Neutron Radiography for the Analysis of Plant–Soil Interactions

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Neutron radiography (NR) can be used to quantify the spatial distribution of water in the soil–plant system with high precision and good spatial resolution. This property of neutron imaging results from the high interaction probability of hydrogen nuclei with slow neutrons. If there is a sufficient difference between the water content of the soil and roots, neutron radiographs can reveal plant roots and show root development. NR is noninvasive, and the radiation dose needed to image plant roots in soil does not affect plant development. Quantification of the soil's water content often requires correction for neutron-scattering artifacts. Root visibility is proportional to root thickness, and is inversely related to the width of the sample container and the water and organic matter contents of the ambient soil. Ideally, the soil should have low organic matter content and low water content but still permit the normal development of plant roots. Currently, the availability of neutron-imaging facilities limits the widespread application of NR to soil and root studies. However, technological development and increased investment will result in NR becoming a standard method for some soil–plant analyses.

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1 INTRODUCTION

Terrestrial plants provide humanity with food and oxygen. Above- and belowground plant processes are equally important: while assimilation through photosynthesis occurs in the aboveground portions, the uptake of water and mineral nutrients occurs belowground. We have a limited understanding of some belowground processes because soil hinders the observation of plant roots. It is difficult to measure root development and water flux in the root zone without disturbing root growth or using artificial systems. Existing techniques include minirhizotrons, which are transparent plastic tubes inserted into the ground to view the roots, e.g. by using a video camera.(1) Minirhizotrons interfere with the root environment and only provide an incomplete picture. X-ray radiography has insufficient contrast to reveal root–water interactions.(2)

Willatt et al.(3) showed that NR could reveal roots and root zone processes, without greatly perturbing the system, thus allowing sequential measurements. This is a critical advantage of NR over other techniques. However, the available technology in the 1970s gave images of insufficient quality and exposed the plants to radiation doses that were potentially harmful. Recent technological developments, especially improved beam collimation, detection systems, and image-processing techniques, have allowed the production of images with much higher contrast and spatial resolution while reducing the plants' radiation exposure. These advances open up the possibility of using this technique to study root system development in soils and simultaneously monitor soil moisture distribution in near real time. Here, we describe the state of the art of NR as it relates to the analysis of plant–soil interactions.

2 NEUTRON RADIOGRAPHY CONFIGURATION

Figure 1 shows the configuration of a typical NR facility. NR requires a neutron source, which may be a reactor, the target of an elemental particle accelerator, or a neutron-emitting isotope. Thermal (12–100 meV) and cold (0.12–12 meV) neutrons are preferable for NR investigations. Therefore, epithermal, intermediate, and fast neutrons from the neutron source must be slowed

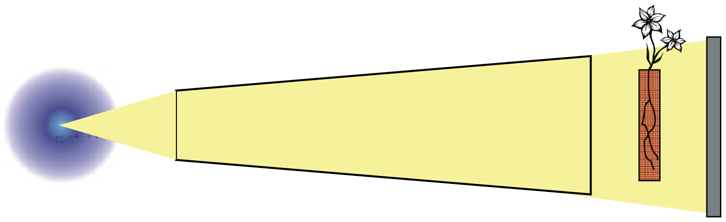


Figure 1 Configuration required for neutron imaging: (a) neutron source, (b) collimator, (c) sample, and (d) detector.

down using a moderator such as heavy water. Neutrons enter a collimator that forms a neutron beam with specific geometric properties. The collimator may also contain filters that modify the energy spectrum of the beam or reduce the beam's content of γ rays. The neutron beam is transmitted through the sample onto a plane position-sensitive detector, which is usually a scintillation screen. A charge-coupled device (CCD) camera coupled to the detector records a two-dimensional image that is a projection of the object on the detector plane. The advent of high-resolution digital cameras has allowed fast imaging and increased resolution. Digital imaging techniques that use a CCD camera combined with image-processing tools nowadays yield quantifiable images with a resolution of about 100 µm. Lehmann et al.[4] obtained a much higher spatial resolution with a special setup for microtomography. The required exposure time for such images is in the order of seconds. Images acquired using imaging plates or films (used until 1995) required several minutes of exposure to the potentially damaging neutron beam. The radiation dose received per image using modern techniques is about 0.003 mSv,[5] which is some two orders of magnitude less than the minimum value of 0.2 mSv h^{-1} found to affect plant growth.[6]

3 NEUTRON INTERACTIONS WITH THE PLANT–SOIL SYSTEM

NR is based on the Beer–Lambert exponential law of attenuation of radiation passing through matter[7]:

$$I = I_0 \exp(-\Sigma_{\text{sample}} d) \qquad (1)$$

where I is the attenuated radiation (neutron) flux (cm^{-2} s^{-1}), after an incident neutron flux I_0 passes through a material of thickness d (cm) with an attenuating coefficient Σ (cm^{-1}), which is a characteristic of the material. The attenuation coefficient, also called the *macroscopic cross section*, is related to the tabulated microscopic cross section σ (cm^2) as

$$\Sigma = N\sigma \qquad (2)$$

with a nuclear density N (M). When a sample is placed in a neutron beam, heterogeneities in the composition and thickness of the sample result in variations in the intensity of the transmitted beam. Unlike X rays, neutron radiation interacts with atomic nuclei. There is no systematic change in the neutron attenuation coefficient with atomic number or mass. Each isotope has a specific neutron cross section, σ, which is also energy dependent. Hydrogen has a neutron cross section some 10 times greater than deuterium and also greater than many other elements in the soil–plant system.

NR reveals structures in plant–soil systems owing to differences in the Σ values of the system's components. Table 1 shows a list of the chemical elements in the plant–soil system, along with their abundances and relative neutron cross sections. In both plant and soil, hydrogen is responsible for more than 90% of the neutron attenuation.

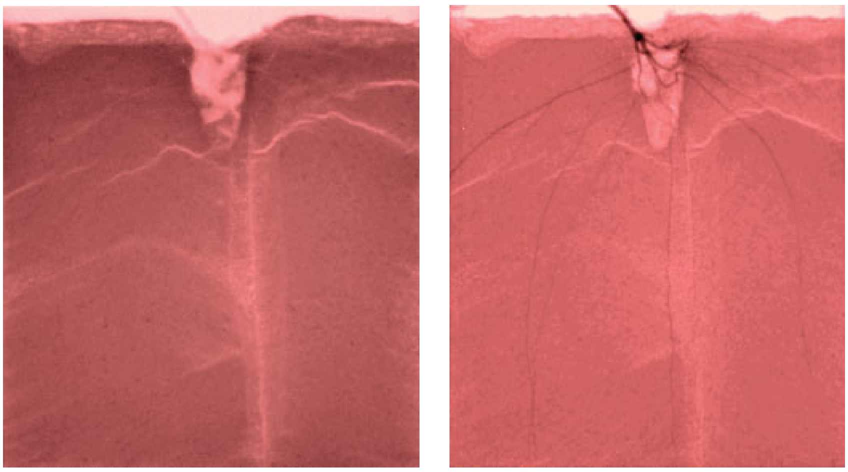
Some hydrogen is associated with organic molecules in the system; however, most hydrogen is water borne. Plant roots may thus be distinguished from soil due to their higher water content (θ). The gravimetric water content, θ ($g\,g^{-1}$) of plant roots generally ranges between 0.7 and 0.95 $g\,g^{-1}$, while that of soils at field capacity usually ranges between 0.12 and 0.3 $g\,g^{-1}$. The structures that NR reveals in the soil–plant system are sensitive to θ.

The high neutron attenuation coefficient of hydrogen is an important advantage of NR over X rays when applied to soil–plant system because the difference in water content allows the visualization of roots. The X-ray attenuation coefficients of root and soil components are less distinct (data not shown); therefore, the resulting radiograph has less contrast. Figure 2 shows that NR provides a better contrast between roots and soil than X rays.

The attenuation coefficient (Σ) results from two types of neutron interactions with matter: absorption (Σ_a) and scattering (Σ_s) (Figure 3a). Hydrogen attenuates neutrons primarily by noncoherent elastic scattering.[11] Neutron scattering causes deviations from the exponential law of attenuation for thicker samples (more than a few millimeters) because some neutrons are multiple scattered into the detector plane, thus producing

Table 1 Chemical elements, listed in order of abundance, in the plant[8]–soil[9] system, along with their relative neutron attenuation coefficients.[10] The plant and soil are assumed to have water contents of 0.8 and 0.2 g g^{-1}, respectively

	Plant (mol kg^{-1})	Soil (mol kg^{-1})	Element Σ (cm^{-1})	Plant neutron attenuation (cm^{-1})	Soil neutron attenuation (cm^{-1})
Hydrogen	100	24	3.4	344	82
Oxygen	50	35	0.17	8.5	6
Silicon	71E−4	9.3	0.11	<0.1	1.02
Carbon	7.5	1.1	0.56	4.2	0.6
Aluminum	7.4E−4	2.1	0.1	<0.1	0.21
Potassium	5.1E−2	0.93	0.06	<0.1	<0.1
Sodium	8.7E−4	0.82	0.09	<0.1	<0.1
Nitrogen	0.21	3.6E−2	0.43	<0.1	<0.1
Calcium	2.5E−2	0.19	0.08	<0.1	<0.1
Iron	3.6E−4	0.19	1.2	<0.1	0.22
Magnesium	1.6E−2	7.8E−2	0.15	<0.1	<0.1
Phosphorus	1.3E−2	1.2E−2	0.12	<0.1	<0.1
Titanium	4.1E−6	2.4E−2	0.6	<0.1	<0.1
Sulfur	6.2E−3	2.3E−3	0.06	<0.1	<0.1
Manganese	1.8E−4	2.8E−3	1.2	<0.1	<0.1
Chlorine	5.6E−4	1.6E−3	1.3	<0.1	<0.1
Zinc	6.1E−5	5.3E−4	0.35	<0.1	<0.1
Boron	3.7E−4	1.8E−4	102	<0.1	<0.1
Copper	1.9E−5	1.6E−4	1.1	<0.1	<0.1
Molybdenum	2.1E−7	5.2E−5	0.52	<0.1	<0.1
Total	158	74		356	91



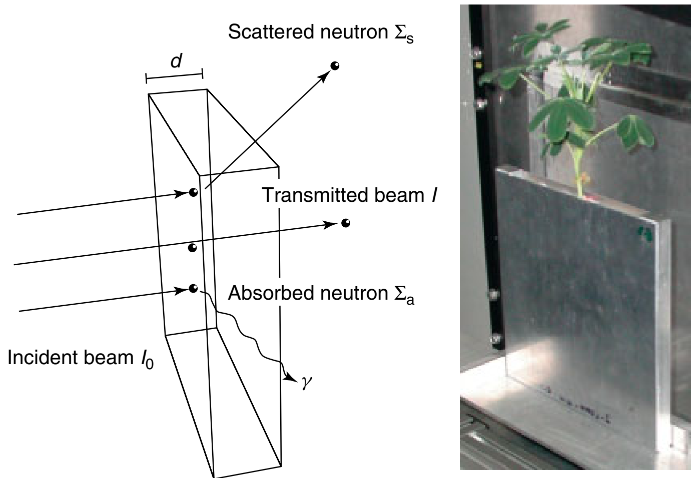
(a) (b)

Figure 2 Comparison of contrast between roots and soil in X-ray (120 keV) radiograph (a) and neutron radiograph (b) of the same sample. While soil heterogeneity and soil cracks are more visible in the X-ray radiograph, neutron radiography provides better contrast between soil and roots.

an artificially high signal. While the significance of neutron scattering is of little importance in the detection of roots in soil, it can be problematic when quantifying θ of the system. Algorithms such as the quantitative neutron imaging (QNI)[12] correct for the nonlinearity arising from neutron scattering. The QNI iteratively reconstructs the image by overlapping point-scattered functions calculated using a Monte Carlo simulation.

4 CONFIGURATION OF SOIL–PLANT SYSTEM FOR OPTIMAL IMAGING WITH NEUTRON RADIOGRAPHY

A neutron radiograph is the result of all the neutron attenuation processes that occur when the neutron beam passes through a sample. The Σ value of the soil depends on θ and the neutron attenuation properties of its solid components. Soils that are high in iron or organic matter



(a) (b)

Figure 3 Neutron interactions with the sample (a) and an example of a plant sample (*Lupinus albis*) mounted in front of the detector (b). The dimensions of the sample container are 150 mm × 150 mm × 12 mm.

Table 2 Properties of various plant growth media with regard to NR

	Bulk density ($g\,cm^{-3}$)	Water content at 1 bar ($g\,g^{-1}$)	Σ (dry material) (cm^{-1})	Σ (water content at −1 bar) (cm^{-1})	Notes
Perlite	0.125	1.08	0.1	4.5	–
Porous glass beads	0.49	0.16	1.6	2.5	High Σ due to boron in glass
Ferrous mine tailings	1.4	0.08	1	1.6	–
Loam	1.2	0.17	0.5	1.5	–
Peat	0.58	0.09	0.75	1.4	–
Loamy sand	1.3	0.09	0.3	0.75	Normal root development
Fine quartz sand	1.5	0.01	0.25	0.35	Root development perturbed
Coarse quartz sand	1.45	0.01	0.25	0.3	

are unsuitable for NR because their high Σ values obscure the visualization of plant roots. The value of θ of the soil at the time of measurement should be as low as possible without inducing water stress in the plants. In practice, this represents the soil's θ at a water potential of −1 bar. Silica sands have a low inherent Σ and a low θ at −1 bar. However, Menon et al.[5] showed that the high density and sharp edges of this material perturb normal root growth. Moradi et al. (unpublished data) tested a variety of plant growth media for their suitability in NR (Table 2).

Table 2 shows that loamy sand, which permits normal root development, has a relatively low Σ at a θ at a water potential of −1 bar. Loamy sand has a higher θ at field capacity (ca. 0.35 $g\,g^{-1}$) than quartz sand (ca. 0.15 $g\,g^{-1}$). Plants can thus be left longer in loamy sand without irrigation.

Root visibility is proportional to root thickness, and inversely related to the width of the sample container.[13–17] The minimum detectable root thickness increases exponentially as the thickness of the soil profile increases. Moradi et al. (unpublished data) showed that using the loamy sand in Table 2, with a soil θ of 0.16 $g\,g^{-1}$ (ca. −1 bar) and an average root θ of 0.85 $g\,g^{-1}$, the minimum detectable root thickness R (mm) is related empirically to the slab thickness T (mm) according to the function:

$$R = 0.0034T^{1.68} \tag{3}$$

Roots are most easily visualized in thin containers. However, this condition is not conducive to plant growth since the walls of the container restrict root development. Therefore, one needs to find a balance between the ease of root visualization and the restriction of normal plant development.

For many herbaceous species, a soil profile with a thickness of 12 mm provides enough space for relatively normal root development.(5) This gives a minimal detectable root thickness of 0.22 mm (Equation 2). Such a setup allows the visualization of the skeleton of the root system; however, fine roots (<2 mm in diameter) are undetectable.

5 PRACTICAL CONSIDERATIONS

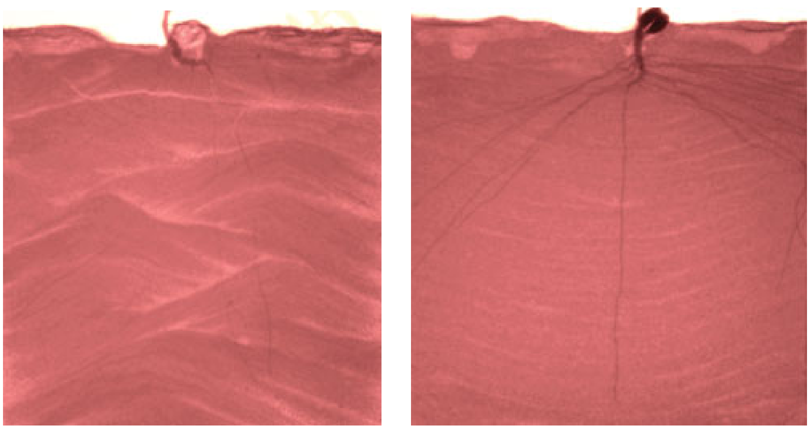
The sample container should be made of a material with a low Σ, such as aluminum ($\Sigma = 0.1\,\mathrm{cm}^{-1}$). Importantly, the system should not contain high concentrations of cobalt, which can form the persistent radioactive isotope Cobalt-60, with a half-life of about five years, upon exposure to neutron radiation.

Filling the sample container with soil as homogeneously as possible provides better contrast for root visualization. Filling from the side of the container rather than from the top results in less structural heterogeneity associated with variations in the particle size distribution and thus pore size distribution, which are visible in the resulting neutron radiograph because of variations in soil water content. Figure 4 shows the effect of filling patterns caused by pouring soil from the top of the container.

The suitability of NR to investigate root systems differs among plant species. As described earlier, thick roots with a high θ are more easily resolved than finer, drier roots. In a 10-mm-thick soil profile, the minimum detectable root size is ca. 0.16 mm (Equation 2). However, many plant species produce finer roots, which would not be visible in this system. Decreasing the thickness of the soil profile may permit the visualization of these roots, but may cause unnatural root growth patterns due to confinement. Moradi et al. (unpublished data) reported good root visibility in some members of the Fabaceae and Asteraceae families, whereas resolution was insufficient in Brassicacae, Solonaceae, and Poaceae. In principle, larger species such as small trees could be investigated using NR. However, this would require a larger container for nonperturbed growth. Consequently, the spatial resolution would decrease and one could only resolve large structural roots.

The water content of the soil and the plant should be monitored so that the soil water content is low enough at the time of imaging to provide adequate contrast yet not so low that the plant becomes water stressed. If a series of measurements are to be taken, then one can calculate the soil θ in the zone of interest from the results of each radiograph. This requires scattering correction and a water quantification algorithm calibrated for the particular plant–soil system. Water quantification using NR has the advantage over a gravimetric measurement because it can be used to determine the water content of the soil in the zone of interest, rather than providing an average value of a soil profile that may have a heterogeneous moisture distribution.

The neutron beam formed in the collimator is not perfectly homogeneous. This also holds for the detector system. Therefore, there are spatial variations in intensity of the resulting radiography that are caused by the beam, rather than the sample. This requires that each radiograph be corrected by normalizing the image by a "flat field" or open-beam image with no sample. Similarly, noise generated by the camera assembly should be removed. A



(a) (b)

Figure 4 Soil heterogeneity resulting from filling patterns can affect the image quality. The radiographs show the roots of *Cicer arietinum* in identical soil with high heterogeneity (a) and low heterogeneity (b).

standard first step in image analysis is normalization, i.e. to transform recorded raw information relative values:

$$I' = NORM\frac{I_{\text{raw}} - I_{\text{dark}}}{I_{\text{openbeam}} - I_{\text{dark}}} \quad (4)$$

where I_{raw} is the image as registered by the camera, I_{dark} is the dark noise image without the beam, I_{openbeam} contains the spatial field variation of the beam without object, and NORM is a factor to bring the resulting value into the valid range of the image-processing tools.

The background gray-level intensity within a neutron radiograph, caused, for example, by variations in the soil water content, may vary so much that a single global threshold cannot differentiate the roots satisfactorily. Menon et al.(5) overcame this problem by modifying an algorithm originally developed for resolving blood vessels in retinal images.(18)

6 APPLICATION

The application of NR can enhance the study of root development and root–soil interactions by revealing the location of roots over time without disturbance. Plant root development is a function of the plant species and the nature of the soil into which they penetrate. Soil components, such as organic matter, nutrients, and contaminants, occur heterogeneously. NR is an ideal tool to study how roots interact with patches of low or high concentration. When roots encounter a patch or discontinuity in soil, they may proliferate, wither, or continue growing unaffected. Figure 5(a) and (b) shows how NR can reveal plant responses to a patch of nickel, a toxic heavy metal, in soil. Such experiments, for example, may reveal species that avoid contaminant hotspots and reduce the risk of plant contaminant uptake. Similarly, understanding the mechanisms by which crop plants interact with patches of nutrients may aid the development of treatments to improve crop, and subsequently human, nutrition.

Perhaps more importantly, NR can quantify the spatial distribution and flux of water in the plant–soil system in near real time, in combination with root imaging. This permits the study of root water uptake and the effect of roots on the passage of water through soil. Figure 6 shows a series of images detailing water infiltration into a profile and water uptake by plant roots. Such studies have a wide variety of potential applications, such as selecting vegetation to minimize leaching from contaminated sites and the optimization of irrigation and fertilization systems.

7 CONCLUSIONS AND OUTLOOK

The quantification of root mass and soil water content using NR of plant–soil interactions has considerable scope for improvement, particularly, the processing of raw images obtained at the neutron facility. Refinement of root segmentation algorithms described by Menon et al.(5) would greatly enhance the accuracy and precision of the technique. One obvious drawback of NR is that, at present, it requires access to a specialized facility, of which there are only a few available worldwide with the desirable performance. Competition for beam time is fierce, since NR finds applications in many fields of science. However, the usefulness of NR indicates that it may follow the same pattern of development as synchrotron radiation facilities. Initially, financial

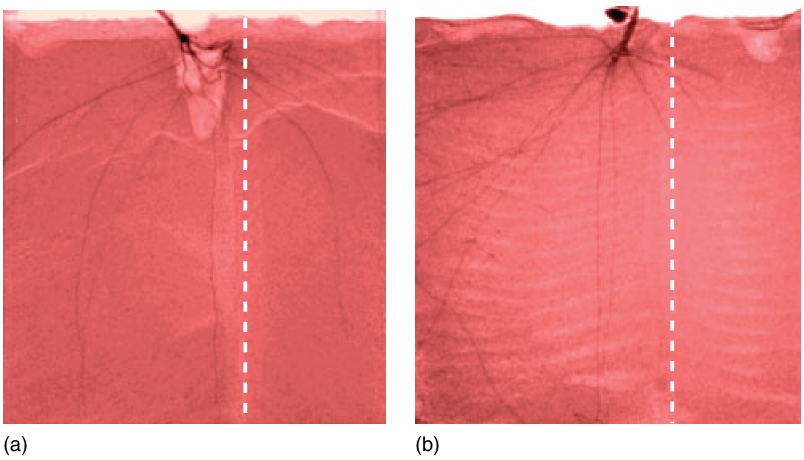


Figure 5 Neutron radiographs of two 150 mm × 150 mm × 120 mm slabs filled with sandy loam. The area to the right of the dotted line was spiked with 125 mg kg^{-1} Ni. The roots of *Cicer arietinum* (b) avoid the high-Ni zone, while the roots of *Berkheya coddii* (a) are unaffected. Neither plant showed any differences in the aboveground portions relative to their respective controls.

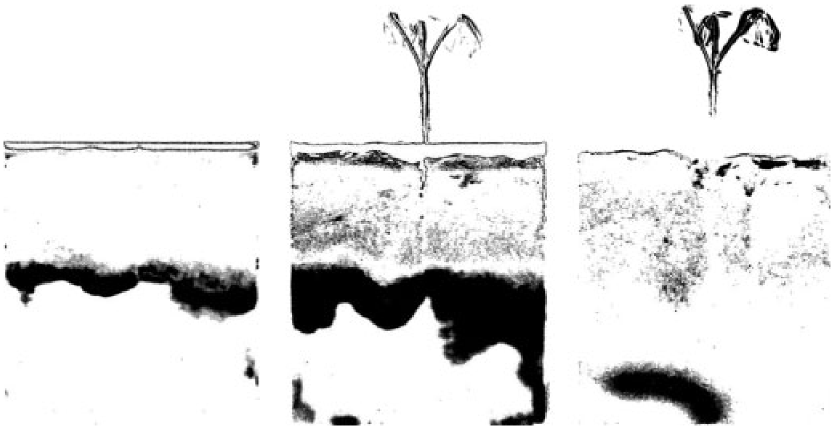


Figure 6 The change in water content (both positive and negative) of a 150 mm × 150 mm × 120 mm container, in which *Lupinus albus* was growing, after the infiltration of 10 mL of water ((a) 1–2 min, (b) 2–5 min, (c) 28–40 min). The wet front shows up as a dark band, while root water uptake is visible in b and c as a discontinuous gray area.

and technical constraints limited their application, but their usefulness ensured subsequent technological development and capital injection, and thus they became commonplace and the standard equipment for some analyses.

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