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Combining Neutron and Magnetic Resonance Imaging to Study the Interaction of Plant Roots and Soil

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Abstract

The soil in direct vicinity of the roots, the root-soil interface or so called rhizosphere, is heavily modified by the activity of roots, compared to bulk soil, e.g. in respect to microbiology and soil chemistry. It has turned out that the root-soil interface, though small in size, also plays a decisive role in the hydraulics controlling the water flow from bulk soil into the roots. A promising approach for the non-invasive investigation of water dynamics, water flow and solute transport is the combination of the two imaging techniques magnetic resonance imaging (MRI) and neutron imaging (NI). Both methods are complementary, because NI maps the total proton density, possibly amplified by NI tracers, which usually corresponds to total water content, and is able to detect changes and spatial patterns with high resolution. On the other side, nuclear magnetic resonance relaxation times reflect the interaction between fluid and matrix, while also a mapping of proton spin density and thus water content is possible. Therefore MRI is able to classify different water pools via their relaxation times additionally to the water distribution inside soil as a porous medium. We have started such combined measurements with the approach to use the same samples and perform tomography with each imaging method at different location and short-term sample transfer.

Keywords: contrast agent; root structure; soil water; tomography; water uptake

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1. Introduction

The investigation of processes proceeding at the root – soil interface is crucial for the understanding of how plants interact with soil and vice versa. This interface is essentially a part of the soil that is modified actively and dynamically in many ways by root activities and associated microorganisms. Present knowledge about it was mostly gained using optical observations and sampling in special two-dimensional plant growth containers. Studies were mainly concerned with its chemical, biochemical and microbial composition and their changes due to nutrient uptake. Less is known about its water transport properties and its impact on water fluxes, for example on root water uptake. Water fluxes appear along gradients of hydraulic and osmotic potential in the soil, and also in the plant. Resulting from that via the retention curve relation, in homogeneous soils a depletion of water around roots taking up water is expected (Gardner, 1960). However, some studies reported increasing water content towards roots (Nakanishi et al., 2005; Tumlinson et al., 2008; Carminati et al., 2010; Moradi et al., 2011). A general hypothesis to explain these contradictions is the dynamic nature and specific hydraulic properties of the root – soil interface, which are different from bulk soil and also may show hysteresis effects and changing properties. First experimental and numerical approaches have indicated the possible impact of hydraulic properties of the rhizosphere on root water uptake (e.g. Bengough, 2012).

Water fluxes are difficult to measure in soil, especially without disturbing them. Therefore, it is tempting to investigate them non-invasively and preferable 3D, which requires tomographic imaging methods such as neutron imaging (NI), magnetic resonance imaging (MRI) or X-ray tomography. A leap forward in this direction can be the combination of these modern imaging techniques in such a way that the weakness of one method is compensated for by the strength of the second one. This idea led us to a joint approach combining neutron imaging and magnetic resonance imaging on the same samples.

2. Materials and Methods

Imaging the root-soil interface requires samples that fit the field of view of the imaging station and are prepared with materials that are suited for both imaging methods, especially the container walls. The typical size of samples of some centimeters in thickness and often also lateral size imply that only relatively small and young plants can be used, if an undisturbed root system is desired.

2.1. Plant Samples

Lupine (*Lupinus albus*), chickpea (*Cicer arietinum*) and maize (*Zea mays*) were prepared for the imaging experiments. After stimulating germination the seeds were planted on top of the sand prepared in the sample containers as porous medium. In a plant growth chamber with controlled environmental conditions the plants then grew stem and roots. Water was added as needed during that period. After about two weeks the plant-soil samples were taken for image experiments. During the experimental period of some days the plant-soil samples were kept under similar conditions (Fig. 1) at the imaging facility, but water was irrigated only in defined quantities.

Some samples received infiltration of a certain amount of Gd-DTPA solution in concentrations up to 200mM. Those samples were tomographed before the infiltration, then transmission images were taken to follow the infiltration process, then another tomography, or several, followed.

Fig. 1. Lupine and maize plants grown in containers for neutron transmission imaging and illuminated by growth lamp in a growth cabinet providing a day-night cycle of light, air temperature and air humidity.

2.2. Neutron imaging

Another approach to image water in soils in 2D and 3D, that has been established more and more during the last years, is based on the neutron transmission imaging and neutron tomography. Imaging was performed at the NEUTRA station and the ICON station of SINQ at the Paul Scherrer Institut, Switzerland. Samples were either prepared for transmission imaging in thin slabs of aluminum or boron free glass, and for tomographies as glass cylinders of 2 to 3 cm inner diameter. In this paper, we exemplarily show results obtained in a measurement campaign at NEUTRA. For each tomogram measured, a sample was rotated stepwise over an angular range of 180°. A radiographic set of 360 projections, 6 flat- and 6 darkfield images was acquired. The exposure time was 25 s for a single radiograph, which corresponds to an acquisition time of about 4 hours for an entire tomogram. The pixel size of radiographs were 65 µm, while the physical resolution was around 200 µm.

Focus of the experiment was testing the feasibility of using Gd as a contrast agent for neutron imaging parallel to MRI experiments. A dilution series (Fig. 2) confirmed that different Gd concentrations can yield sufficient contrast differences to distinguish to some degree concentrations in that range.

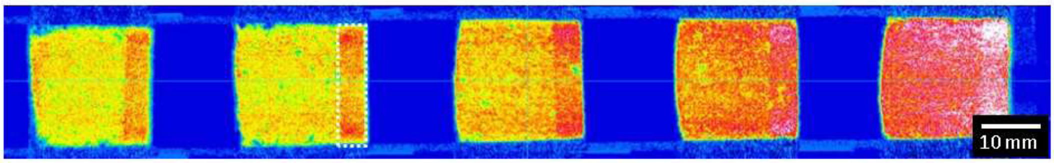

Fig. 2. Dilution series of Gd-solutions in a porous medium (cross-sectional view) imaged by neutron tomography at the NEUTRA station. In real the different samples were stacked vertically above each other, but are plotted here turned by 90°. A suction plate (marked by dashed white borderline) is visible at the bottom with constantly high attenuation, but otherwise attenuation is increasing with Gd-concentration in solution inside the porous medium (from left to right; 20 mM, 50 mM, 100 mM, 150 mM and 200 mM).

The main experiments were performed to obtain tomographies of the samples mainly for identification of the root system. Then time-series of neutron transmission images were collected after infiltration of Gd-solutions in a sample.

2.3. Magnetic Resonance Imaging

High field [1]H-MRI experiments were performed at FZJ Jülich, Germany, in a MRI greenhouse, similar as described by Pohlmeier et al. (2008) and Pohlmeier et al. (2013). Different tomographies were taken of the samples. 1) The root system architecture was monitored by a moderately T_2-weighted spin echo pulse sequence which profits from the reduction of the more rapidly decaying signal from soil. Measurement parameters: echo time = 5ms, repetition time = 2s, FOV: 80x80mm, matrix size 256x256, resolution 0.3x0.3mm, slice thickness 2mm.
2) The monitoring of Gd plumes in porous media uses the fact that longitudinal relaxation times (T_1) and transversal relaxation times (T_2) of H_2O depend on pore properties in soil and the presence of paramagnetic contrast agents, e.g. Gd^{3+}. This can be described by

$$\frac{1}{T_{1,2}} = \frac{1}{T^{b}{}_{1,2}} + \rho_{s1,2} \cdot \frac{S}{V} + R_{1,2} \cdot c(Gd^{3+}) \quad , \tag{1}$$

where R is the relaxivity of Gd as contrast agent, ρ_s is the surface relaxivity, S/V represents pore surface to pore volume ratio. This can be used to track water flow by applying contrast agents and following their transport over time (e.g. Haber-Pohlmeier et al., 2010). Please note that in (1) the second term on the right describes the sensitivity of the relaxation times for the pore sizes, and no acceleration of T_2 by diffusion in internal gradients is considered.

3. Results

The results presented in the following originate from a part of the campaigns performed in 2014 and further evaluation and interpretation will be added in the future.

3.1. Neutron transmission and tomography

Figure 3a shows a radiographic image of the root system of a lupine plant that has been infiltrated by a Gd-solution from top. The tracer was injected via a syringe into a near-surface layer of the soil close to the root. From the point of injection it spread out into the soil, appearing by its high neutron attenuation as dark cloud covering the topmost region of the root system. In Figure 3b the primary and secondary roots are tracked using the semi-automated root image analysis program SmartRoot in order to determine root morphological parameters relevant for water uptake from soil such as root mean length and diameter, insertion angle or interbranch distance. Figure 3c provides a three-dimensional rendering of the root morphology of the same plant. The image represents the result of a tomographic scan of the root system at a soil water saturation of 0.08 before tracer injection. It promotes a good impression on the three-dimensional root orientation and the morphological status of root development. To render the reconstructed sample volume in 3D the visualization software VG StudioMax was employed.

3.2. Magnetic Resonance Imaging

Before neutron imaging of the plant-soil samples at the Paul Scherrer Institut MRI experiments on the same plant-soil samples had been performed at FZJ Jülich. Figure 4 shows cross sections of the same sample taken by MRI (a-d) and the corresponding cross sections as derived by neutron tomography (e-h).The tomographic slices represent cross sections of the root system taken at soil depths of 8, 10, 12, 14 mm. The lupine roots are clearly visible in both the MRI and neutron images. Roots with a diameter of about 0.3mm are clearly distinguishable from the soil matrix at a water saturation of 0.26. In contrast to NI, the MRI images locally show dark zones around part of the root system at the interface between root and soil matrix. These zones have been reported earlier and interpreted as water depletion zones (MacFall et al., 1991). However, this is in contrast to NI results (Moradi et al. 2011) postulating water enrichment in the rhizosphere. The decreased signal of T_2 weighted MRI sequences in the rhizosphere can be due to locally faster T_2 relaxation, caused by smaller local pore size or presence of mucilage. Further clarification of the effect of such structures on MRI and NI images is part of the ongoing project.

Owing to the higher spatial resolution the root morphology appears more detailed in the neutron images. Also, when measuring the roots at MRI instruments the soil water content was significantly higher than during the subsequent neutron experiments.

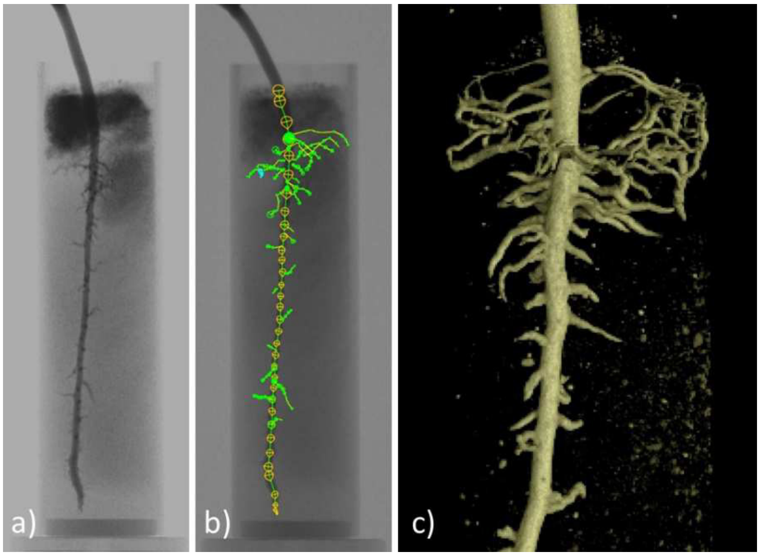

Fig. 3. (a) Image of a sample with a lupine plant, after infiltration of a Gd-solution from top, imaged by neutron transmission at the NEUTRA station. Dark regions indicate low neutron transmission, bright regions high neutron transmission; (b) Tracking of the root system via the semi-automated root image analysis program SmartRoot (Softpedia). (c) Perspective 3D-view on the root system using the software VG StudioMax.

3.3. Discussion

First results of the neutron imaging experiments showed the suitability of Gd as tracer substance for visualizing plant root soil interactions. As shown in a dilution series (see Fig. 2) the contrast to water in the neutron absorption increases clearly with increasing Gd concentration of the solution, at least in the tested range. Furthermore, Gd has already been proven and is used as contrast enhancing tracer for MRI experiments, also on root-soil samples (Haber-Pohlmeier et al., 2010). Together this offers an excellent opportunity for a joint investigation of the rhizosphere with MRI and NI, using very similar experimental procedures, but yielding complementary information on water status in the rhizosphere. The high spatial resolution of neutron imaging and its sensitivity for water can be exploited for the 3D analysis of the root morphology (see Fig. 3) and detailed mapping of three dimensional water content at the root soil interface and the surrounding soil. MRI yields complementary information about the dynamics of water in the underlying soil structures by relaxation time weighted images and relaxation time maps. In Fig. 4 the influence of the heterogeneity in pore structure as well as the absolute water content is reflected by different grey values of the T_2-weighted MRI images.

The comparison of the MRI and NI images presented in Fig. 4 indicates the decrease of water content in the soil due to the time period of two days between the MRI and NI experiments. This underlines the importance of minimizing time intervals for transport as well as plant stress and sample disturbances caused by transport over large distances.

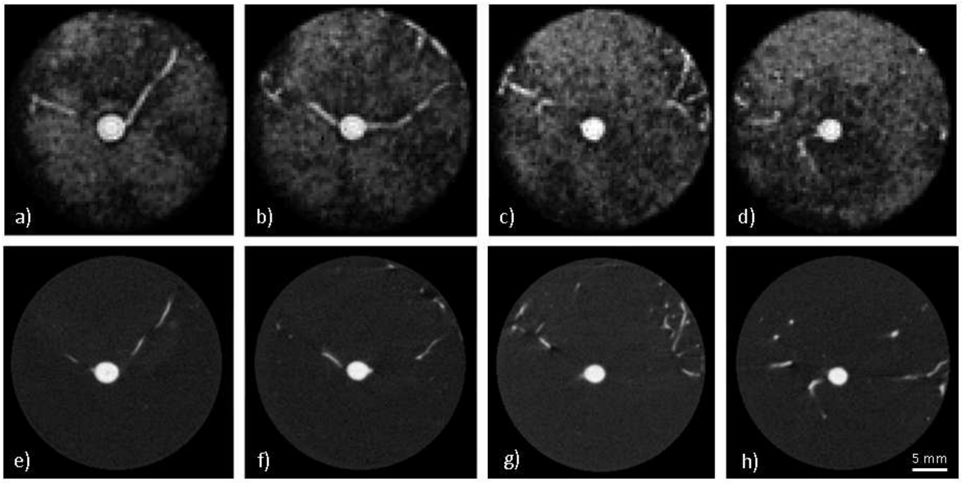

Fig. 4. Tomographic slices at different depths of 8, 10, 12, and 14 mm below soil surface showing the same cross sections of a lupine root imaged by magnetic resonance imaging (a-d) and neutron tomography (e-h). Water saturation of the soil matrix was 0.26 during MRI- and 0.08 during NI-measurement.

4. Conclusions

We have started to combine for the first time the detailed, three-dimensional imaging of the system plant root - root-soil interface - soil by means of NI and MRI. For a start a conventional high-field MRI scanner was used and long range transport to the neutron imaging facility of PSI was undergone, though this implies changes in the samples between imaging with the two different tomographic methods. Investigated systems were young lupine and maize plants in fine sand as soil under steady state conditions and during desiccation – rewetting cycles.

These first results show that a design of samples, soil and water content for joint use is possible and actually the same samples could be used for a sequence of MRI and NI covering a few days during a transpiration period. The root structure could be identified by both tomograms, but with different image properties, besides different water content driven by the drying of the samples in the elapsed time period. Gd in solution can not only be applied as contrast enhancing tracer, as is well known, but actually also for neutron imaging yielding substantial contrast. However, the tracking of water fluxes or the transfer of Gd into roots and inside the roots will need further investigations, but seems possible based on these first experimental results.

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