FOCUSED REVIEW

Quantification of root water uptake and redistribution using neutron imaging: a review and future directions

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SUMMARY

Quantifying root water uptake is essential to understanding plant water use and responses to different environmental conditions. However, non-destructive measurement of water transport and related hydraulics in the soil–root system remains a challenge. Neutron imaging, with its high sensitivity to hydrogen, has become an unparalleled tool to visualize and quantify root water uptake *in vivo*. In combination with isotopes (e.g., deuterated water) and a diffusion–convection model, root water uptake and hydraulic redistribution in root and soil can be quantified. Here, we review recent advances in utilizing neutron imaging to visualize and quantify root water uptake, hydraulic redistribution in roots and soil, and root hydraulic properties of different plant species. Under uniform soil moisture distributions, neutron radiographic studies have shown that water uptake was not uniform along the root and depended on both root type and age. For both tap (e.g., lupine [*Lupinus albus* L.]) and fibrous (e.g., maize [*Zea mays* L.]) root systems, water was mainly taken up through lateral roots. In mature maize, the location of water uptake shifted from seminal roots and their laterals to crown/nodal roots and their laterals. Under non-uniform soil moisture distributions, part of the water taken up during the daytime maintained the growth of crown/nodal roots in the upper, drier soil layers. Ultra-fast neutron tomography provides new insights into 3D water movement in soil and roots. We discuss the limitations of using neutron imaging and propose future directions to utilize neutron imaging to advance our understanding of root water uptake and soil–root interactions.

Keywords: attenuation coefficient, convection, diffusion, radiography, root hydraulics, root water uptake, tomography.

INTRODUCTION

Edaphic stress has been recently reported as the dominant constraint on vegetation productivity globally (Liu et al., 2020). The projected drought events due to climate change could further limit plant growth and production (Ben-Ari et al., 2018; Dai, 2013). Therefore, there is an urgent need to secure sustainable crop production with decreasing water availability (Marris, 2008). Addressing this challenge requires a deep understanding of how plants take up water and respond to water stress, which facilitates the identification of appropriate below-ground traits and the

development of optimal irrigation strategies. Hence, how and where water flows from soil towards roots and within roots are not only fundamental questions to explore but also direct implications for food production under various environmental conditions (Ahmed et al., 2018a).

Roots have a central role in plant water and nutrient uptake. Root length density and distribution are considered to be important traits affecting water uptake. However, profuse and deep roots do not necessarily translate into more uptake, especially under dry conditions (Zaman-Allah

et al., 2011). This is because water uptake is a complex process that depends on the hydraulic conductivity of the soil, the soil–root interface, and the radial and axial conductance (Alm et al., 1992; Draye et al., 2010; Landsberg & Fowkes, 1978; Meunier et al., 2018b; North & Nobel, 1996; Steudle & Peterson, 1998). Measuring root hydraulic properties in the soil is still a challenge due to the opacity of soil and inaccessibility of the roots. Hence, different experiments have been designed to measure such hydraulic properties in plants grown in hydroponic, artificial cultures and under aeroponic conditions (Amodeo et al., 1999; Melchior & Steudle, 1993; Varney & Canny, 1993; Zwieniecki et al., 2002).

Earlier studies suggested that the results obtained using these artificial cultures are remarkably different from those performed in soil (Varney & Canny, 1993). As the soil dries, the hydraulic conductivity of the bulk soil and the soil–root interface drop drastically (Cai et al., 2021, 2022; Zarebanadkouki et al., 2016a), which have evident impacts on root hydraulics and the root water uptake profile (Abdalla et al., 2021, 2022; Cai et al., 2018; Carminati, 2012; Carminati et al., 2011; Huang & Nobel, 1993; Javaux et al., 2008; McCully, 1995). Furthermore, heterogeneity of the soil moisture distribution and the interplay between soil moisture and root development introduce additional complexity to estimate local root water uptake (Ahmed et al., 2018c; Cai et al., 2018; Carminati et al., 2010; Moradi et al., 2011). Based on the hydraulic network in the soil–root–plant continuum, several root water uptake models with different degrees of complexity have been developed (Couvreur et al., 2012; de Jong van Lier et al., 2008; Doussan et al., 1998; Javaux et al., 2008). However, these models are still not widely used due to the lack of experimental data for validation and parameterization. Therefore, there is an urgent need for accurate water uptake measurements in different soils and climate conditions.

The advent of non-invasive imaging techniques in plant science, for instance, magnetic resonance imaging (MRI) (Pohlmeier et al., 2009), nuclear magnetic resonance (NMR) (Bottomley et al., 1986), X-ray computed tomography (CT) (Koebernick et al., 2014), light transmission (Doussan et al., 2006), and neutron radiography and tomography (Oswald et al., 2008), has opened new avenues to visualize the root system architecture and water dynamics in soil and roots. Compared to other imaging techniques, neutron imaging has the advantage of being highly sensitive to hydrogen. Hence, it is possible to quantify small changes in water content in both soil and roots (Moradi et al., 2009). Furthermore, in combination with isotopes, i.e., deuterated water (D_2O), neutron radiography and high-speed tomography provide real-time visualization of water flow in soil and roots at a high spatial and temporal resolution (Ahmed et al., 2018c; Matsushima et al., 2008; Tötzke et al., 2017; Zarebanadkouki et al., 2012). Over the last decade, neutron imaging has become an effective tool to investigate soil–

plant water relations (Brodersen, 2013; Kaestner et al., 2016; Tengattini et al., 2021). Here, our objectives were: (i) to review the recent advances in utilizing neutron radiography and tomography to visualize and quantify root hydraulic properties and water transport in the soil–root system; (ii) to discuss the limitations of using neutron imaging in soil–plant water relations; and (iii) to propose future research directions in exploiting neutron imaging to advance our current understanding of root water uptake and soil–root interactions.

PRINCIPLE OF VISUALIZING WATER FLOW USING NEUTRON IMAGING

In neutron imaging, a neutron beam is attenuated by the object (e.g., sample) when passing through it due to absorption and scattering in the sample (Kaestner et al., 2008; Strobl et al., 2009). A scintillator screen converts the shadow image of the neutron beam to visible light. The light from the scintillator is deflected by a mirror and captured by a highly sensitive camera (Figure 1a). The neutron beam attenuation depends on sample thickness, density, and material combination. Neutrons interact with matter on the level of atomic nuclei instead of the electron shells like X-rays. Therefore, the neutron attenuation coefficient varies strongly even between elements with similar atomic numbers and even between isotopes of the same element. For example, H_2O has a high attenuation coefficient (3.65 cm^{-1}) (Borgschulte et al., 2016) compared to sandy soils (92% sand, 0.13 cm^{-1}) and D_2O (0.61 cm^{-1}) (Kaestner et al., 2008; Zarebanadkouki et al., 2012). Sandy soils are used in soil–root interaction experiments mainly because they have: (i) high hydraulic conductivity and small amounts of strongly bound water and (ii) a low attenuation coefficient (Koestel et al., 2022). The difference in attenuation coefficient between H_2O, D_2O, and soil is typically used to quantify the amount of water in the samples using time series of neutron images in 2D (Figure 1b,c). For 3D imaging, i.e., tomography, calibration measurements are conducted to specify the soil water content. Therefore, the grayscale value of each soil voxel can be converted to a 3D water map of rhizosphere and bulk soil (Tötzke et al., 2017). The primary neutron–water interaction is scattering. The scattered neutrons introduce a bias to the measured neutron intensity. Therefore, it is of utmost importance for the quantification accuracy to remove biases caused by neutrons scattered from the sample and instrument background (See Section Limitations of Applying Neutron Imaging Technique for details; Boillat et al., 2018; Carminati et al., 2019).

QUANTIFYING WATER TRANSPORT IN SOIL AND ROOT USING NEUTRON RADIOGRAPHY

Quantification of local root water uptake

Matsushima et al. (2008) combined neutron radiography and D_2O to visualize root water uptake in tomato plants
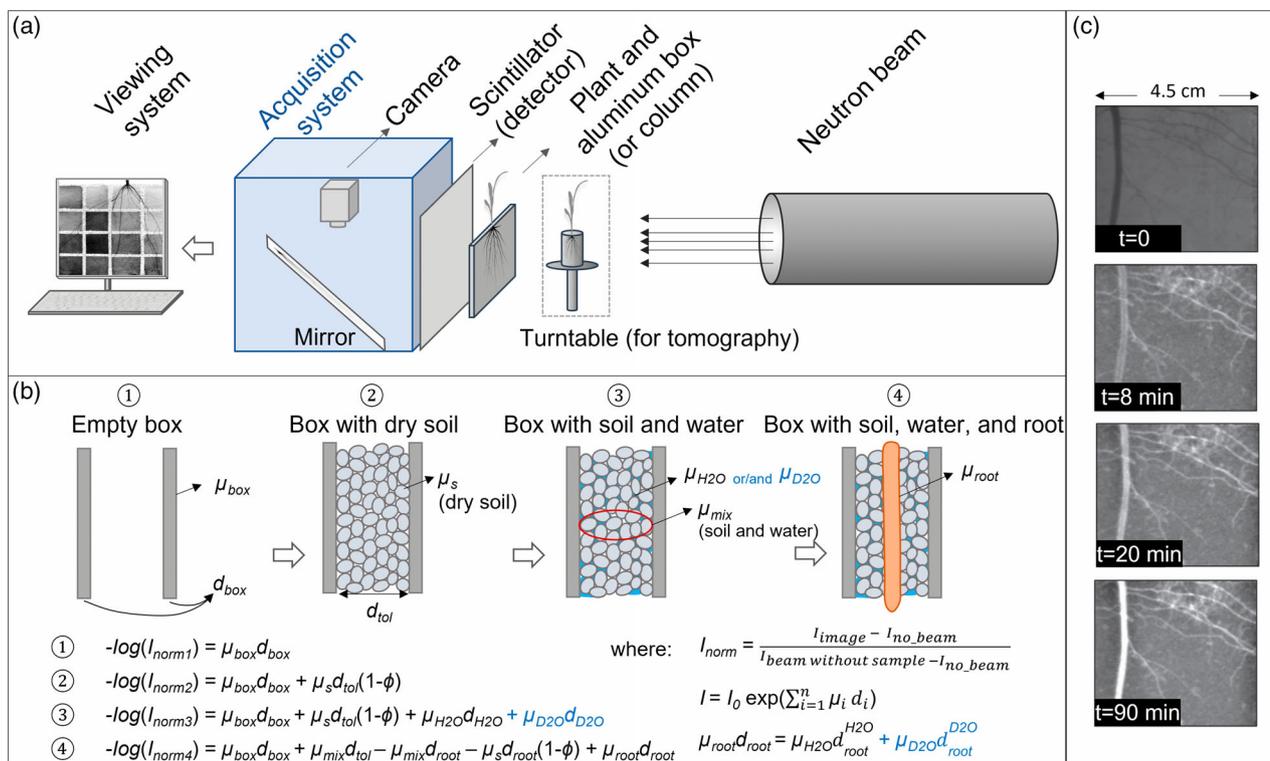
Figure 1. Principle of measuring water distribution in soil and roots using neutron radiography. (a) Sketch of a neutron imaging system. To minimize blurring, the sample is located a few centimeters in front of the detector. The image is taken by a highly sensitive camera (charge-coupled device [CCD] or scientific complementary metal-oxide semiconductor [sCMOS]). The container for neutron radiography is usually a flat rhizobox with a thickness of around 10–15 mm made of aluminum or boron-free glass. For neutron tomography, it is a round column with a diameter of 2–3 centimeters considering the effect of attenuation. (b) The principle of image analysis to quantify water by neutron radiography. Neutron attenuation across a medium follows the Beer–Lambert law. The attenuation is a function of the attenuation coefficient and the thickness of each element in a sample (equations are given below the drawings). The detailed procedure of the analysis can be found in Zarebanadkouki et al. (2012) and Ahmed et al. (2016b). I_0: intensity of the incident neutron beam (cm^{-2} sec^{-1}), I: attenuated intensity, I_{norm}: normalized beam intensity, μ: attenuation coefficient (cm^{-1}), d: thickness (cm), Φ: soil porosity. (c) Exemplary neutron radiographs showing D_2O transport in lupine (*Lupinus albus*) roots (after Zarebanadkouki et al., 2013).

(*Solanum lycopersicum* L.). Oswald et al. (2008) followed a similar approach to investigate root water uptake of lupine (*Lupinus albus* L.) and maize (*Zea mays* L.) and found that root water uptake was (i) not uniform along the root and (ii) affected by root distribution and soil structure. Although these earlier studies were carried out with a relatively low temporal resolution (15 sec to 5 min), they provided the first examples of utilizing neutron radiography to qualitatively visualize root water uptake for roots growing in soil.

Using a higher temporal resolution (10 sec per radiograph), Zarebanadkouki et al. (2012) combined neutron imaging, D_2O injection, and a simple 1D **diffusion–convection** model (see glossary) to visualize and quantify radial (from soil to root) and axial (along the root system) flow in lupine roots. The authors divided the soil into compartments using 1-cm-thick layers of coarse sand as a capillary barrier to keep the D_2O within a limited volume. For radial flow, D_2O enters roots via diffusion (due to differences in concentration) and convection, which depend on the **diffusion permeability** of the root tissue and the water potential gradient between soil and xylem, respectively. The D_2O

diffusion coefficient was obtained by fitting the dynamics of D_2O concentration in the roots using the 1D diffusion–convection model and the radiographs taken at night when transpiration is negligible. The convective part of the radial flow (i.e., the actual root water uptake) was obtained by fitting the changes in D_2O concentration during the day, assuming that the diffusion coefficient is identical to that at night. The axial flow, largely driven by gradients in water potential, was calculated from radial flux and D_2O concentration in the root stele downstream of the D_2O injection compartment. There will still be low axial diffusion (flux: 10^{-9}–10^{-7} m sec^{-1}), but it is negligible in comparison to the magnitude of convective flow (flux: 10^{-5}–10^{-4} m sec^{-1}).

In a follow-up study, Zarebanadkouki et al. (2013) used the same species and found that radial and axial flow rates dropped along an individual root from the proximal to the distal part (Figure 2a). However, the reasons for the reduction in both flow rates are different. The decrease in radial flow might result from a decline in dead cortical cells (North & Nobel, 1996) and radial hydraulic conductivity

(Doussan et al., 2006). The reduction in axial flow could be due to slower maturation of late metaxylem vessels in the distal part (McCully & Canny, 1988; Varney & Canny, 1993; Wang et al., 1991), especially for lateral roots. Furthermore, Zarebanadkouki et al. (2013) observed higher radial flow rates in the lateral roots in the upper layers compared to the lower layers. This observation is in agreement with results reported by Dara et al. (2015), who also used neutron radiography to observe root water uptake in lupine. This difference in water uptake between upper and lower layers probably resulted from the dissipation of water potential from shoot downward to root. Note that this difference and the location of water uptake along the root might change as the soil dries in the upper soil layers. The studies using neutron imaging mentioned above directly support the modeling studies considering radial and axial flow (Doussan et al., 1998; Landsberg & Fowkes, 1978) and studies with destructive methods applied to the roots of different species (Frensch & Steudle, 1989; Zwieniecki et al., 2002).

Effect of root types on water uptake

Variations of radial and axial hydraulic conductivities could result from the difference in root maturation, membrane permeability (in terms of aquaporin), xylem vessel size, and vessels (Frensch & Steudle, 1989; Huang & Eissenstat, 2000; Steudle & Peterson, 1998; Varney & Canny, 1993; Vetterlein & Doussan, 2016). Therefore, root water uptake does not only vary along individual roots but also differs between different root orders and root types. In this section, we compare the water uptake of lupine and maize, which are typical representative plant species with contrasting root system architecture, namely a taproot system versus a fibrous root system.

For lupine, Zarebanadkouki et al. (2013) used neutron radiography and D_2O injection to show that water was mainly taken up first by lateral roots (like 'streams') and then transported upward to the shoot through the taproot (like 'rivers') (Figure 2a). This differentiation in water uptake patterns between root types was supported by the difference in root anatomy and nocturnal diffusion. The taproot had larger and more abundant vessels, which resulted in a higher axial hydraulic conductance. In contrast, lateral roots were more permeable than the taproot in the radial direction, and hence diffusion rates were much higher, and D_2O entered lateral roots faster (Zarebanadkouki et al., 2013). A lower ratio of radial to axial hydraulic conductivities of the taproot may determine the
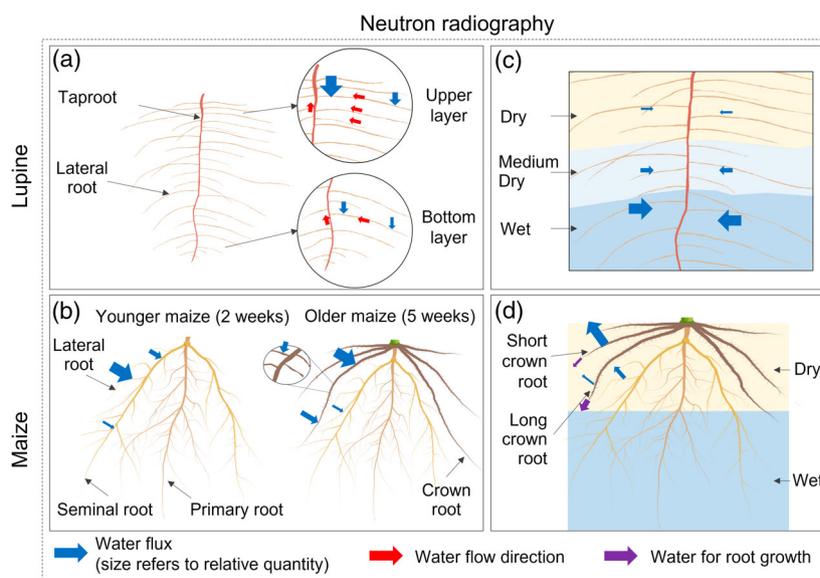
Figure 2. Overview of the main results of neutron radiography studies on root water uptake and redistribution. (a) Radial water flow to individual lupine roots in upper and lower soil layers (redrawn after Zarebanadkouki et al., 2013). Within laterals, the radial flow decreased from the proximal to the distal part (blue arrow). The flow rate was higher in lateral roots in the upper soil layers compared to the bottom soil layers. Under uniform soil moisture distribution, water was first taken up by lateral roots and then transported to the taproot (red arrow). (b) Radial water flow into roots of young and mature maize root systems (redrawn based on Ahmed et al., 2016b; Ahmed et al., 2018c). In 2-week-old maize, water was mainly taken up by laterals and then transported to the seminal and primary roots (indicated by the arrow size). However, in 5-week-old maize, crown/nodal roots and their lateral roots were the primary locations for water uptake. In contrast to the seminal roots, crown/nodal roots were able to take up water also from their distal segments. (c) Under non-uniform soil moisture distribution, compensatory water uptake was observed (redrawn after Dara et al., 2015). Water uptake increased in the wetter soil compartment and decreased in the drier compartment. (d) Hydraulic redistribution in soils with non-uniform soil moisture distribution in maize (redrawn after Hayat et al., 2020). The proportion of redistributed water from the long crown roots was greater than that from the short crown roots, while the proportion from the lateral roots was intermediate between the crown roots (blue arrow). Some of the redistributed water flowed towards the crown root tips to maintain their growth during the night. This effect is more pronounced for the long crown roots than for the short ones (purple arrow).

particular function of 'collecting water' from the lateral roots (Bramley et al., 2009; McCully, 1995).

On the other hand, in maize, observations with neutron radiography revealed that water uptake varied not only between root types but also, interestingly, between roots of different ages (Ahmed et al., 2016b; Ahmed et al., 2018c). Ahmed et al. (2016b) found that in 2-week-old maize, lateral roots were the primary location for water uptake, whereas, similar to the taproot in lupine, seminal and primary roots mainly transported the water to the shoot (Figure 2b). Furthermore, the distal parts of primary and seminal roots barely contributed to the water uptake. The latter is in line with earlier studies on maize (Varney & Canny, 1993; Wang et al., 1991). The authors explained these results by a slower xylem development and/or the greater water potential dissipation along the root (see Ahmed et al., 2016b for details).

However, in 5-week-old maize, water was mainly taken up by the crown roots and their laterals (Ahmed et al., 2018c). The laterals of seminal roots, which were the main location of water uptake in younger plants, had a minor, if any, contribution to root water uptake. Interestingly, in contrast to the seminal roots, crown roots were able to take up water also from their distal segments (Figure 2b). A possible interpretation is that high hydraulic axial conductance related to more and larger xylem vessels (Tai et al., 2016) along the whole crown root results in a low dissipation of water potential along the root. In fact, crown roots develop shorter and fewer lateral roots than seminal roots, allowing a more uniform water potential along the root (Ahmed et al., 2018c). The vital role of crown (or nodal) roots has also been noted in other species such as barley (*Hordeum vulgare*), wheat (*Triticum*), millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolor*) (Krassovsky, 1926; Rostamza et al., 2013; Sallans, 1942). These findings underline the importance of considering the differences in hydraulic properties between root types.

Compensatory water uptake and hydraulic redistribution within roots

Compensatory water uptake has been observed in soils with heterogeneous moisture distribution. Using neutron radiography, Dara et al. (2015) showed a 100% increase in water uptake from roots in a wetter soil compartment while transpiration of the whole plant (3-week-old lupine) decreased by 40% due to water stress (Figure 2c). The observed compensatory uptake is in agreement with results reported by McLean et al. (2011), who measured increased water uptake and a transient increase in root hydraulic conductance and aquaporin expression in woody seedlings during partial root-zone drying. The results reported by Dara et al. (2015) provided direct evidence. The data from the latter study could be used to

parameterize root water uptake models considering a non-uniform soil moisture distribution and compensatory uptake (e.g., Couvreur et al., 2012).

When soil moisture is not uniformly distributed, water can be transported from moist to dry soil regions through roots due to the gradients in water potential within the soil, which is called hydraulic redistribution. Although hydraulic redistribution is well documented for both woody and herbaceous species (Caldwell et al., 1998; Jackson et al., 2000; Neumann & Cardon, 2012; Prieto et al., 2012), the changes in soil moisture through this process are rather exiguous to be detected by typical soil sensors (Cai et al., 2018; Thomas et al., 2020). Only a few studies have non-destructively measured variations in the isotopic composition of soil water using the stable water isotope H_2O^{18} (Meunier et al., 2018a; Zegada-Lizarazu & Iijima, 2004). Hence, quantifying hydraulic redistribution non-invasively *in situ* remains challenging. Using neutron radiography and D_2O injection, Warren et al. (2013) visualized hydraulic redistribution in the roots from the soil surface to the lower soil layers. Similarly, Hayat et al. (2020) used neutron imaging to visualize and quantify hydraulic redistribution in soil and roots (Figure 2d). Interestingly, the authors found that hydraulic redistribution depended on root type, and a fraction of redistributed water was utilized to maintain the growth of crown/nodal roots (found in the dry soil layer) (Figure 2d). The redistributed water in the drier top layer might also be used for nutrient mobilization and increased rhizosphere microbial activity (reviewed by Neumann & Cardon, 2012; Prieto et al., 2012). On the other hand, the redistributed water used to sustain the growth of nodal roots might underline the importance of their role in water uptake, as these roots have a greater potential for water uptake than other root types (Ahmed et al., 2018c). It is still unclear whether plants grow nodal roots towards the wet patches for better water extraction or if it is a strategy to develop this specific root type to take up future water from rain/irrigation events. Further investigations are required to answer this question.

VISUALIZING WATER TRANSPORT IN 3D USING NEUTRON TOMOGRAPHY

Moradi et al. (2011) used neutron tomography to visualize and quantify the 3D soil water content distribution in the rhizosphere. The authors successfully showed fine root structure and quantified the 3D gradients in soil moisture in the rhizosphere at high spatial resolution (up to 56 μm at a voxel size of $13 \times 13 \times 13$ μm^3). However, each scan took around 6 h due to the limited light output of the high-resolution scintillator screen. Recent developments of neutron tomography have greatly reduced the acquisition time per 3D image from hours to 1.5 sec (Kaestner et al., 2016; Tötzke et al., 2019; Zarebanadkouki et al., 2015), without severely compromising image quality while achieving

physical spatial resolutions of 100–200 μm (at a voxel size of $100 \times 100 \times 100$ μm^3). High-speed neutron tomography opened a unique avenue to visualize water transport in soil and roots in 3D. For instance, Tötzke et al. (2017) demonstrated that high-speed neutron tomography could capture soil water movement and root system architecture within seconds, providing detailed insights into the 3D water dynamics of the root–soil system (Figure 3a). Using this technology, Tötzke et al. (2021) observed non-uniform water uptake in the root system of 2-week-old maize. The water was mainly taken up by the primary root (Figure 3b), in contrast to the observations of Ahmed et al. (2016b), where the water was mainly taken up by the lateral roots. Note that the volume of the soil column in Tötzke et al. (2021) was 3.8% of the rhizobox in Ahmed et al. (2016b). The smaller growth volume could have a greater impact on root development, for instance, shorter root length and fewer lateral roots (see the next section). These laterals might have been too fine to be observed. Hence, these findings may not necessarily be different since the water taken up by lateral roots is transported to the primary root.

LIMITATIONS OF NEUTRON IMAGING TECHNIQUES

Neutron imaging has some limitations that need to be considered in water transport investigations in soil and roots. The first limitation is soil texture. The substrates in most studies were quartz sand (sand proportion >90%) because it contains little hydrogenous material and few metallic minerals and has a relatively low attenuation coefficient in both wet and dry conditions (Moradi et al., 2009; Tengattini et al., 2021). The absence of hydrogenous material facilitates the visualization and segmentation of roots and reduces the uncertainty in quantifying soil water fluxes. Loamy, clayey, and natural sandy soils may have a relatively high content of organic matter, which contains many hydrogenous materials. Loamy and clayey soils often contain a relatively large fraction of clay minerals and thus a significant amount of strongly bound water. These soils increase the attenuation coefficient and reduce the contrast

between soil and roots. Furthermore, these soils also reduce the penetration depth of neutrons, decreasing the possible sample thickness. Mawodza et al. (2020) used sieved but repacked sandy loam soil with a heterogeneous structure and found that this soil added complexity to image processing, e.g., estimation of soil moisture and root segmentation. It also suggested that quantifying water distribution and root water uptake using neutron imaging in naturally structured soil will be more challenging. Furthermore, during soil drying, soils with a certain proportion of clay may shrink and create cracks, which complicates the image registration when considering time series and affects water distribution and root growth.

Secondly, the soil water content should be controlled depending on the objectives of the particular study. Experiments with D$_2$O injection require relatively moist soil conditions to quantify root water uptake because the injection should not change the range of soil water potential under these conditions, particularly in sandy soil (Ahmed et al., 2016b). If the soil is too dry (e.g., <0.1 cm^3 cm^{-3} in sandy soil), D$_2$O injection would increase the soil water content and thus alter the water potential gradient between soil and roots, which would affect water flow from soil to roots. At the same time, the soil water content should not be too high, e.g., 0.3 cm^3 cm^{-3} in sandy soil, as this would reduce the neutron transmission signal, degrade image contrast, and make accurate water quantification impossible. For studies without D$_2$O injection, low soil moisture content (0.05–0.1 cm^3 cm^{-3}) is beneficial as it provides optimum contrast conditions (Ahmed et al., 2016a; Ahmed et al., 2018b). Note that the soil must not be too dry, as otherwise roots start shrinking and desiccating (Duddek et al., 2022), which may cause them to vanish from the neutron images.

Thirdly, in quantifying radial flow using the diffusion–convection model, the diffusion coefficient of individual roots is assumed to be identical during night and day. However, diffusion in root tissue may vary in transpiring plants due to differences in aquaporin activities (Maurel et al., 2016; Pou et al., 2013; Vandeleur et al., 2009).

Neutron tomography
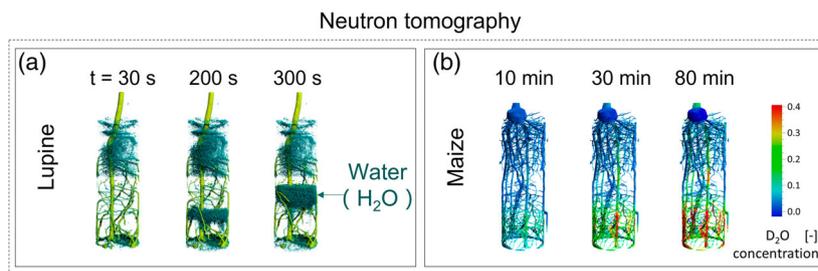
Figure 3. Overview of the main neutron tomography studies on root water uptake in lupine and maize. (a, b) Time series of water movement and distribution after D$_2$O injection at the bottom of the soil column for lupine (after Tötzke et al., 2017) and maize (after Tötzke et al., 2021), respectively. Images in (a) show the formation and ascent of the H$_2$O front within 6 min after D$_2$O injection. Images in (b) show the accumulation of D$_2$O in the root system within 80 min after D$_2$O injection. The soil columns in (a) and (b) had a diameter of 27 mm and a height of 100 mm.

Hence, the diffusion coefficient during the day may be underestimated. Note that the diffusion coefficient is a fitting parameter in the model and the underestimation may be compensated by overestimating convective flux when fitting variations of D_2O in the roots. In other words, this assumption of an identical diffusion coefficient during the day and night might result in an overestimation of local root water uptake during the day (Ahmed et al., 2016b). Therefore, it would be worthwhile investigating the effect of aquaporin activities on the diffusion coefficient and convective flux and hence local water uptake.

Fourthly, high spatial resolution does not usually come with high temporal resolution in neutron imaging. This involves beam intensity, scintillator, and camera. The beam intensity is a very important parameter that mainly depends on the power of the source (reactor or spallation) and the quality of the beam guide. The number of captured neutrons ultimately decides the signal-to-noise ratio in the images. The detectability of the roots also depends on the contrast-to-noise ratio between soil matrix and roots. The efficiency of the scintillator plays a role here since it converts the attenuated neutron intensity to visible light, but the requirements are contradictory for high temporal or spatial resolution, respectively. A thinner scintillator with a low light output is usually applied for high spatial resolution because it can catch as many photons as possible with sensitive optics and camera (Tengattini et al., 2022). By contrast, a thicker scintillator is typically chosen for high temporal resolution because it can catch more neutrons and brings higher luminosity to the images (but also with more diffuseness). Regarding the camera, for high spatial resolution a sensitive camera is required, but for temporal resolution it is of higher importance to focus on short readout times. With current technology and available neutron fluxes, optimizing the detector system to simultaneously meet the requirements of high spatial and temporal resolution remains a challenge. The final option is to increase the neutron flux, which can be achieved by selecting a more powerful source or, to some degree, by changing the beamline optics, e.g., increasing the aperture or using a neutron guide. With a higher neutron flux either the exposure time can be decreased or the spatial resolution can be increased (using a smaller beam aperture) (Tötzke et al., 2017). The former is important for high-speed measurements, and the latter is important for high-resolution measurements.

Fifthly, the size of the plant container, and hence the available volume for root growth, is limited to ensure sufficient beam transmission through the root–soil sample. For 2D measurements, this limitation refers to the thickness of the rhizotron slab, which determines the length of the neutron transmission path. The thickness of plant containers designed for 2D measurements usually ranges from around 10 to 15 mm (Esser et al., 2010; Zarebanadkouki

et al., 2012). Moradi et al. (2009) showed that the detection limit of the root diameter increases exponentially with container thickness for constant spatial resolution and field of view (FOV). With increasing thickness, the attenuation signal of soil inside the container becomes more dominant, and below a specific root diameter-to-soil thickness ratio, the root signal disappears in the background signal (soil). It is worthwhile mentioning that there is a general tradeoff between spatial resolution and FOV: the larger the FOV, the coarser the resolution, i.e., the less detailed the image of water transport between soil and root. For larger samples, where the lateral container dimensions may even exceed the beam size, image stitching may solve this problem. However, this increases the complexity of the image post-processing, and it is not suitable for dynamic observations. Plant containers designed for neutron tomography measurements are mostly cylinders for uniform transmission over the entire angular range of the scan with diameters usually not exceeding 30 mm and a height of about 100 mm (Figure 3) (e.g., Moradi et al., 2011; Tötzke et al., 2017). Usually, the size of FOV is adjusted to capture the entire plant container, e.g., approximately 100×100 mm, which corresponds to a spatial resolution ranging from 50 to 200 μm depending on the acquisition speed, neutron flux, and other technical imaging conditions of the respective neutron station. Recent advances in detector techniques and high neutron flux (improved signal-to-noise ratio), which is mainly available with strong neutron sources, have considerably increased the spatiotemporal resolution power (e.g., Tengattini et al., 2020; Tötzke et al., 2019). The smallest detectable root diameter depends on the soil moisture (determining the transmission signal and the image contrast between root and soil) and the spatial resolution of the respective measurement. For plant containers with a diameter of 3 cm, the detection limit at moderate soil moisture usually ranges between 0.1 and 0.2 mm (e.g., Mawodza et al., 2020; Zarebanadkouki et al., 2019). Much finer roots (e.g., ≤0.05 mm) can be detected using high-resolution detector setups. The higher resolution comes at the expense of a smaller FOV and thus also a smaller plant size. The current findings were mainly obtained from young plants (e.g., around 1 month old). Further investigations are required to determine whether the results are generalizable to mature plants.

Sixthly, scattering artifacts are not often considered. Water attenuates neutrons mainly by scattering. This means the detector not only receives straightly transmitted neutrons but also scattered ones that produce a bias in the grayscale values of neutron images (Moradi et al., 2013). Injecting D_2O changes the soil water distribution and results in a dynamic redistribution of the scattering bias. Hence it changes the correlated distribution of scattered neutrons and has a potential impact on the quantification of water transport, especially when considering small

changes in D_2O concentration in the root. Therefore, dynamic scattering corrections are required to improve the quantification accuracy. The Paul Scherrer Institute (PSI; Villigen, Switzerland) has developed an approach for neutron radiography using a black body grid during image capture to estimate the spatial distribution of scattered neutrons for the specific sample under study (Boillat et al., 2018; Carminati et al., 2019). However, scattering correction of dynamic 3D measurements for neutron tomography remains a major challenge.

Lastly, neutrons and D_2O have some effects on plant activity. For instance, neutron irradiation was shown to affect gene expression in Arabidopsis (Fortunati et al., 2010) and chlorophyll concentration in wheat (Hanafy & Mohamed, 2014). D_2O was reported to impact protein synthesis in winter rye (*Secale cereale*. L. ev. Winter) (Waber, 1975) and germination of barley (Bhandarkar et al., 1971). The impact of D_2O on plant growth is concentration- and time-dependent. Salvagno (2013) found that Arabidopsis did not show a difference in plant development after 7-week growth in natural water and 10% D_2O but exhibited slower growth and discolored leaves in 60% D_2O. In a solution with 86.7% D_2O, root growth of *Kalanchoe* seedlings was remarkably inhibited after 10 h compared to the ones grown in natural water (Pratt & Curry, 1937). The amount of D_2O injected into the samples was a few milliliters and the measurements of root water uptake or hydraulic redistribution were taken only in a time span of hours, which may not introduce a significant effect on plants from neutron radiation and D_2O. However, after several hours of neutron irradiation, the plants, including the soil columns, are activated and are therefore subject to strict radiation protection regulations. The activated plant samples must be stored safely in the neutron facility, usually for several days to weeks, until their radiation level falls below the legal limit, and they cannot be used for further analysis during this time. The activity decay time depends on the captured neutron dose, which varies with neutron source flux and experiment duration.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Neutron imaging, including radiography and tomography, has become a unique and vital tool for investigating *in vivo* water transport in the soil–root system (Box 1). In combination with D_2O injection, which has a strong contrast compared to H_2O, and a diffusion–convection model, root water uptake and hydraulic redistribution in root and soil can be visualized and quantified. Furthermore, neutron imaging has been utilized to visualize non-uniform water uptake along an individual root and variations in water uptake between different root orders and different root architectures. Hence, neutron imaging does not only improve our understanding of root functions in water uptake but also offers opportunities to explore root

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> **BOX 1.** Bullet points
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> 1. With the advantage of being highly sensitive to hydrogen, neutron imaging has become an unparalleled technique to visualize and quantify root water uptake *in vivo*.
> 2. Root water uptake is not uniform along individual roots and soil depths, and water uptake patterns are remarkably impacted by root type and age.
> 3. Under non-uniform water distribution conditions, hydraulic redistribution and compensatory water uptake were observed during night- and daytime, respectively.
> 4. The development of fast neutron tomography opens new avenues to explore water flow in soil and roots in 3D.
> 5. The application of neutron imaging in root water uptake is limited to young plants (e.g., around 1 month old).

development and responses to different environmental conditions.

Nevertheless, there are still open questions on how neutron imaging can be used to advance our understanding of soil–root interactions and local root water uptake (Box 2). Recent studies have shown that root hydraulics (Cai et al., 2021) and root diameter (Lippold et al., 2021) differ between contrasting soil textures. It would be of high interest to use neutron imaging to investigate whether plants adapt root development (e.g., root diameter, length, permeability, and xylem vessel) and root hydraulics to different soil conditions. During soil drying and rewetting, roots and root hairs may shrink and swell (Cai & Ahmed, 2022; Carminati et al., 2009; Duddek et al., 2022). How roots respond to such processes, e.g., variations of water flow and root hydraulics, remains unclear. Bimodal imaging, for instance, using neutrons and X-rays (Kaestner et al., 2017) on the same sample offers information on soil structure, root distribution, and water distribution. Using neutrons and **planar optodes** (Rudolph-Mohr et al., 2021) offers information on root architecture, water distribution, and soil oxygen and pH status simultaneously. Thus, the combination of different imaging techniques could be an ideal tool to explore such questions. Additionally, arbuscular mycorrhizal fungi (AMF) were shown to enhance soil–plant hydraulic conductance in dry conditions (Abdalla & Ahmed, 2021). How AMF impact root conductance and water uptake remains unclear, but this could be explored using neutron imaging.

Quantification of root hydraulics in different plant species and soil conditions offers various data that could be utilized to calibrate and parameterize sophisticated 3D

BOX 2. Open questions

1. Can we use neutron radiography and/or tomography to quantify the effect of root shrinkage and swelling on root hydraulic properties and local water uptake?
2. Can bimodal imaging, combining neutron and X-rays, be used to overcome the impact of soil textures on the visualization of water uptake and soil–root interactions?
3. Are the results observed in young plants via neutron imaging generalizable to mature plants and contrasting species?
4. Can we use a combination of neutron imaging and D_2O to measure the effects of arbuscular mycorrhizal fungi (AMF) on root water uptake?
5. Can we use high-resolution neutron tomography with D_2O injection to parameterize root water uptake models at the cellar scale, e.g., MECHA (Couvreur et al., 2018)?

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models of root water uptake (Doussan et al., 2006; Javaux et al., 2008). For instance, by combining neutron images and a root architecture model (Doussan et al., 1998), Zarebanadkouki et al. (2016b) estimated hydraulic conductivities of the taproot and lateral roots of lupine, which has been replicated with other plant species (Meunier et al., 2018b). Moreover, high-speed neutron tomography provides more detailed information on water dynamics in soil and roots, and its application to water flow is just in the emerging stage. It has high temporal resolution and produces huge 3D data sets (Tötzke et al., 2017), which requires efficient imaging processing tools and probably also adequate models to explore and interpret the data. Recently, a root water uptake model based on hydraulics was developed at the cellular scale, namely MECHA (Couvreur et al., 2018). It will be highly interesting to investigate whether a microscopic (cellular-scale) root water uptake model could interpret water flow measured with neutron tomography and, in turn, whether such a model could be parameterized using high-resolution neutron tomography and D_2O injection.

AUTHOR CONTRIBUTIONS

MAA conceived the idea of the review. GC and MAA conducted the conceptualization. GC wrote the initial draft with input from MAA, CT, and AK.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

No new data were generated or analyzed in this study.

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