrated, and that the contribution of groundwater was apparently negligible, despite capillary rise in the RF might likely affect the soil moisture in the deepest soil layers, where some roots were also present. Interestingly, we observed that xylem water was isotopically very different from irrigation water, apparently suggesting that trees under the tested field conditions did not directly access this source. We related this 'hidden' tracer signature of irrigation water to the effects of soil evaporation that strongly modifies its original isotopic composition: irrigation and rainwater infiltrate into the soil and mix with isotopically fractionated soil water, and trees take up a mixture of waters with different isotopic composition compared to that of the original irrigation source.

This work contributes to gain new ecohydrological knowledge on soil and water uptake dynamics in mountain apple orchards. However, further research steps are necessary to quantify the proportion of irrigation and rainwater used by trees. In this context, isotope sampling at higher temporal frequency is advisable to observe ecohydrological dynamics at finer time scales and to validate our conceptual model of water uptake strategies. Furthermore, labelling experiments are a possible way to thoroughly explore the sources of root water uptake and their temporal variability in these Alpine orchards; these experiments should be carried out at different times during the growing period and possibly coupled with the application of a numerical model able to simulate the isotopic transport in the plant.

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AUTHOR CONTRIBUTIONS

Daniele Penna: conceptualisation, investigation, formal analysis, visualisation, writing—original draft preparation. Damiano Zanotelli: investigation, formal analysis, writing—review and editing. Francesca Scandellari: investigation, formal analysis, writing—review and editing. Agnese Aguzzoni: investigation, visualisation, writing—review and editing. Michael Engel: investigation, writing—review and editing. Massimo Tagliavini: conceptualisation, funding acquisition, project administration, writing—review and editing. Francesco Comiti: conceptualisation, funding acquisition, project administration, writing—review and editing.

DATA AVAILABILITY STATEMENT

The isotopic dataset is freely available upon request to the corresponding author.

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SUPPORTING INFORMATION

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