On-the-fly neutron tomography of water transport into lupine roots

Mohsen Zarebanadkouki\textsuperscript{a,}\textsuperscript{*}, Andrea Carminati\textsuperscript{a}, Anders Kaestner\textsuperscript{b}, David Mannes\textsuperscript{b}, Manuel Morgano\textsuperscript{b}, Steven Peetermans\textsuperscript{b}, Eberhard Lehmann\textsuperscript{b}, Pavel Trtik\textsuperscript{b}\textsuperscript{*}

\textsuperscript{a}Division of Soil Hydrology, Georg-August University of Goettingen, Goettingen, Germany  
\textsuperscript{b}Paul Scherrer Institut, Villigen, Switzerland  
\textsuperscript{*}Corresponding authors: mzareba@gwdg.de, pavel.trtik@psi.ch

Abstract

Measurement and visualization of water flow in soil and roots is essential for understanding of how roots take up water from soils. Such information would allow for the optimization of irrigation practices and for the identification of the optimal traits for the capture of water, in particular when water is scarce. However, measuring water flow in roots growing in soil is challenging. The previous 2D experiments (Zarebanadkouki et al., 2012) have not been sufficient for understanding the water transport across the root and therefore we employed an on-the-fly tomography technique with temporal resolution of three minutes. In this paper, we show that the series of on-the-fly neutron tomographic experiments performed on the same sample allow for monitoring the three-dimensional spatial distribution of D\textsubscript{2}O across the root tissue. The obtained data will allow us to calculate the convective and diffusive transport properties across root tissue and to estimate the relative importance of different pathways of water across the root tissue.

1. Introduction

Supply of water and solutes to the shoot are one of the essential functions of roots. The flow of water from the soil into the roots sustains the evaporative demand of plants and prevents plants from wilting. Water is becoming
the main limiting factor for plant growth and food production in many agricultural systems and an efficient and sustainable management of the earth water resources is of fundamental importance to feed the growing world population (Gallagher et al. 1976; Oki and Kanae 2006; Piao et al. 2010). Knowledge of the location of root water uptake will reveal the function of different root segments and root architecture in water uptake. This information will allow us to identify optimal traits for the capture of water from the soil under water shortage and optimize irrigation practices.

Despite the numerous studies of root water uptake, ranging from anatomical studies of root tissue during the plant development and in response to drought stress (Huang and Nobel 1993; Steudle and Peterson 1998; Huang and Eissenstat 2000; Enstone et al. 2002; Macarisin et al. 2009), molecular studies of the expression of aquaporins (Ranathunge et al. 2004; Törnroth-Horfield et al. 2006; Knipfer et al. 2011), detailed measurements of root hydraulic conductivities (Frensch and Steudle 1989; Zwieniecki et al. 2002), up to architectural models of root water uptake (Doussan et al. 2006; Javaux et al. 2008), there are still big challenges in understanding how roots take up water from soils. The main difficulty consists in measuring the water fluxes and the root hydraulic conductivity in situ. This means that the intrinsic heterogeneity of the soil cannot be neglected. However, up to date, most of data on location of root water uptake were obtained from roots grown in hydroponics or indirectly from monitoring and modelling water distribution in soil.

Recent advances in imaging water and root distribution in soil allowed in situ quantification of water content changes in the soil around the roots of transpiring plants (Pierret et al. 2003; Garrigues et al. 2006; Carminati et al. 2010; Moradi et al. 2011; Stingaciu et al. 2013). Although these studies provided very valuable information on root and water distribution in soils, a method to directly measure the water fluxes was still missing.

Recently, Zarebanadkouki et al. (2012; 2013) introduced a novel technique to measure local flow of water into roots of transpiring plants growing in soil. The technique consists of monitoring and modelling the transport of deuterated water (D$_2$O) into roots. Due to a large difference in the neutron attenuation coefficient of H$_2$O versus D$_2$O for cold neutrons (µ$_{H2O}$ = 5.73 cm$^{-1}$, µ$_{D2O}$ = 0.68 cm$^{-1}$ based on Hassanin 2006), the transport of D$_2$O into the plant roots leads to a significant negative contrast in the resulting images. D$_2$O was injected in the soil and its transport through the roots was monitored by means of time-series neutron radiography. In Zarebanadkouki et al. (2014), a diffusion convection model was employed to quantify the diffusive and convective transport of D$_2$O into the roots. The model was solved numerically to calculate the diffusion coefficient of the root tissue and the convective fluxes of water across the root-tissue (radial flow) and along the root xylem (axial flow). The diffusion coefficient and the convective transport of D$_2$O were estimated by inverse simulation of D$_2$O transport into roots. The model included diffusive and convective transport of D$_2$O along different pathways across the root tissue: the apoplastic and the cell-to-cell pathway (Steudle and Peterson 1998). The apoplastic pathway occurs along the cell walls and the extracellular space. Although its cross section is small (three percent of the total cross-sectional area, according to Fritz and Ehwald (2011) for maize), water flow in the apoplastic pathway does not involve crossing of cell membranes and it is expected to be less resistant to water flow. However, the relative importance of the apoplastic and cell to cell pathways remains a matter of debate (Steudle, 2000; Knipfer and Fricke, 2010b).

The model introduced in Zarebanadkouki et al. (2014) includes different pathways of water across the root and has the potential to reveal the relative importance of the different pathways of water. However, application of this model to reveal the relative importance of the different pathways of water requires detailed 3D information on the temporal distribution of D$_2$O across the root tissue. In this study, we tested the feasibility of an on-the-fly neutron tomography measurement to monitor the spatiotemporal distribution of D$_2$O across the root tissue.

2. Materials and Methods

2.1. Plant and soil preparation

We grew six lupines in graphite cylindrical plant pots (a diameter of 27 mm and a length of 100 mm) filled with a sandy soil collected from the artificial catchment Chicken Creek near Cottbus, Germany. The soil (sieved to < 2 mm) consisted of approximately 92% sand, 5% silt and 3% clay. The soil hydraulic properties are given in Carminati et al. (2010). The sand was poured into the containers through a sieve to favour a homogenous sand
deposition and limit soil layering. The average bulk density of the packed soil was 1.4 g cm⁻³.

The seeds were germinated for two days on moist filter paper (details are given in Zarebanadkouki et al. (2012)). The seedlings were placed into the soil at a depth of 1 cm. When the plants were three days old, we added 1 cm layer of quartz gravel (grain diameter of 2 mm) on the top of the soil to minimize evaporation. The plants were grown with a photoperiod of 14 hours, light intensity of 300 μmol m⁻² s⁻¹, day/night temperature of 25°C/19°C, and relative humidity of 60%. During the first four days after planting, the samples were irrigated every day from the top. Then, we irrigated the plants every fourth day by immersing the samples in a water table of 3 cm from the bottom. This procedure resulted in an average soil water content of 0.30 m³ m⁻³.

The neutron tomography measurements started when the plants were 10 days old. Prior to the measurement, plants were placed in the imaging station and the transpiration was monitored gravimetrically by weighing the samples at two different times with an interval of three hours. The daytime transpiration was 0.4 cm³ h⁻¹ and it was negligible at night-time.

2.2. Neutron tomography

Neutron tomography is an excellent technique for in situ and non-invasive assessment of the spatiotemporal distribution of water (Kaestner et al., 2011; Moradi et al., 2011). The technique consists of passing a neutron beam through a sample while the sample is rotated. The transmitted neutron beams are captured in a series of 2D radiographs. The transmitted neutron beams under each angle carry information about the attenuation of neutrons by the material due to the composition and the thickness of the sample. To obtain the volumetric information about the distribution of neutron attenuation in the sample a sufficient number of projections at different angular views are needed. The 3D map of attenuation coefficient can be then reconstructed using the filtered back projection algorithm.

The neutron tomography was performed at the beamline BOA at the Paul Scherrer Institute (PSI), Villigen, Switzerland (Morgano et al. 2014). The neutron beam was conditioned using 20 x 20 mm aperture followed by two flight tubes of 4.7 m (2.1 m and 2.6m in length). A 50 μm ⁶LiF/ZnS scintillator screen of the detector was placed at 5.25 m downstream the aperture. The light produced by the scintillator was collected - after mirror and a 50 mm lens - by a sCMOS camera (ORCA Flash 4.0, Hamamatsu).

The samples were placed on a rotational stage whose axis of rotation was placed at about 20 mm upstream the scintillator screen. The rotational stage available at BOA beamline allows for maximum four 360-degrees rotations. Consequently, the experiments we performed in such a way that the samples were tomographed once before D₂O injection. Then, the D₂O was injected into the sample system and after stabilization of the D₂O-front (after circa 15 s) the series of on-the-fly tomographies was started. Throughout all the experiments, the sCMOS detector was set to acquire images of 768 (width) x 2048 (height) pixels, the exposure time was set to be 1 second, the rotation rate of the sample stage was set at 1 degree per second and the proton current at the SINQ target was about 1.43 mA, which corresponds to flux approximately 1.0 x 10⁷ neutrons cm⁻² s⁻¹ at the sample position.

Consequently, the temporal resolution of the series of on-the-fly neutron tomographies (from the angular view of 0 to 180 degrees) was 3 minutes. While the nominal pixel size of the acquired images corresponded to 51.1 μm, the spatial resolution in 2D is about 150 micrometres as assessed visually from the image of PSI test pattern Gd-based Siemens star (see Figure 1) (Grünzweig et al., 2007). The spatial resolution of the resulting 3D images is further affected by the motion artefacts due to (i) dynamic process inherent to the sample (ii) the on-the-fly acquisition process. In such test arrangement, the estimated neutron flux is about 260 neutrons per pixel (per second), the acquired flat field image images show about 1150 counts per pixel above the offset.

2.3. Image processing

The CT projection data set consisted of 181 neutron projections obtained from the angular view from 0 to 180 degrees, which were reconstructed into a 3D volume using Octopus software (Castro et al. 2006). Prior to reconstruction, the neutron radiographs were normalized for the flat field, dark current and beam variation through
the radiographs. Based on the exponential law of Beer-Lambert, the neutron attenuation in each pixel of the 2D radiographs can be related to the sum of the weighted neutron attenuation along the thickness of the sample.

\[ I = I_0 e^{-\sum_{i=1}^{i=n} \mu_i d_i} \]  

(1)

where \( I \) is the intensity of the attenuated neutron beam [number of neutrons cm\(^{-2}\) s\(^{-1}\)], \( I_0 \) is the intensity of the incident neutron beam [number of neutrons cm\(^{-2}\) s\(^{-1}\)], \( d_i \) (m) is the thickness of the \( i \)-material composing the sample, and \( \mu_i \) [cm\(^{-1}\)] is the macroscopic neutron attenuation coefficient, which describes the probability of neutron interactions with the materials per unit of thickness.

Octopus uses a filtered back projection algorithm to reconstruct the spatial distribution of neutron attenuation in horizontal slices perpendicular to the sample rotation axis. After reconstruction, we obtained the voxel-wise neutron attenuation coefficient of the sample. The voxel-wise neutron attenuation coefficient for the voxel containing soil is

\[ \mu_i = \mu_{H_2O} F_{H_2O} + \mu_{D_2O} F_{D_2O} + \mu_{soil} F_{soil} \]  

(2)

and for the voxel containing root is

\[ \mu_i = \mu_{H_2O} F_{H_2O} + \mu_{D_2O} F_{D_2O} + \mu_{tissue} F_{tissue} \]  

(3)

where \( \mu \) is the linear neutron attenuation coefficient, \( F \) is volumetric fraction of the different phases in the voxel and subscripts H\(_2\)O, D\(_2\)O, soil and tissue refer to water, D\(_2\)O, soil and root tissue, respectively.

3. Results

Figure 2a shows a neutron radiograph of a 10-day old lupine before D\(_2\)O injection. This radiograph is taken as the angular view of zero degree and shows the average neutron attenuation along the thickness of the sample. This image is normalized for flat field and camera dark current. We used 181 projections obtained by scanning the sample at the angular view from 0° to 180° to reconstruct the volumetric neutron attenuation in the sample. A 2D horizontal cross section at different depth is shown in Figure 2b-d. After reconstruction, the root system was easily segmented from the soil due to the high contrast in neutron attenuation between roots and soil. Figure 2e shows the
three dimensional architecture of the root system of a 10-day old lupine. Note that in this figure the neutron attenuation in the pixels containing soil is cut off.

Figures 3a and 3b show two 2D horizontal cross sections at a depth of 3cm of two samples at different times after D$_2$O injection. Figures 3a refers to sample scanned during the day, and Figure 3b to sample scanned during night-time, respectively. In these images, the more red is the value of pixels the higher is the neutron attenuation (the higher the H$_2$O content). After D$_2$O injection, the neutron attenuation in the soil and in the root decreased. Figures 3c and 3d show the ratio between 2D horizontal cross sections at respective times after D$_2$O injections and the one before D$_2$O injection. These images show the change in neutron attenuations after D$_2$O injection. The changes in neutron attenuation are due to the replacement of H$_2$O with D$_2$O and show the transport of D$_2$O into the root. We were able to observe that the changes in neutron attenuation in the roots were clearly faster during day than during night-time. Interestingly, the changes in neutron attenuation were much faster in the lateral roots than in the taproot. This applies to both, day and night measurement (see Figure 3c).
Fig 3. Horizontal cross section of a 10 day old lupine at depth of 4 cm. Figures a and b show neutron attenuation at different times after D$_2$O injection during day and night, respectively. In these images, the colour bars refer to neutron attenuation. Figures c and d show the difference between neutron radiographs at different times after D$_2$O injection and the one taken before D$_2$O injection. In these images, the colorbar shows the change in neutron attenuation after D$_2$O injection. Neutron attenuation was initially higher in the root tissue during night then during daytime.

4. Discussion & Conclusions

This study builds upon our recent neutron radiography experiments with D$_2$O where we measured the local flow of water into the roots of transpiring plants growing in soil. The limits of our former studies were that in the radiography mode we could not resolve the profile of D$_2$O across the root tissue. Here, we performed series of on-the-fly neutron tomographies with temporal resolution of three minutes (for a full tomography). The reconstructed volumes were sufficiently accurate to quantify the changes of D$_2$O across the root tissue. This study proves that on the fly tomography can be successfully used to study the flow of water across the root tissue in three dimensions at
sufficiently high spatial and temporal resolution. The success in our 3D visualization of D₂O transport across the root tissue opens new avenues to study the relative importance of the apoplastic and cell-to-cell pathways in root water uptake. Such information is urgently needed for understanding the physical mechanisms controlling water, solute and hormone transport in the roots.

5. References

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