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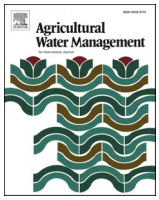

Estimating uptake and internal transport dynamics of irrigation water in apple trees using deuterium-enriched water

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ABSTRACT

With future climatic scenarios foreseeing increased crop water demands and reduced irrigation water availability, a deeper knowledge on the dynamics of water uptake and translocation inside plants is needed. Little is known about the time interval existing between the irrigation water supply and the presence of irrigation water inside trees, and whether the dripper localization can affect water uptake and translocation dynamics. Another research gap concerns the redistribution of irrigation water in the canopy following irrigation localized to one side of the tree.

A field and a pot experiment were designed to gain more insight into this context. In the field experiment, we tested the effect of different drip irrigation layouts on the extent and velocity of water uptake by apple trees. Trees were irrigated using deuterium-enriched water using one, two, or four drippers per tree. Samples were collected from different heights in the canopy at regular intervals following the irrigation event. In the pot experiment, the soil was saturated with labelled water and samples were collected at different time intervals and heights along the tree stem. Labelled water was detected in the lowest stem section of potted trees after 1 h from irrigation. In field-grown trees, labelled water appeared in the shoots after 4 h and 6 h in the bottom and top part of the canopy, respectively. By increasing the number of drippers per tree, the fraction of irrigation water in the shoots increased accordingly. However, uptake and transport velocity were unaffected by the number of drippers, averaging 0.60–0.65 m h^{-1}. In trees that were irrigated from one side only, irrigation water could be found on the opposite side in the top part of the canopy. Our results suggest that the localization and amount of irrigation water can significantly influence root water uptake in apple trees.

1. Introduction

Climate change will likely increase crop water demand and reduce the availability of water for irrigation (Elliott et al., 2014). Adaptive strategies are therefore needed to maintain agricultural production at satisfactory levels in terms of quality and quantity (Fischer et al., 2007).

South Tyrol, in Northern Italy, accounts for one of the major apple growing regions in Europe and worldwide, comprising roughly half of the Italian apple production (Zanotelli et al., 2019). Apple orchards are frequently equipped with drip irrigation, which is considered among the most efficient irrigation systems (Batchelor et al., 1996; Bravdo and Proebsting, 1993). However, the full water saving potential of drip irrigation is only exploited by proper irrigation management (Van der Kooij et al., 2013). In this light, water saving irrigation strategies, such as regulated deficit irrigation (Ebel et al., 1995) and partial rootzone drying (PRD) (Leib et al., 2006; O'Connell and Goodwin, 2007) are being studied and implemented (Ben Abdelkader et al., 2022b).

These irrigation strategies fit in the definition of precision irrigation, namely an approach taking into consideration the temporal and spatial variations in soil properties and crop characteristics to maximize water savings and minimize environmental impact. Such goals are achieved by scheduling irrigation based on the crop requirements and by precisely delivering irrigation water close to the plants' roots (Abioye et al., 2020). For tree crops, most of the previous studies on irrigation management have focused on improving irrigation on a temporal scale (i.e., activating irrigation only at times when it is actually necessary). Spatial distribution patterns of irrigation water, in terms of vertical and horizontal spreading from the drip emitter, have been widely investigated,

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mainly by numerical simulations (Elmaloglou et al., 2013; Skaggs et al., 2010). However, only few studies have examined the irrigation water distribution from drip emitters in orchards in relation to the trees' rootzone. In apple production areas, where rainfall distribution is relatively homogeneous over the course of a growing season, natural precipitation may suffice to maintain adequate and spatially homogeneous soil moisture levels for long periods (Ucar et al., 2023). In these conditions, it is likely that the root system of each tree expands well beyond the soil volume that can be wetted by drip irrigation (Sokalska et al., 2009). As a consequence, especially in prolonged periods of scarce rainfall, a single dripline could be able to wet only a portion of the root system and thus be insufficient to fully satisfy the requirements of apple trees. It would be therefore important to know whether the spatial localization of irrigation water around trees can influence the extent of tree water uptake, and how the water absorbed by a limited fraction of the trees' roots, following localized drip irrigation, redistributes within the tree canopy.

The localization of irrigation water could also affect the time interval between irrigation and water uptake by the plants. Drip irrigation systems are frequently used also for fertigation, i.e., the distribution of nutrients dissolved in the irrigation water (Bar-Yosef, 1999). This approach allows to precisely supply nutrients to the plants according to their requirements, and also to rapidly recover from possible nutrient deficiencies (Haynes, 1985; Neilsen et al., 2004). Despite the extensive literature dealing with drip irrigation and fertigation, still little is known on the time interval occurring between irrigation and the arrival of irrigation water in tree crops. Aguzzoni et al. (2022) provided first estimates but in their study irrigation water was distributed homogeneously over the soil. To the best of our knowledge, information reflecting real field conditions is still missing.

Several methods that monitor tree sap flow or tree water status can readily detect the response of trees to rapid changes in soil water availability (e.g., following rain or irrigation events) (Blanco and Kalcsits, 2021; Burgess et al., 2001). When plants experience limitations in water availability, a sudden increase of sap flow rate could be directly linked to the previous irrigation, thus allowing to estimate the time interval between irrigation and tree response. However, sap flow and other plant status methods are not able to distinguish between irrigation water and other water sources, and will likely underestimate the plant's response to irrigation when the soil is already wet. Under non-limiting soil water conditions, isotopic tracers can be used to differentiate between water sources, either by tracing a water-soluble compound (Quiñones et al., 2012) or the water molecule itself.

Stable isotopes of hydrogen and oxygen in water are a widely utilized tool in ecohydrological studies in forest and agro-ecosystems (Penna et al., 2020; Sprenger et al., 2019; Vargas et al., 2017). In particular, the use of isotopically enriched water allows to trace the movement of a labelled water source (e.g., irrigation water) through the soil-plant-atmosphere continuum (Penna et al., 2018; Seeger and Weiler, 2021) and to assess its contribution to the overall plant water uptake (Rowland et al., 2008; Penna et al., 2021; Aguzzoni et al., 2022). By repeatedly sampling the trees after the distribution of isotopically labelled irrigation water, it is possible to extrapolate its travel time through the plants and the velocity of water flow within plants (James et al., 2003; Meinzer et al., 2006; Mennekes et al., 2021). Most of the ecohydrological studies to date have been performed in forest environments, with many others focusing on agricultural settings but only a few considering tree crops.

In the present study, we applied deuterium-enriched irrigation water to apple trees in field and pot conditions, addressing the following research questions:

a) How does tree water uptake vary by increasing the amount of irrigation water distributed through an increasing number of drippers?
b) What is the transit time between drip irrigation and the arrival of irrigation water at different heights in the tree canopy?
c) How does the water supplied to only one side of the tree redistribute within the tree canopy?

2. Materials and methods

2.1. Field experiment – Water uptake in trees subjected to different irrigation treatments

2.1.1. Experimental site

The experimental activities were conducted following two approaches: a field experiment in an apple orchard and an experiment in a greenhouse with potted trees.

The field test was conducted in summer 2021 in a 15-year-old experimental apple orchard (cv. Nicoter Kanzi® on M9 rootstock) located close to Ora/Auer (Bolzano province, Italy; N46.3433 E11.2788). Planting distances were 3.0 m x 0.8 m, corresponding to a density of 4167 trees ha^{-1}. Trees were trained as slender spindle with a height of roughly 3.5 m, limited by the presence of black anti-hail nets. Soil type in the orchard is sandy loam (54% sand, 41% silt, and 5% clay). Soil bulk density in the first 0–60 cm soil layer was 1.37 ± 0.12 kg L^{-1}. Long-term measurements with piezometers installed close to the orchard site demonstrated that the groundwater table lies around 2.3 m below soil surface. Three sets of trees corresponding to three irrigation treatments, distributed according to a randomized block design with four blocks located on different rows, were used: 1) single drip line with two drippers per tree (SL2); 2) double drip line with four drippers per tree (DL4); 3) partial root-zone drying with one dripper per tree irrigating only one half of the rootzone at a time (SL1). The study orchard has hosted an irrigation trial since 2019, using the same dripper layout as in the present study. All driplines had 40 cm spaced drippers with a flow rate of 2.3 L h^{-1}. Digital tensiometers were installed to continuously monitor the soil water potential (SWP), and irrigation automatically started when soil water potential reached a threshold of -300 hPa. In SL1, the irrigated side was switched each time SWP in the "dry" side reached -600 hPa. Irrigation frequency in the different growing seasons varied depending on rainfall, averaging 10 irrigation events per year. Additional details can be found in Ben Abdelkader et al. (2022b). A soil water retention curve for the orchard soil was calculated in 2019 by contrasting soil water potential values measured by digital tensiometers (Ben Abdelkader et al., 2022b) and soil water content values measured by TMS-4 probes, both installed at 25 cm depth (SM Fig. 1). The orchard was equipped with a mini meteorological station allowing continuous control of the microclimatic conditions.

2.1.2. Labelled water supply

The experiment was conducted on two typical late summer days (September 1st and 2nd, 2021), with clear sky and T_{max} close to 30 °C (see SM Fig.2 for further details).

A total of 12 trees (one tree per irrigation treatment and per block, i.e., four trees per irrigation treatment) were selected. A by-pass was installed three weeks before the experiment to avoid irrigation on the selected trees and achieve a homogenous soil moisture content among treatments before starting the experiment. A total rainfall of 31.4 mm distributed over three rain events was recorded in the three weeks before the experiment.

Labelled irrigation water was prepared shortly before the experiment by adding 5.0 mL of isotopically heavy water (99.9% ^2H_2O) to 3 L of store-bought still mineral water by means of a micropipette. Two bottles were prepared for each dripper. A representative subsample of the labelled irrigation water was collected to measure the hydrogen isotope ratio (δ^2H), showing a value equal to 12050 ± 125‰.

The three drip irrigation treatments (SL2, DL4 and SL1) were manually reproduced by mounting the bottle containing the labelled water on the top of an adjustable self-watering spike allowing to control the flow. The floater was removed from the dripper during the experiment to increase the water flow rate, achieving an adequate flow of

3 L h^{-1} for each dripper.

In SL2, two drippers were positioned 20 cm from the trunk, along the tree row, on opposite sides of the tree. In SL1, the dripper was positioned as in SL2, but on one side only. In DL4, four drippers were arranged around the tree forming a square, with the tree in the centre and 20 cm away from the middle point of each side of the square (dripper ca. 28 cm from tree). Each dripper supplied 3 L of labelled water. The dripper layout in the different treatments is depicted in Fig. 1.

2.1.3. Shoot and fruit sampling

Before the experiment, 12 shoots within each tree were selected at two heights and then tagged (six shoots per tree and per height). Bottom shoots were selected from branches departing at a height of 1.5 m from the ground, with an average total distance of 1.49 ± 0.22 m separating the shoot base from the ground. Top shoots originated from branches inserted at 3.0 m of height with an average total distance of 2.86 ± 0.17 m from the shoot base to the ground.

In SL1, the number of samples was doubled as shoots were collected both from the irrigated side and the dry side of the tree, within each height.

The shoot sampling was performed one hour after the end of the labelled water supply and was repeated at 2, 4, 6, 8, and 32 h after the irrigation. The leaves and the shoot bark were removed, and the wood collected for the H isotope ratio analysis. Shoots were further cut into shorter sections to make them fit inside the 12 mL Exetainer® vials (Labco Ltd., UK), which were sealed with a screw cap with rubber septum.

Fruits were sampled from SL2 trees only: two fruits per tree were collected at each sampling time (one in the bottom and one in the top part of the canopy). Fruit sampling was carried out at 2, 4, 6, 8, 10, 22, and 32 h after the irrigation. Whenever possible, we selected fruits located at the base of the selected shoot (fruit and shoot originating from the same mixed bud); if no fruit was present, we selected the closest fruit to the base of the selected shoot. After picking the fruit, a slice was cut from each apple (going from the surface all the way to the core), peeled, and subdivided in smaller slices to make it fit in the vial, as described for the shoot samples.

To minimize water loss and potential isotope fractionation, all samples were stored in a cooled container and then frozen until processing (Millar et al., 2022).

2.1.4. Soil sampling

To avoid any disturbance to the soil around the trees used for shoot and fruit sampling, the dynamics of irrigation water in the soil were assessed on four additional trees belonging to treatment SL2: one hour after the end of irrigation with labelled water, soil samples were collected below each dripper and 20 cm outwards from it, towards the centre of the orchard alley (Fig. 1). After the last shoot sampling (32 h after irrigation), a second soil sampling was performed, following the same scheme, directly beneath the trees used for the labelling experiment.

Sampling was carried out using a soil auger, up to 60 cm depth divided into three layers of 20 cm each. From each soil layer, two representative samples were collected. The first sample was transferred to airtight vials, sealed, frozen, and stored until processing for the isotope analyses. The second one was placed in plastic bags and used to measure the gravimetric water content after 48 h of oven drying at 105 °C.

Soil samples were then subjected to water extraction followed by analysis of the isotopic composition.

2.1.5. Root sampling

At the end of winter following the experiment (8th March 2022) soil samples below the trees that were used for shoot sampling were collected to assess root density. Soil cores (single layer 0–40 cm depth, diameter 5 cm) were collected in correspondence of the drippers for all trees used in the labelling experiment. On three trees belonging to the DL4 treatment, additional soil cores were collected in other positions; a schematic diagram of the position of soil cores relative to the tree trunk is provided in the Supplementary Material (SM Fig.3).

Soil samples were transferred to plastic bags and stored frozen until analysis. The samples were then sieved to isolate the roots, which were further subdivided into fine roots (diameter < 2 mm) and coarse roots (> 2 mm) and weighed. A subsample of both fine and coarse roots was oven dried (65 °C, 48 h) and used to determine the root dry weight.

2.1.6. Estimation of sap flow velocity

Two approaches were used for estimating tracer velocity. The first approach followed the method reported by Meinzer et al. (2006): the δ^2H value of every single shoot was normalized to the maximum δ^2H value reached in the tree. We calculated the tracer velocity (cm h^{-1}) dividing the root-shoot distance (cm) by the time needed for the tracer to reach 10% of its maximum δ^2H value (Meinzer et al., 2006). We assumed as maximum δ^2H values those recorded at the last sampling (32 h after irrigation), based on Aguzzoni et al. (2022), who found that δ^2H shoot values reached a peak and leveled off after 24 h from irrigation in a similar field experiment in an apple orchard. Root-shoot distance was obtained by adding 30 cm (an assumed average root depth in the studied orchard) to the measured distance between ground level and the base of each sampled shoot.

The second approach consisted in determining the first sampling time at which the shoot δ^2H values were significantly different from the δ^2H values recorded before irrigation. To derive tracer velocity, the root-shoot distance was divided by such a time.

2.1.7. Pot experiment

Twelve 2-year-old, bare-rooted apple trees (cv. Reanda, rootstock M9) were potted in 23 L pots filled with a silty loam soil (28% sand, 55% silt, 17% clay) and transferred in an open area under a transparent shelter. Before the experiment, the trees were constantly irrigated to

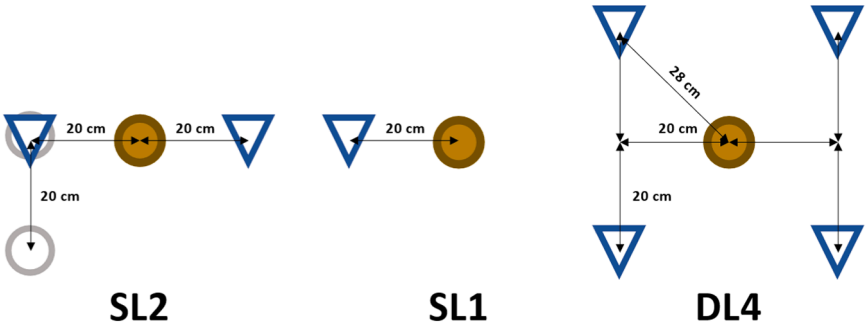

Fig. 1. Positions of drippers and soil cores for soil moisture and isotope analysis - triangles indicate the drippers and grey circles indicate soil sampling positions relative to the tree trunks (brown circles); SL1, SL2, and DL4 indicate the irrigation treatments.

avoid any water stress. Since the main goal was to determine water flow in vegetative organs, flowers were removed manually to avoid interference from developing fruitlets.

The experiment was carried out on July 6th, 2021. The soil in each pot was tilled superficially to facilitate water infiltration and a metal ring was placed under each pot to allow excess water to percolate. Pots were weighed before starting the experiment. ^2H enriched irrigation water was prepared by adding 10 mL of ^2H_2O (99.9%) to 40 L of tap water ($\delta^2H = 1631‰$). Control samples were collected just before irrigation, sampling one lateral branch (50–100 cm above the graft union) from three trees selected without any specific criteria.

All trees were irrigated with enriched water (4 L per pot) starting from 9:15AM, distributed gradually to allow infiltration in the soil. Irrigation ended at 10AM. After irrigation, pots were covered with plastic film and aluminium foil to avoid evaporation from the soil surface.

Plant samples were collected at 1, 2, 4, and 8 h after the end of irrigation. Before each sampling, pots were weighed again to estimate tree water uptake and transpiration. For each sampling, a 5 cm section of the xylem was collected from the stem of three trees. For each tree and at all sampling times, stem sections were collected from four different positions along the tree axis: few centimetres below the graft union (0 cm), and 50 cm, 100 cm, and 150 cm above the lowest position.

Xylem samples for isotope analysis, after bark removal, were placed in airtight glass vials, with screw cap and rubber septum, sealed and frozen until further treatment to avoid evaporation and potential isotope fractionation due to the high ambient temperature at the time of sampling (Allen et al., 2019; Poca et al., 2019). Meteorological conditions were monitored by a weather station installed close to the experiment location. Air T_{max} on the experimental day was slightly above 30 °C (SM Fig.4).

2.1.8. Water extraction from samples

Water was extracted from tree and soil samples by cryogenic vacuum distillation described by Koeniger et al. (2011), with slight modifications. The vials filled with frozen samples were connected through capillaries to empty vials. Vacuum distillation took place at 200 °C for 15 min with the vaporizing water cold trapped in the empty vials. After defrosting at room temperature, in sealed conditions, the water fraction was sampled for subsequent isotope analysis.

After extraction, sample weights were compared to the oven-dried weights (105 °C, 24 h) determining a water extraction efficiency higher than 99%. Hence, bias due to incomplete water extraction were excluded (Araguás-Araguás et al., 1995; Bowers et al., 2020).

2.1.9. Isotope analysis

The hydrogen isotope composition of the enriched irrigation water and of soil water extracts was measured using a Picarro cavity ring down spectrometer (CRDS L2130-I, Picarro Inc.) equipped with a vaporizer unit for liquid water injection (vaporization module A0211, Picarro Inc.) and an autosampler (A0325, Picarro Inc.). Results were processed using the Picarro's ChemCorrect post-processing software package. Memory effect was minimized following the procedure described in Penna et al. (2012). Due to the presence of organic contaminants, water samples extracted from tree organs were not suitable for the hydrogen isotope analysis at the CRDS and were analysed at the mass spectrometer. For each sample, 0.2 mL of extracted water was transferred into a vial together with a Pt stick as catalyst and flushed with an equilibration gas (2% H_2 in helium) for 40 min. After equilibration, the vial headspace was analysed with a Gas Bench II (Thermo Scientific) coupled to a Continuous Flow Isotopic Ratio Mass Spectrometer (CF-IRMS, Delta V Advantage Conflo IV, Thermo Scientific).

Isotope ratios were expressed in delta (δ) notation, relative to the VSMOW international standard (Vienna Standard Mean Ocean Water), and reported in parts per thousand (‰). The precision, expressed as twice the standard deviation of multiple standard injections, was < 1.5‰ at the CRDS and < 3.0‰ at the IRMS. Since a previous study demonstrated the result consistency between CRDS and IRMS measurements of water samples collected in an apple orchard and analysed in the same laboratory (Penna et al., 2021), results of the hydrogen isotope ratio were combined without further corrections.

2.2. Mixing models

A two end-member mixing model (Aguzzoni et al., 2022; Pinder and Jones, 1969) was applied to quantify the fraction of ^2H-enriched irrigation water present in soil, shoots, and fruits, in both experiments. Stable isotope composition measurements of soil and plant water have been performed in the studied orchard since several years. Isotopic composition of xylem samples collected at eye level from secondary branches was variable over time, with a general increasing trend from the beginning to the end of the growing season. In 2021, the average δ^2H value for xylem, calculated for the entire growing season (March--November), was $-61.4 \pm 14.4‰$ (n = 77). This value increased to $-52.8 \pm 4.5‰$ (n = 6) when only the period of our experiment was considered. Soil water δ^2H values in the days before the experiment were equal to $-47.4 \pm 13.5‰$ (n = 8).

We assumed that the sampled soil water was composed by pre-irrigation soil water and irrigation water. Similarly, for shoot (or fruit) water, we assumed that the sampled shoot (or fruit) water was composed by pre-irrigation shoot (or fruit) water and irrigation water. Shoot water was assumed to have the same isotopic signature as the shoot (or fruit) water of control trees. Pre-irrigation and irrigation water together correspond to the totality of the water present in the soil, shoot, and fruit samples.

Based on these assumptions, we applied the mixing model as follows:

$$F_{IW\ \ (shoots,fruits)} = \frac{\delta^2 H_{irrigated\ \ tree} - \delta^2 H_{control\ \ tree}}{\delta^2 H_{irrigation\ \ water} - \delta^2 H_{control\ \ tree}} \quad (1)$$

where F_{IW} is the fractional contribution of irrigation water (IW) to the total water sampled from the shoots or fruit in the irrigated trees, and δ^2H represents the isotope composition of shoot or fruit water.

The same approach was used to determine the fraction of soil water composed by irrigation water:

$$F_{IW\ \ (soil)} = \frac{\delta^2 H_{irrigated\ \ soil} - \delta^2 H_{control\ \ soil}}{\delta^2 H_{irrigation\ \ water} - \delta^2 H_{control\ \ soil}} \quad (2)$$

where F_{IW} is the fractional contribution of irrigation water to soil water, and δ^2H represents the isotope composition of soil water.

2.3. Statistical analysis

^2H abundance data displayed a rather large variability. δ^2H values did not meet the assumptions of normality, so they were first transformed into positive values and then log-transformed. After that, a linear mixed-effects model was fitted to the data and multi-factor ANOVA was applied to test the effects of the different factors. In the field experiment, treatment, sampling position and time after irrigation, and block were regarded as fixed factors, whereas the tree (subject) was considered a random factor. Data from SL1 treatment were subjected to an additional analysis to test the differences in isotopic enrichment between two tree sampling sides (from the canopy above the dripper or above the non-irrigated side, SL1 Fig. 1). Fixed factors in the pot experiment were sampling time (hours after irrigation) and sampling height, with the tree (subject) as random factor. Root distribution was analysed for the effects of treatment, position, and block (fixed), and tree (random). The significance threshold was set at $p < 0.05$. Statistical analysis was performed in the R environment (R Core Team, 2021). Means separation was performed by applying Tukey's method for multiple comparisons. Data are presented as mean ± standard deviation, unless otherwise

specified.

3. Results

3.1. Soil water dynamics

The soil gravimetric water content below and around drippers just after the irrigation event and after 32 h is depicted in Fig. 2. Similarly, Fig. 3 reports the isotopic composition and the fraction of irrigation water present in the soil water at different depths and positions around the drippers.

In proximity of the dripper, soil moisture and δ^2H values sharply increased following the irrigation (Fig. 2 and Fig. 3). One hour after irrigation, soil moisture was higher in the top 20 cm of soil compared to the deeper soil layers below the dripper, whereas soil moisture was more uniform over the entire profile at 20 cm from the dripper. This pattern is reflected also by the δ^2H values of soil, with virtually all the labelled water located in the shallowest soil layer below the dripper. After 32 h from irrigation, soil moisture was more evenly distributed, reaching the deepest soil layer (40–60 cm depth) and soil volumes spaced 20 cm from the drippers. The maximum δ^2H value was still registered close to the dripper, with only small enrichment recorded for deeper soil layers or 20 cm away from the dripper. This is also reflected by the fraction of irrigation water (F_{IW}) present in the different soil layers after 32 h: irrigation water accounted for more than 70% of soil water in the uppermost soil layer below the dripper, while this share was only 6% in the layer of 20–40 cm depth below the dripper and 5% in the uppermost 20 cm at 20 cm distance from the dripper (Fig. 3). The presence of irrigation water in other positions (deeper than 40 cm below the dripper and deeper than 20 cm away from the dripper) was negligible, with a calculated fraction of irrigation water not exceeding 2%.

3.2. Root distribution

The average root density below the dripper was not significantly different among treatments (Table 1). Considering DL4, we could detect a general pattern of decreasing root density at increasing distances from the trunk, but the differences were not statistically significant (data not shown). Our root density data are consistent with an extensive survey of apple tree root distribution in the same orchard carried out in autumn 2018, reported in the Supplementary Material (SM Fig.5).

3.3. Tree water dynamics

The temporal evolution of shoot δ^2H values after irrigation is reported in Fig. 4 (results of ANOVA are reported in SM Table 1). A progressive isotopic enrichment over time is visible in all treatments and at both sampling heights. A clear time lag between the two sampling positions is especially evident in SL2 and DL4. The isotopic composition of shoot water was higher ($p < 0.05$) than pre-irrigation values in the bottom part of the canopy after 6 h for SL1 trees and after 4 h for SL2 and DL4 trees. In the top part of the canopy, a significantly higher isotopic composition was found after 8 h for SL1 trees and 6 h for SL2 and DL4 trees. At 1 and 2 h from irrigation, no significant differences were found between treatments. In the bottom part of the canopy, starting from 4 h after irrigation onwards, SL2 and DL4 trees showed a similar isotopic enrichment and significantly higher than SL1, in line with the fact that trees belonging to those treatments had received more labelled irrigation water. In the top part of the canopy differences were detected between DL4 and SL1 from 6 h after irrigation onwards, whereas SL2 had intermediate values. After 32 h from irrigation, DL4 and SL2 showed similar and significantly higher values than SL1 (SM Table 2). Significantly different values between the bottom and top part of the canopy could be detected at 4 and 6 h and at 4, 6, and 8 h after irrigation for SL2 and DL4, respectively. In all treatments, we could not detect any difference between the two positions at the end of the experiment (32 h after irrigation).

The relative contribution of irrigation water to total shoot water at the end of the experiment (32 h after irrigation) reached values of around 3.5%, 2%, and 1%, for trees belonging to DL4, SL2, and SL1, respectively (Fig. 4).

When trees were irrigated by only one dripper (SL1), the δ^2H values of shoots sampled from two opposite sides of the tree were compared (Fig. 5). In the bottom part of the tree (1.5 m), we could detect slightly higher values in the tree side directly above the dripper (drip) compared to the opposite side (no-drip), with significant differences at 6 h and 32 h from irrigation. δ^2H values of both sides were similar in the top part of the canopy and at all the other sampling times.

We also measured the isotopic composition of apples (data not shown). The δ^2H values of fruit samples ranged from − 91.9‰ to − 27.9‰ (mean −62.7‰) and remained constant over the course of the experiment. Accordingly, the fractional contribution of irrigation water to apples was null at all sampling times.

The velocity of water uptake and transport within the sap flow was

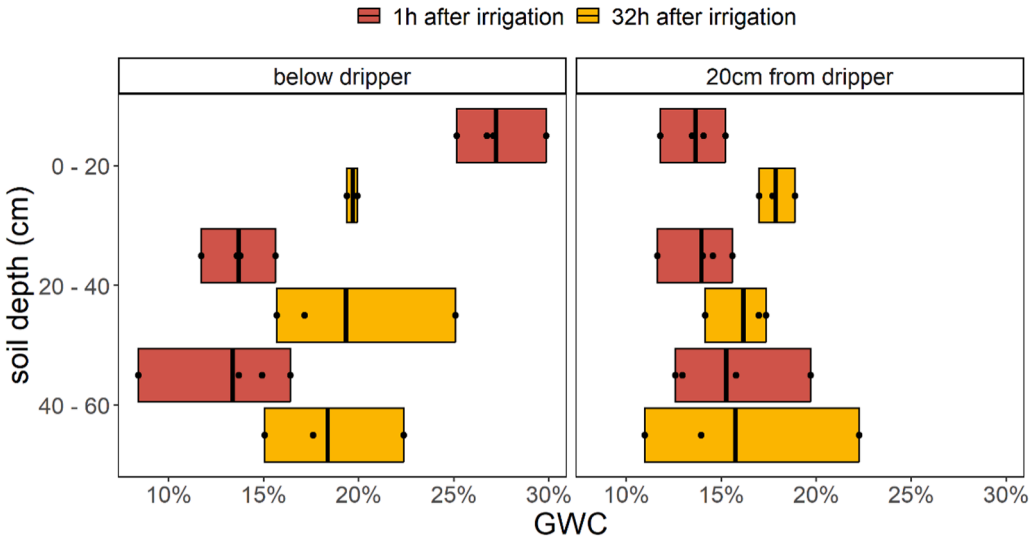

Fig. 2. Field experiment - Soil gravimetric water content at different depths and positions around the dripper. Data refer to treatment SL2 only (n = 4 and n = 3 for the sampling at 1 h and 32 h after irrigation, respectively). Bars span from the minimum to the maximum values, with the central line indicating the mean. Points refer to individual measurements. GWC: gravimetric water content.

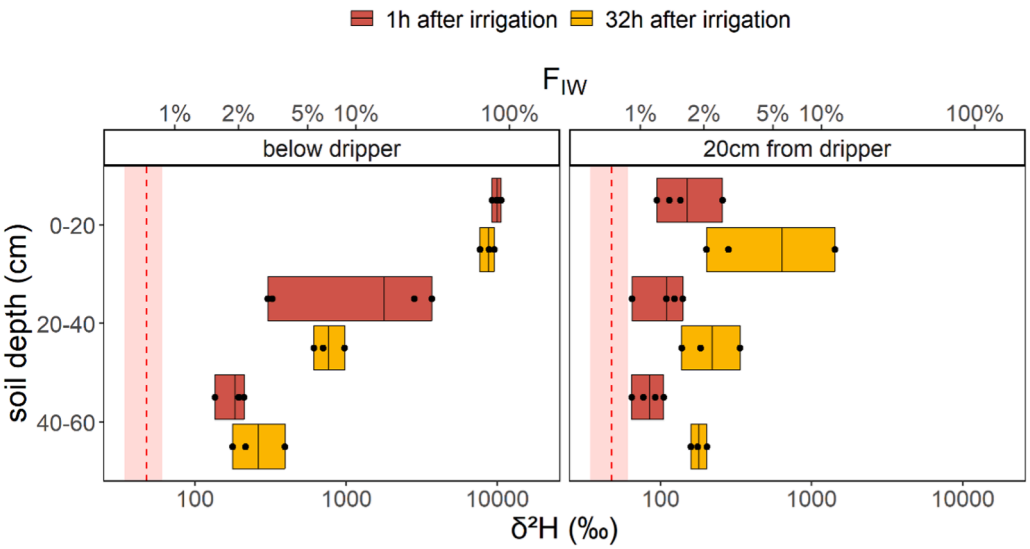

Fig. 3. Field experiment-- Isotopic composition (δ^2H) and fraction of irrigation water (F_{IW}) in soil water at different depths and positions around the dripper. Data refer to treatment SL2 only (n = 4 and n = 3 for the sampling at 1 h and 32 h after irrigation, respectively). Bars span from the minimum to the maximum values, with the central line indicating the mean. Points refer to individual measurements. The red dashed line represents the mean control value measured before irrigation, with the shaded area extending ± 1 standard deviation around the mean.

Table 1
Average density (g dry weight L^{-1}) of fine (< 2 mm) and coarse (> 2 mm) roots in the 0–40 cm layer below the drippers in the different treatments (mean ± standard deviation).

Treatment	Fine root density g DW L^{-1}	Coarse root density g DW L^{-1}
SL1	0.184 ± 0.089	1.120 ± 0.742
SL2	0.314 ± 0.183	2.370 ± 3.644
DL4	0.175 ± 0.116	1.548 ± 2.692
	n.s.	n.s.

calculated following two methods and the results are summarized in Fig. 6. The two estimates produced consistent results both between treatments and between sampling heights, ranging from 44.4 to 65.8 cm h^{-1}. Sap flow velocity estimates calculated for the top and bottom shoots were similar (64.4 ± 12.6 cm h^{-1} and 59.9 ± 17.7 cm h^{-1}, respectively). The error bars for the "first arrival" method only represent the between-tree variability in shoot-to-ground distance, since the method only allowed to calculate one transit time for all trees in the same group (see Section 2.1.6).

3.4. Pot experiment

The δ^2H values and the fraction of irrigation water in xylem water at

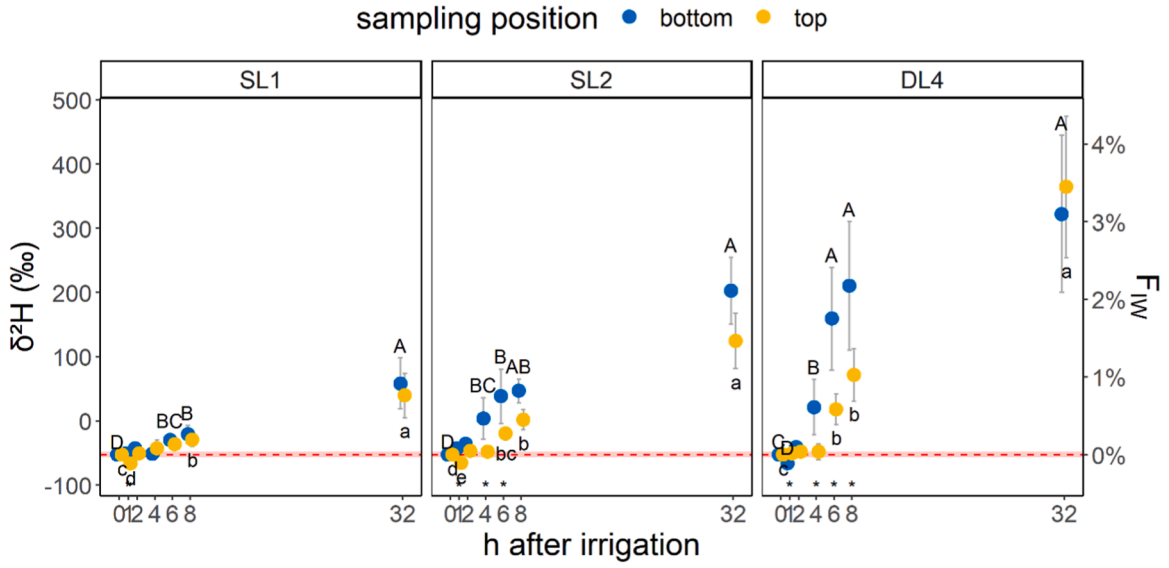

Fig. 4. Field experiment - Isotopic composition (δ^2H) and fraction of irrigation water (F_{IW}) in shoots at different heights and at different sampling times for trees subjected to different treatments. Points represent mean values, with the error bars indicating ± standard error (for treatment SL1, points are the average of both sides of the canopy). Letters indicate differences ($p < 0.05$) among sampling times within each treatment and height (uppercase and lowercase letters refer to bottom and top positions, respectively). Letters are only shown for points which are significantly different from the control values. Asterisks denote significant difference ($p < 0.05$) between the two sampling positions within each sampling time. The red dashed line represents the mean isotopic composition of shoots before irrigation, with the shaded area representing ± 1 standard deviation.

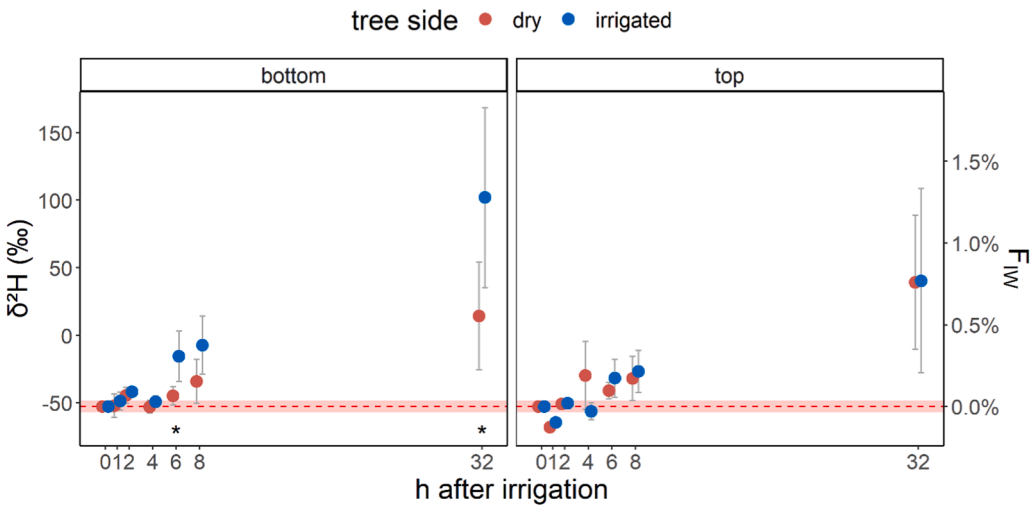

Fig. 5. Field experiment - Isotopic composition (δ^2H) and fraction of irrigation water (F_{IW}) at different heights (bottom, left panel, and top, right panel) and at different sampling times in shoots from the two opposite sides of the canopy in treatment SL1. "Irrigated" refers to the part of the canopy above the dripper; "dry" refers to the opposite side, see Fig. 1 (SL1). Asterisks denote significant difference ($p < 0.05$) between tree sides at each sampling time. Points represent mean values, with the error bars indicating ± standard error. The red dashed line represents the mean isotopic composition of shoots before irrigation, with the shaded area representing ± 1 standard deviation.

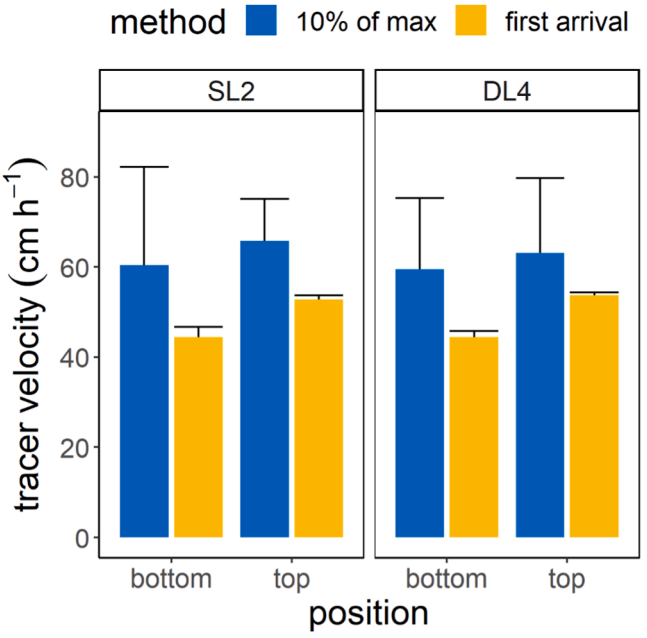

Fig. 6. Field experiment - Sap flow velocity for different heights calculated according to Meinzer et al. (2006) ("10% of max", blue bars) and based on the first sampling time at which the isotopic composition was different from control values ("first arrival", yellow bars). Values are represented as mean ± standard deviation.

different sampling times and at different heights along the tree trunk are presented in Fig. 7 (results of ANOVA are reported in SM Table 3). The δ^2H enrichment showed an increasing pattern from 1 to 8 h after irrigation at all heights. The bottom parts of the trunk (0 cm and 50 cm) displayed a notable increase in δ^2H already 1 h after the irrigation, but the difference was significant for the position at 0 cm only, while at 50 cm the difference was significant after 2 h from irrigation. A significant enrichment at 100 cm and 150 cm could be detected at 2 h from irrigation. At 4 h after irrigation, the isotopic composition of xylem water was similar among all sampling heights. The isotopic composition at the last sampling (8 h) did not vary considerably.

By considering the time needed for the isotopic composition to become significantly different from the control values (measured before irrigation) in the highest position along the trunk (150 cm, 2 h; Fig. 7), we could estimate a sap flow velocity of 75 cm h^{-1}.

Although the soil was saturated with labelled water following the irrigation, the fraction of irrigation water in the xylem never reached, on average, values above 30%.

4. Discussion

4.1. Effects of different dripper configurations and irrigation amount on water uptake

Immediately after the irrigation event, soil moisture in the top layer of soil directly below the dripper was higher compared to deeper soil layers and to the soil portions located at 20 cm distance from the dripper. Soil moisture redistributed laterally and penetrated to deeper soil layers over time. Some extent of percolation from the upper (0–20 cm) to the lower (20–40 cm) soil layer was likely, since the soil water content in the upper soil layer immediately after irrigation (Fig. 2) was very close or above field capacity (SM Fig.1) (Ben Abdelkader et al., 2022b). Interestingly, the labelled irrigation water (Fig. 3) remained relatively confined to the soil volume underneath the dripper even after 32 h from irrigation. Comparing data in Fig. 2 and Fig. 3, it appears that the spread of the labelled irrigation water did not match the changes in soil moisture, since the soil moisture increase was proportionally larger compared to the increase in δ^2H values. We speculate that the recently provided irrigation water (with a highly enriched isotopic composition) was able to displace the previously present soil water (with a natural isotopic composition), pushing it down towards deeper soil layers, following a sort of piston-type water flow in the soil (Gazis and Feng, 2004). Under such circumstances, the mixing between these two water pools was rather limited during the period of observation.

One of the main questions of this work was to test whether different dripper configurations around the trees, delivering increasing amounts of irrigation water, could modify the extent and velocity of irrigation water uptake. Knowledge on the time interval between the delivery of irrigation water and its uptake and redistribution within cultivated trees could be valuable, for example when considering the recovery from drought. A short time interval would imply that trees can benefit from irrigation nearly immediately. In highly specialized crops such as apple

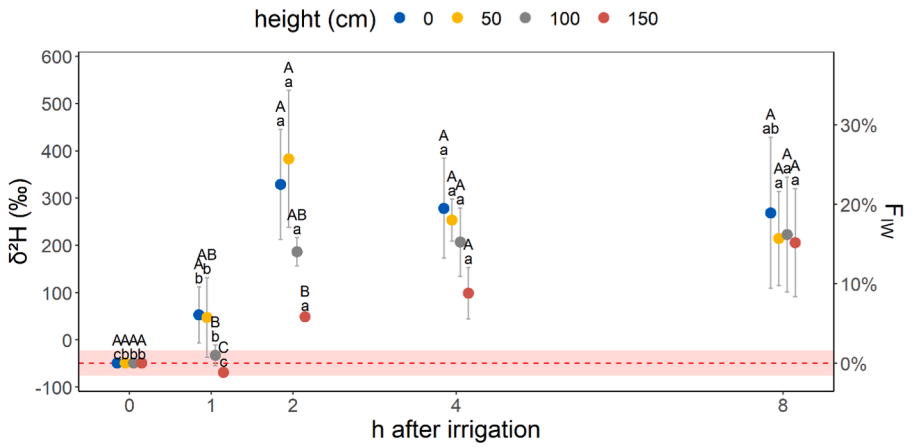

Fig. 7. Pot experiment. Isotopic composition (δ^2H, left axis) and fraction of irrigation water (F_{IW}, right axis) in xylem water at different sampling times and at different heights along the stem (points represent mean ± SE, n = 3). Uppercase letters indicate differences ($p < 0.05$) among heights within each sampling time, lowercase letters indicate differences among sampling times within each sampling height. The red dashed line represents the control value measured before irrigation (mean $\delta^2H = -49.3‰$), with the shaded area extending ± 1 standard deviation around the mean.

orchards, irrigation water is frequently used to deliver nutrients to the trees (fertigation). Thus, the time interval between irrigation and water uptake would be useful also for nutrient management purposes. During the summer preceding the field experiment, the differential water supply methods (SL1, SL2 and DL4) did not significantly affect the water status of apple trees, suggesting that DL4 trees could have been over-watered (Ben Abdelkader et al., 2022b). This was likely due to frequent summer rainfall, which provided homogeneous and adequate soil moisture levels before the experiment. In our field experiment, the labelled irrigation water in the shoots located in the bottom part of the canopy could be detected after 4 h from irrigation. Here, the two largest amounts of irrigation water supplied through two (SL2) or four drippers per tree (DL4) also determined the highest fraction of irrigation water in the shoots (3.5% and 2% in DL4 and SL2, respectively, compared to 1% in SL1). In a similar experiment with field-grown apple trees, where labelled irrigation water was supplied mimicking a sprinkler system (40 mm), Aguzzoni et al. (2022) first detected irrigation water in the shoots after 2–4 h from irrigation, although they found higher F_{IW} values, up to 7.5%, in line with the larger volume of irrigation water as compared to our experiment. It can be concluded that by increasing the number of drippers and delivering a proportionally larger amount of water, the irrigation water was made available to an increasingly larger proportion of the root system, ultimately resulting in greater uptake. Under the current climate change scenarios, the resulting water uptake increase might be crucial to offset the increasing atmospheric evaporative demands, which especially occur during the summer heat waves. If irrigation water is sufficient, systems able to wet a larger fraction of the roots (e.g., single drip lines with narrower dripper spacing, multiple drip lines, or microsprinkler) would result in an increased irrigation water uptake by trees, in particular in coarse-textured soils like the one in our study orchard.

We have not measured soil moisture before the water supply, but soil water content data reported in Fig. 2 at 20 cm distance after one hour from the labelled water supply (around 13–14% w:w) provide an indication of the soil moisture to which the roots almost unaffected by the deuterium tracer (F_{IW} less than 2%) were exposed. Considering the soil bulk density of 1.37 kg L^{-1} and the water retention curve experimentally determined for the orchard soil (SM Fig.1) it is clear that while roots under the drippers took up labelled water, the water uptake by the remaining part of the root system was not limited by water scarcity, being the water potential values higher than − 50 kPa, which would exclude water stress in all treatments.

Our experimental design did not allow us to disentangle the effects of the amount of irrigation water from its spatial distribution through an increasing number of drippers. Future research should investigate whether delivering the same amount of irrigation water per tree using a different number of drippers could affect the irrigation water use efficiency.

The isotopic composition in the top canopy of field-grown trees reached a significant difference from control values with a 2 h delay compared to the bottom part of the canopy, irrespective of the treatment (Fig. 4). So, even if the time of first detection of irrigation water in the trees was seemingly affected by the amount and localization of irrigation water, the temporal dynamics of redistribution within the trunk were rather uniform.

4.2. *Estimation of sap flow velocity*

Sap flow density and sap flow velocity are important physiological variables to consider when assessing water fluxes in the soil-tree-atmosphere continuum. Nowadays, electronical sensors are available to monitor sap flux, based on different working principles (Burgess et al., 2001; Cohen et al., 1981; Nadezhdina, 2018). One drawback of such method is the within-tree variability in sap flow measurements, with notable differences depending on probe insertion depth (radial variability) and azimuthal position around the trunk (Cohen et al., 2012). Moreover, sap flow sensors can underestimate the actual sap flow velocity (James et al., 2003; Meinzer et al., 2006). The use of an isotopic tracer could minimize the effects of local variability in the tree vascular system and allow for a better estimate of sap flow. Which method should be used to evaluate the tracer transit time is still a matter of debate (Mennekes et al., 2021). In line with previous studies (Schwendenmann et al., 2010; Gaines et al., 2016), we followed the approach proposed by Meinzer et al. (2006) and estimated the tracer arrival time as the time at which the xylem isotopic composition reached 10% of the maximum value. Due to the limited duration of our experiment (last sampling 32 h after irrigation), we considered the value at the last sampling as the maximum, in accordance with Aguzzoni et al. (2022), who found that the δ^2H values of shoot axes peaked and levelled off after 24–48 h from a labelled irrigation water supply in a similar experiment with field-grown apple trees. As an alternative to this method, we explored a "statistical" approach, by comparing the δ^2H values at each sampling time to those measured before the irrigation. The first sampling time at which the difference was significant was considered as the tracer arrival time and it was used to calculate tracer velocity. This method slightly underestimated the results of the former approach (Fig. 6). Limitations in our method include the limited sample size (n = 4 for each combination of treatment, sampling position, and sampling time), coupled with a large

among-tree variability. In addition, this method is "discrete", i.e., only times in which sampling was performed can be considered as tracer arrival times. It follows that larger sample size and more frequent samplings are needed when applying this approach to more precisely estimate the tracer arrival time. In a recent study, applying in situ methods, tracer arrival time was determined as the first moment after the delivery of labelled water at which the δ^2H value was above the range of values measured during the two days prior to the labelling (Mennekes et al., 2021). This latter approach makes the assessment less arbitrary than that proposed by Meinzer et al. (2006), but it requires very dense datasets which are only obtainable with in situ techniques. Despite the increasing interest towards in situ measurements, we argue that destructive samplings will still be largely applied in future studies due to their relative simplicity, in spite of their problems related to temporal and spatial resolution. For this reason, we highlight the need for a common approach to estimate travel times based on isotopic data.

The sap flow velocity estimates found in our study are rather homogeneous regardless the approach used, the tree height considered, and the fact that data come from both field-grown and potted trees. Data of sap flow velocity (0.60–0.65 m h^{-1}, Fig. 6) are in line with data obtained in apple trees by means of sap flow sensors (Ben Abdelkader et al., 2022a; Green and Clothier, 1988). It should be noted that the estimates reported in Fig. 6 are calculated from the time since irrigation end, thus they also included the time needed for irrigation water to travel from the soil surface to the roots and the time for water uptake by the roots. However, since we sampled two different positions within the trees, we could also get a separate estimate considering only the xylem transport within the tree stem and branches. Fig. 4 clearly shows that the δ^2H values at 8 h after irrigation in the top of the canopy were nearly the same to those at 6 h in the bottom of the canopy, suggesting that the tracer took around 2 h to move from the bottom (1.49 m) to the top of the canopy (2.86 m), thus at a velocity of about 0.69 m h^{-1}.

4.3. Redistribution in the canopy of highly localized irrigation water

A further aim of our research was to assess the redistribution of irrigation water in trees in which irrigation water was delivered to only one fraction of the soil volume explored by tree roots, reproducing the conditions experienced by trees under the partial rootzone drying technique. Partial rootzone drying has been applied to several crops, including apple, to increase water use efficiency (Ben Abdelkader et al., 2022b; Caspari et al., 2004; Leib et al., 2006). This irrigation strategy implies the supply of water to a limited fraction of the tree root volume, similar to what we have experimentally simulated in the SL1 treatment. In the bottom part of the tree (1.5 m height), the labelled irrigation water was more concentrated in the dripper side as compared to the opposite side, located above a non-irrigated soil portion (Fig. 1 and Fig. 5). We speculate that this result depends on the direct vascular connections between the roots and the portion of the canopy located on the same side of the tree (McElrone et al., 2021; Nadezhdina, 2010; Schulte and Costa, 2010). As sap flow is governed by water potential gradient (Franks and Brodribb, 2005; Tyree and Zimmermann, 2002), the axial flow should be predominant on the radial or tangential flow in the lower part of the canopy, where no or few branches were present. Interestingly, no differences in the labelled water abundance were found between the two sides of the tree canopy at 3 m height, suggesting that exchanges occurred between vessels in the portion of trunk above 1.5 m. An isotope-based study on beech trees revealed a certain degree of communication between sapwood and heartwood (Fabiani et al., 2022) and other studies on several tree species have shown that interactions between vessels increase moving from the bottom to the top part of the tree (Nadezhdina, 2010 and citations therein). We conclude that apple trees display a certain degree of functional hydraulic sectoring, a behaviour that was recently described for grapevine (McElrone et al., 2021). This could have consequences on the availability of water and xylem-mobile nutrients in the different parts of the canopy. According to our findings, however, such consequences would be limited to the lower part of the canopy. It is thus unlikely that the adoption of a well-managed partial rootzone drying irrigation strategy would lead to water or nutrient deficits at the whole tree scale.

4.4. Limitations of cryogenic vacuum distillation

In the field experiment we observed an increasing pattern of isotopic composition in the xylem samples even if the fraction of irrigation water remained low (around 3.5% in DL4), likely due to the fact that irrigation water only reached a fraction of the rootzone and the provided amount of irrigation water was insufficient to saturate the xylem vessels of the trees. On the contrary, in the pot experiment the soil was entirely saturated with labelled irrigation water, so the entire root system could potentially absorb labelled water. Even under the pot conditions, however, the maximum δ^2H value of tree water was far below that of soil water and the fraction of irrigation water in shoots reached a plateau soon after the irrigation (around 20–30%). A similar result was obtained in a previous study on apple trees (Aguzzoni et al., 2022), where the authors inferred that the labelled irrigation water would only partially mix with resident water in the plant tissues, suggesting a strong compartmentalization of water within the trunk and shoot cells. Cryogenic vacuum distillation is known to extract the totality of water present in the sample (Koeniger et al., 2011), thus it is not able to selectively retrieve the water present inside the xylem vessels (Barbeta et al., 2022; Millar et al., 2018). It is therefore possible that in our pot experiment the xylem vessels were entirely filled with labelled irrigation water, but this observation was masked by the presence of significant amounts (about 60–70%) of pre-irrigation water outside of the xylem vessels, which did not mix with water in the transpiration stream. This highlights the need for suitable water extraction methods to assess the composition of water inside xylem vessels only, which is one of the aims of many ecohydrological studies recently published (Barbeta et al., 2022; Penna et al., 2021; Zuecco et al., 2022).

5. Conclusions

In this study, the application of isotopically labelled irrigation water allowed to assess the velocity and extent of water uptake in apple trees subjected to increasing amounts of irrigation water delivered by an increasing number of drippers. In our field experiment, the enriched irrigation water in the soil remained relatively close to the dripper in the 32 h following irrigation.

Trees irrigated by two and four drippers per tree took up larger amounts of irrigation water compared to those irrigated over a single dripper. Irrigation water could be detected in the shoot axes in the bottom part of the canopy already after 4 h from the irrigation, and after 6 h it was visible in the top part of the canopy.

The use of water isotopes as tracers to estimate the velocity of sap flow yielded results (0.60–0.65 m h^{-1}) that are consistent both in field and pot conditions. The velocity of water transport within the tree was not affected by the irrigation treatment. Our results underline the validity of isotope techniques in assessing sap flow velocity in trees and in tracing water movement in soil and plants.

The distribution of the labelled irrigation water between the part of the canopy above the portion of roots receiving the irrigation water and the opposite part of the canopy suggests that apple trees display a functionally sectored xylem, similarly to what was recently found in other crop species. However, differences could only be detected in the bottom part of the canopy, confirming that even if water (and possibly mobile nutrients dissolved in it) is distributed to a limited fraction of the roots, it is then redistributed rather homogeneously to the entire tree canopy. This highlights the suitability of highly localized water distribution systems, like partial rootzone drying.

Tracing experiments aiming at maximizing irrigation water efficiency could further contribute to determine the optimal way of

delivering water to the trees.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2023.108532.

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