



Advancing root architecture analysis: 3D neutron imaging of plants grown in slab rhizotrons

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Abstract

Background and aims Root system architecture (RSA) shapes biogeochemical concentration patterns in the rhizosphere. Root-soil studies are often conducted on plants cultivated in rectangular rhizotrons, including when using 2D hydrochemical analysis methods. However, roots naturally expand in three dimensions, with the rhizosphere extending accordingly. Three-dimensional neutron imaging can enhance interpretation of such studies, yet imaging flat, slab-shaped rhizotrons is technically challenging. This study presents a methodological comparison between conventional neutron tomography (NT) and neutron computed laminography (NCL) to assess whether NT under high-flux conditions can achieve image quality sufficient for 3D root

segmentation, comparable to NCL, without requiring tilting of the rotation axis.

Methods NT and NCL were applied to maize plants grown in rectangular rhizotrons. Imaging artifacts and their impact on root segmentation were assessed for two plants representing low and high soil moisture conditions suitable for neutron imaging.

Results Both methods produced 3D tomograms of comparable quality across the tested moisture range, enabling effective segmentation of primary and seminal roots. Lateral root detection was more challenging and depended on soil moisture. NCL captured a greater number of horizontally oriented lateral roots while NT was more effective in resolving vertically oriented roots.

Conclusions NCL is not required to resolve 3D RSA of maize plants in flat rhizotrons. Under high-flux neutron beam conditions, NT is preferable as it simplifies sample handling, reduces plant stress, avoids soil water redistribution and enables direct integration with timeseries of 2D chemical and neutron radiographic imaging.

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Introduction

The root system architecture (RSA) of a plant is determined by a combination of genetic factors and environmental influences (Rogers and Benfey 2015). The chemical and physical conditions of the soil, as well as the spatial availability of resources (nutrients, water), play a crucial role in shaping the RSA (Pierret et al. 2007). Conversely, the position, function and type of the roots have substantial impact on the characteristics of the soil, for example its chemical gradients, water distribution, and the presence of mucilage or organic carbon compounds (Hinsinger et al. 2009). Measuring RSA alongside biogeochemical dynamics in the root zone, such as pH, enzyme distribution, gas concentrations and moisture distribution, is critical for comprehending plant resource acquisition, stress adaptation, rhizosphere interactions, and carbon cycling. To that end, experimental methodologies that integrate both the observation of biogeochemical patterns within the root zone and the spatial configuration of the root systems (the RSA), are required.

Non-invasive imaging techniques, particularly neutron imaging with its high sensitivity for water, offer accurate options for capturing RSA, also in combination with the analysis of root water uptake and water content distribution in soil, via 3D Neutron Tomography (NT) (Moradi et al. 2011; Tötze et al. 2021a, 2017) or 2D neutron radiography, (Ahmed et al. 2016; Dara et al. 2015; Zarebanadkouki et al. 2013). For in-situ observation of chemical properties and dynamics within the rhizosphere, e.g. the distribution of gases (O_2 , CO_2), pH, enzymes, and other root exudates, imaging methods are also widely applied. Common techniques include planar optodes (Li et al. 2019), diffusive gradients in thin-films (Santner et al. 2015), ^{14}C imaging (Holz et al. 2019) and zymography (Bilyera and Kuzyakov 2024; Zhang et al. 2023). These methods are, however, constrained to two-dimensional applications. Given that the rhizosphere can extend several centimeters around individual roots (Kuzyakov and Razavi 2019) and that plant root systems do not naturally form a 2D structure,

there is a clear need to methodically link 2D chemical imaging with 3D information on RSA. However, this presents a significant technical challenge: While the aforementioned chemical imaging techniques are well established and provide valuable insights into the biogeochemistry of the rhizosphere, their inherent two-dimensional nature necessitates plant cultivation within rectangular rhizotrons with a large front plate, to allow sensors (e.g. planar optodes or membranes) to be placed in contact with the roots and the soil surface. Even if root growth along the rhizotron front is forced by tilting the system during plant growth, spatially distant roots can still confound interpretation of data captured at the sensor-root-soil interface (Rudolph-Mohr et al. 2021), and roots growing on the surface may have modified properties. Conversely, 3D root system segmentation from neutron tomography is most effective for plants grown in small-diameter cylindrical soil columns. First attempts to scan rectangular rhizotrons in 3D were conducted by Esser et al. (2010) via tomographic measurements with thermal neutrons; however, the quality of the presented images appeared not adequate for accurate root segmentation. This can be attributed to unfavorable image statistics resulting from the substantial attenuation of the neutron beam when it passes through the longer dimension of the rhizotron.

An effective approach to overcome these limitations for specimens with flat geometry (high aspect ratio) is neutron computed laminography (NCL), an adapted tomographic imaging technique in which the axis of rotation is tilted to ensure uniform transmission from all projection angles to minimize missing information (i.e. wedges in the Fourier domain for limited-angle tomography) and other artifacts during reconstruction (Helfen et al. 2011b; Salvemini et al. 2015; Xu et al. 2012). Until recently, we considered NCL the only viable approach for capturing the 3D architecture of root systems grown in slab-shaped flat rhizotrons, given the limited neutron flux density at available imaging facilities. NCL was successfully integrated into a 2D imaging setup for plant root experiments, which included 2D imaging of O_2 patterns with via planar optodes and soil water

distribution by cold neutron radiography (Bereswill et al. 2023; Rudolph-Mohr et al. 2021). It was shown that NCL can capture the three-dimensional RSA of maize plants enabling 3D segmentation of roots and analysis of root O_2 uptake patterns as influenced by roots located up to 7.5 mm from the planar optodes (Rudolph-Mohr et al. 2021).

However, a significant drawback of using NCL in plant experiments is its requirement to tilt the sample during scanning, which can induce redistribution of water and air in soil pores, and may consequently influence exudate patterns or microbial processes. Consequently, when combining 3D NCL with 2D chemical imaging and 2D soil water distribution measurements, NCL must be performed either before or after the acquisition of time series data. This prevents truly simultaneous observation of root system architecture in 3D alongside 2D patterns and makes NCL unsuitable for obtaining 3D information on undisturbed soil water content. Compared to standard NT setups, NCL requires specific technical adaptations, such as a goniometer for sample tilting and a custom sample holder for rotation along the inclined axis (Helfen et al. 2011b). Furthermore, NCL reconstruction must account for 3D geometry imposed by the tilted axis (Helfen et al. 2011a), unlike conventional NT, where parallel-beam data allow simpler reconstruction of independent 2D slices.

Recent developments in neutron imaging, driven by the commissioning of advanced high-flux instruments at stronger neutron sources, are significantly expanding experimental capabilities for plant research. Notably, the NeXT neutron tomography station provides a tenfold increase in neutron flux compared to the CONRAD II instrument, which was used for earlier NCL measurements by Rudolph-Mohr et al. (2021). Specifically, NeXT achieves a flux of 3×10^8 neutrons $\text{cm}^{-2} \text{s}^{-1}$ at a collimation ratio of L/D 333 (Tengattini et al. 2020), whereas CONRAD II delivered only 2.4×10^7 neutrons $\text{cm}^{-2} \text{s}^{-1}$ at an L/D of 350 (Kardjilov et al. 2016; Rudolph-Mohr et al. 2021). This substantial gain in flux not only enables higher-resolution (Tengattini et al. 2022) and faster imaging (Tötzke et al. 2019), but also facilitates three-dimensional imaging of geometrically challenging samples, such as flat-shaped rhizotrons.

The objective of this study was to evaluate whether conventional neutron tomography (NT), performed

under the high-flux imaging conditions provided by the NeXT tomograph, can achieve image quality sufficient for three-dimensional root segmentation in rectangular rhizotrons—comparable to that obtained with neutron computed laminography (NCL)—without requiring tilting of the rotation axis and thereby avoiding associated disadvantages for plant experiments.

To address this, we conducted comparative imaging of maize root systems grown in slab rhizotrons in sandy soils. We measured each plant with both techniques at different soil moisture contents, to define the best operational setting, by i) assessing image quality and potential reconstruction artifacts from NT versus NCL, ii) comparing segmentation of the root systems from both imaging approaches and iii) exploring to which extent soil water content limits the application of NT and NCL for capturing 3D RSA of plants grown in slab-shaped rhizotrons.

Methods

Sample preparation

Nine rectangular, slab-shaped boron-free glass rhizotrons (150 mm $\times$ 150 mm $\times$ 15 mm) were filled with sandy soil (92% sand, 7% silt and 3% clay, sieved to < 2 mm). One optode sensor foil similar to the ones described in Rudolph-Mohr et al. (2021) was glued to the front and back window, in order to mimic transmission and scattering conditions as in previous experiments where NCL was combined with optode imaging. One pre-germinated maize seed (*Zea mays*) was planted per rhizotron and the soil was watered with a nutrient solution containing an NPK ratio of 7:3:6 supplemented with micronutrients. The fertilizer was applied at a dilution ratio of 1:166 (v/v, fertilizer:water). The rhizotrons were then sealed for transport, and sent to the Institut Laue-Langevin (ILL, Grenoble, France) where the plants were grown in a plant growth chamber located next to the beamline for the following 21 days under the following controlled conditions: a 14-h photoperiod with a light intensity of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with the light intensity gradually increased from 0 to 100% between 6:00 a.m. and 8:00 a.m., and subsequently decreased between 6:00 p.m. and 8:00 p.m. A constant temperature of 25 °C was maintained during the day and 19 °C at night,

with a relative humidity of 60%. Water and nutrient solution were applied regularly to maintain soil water content at $0.25 \text{ cm}^3 \text{ cm}^{-3}$.

Imaging techniques: neutron tomography and neutron computed laminography

The imaging experiments were performed at the neutron and X-ray tomography instrument “NeXT” at the ILL. A curved neutron guide delivers cold neutrons from the ILL’s 58.3 MW high-flux reactor to the imaging station, where for performing the tomography the sample is placed onto a rotation stage in front of a neutron detector at a distance of 10 m from a group of pinholes (Tengattini et al. 2020). Using a pinhole with 15 mm in diameter, the neutron flux at the measuring position is about $10^8 \text{ neutrons cm}^{-2} \text{ s}^{-1}$ at a L/D ratio of 665. The medium-resolution detector consists of a 200 μm thick $^6\text{LiZnS:Ag}$ scintillator screen (converting incident neutrons into visible light) and a sCMOS camera (Hamamatsu Orca Flash 4V2) combined with a high aperture Canon photographic lens (aperture $f/1.2$, focal length 50 mm) (Tötzke et al. 2019). The effective field of view of the 2D detector can be adjusted by modifying the position of the imaging lens (and camera) with respect to the scintillator plane. A field of view of approximately $160 \times 160 \text{ mm}^2$ was used, corresponding to a nominal detector resolution of 78 μm . Considering geometric blurring ($\sim 150 \mu\text{m}$) and scattering effects, the effective spatial resolution under the given experimental conditions is estimated at about 200 μm .

We conducted comparative measurements using both conventional tomography and laminography modes. The key imaging parameters are summarized in Table (1). The primary distinction between the two setups lies in the alignment of the rotation axis and the orientation of the rhizotron relative to it. In tomography mode, the rotation axis is vertically aligned, perpendicular to the direction of the transmitted neutron beam. The rhizotron is positioned upright on the rotation stage, with the plant’s stem oriented approximately parallel to the rotation axis (Fig. 1A). In contrast, for laminography, the rotation axis is tilted by approximately 30 degrees from the vertical, and the plant’s stem is oriented perpendicular to this inclined axis (Fig. 1B). This permits maintaining an almost constant average neutron transmission through the flat rhizotron since the effective object thickness along

the beam direction does not vary substantially for the different rotation angles. This is illustrated in the animated image series of radiographic projections and the corresponding plot of angular relative transmission in Video S1 and Figure S1 in the Supplementary Information. Prior to imaging, the soil surface was covered with aluminum foil and secured with adhesive tape to prevent soil loss during rotation.

Experimental procedure

At the start of the experiment, the root systems of all plants were inspected by 2D neutron radiography to choose the five best-developed plants for the 3D study (Figure S2 in the Supplementary Information). In the following, we present the results obtained from two plants (“M2” and “M1”) that represent the cases of very low soil moisture and moderately wet soil, respectively. This enables a meaningful comparison of the tomographic methods for samples with moderate versus high attenuation. Sample “M2” was scanned at $0.07 \text{ cm}^3 \text{ cm}^{-3}$ soil moisture vs. sample “M1” measured at $0.17 \text{ cm}^3 \text{ cm}^{-3}$ (Table 1).

The plants were scanned in the conventional tomographic mode (NT) first using the full rotation range (360°). Afterwards, the plants were stored in the plant growth chamber next to the beamline while the imaging set-up for NCL was prepared. One hour prior to the start of NCL, the corresponding sample was weighed to measure the gravimetric water content and watered with an amount to achieve a soil water content as close as possible to the previous NT measurement. Directly before NCL, the plant stems were cut off to allow the sample to be rotated 360° in a tilted position without bending and scratching the scintillator screen. While stem removal halted transpiration, we assumed that root water content did not change substantially during the scan, given the short duration and the expectation of limited osmotic water loss. This (non-mandatory) procedure made the scan easier and is a viable option in general if plants are no longer needed after the scan. Due to the substantial time investment required to equip the beamline for each imaging modality and to establish all the necessary technical parameters, it was impractical to repeatedly switch between NCL and NT for every plant sample in our experiment. Therefore, we first conducted NT on all plant samples and then changed

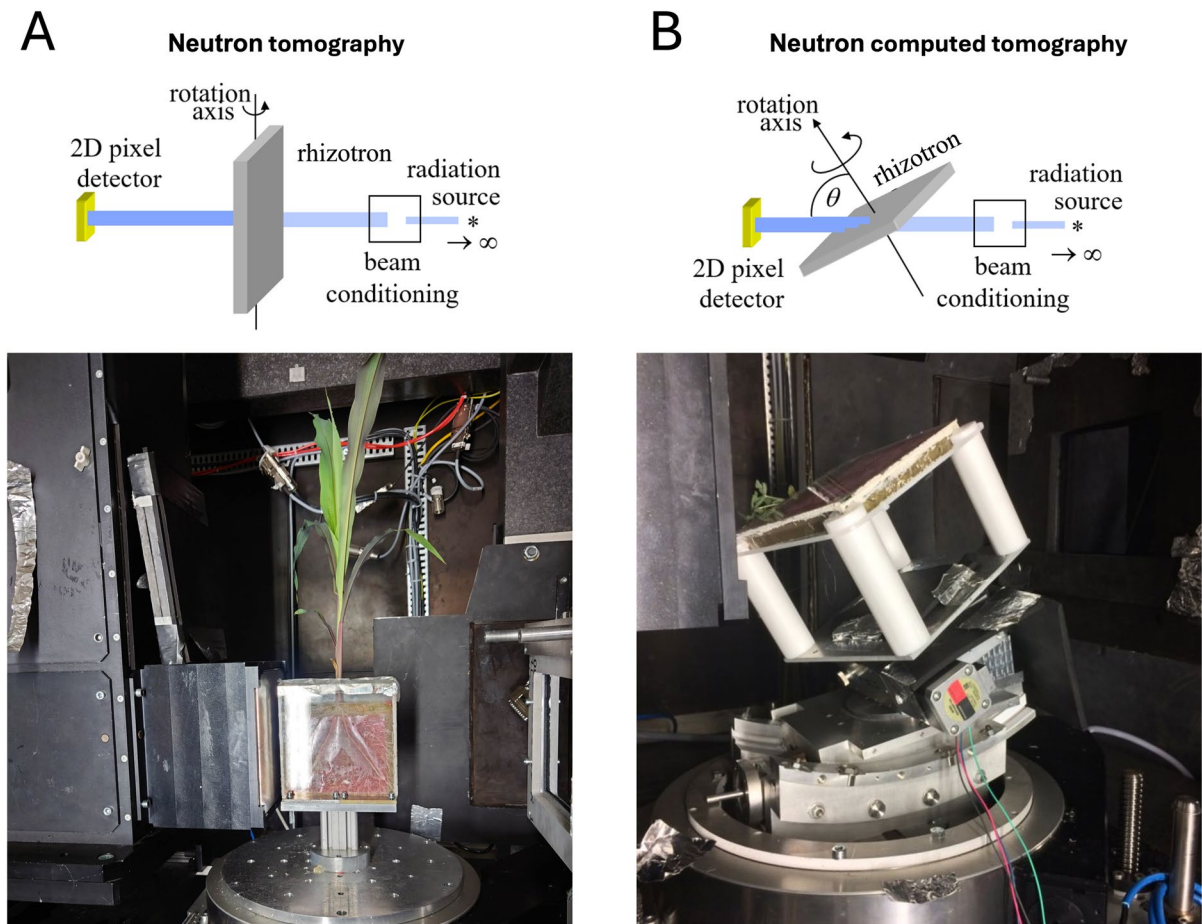


Fig. 1 Experimental setup for comparative neutron imaging: tomography (NT) vs. laminography (NCL). **A**) Schematic illustration of the NT setup and photograph of the rhizotron mounted upright on the rotation stage. The rotation axis is oriented vertically, perpendicular to the neutron beam. **B**)

Schematic illustration of the NCL setup and photograph of the rhizotron mounted on the goniometer. The rotation axis is inclined by approximately 30°, and the rhizotron is oriented accordingly to enable laminographic scanning

Table 1 Summary of key parameters for tomography and laminography scan

Parameter	NT	NCL
Pinhole [mm]; collimation ratio L/D [-]	15; 665	15; 665
Detector rotation axis distance [mm]	95	100
Tilt of rotation axis [°]	0	29.9
Number of projections	2816	2695
Exposure time per projection [s]	3	3
Total scan time [h:min]	2:20	2:15
Pixel size [μm]	78	78
Estimated physical resolution [μm]	~200	~200

the setup to NCL and performed NCL on all samples, which caused a time lag between NCL and NT of 24 to 27 h per plant.

Image processing

The 3D volume data sets were reconstructed using the filtered back-projection algorithm (Helfen et al. 2006; Kak and Slaney 2001). In order to facilitate comparability, the same software was used for the two modalities of NT and NCL. We chose the open-source processing toolkit “tofu” (Fargó et al. 2022) as it provides fast, GPU-accelerated back-projection (Vogelgesang et al. 2016) of 2D parallel-beam data

onto a 3D reconstruction grid (i.e. the volume data set to be reconstructed) and provides the possibility to adjust all relevant geometry parameters for the position and angles of the rotation axis with respect to the 2D detector system. A filter corresponding to the ideal ramp filter in 2D computed tomography was chosen. After reconstructing with 32-bit floating point precision, the voxel data was scaled down to 8 bit in order to facilitate segmentation and analysis. Both types of scans were reconstructed from projection data of a full rotation, without additional filtering such as removal of transmission outliers or the like.

Image segmentation of maize roots and rendering of 3D images were performed using the software “VGSTUDIO MAX” (Volume Graphics, Heidelberg, Germany, version 3.1). A region-growing algorithm was used as a user-interactive segmentation method to extract connected structures—such as roots—based on voxel intensity similarity and spatial continuity. Segmentation was initiated by manually placing a seed point within the root tissue, typically in the center of a root cross-section identified through visual inspection of orthogonal slice views. The algorithm then expanded the region by evaluating neighboring voxels based on a predefined intensity threshold and connectivity criteria. Primary and seminal roots—characterized by relatively high contrast and continuous geometry—were usually segmented in a single pass. In contrast, lateral roots often exhibited lower local contrast and discontinuities due to their fine structure. Therefore, an iterative segmentation approach was applied: seed points were manually repositioned along unsegmented portions of the lateral roots, and region-growing parameters (intensity tolerance and neighborhood radius) were locally adapted to avoid leakage into adjacent structures. Segmented fragments from successive passes were then merged to reconstruct the complete root system.

Root system segmentation was followed by skeletonization in Fiji to obtain quantitative measurements of root length. Signal-to-noise ratio (SNR) was calculated slice-by-slice in Fiji, and contrast-to-noise ratio (CNR) was computed in VGStudio MAX for lateral roots, the most challenging structures to segment due to their small diameter. CNR was calculated as the difference between the mean gray value of segmented lateral roots and the mean gray value of surrounding soil, normalized by the image noise (standard deviation of gray values in a homogeneous background region above the soil). The region of interest (ROI) in

the soil was defined as a shell around the roots (inner boundary: root surface dilated by 2 pixels; outer boundary: dilated by 9 pixels), with any overlap with root volume removed by subtracting the corresponding ROIs.

Results

Image quality and reconstruction artifacts

This section compares the characteristics of the image artifacts for the two recording modes, NT and NCL, at low and moderately high soil moisture. In principle, it is known that 3D detection of soil-grown plant root systems using neutron imaging works best at low soil moisture levels (Cai et al. 2022). Low soil water content allows sufficient transmission of the sample and provides high image contrast between the water-rich root tissue and its soil environment. As soil moisture levels rise sample transmission decreases reducing signal-to-noise ratio and diminished image contrast between the soil and the root. Figure 2 provides cross-sectional images for the maize plant measured at dry conditions. The longitudinal (z-x plane) and transverse (x-y plane) sections through the rhizotron show that the image quality for NT (Fig. 2A) and NCL (Fig. 2B) is in principle of a similarly good quality. Roots are clearly visible as the brightest structure in the images, which is a good prerequisite for segmentation using threshold-based segmentation algorithms. A slight double shadow can be seen in the upper part of the laminography image (Fig. 2B). The source of this artifact is not visible in this slice but is generated by the plant stem from the neighboring image layers. This type of artifact occurs with all highly attenuating plant structures as clearly visible in the orthogonal (x-y) view provided in Fig. 2D: Starting from each root section, there are four diagonal stripe artifacts. They cause the double shadows, which fade and drift apart with increasing distance from the corresponding highly attenuated structure. The diagonal streaks in Fig. 2D leave the horizontal at an angle of 30°, which corresponds to the angle of inclination of the axis of rotation in the laminographic measurement.

The reconstructed 3D image of the NT tomogram does not show such double shadows or corresponding

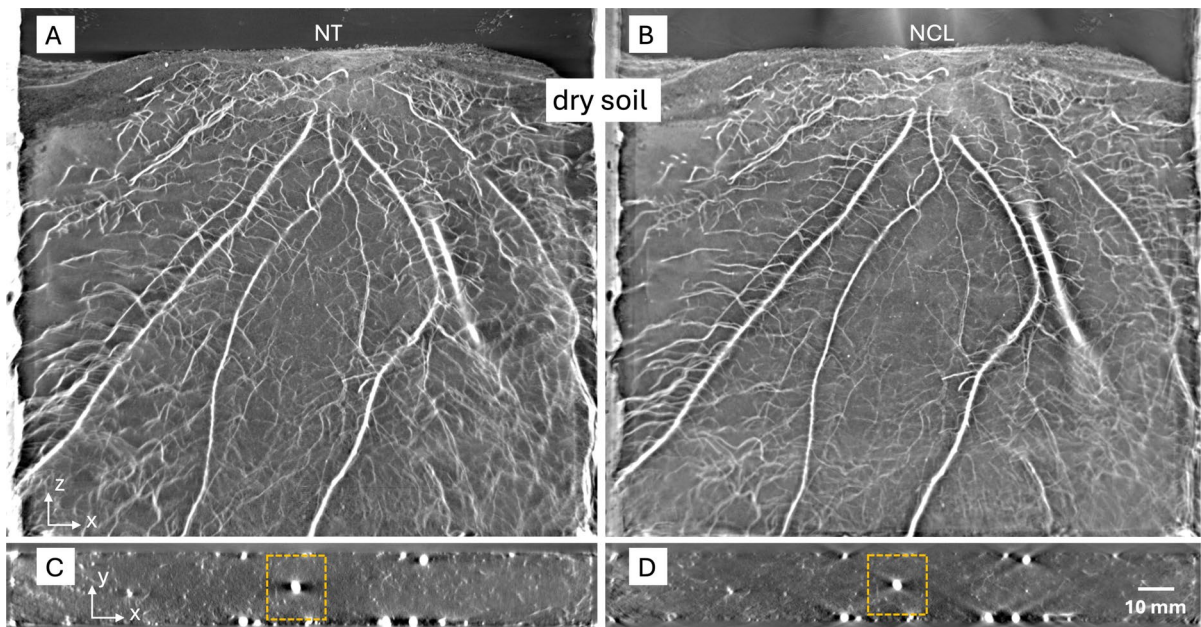


Fig. 2 Root system of the maize plant “M2” measured under dry soil conditions ($0.07\text{cm}^3\text{ cm}^{-3}$) with NT (left) and NCL (right). Roots as the highest attenuating structure appear bright in the images. A) A vertical cross section located close to the front wall of the rhizotron captured by NT. B) The same cross-sectional image but captured by NCL contains a bright double shadow artifact visible in the above ground region caused

by the stem of the plant. The stem itself is not visible in this tomographic slice. C) and D) provide a cross-sectional view from the upper soil region for NT and NCL, respectively. They reveal typical artifact structures around each root cross section, which are shown in more detail for the subregion bordered by the yellow dashed line in Fig. 3

stripe artifacts in the orthogonal view (see Fig. 2A and 2C). However, a similar artifact structure of comparable strength is also superimposed on the soil environment around the highly attenuating root structures. There is a dark shadow area around each cross section of larger roots extending in the x-direction, while a bright halo extends above and below the root cross-section in the y-direction.

This is illustrated for the area surrounding the primary root (dashed rectangle in Figs. 2C and 2D) in the magnified views of Figs. 3A and 3B. Figure 3C shows gray-scale values along the x- and y-directions in this region, normalized to the maximum value within each image subsection. The plots in y-direction, shown as dashed and solid blue graphs, indicate for both image modalities a region of soil with elevated gray values extending approximately 1 mm from the root surface. In x-axis direction, a dark, shaded area of soil appears next to the root, which has a greater extent in the tomography image, i.e., 3 mm (NT) versus 2 mm (NCL). Although these artifacts compromise the reliability of the information on the

water status of the rhizosphere, they appear to be relatively unproblematic for the segmentation of the roots itself. Nonetheless, this could result in a slight change in the shape of the root cross section and complicate the detection of lateral roots, especially if these roots are sandwiched between two larger neighboring roots. These artifacts are apparent when plants are measured under low soil moisture conditions, but less pronounced under elevated soil water content as shown in Figure S2 in the Supplementary Information.

The vertical and horizontal cross-sections of the root system of plant M1, measured at a soil water content of $0.17\text{ cm}^3\text{ cm}^{-3}$, are presented in Fig. 4. Due to the significantly higher moisture content of the soil and the resulting larger neutron attenuation, tomographic imaging of this sample is more challenging. First of all, a direct comparison of the images shows that the roots, especially the seminal root in the center of the image, have clearly grown further. Although this was also the case for sample “M2”, the roots of plant “M1” grew more, likely due to the better water availability to the plant. The image quality

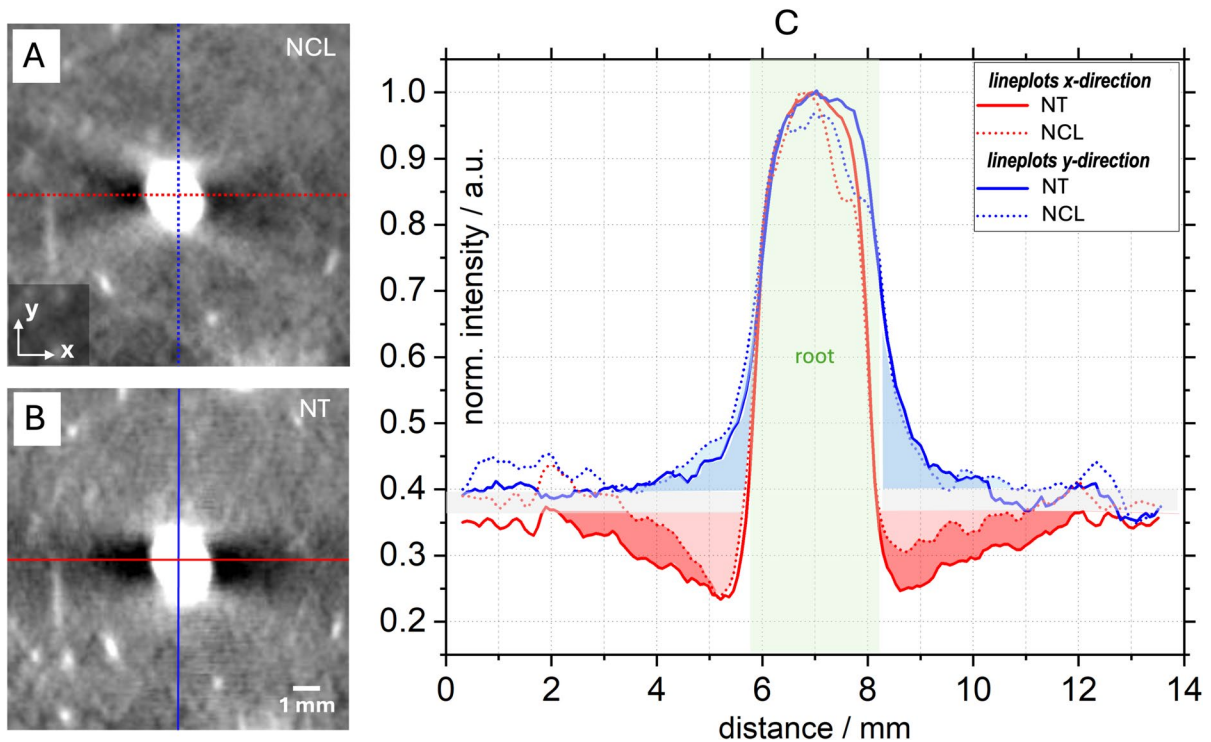


Fig. 3 Line plots through a cross-section of the primary root of plant "M2" reveal how artifacts influence signal intensity in the adjacent soil area (C). The respective cross-sections

are shown in (A) for NCL and in (B) for NT. In x-direction a shadow of 1–2 mm is cast over the soil while a halo surrounds the root in y-direction

is comparable over most of the image area for both scanning techniques and appears sufficient for segmenting at least the larger roots. However, the tomographic image (Fig. 4A) shows that the lateral edge areas are shadowed by the so-called missing wedge artifact. The missing wedge is the region (the lateral region marked by the dashed line in Fig. 4A and 4B) where insufficient structural information is available because the transmission of the sample is poor over a larger angular range of the scan, where the neutron beam must penetrate the long side of the rhizotron. This particular artifact is the main drawback of NT for such rhizotrons and avoided by a laminographic imaging (Fig. 4A and 4C) but requires tilting the axis of rotation.

As an additional descriptive metric of image quality, the signal-to-noise ratio (SNR) was evaluated on a slice-by-slice basis along the frontal (y) direction. For each tomographic slice, the SNR was calculated as the ratio of the mean signal intensity (gray-scale value) within the soil-filled ROI of the rhizotron to

the background noise, defined as the standard deviation of the signal measured in an object-free region above the soil surface. The curves in Fig. 5 clearly distinguish between the dry and wet measurement states for both NT and NCL. This pronounced difference results from the increased neutron scattering in the presence of water, which raises the overall background intensity and thereby reduces the absolute contrast between signal and noise. Figure 5 further shows that NCL consistently yields a lower SNR than NT. This reduction can be attributed to the propagation of streak artifacts and structural blurring along the frontal (y) direction (see Fig. 3a). These artifacts introduce additional signal into regions where none should be present, thereby increasing the standard deviation and background contribution, which in turn lowers the overall SNR.

Root segmentation

Figure 6 shows 3D rendered perspectives of the specimen "M2" measured at soil water content of

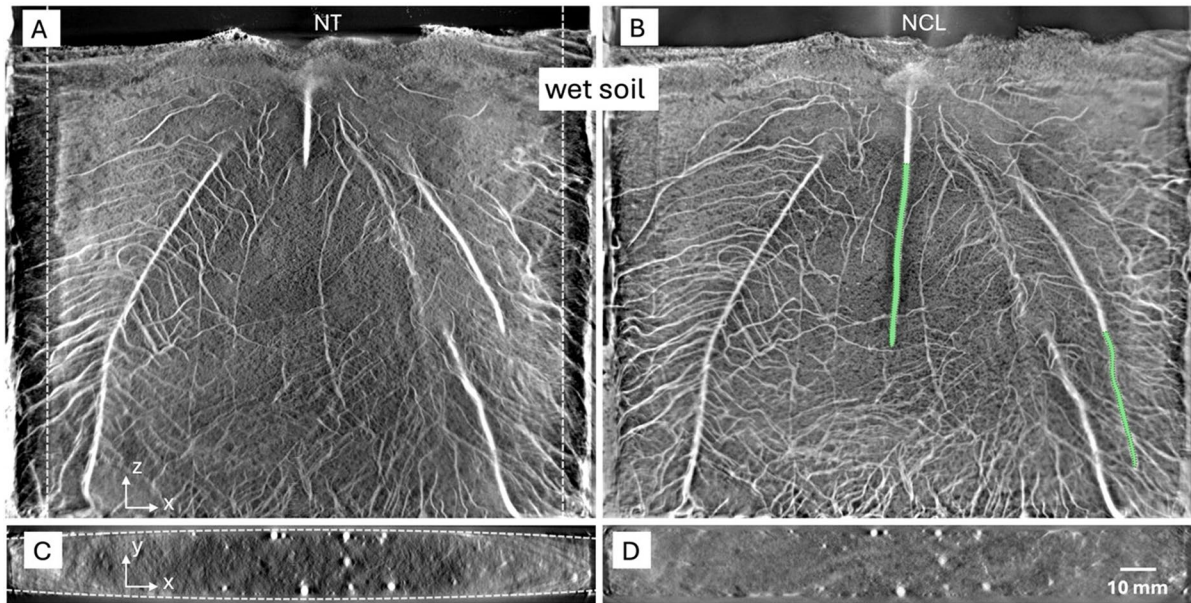


Fig. 4 Root system of the maize plant “M1” measured under moderately wet soil conditions ($0.17 \text{ cm}^3 \text{ cm}^{-3}$). The vertical cross sections captured by **A**) NT and **B**) NCL are located close to the front wall of the rhizotron. The soil appears brighter due to its higher water content. Substantial root

growth occurred between the NT and NCL measurements, as exemplarily indicated by the green marks. **C**) and **D**) Cross-sectional view from the upper soil region: artifacts around roots are still there but masked by the lower contrast between roots and wet soil

$0.07 \text{ cm}^3 \text{ cm}^{-3}$. Due to the low moisture content, the soil appears mostly transparent, revealing a detailed impression of the maize root system architecture. Some bright soil particles with higher neutron attenuation are visible in Fig. 6B and 6E, which appear to have a different specific mineral composition, rather than a higher pore space water content, than the rest of the bulk soil, as has been observed in other neutron imaging studies already (Tötze et al. 2021a, b). Overall, the favorable image contrast conditions allow for relatively easy segmentation of the primary and seminal roots from both imaging modalities. In addition, a large part of the fine lateral roots could be segmented in both images. However, the image contrast between finer lateral roots and soil was significantly weaker, which made the segmentation via region growing a more complicated and time-consuming procedure. It should be noted that further reduction of soil moisture to values below $<0.07 \text{ cm}^3 \text{ cm}^{-3}$ would not improve the situation as the lack of soil water availability would lead to water stress, desiccation or even shrinkage of root tissue and weakened root soil contact (Carminati et al. 2009). This effect would initially be noticeable in the neutron image by the visual

disappearance of lateral roots. Side views of the segmented root systems reveal that only few roots grew right in the center of the rhizotron, while the majority grew not exactly vertically, and thus along the front or rear window of the rhizotron (Figs. 5C and 5H). Further 3D insights into this soil-root system, including the segmented root system of plant “M2”, are shown in Video S2, which is provided with the Supplementary Information.

The second set of measurements (maize plant “M1”) was performed under more moist soil conditions. The lower neutron transmission through the rhizotron is clearly visible in the 3D rendered front views of Fig. 7 (see Fig. 7B for NT and 7E for NCL). The soil appears brighter (opaque) in the image and partially obscures the view of the root system. Despite the poorer contrast ratio between roots and soil environment, main roots are still clearly visible and can be segmented without much difficulty from both the laminographic and tomographic image. In both images, extraction of lateral roots was incomplete and required significantly more effort. It is noticeable that the efficacy of the root segmentation process varied among different image regions which was partly due

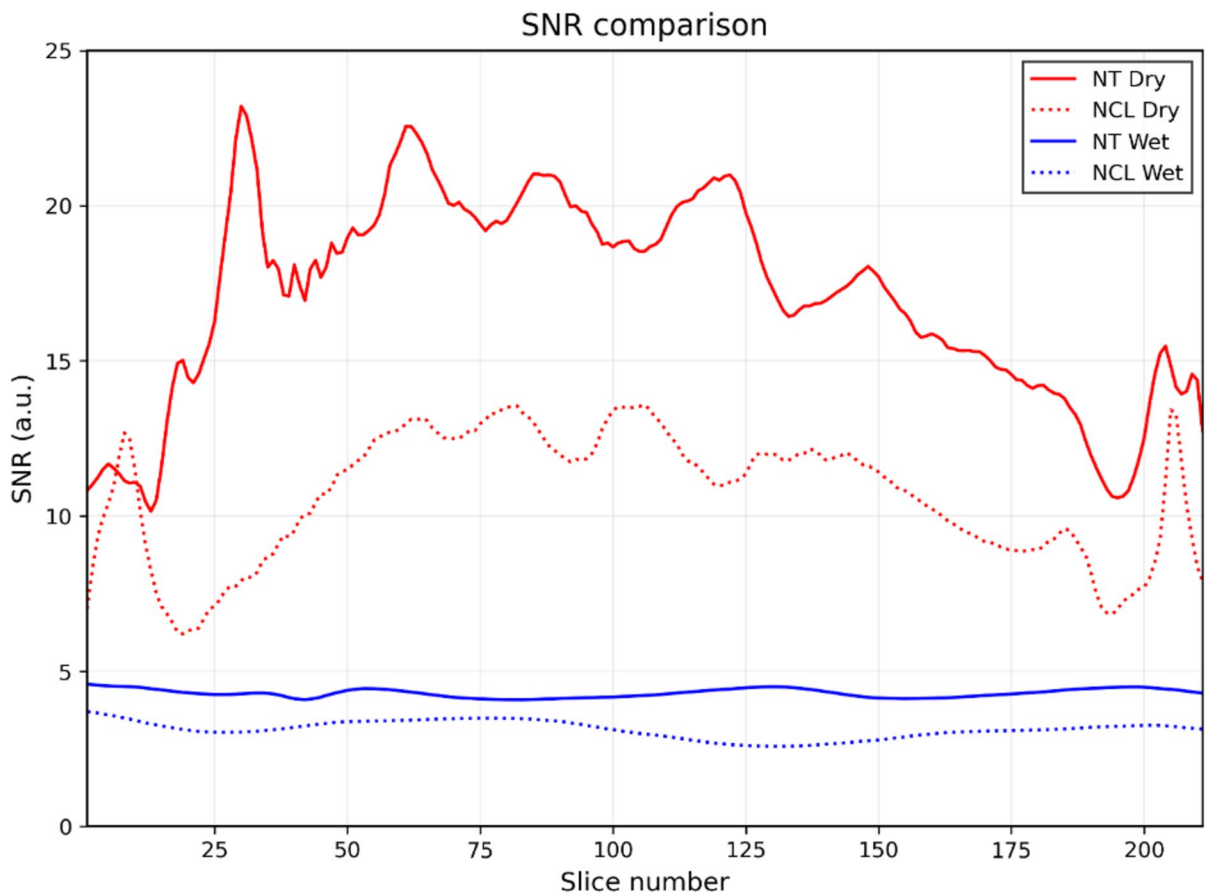


Fig. 5 Slice-by-slice signal-to-noise ratio (SNR) calculated along the frontal (y) axis for the M1 and M2 tomography and laminography datasets

to inhomogeneous soil moisture distribution. The “missing wedge” artifact in the NT image hampered the detection of lateral roots, particularly in the left lateral region of the rhizotron (compare Fig. 7B and 7D vs. 7E and 7G). The elevated soil water content also exerted an influence on the growth rate of the maize roots: in between the NT and NCL measurements the roots grew more compared to plant “M2” and several new seminal roots emerged, even though the time difference between the respective images was very similar (24 h plant “M2” vs. 27 h plant “M1”). Further 3D insights into the rhizotron, including the segmented root system of plant “M1”, are shown in Video S3 V, which is provided with the supplementary material.

The side-by-side comparison of the segmented 3D images in Figs. 6 and 7 provides an initial impression of how effectively the two scanning techniques

captured the fine lateral roots of the maize plants. For a more detailed evaluation, differential and sectional images of the segmented NCL and NT scans were superimposed, as shown in Fig. 8 and in the corresponding animated 3D renderings (Video S4, Supplementary Information). The resulting 3D visualization differentiates the maize root systems into four distinct categories i) roots (white) present in both images, ii) roots (red) visible only in the NCL image, iii) roots (cyan) detected exclusively in the NT image and iv) root sections that emerged posterior to the NT scan and are, consequently, visible only in the NCL image (green).

The roots of maize plant M2 depicted in Fig. 8a are predominantly white, indicating that in conditions of low soil moisture, the majority of the lateral maize roots can be effectively segmented from both the NCL and NT images. In contrast, for the maize

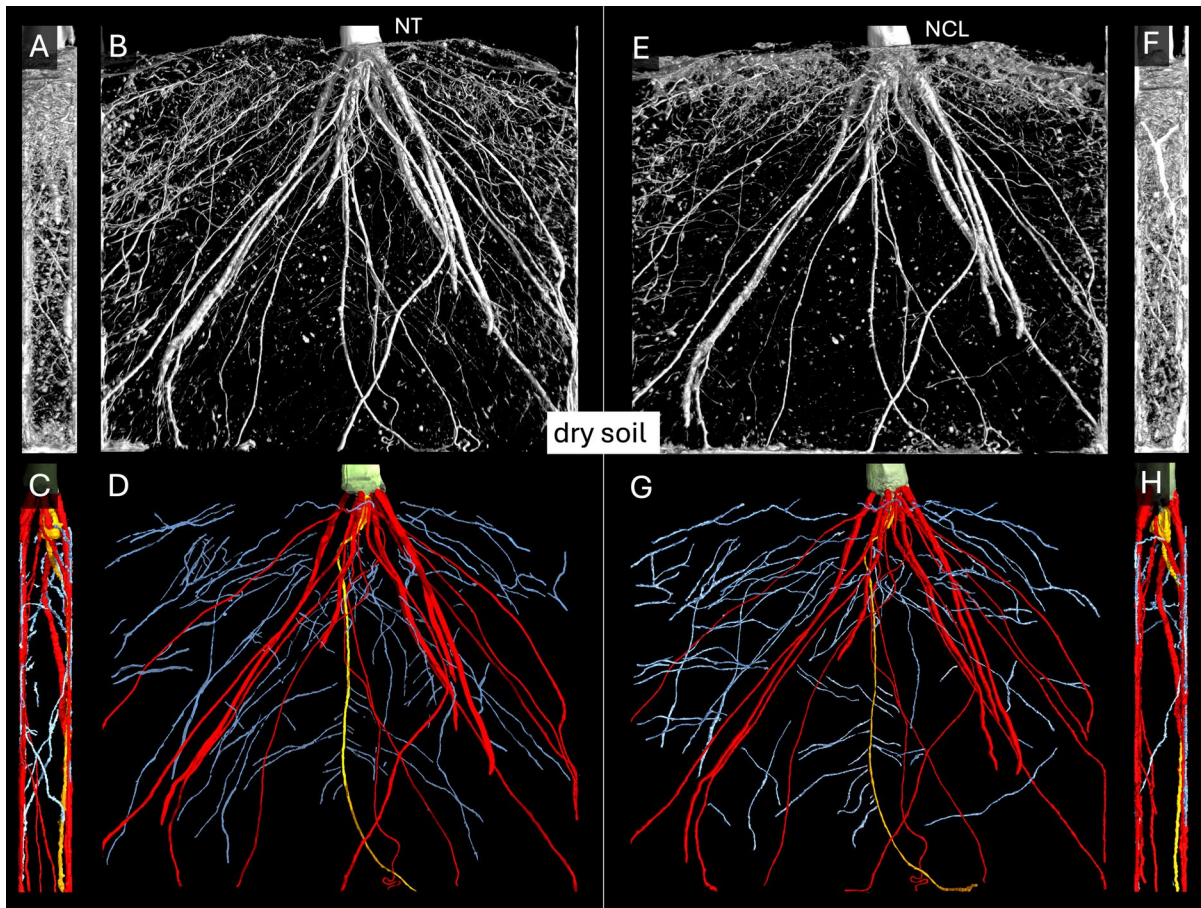


Fig. 6 Virtual extraction of the 3D maize root system of sample “M2” measured at low soil moisture conditions. Top row: 3D rendered side and front views of the reconstructed NT (A and B) vs. the NCL (E and F). Bottom row: 3D rendered side and front views of the segmented root system extracted from

NT (C and D) vs. NCL (G and H). The stem base is colored in green, the primary root and maize seed in yellow, seminal roots in red and lateral roots in light blue. Animated 3D images are provided as supplementary information (Video S2)

plant M1 that was measured under wetter conditions, it is evident that fewer lateral roots could be segmented (Fig. 8b). Most of these fine lateral roots are colored red or cyan, indicating that they could only be extracted from the NCL or NT images, but not both. Furthermore, it is apparent that the roots segmented from NCL image are oriented more horizontally, while the ones extracted from NT scan grew predominantly vertically. This indicates that the methods have a direction-dependent resolution that is related to the orientation of axis of rotation. A significantly greater number of horizontally oriented lateral roots (red) could be segmented from the NCLs. However, the fact that many more lateral roots grown vertically (cyan in Fig. 8) were segmented from NT images but

not NCL images suggests that NCL’s superior horizontal resolution comes at the cost of lower sensitivity to vertical structures.

To complement the visual assessment of the root system, Table 2 compares root lengths determined by skeletonization of the segmented images for the two modalities. For sample M2 (dry), the total root length was 4089 mm in the NT image and 4043 mm in the NCL image, the latter including approximately 61 mm of new root growth. Despite the comparable total root lengths, both imaging modalities failed to capture a substantial fraction of lateral roots. For sample M2, the proportion of missing lateral root length relative to the total lateral root length detected by both modalities

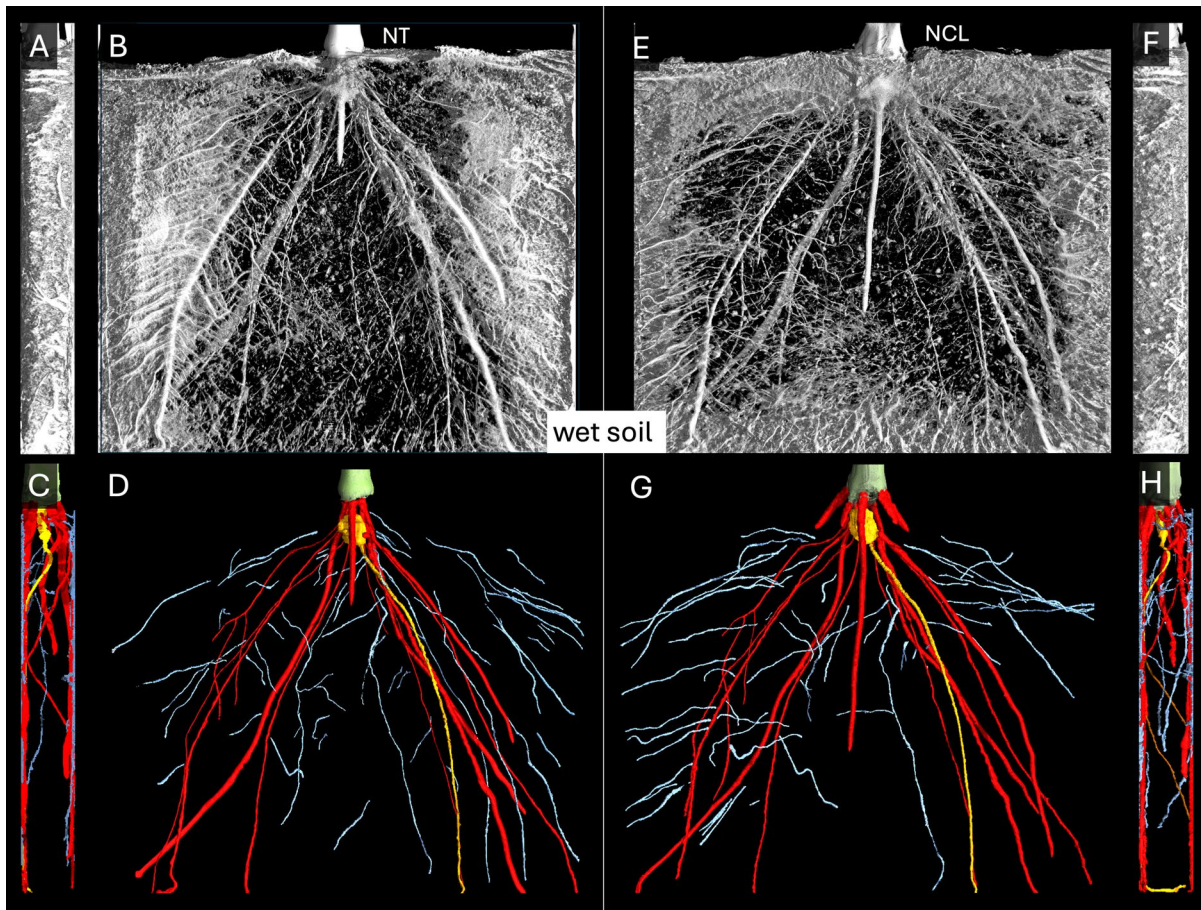


Fig. 7 Virtual extraction of the 3D maize root system of sample “M1” measured at moderately wet conditions at a volumetric soil water content of $0.17 \text{ cm}^3 \text{ cm}^{-3}$. Top row: 3D rendered side and front views of the reconstructed NT (A and B) vs. NCL (E and F). Bottom row: 3D rendered side and front views of the segmented root system extracted from NT (C and D) vs.

NCL (G and H). The stem base is colored in green, the primary root and maize seed in yellow, seminal roots in red and lateral roots in blue. During the one-day time gap between the measurements the root system grew and developed new seminal roots. Animated 3D images are provided as supplementary information (Video S3)

was slightly lower for NT (21.6%) than for NCL (25.2%), indicating a marginally better detection performance of NT. Under wetter conditions (M1), the proportion of root length undetected by either modality increased markedly to 37.6% and 37.5%, respectively. It should be noted that the segmented roots in the overlay image served as a reference baseline rather than an absolute ground truth, implying that the true proportion of undetected root length is likely higher. The higher CNR values for Sample M2 indicate that lateral roots are

more clearly distinguishable from the surrounding soil under lower moisture conditions, reflecting improved root–soil contrast.

Discussion

The high aspect ratio of slab-shaped rhizotrons makes three-dimensional neutron imaging of RSA of the contained plants challenging. In this section, we discuss the results for two maize samples that represent

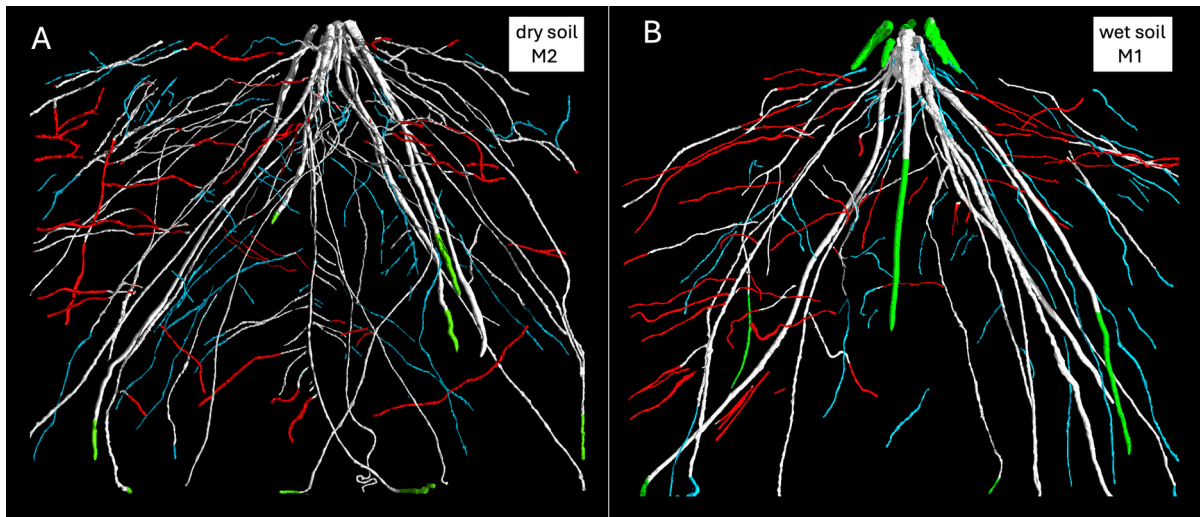


Fig. 8 Combining the intersections and differences of NCL and NT scan images provides a direct visual comparison of the segmentation results of the methods for the root systems of M2 (A) and M1 (B). White roots could be extracted from both the NT and NCL images. Red roots could only be extracted from

the NCL image. Cyan roots could be extracted from the NT image only. Green root segments grew after the NT scan and were virtually extracted by the NCL. Animated 3D images are provided as supplementary information (Video S4)

Table 2 Root lengths (mm) obtained by skeletonization of segmented images for the two imaging modalities (NT and NCL) under dry (M2) and wet (M1) conditions. Length of lateral roots detected exclusively by either modality and val-

ues for the overlay image (NT+NCL) served as a reference to estimate the undetected lateral root fraction. Contrast-to-noise ratio (CNR) analysis indicate higher contrast for lateral roots in the dryer soil (Rodriguez-Molares et al. 2020)

Root length [mm]	Sample M2—dry		Sample M1—wet	
	NT	NCL	NT	NCL
Entire root system	4089	4043	2736	2840
Primary and seminal roots	1788	1846	1306	1409
Lateral roots	2301	2196	1430	1431
Lateral roots detected by NT or NCL only	797	634	802	862
Lateral roots in overlay image (NT+NCL)	2935		2292	
Missing lateral roots compared to overlay [%]	21.6	25.2	37.6	37.5
Root growth		61		190
CNR lateral roots	5.3	5.7	4.0	3.4

the upper and lower ranges of feasible soil moisture contents, as obtained by scanning modes, i.e. NCL versus conventional NT. Due to the constraints imposed by root growth and local differences in soil moisture that developed during the one-day interval between the NT and NCL measurements, the comparative analysis per sample was performed primarily on a qualitative basis.

Comparison of image quality, artifacts and root segmentation of NCL and NT

The high water content of roots makes them easily detectable in soil by neutron probing. However, the high neutron attenuation by plant tissue is also associated with specific artifacts in 3D images, such as dark shadows and halos around stems and thicker

roots. These artifacts are particularly visible at low soil moisture levels and can introduce uncertainty when precise quantification of moisture or chemical gradients relative to the position of the root surface (i.e., the extent of the rhizosphere) is required. This is because the thickness of the root itself and the location of the root-soil interface may not be accurately determined. They also reduce the ability to correctly interpret soil water gradients present in the rhizosphere and increase the likelihood of missing lateral roots within the artifact area. Diagonal streaks, visible in the x–y cross-sectional images of the laminography, cause bright double shadows of the stem and root structures, but were found not to be critical for the segmentation process due to their relatively low intensity. The comparative analysis of the segmented root systems using both NCL and NT suggests that both methods are equally effective in detecting the root system when focusing on the main roots in the rhizotron with low soil moisture.

At elevated soil moisture content, as was the case in the measurement of plant “M1”, the neutron attenuation of the soil was considerably stronger. This not only reduced the image contrast between roots and soil but also decreased the neutron signal resulting in noisier images. This moisture related phenomenon is independent from the tomographic mode used, and in line with the findings from studies applying 3D neutron imaging of plants cultivated in cylindrical containers (Tötze et al. 2021a, 2017). As a result, the root segmentation was more challenging in both conventional NT and NCL compared to the plant “M2” which was measured at low moisture conditions. The quality of the NT image taken at higher soil moisture levels was further compromised by missing-wedge artifacts, which refer to the lack of reliable information (transmission) for the affected angular range of the neutron scan. To some extent these artifacts degrade the quality of images in the lateral areas on the front and back of the rhizotron, which affects regions with high root density.

Overall, using both methods we succeeded to segment the primary and seminal roots, as well as thicker lateral roots. However, due to the limited spatial resolution and the diminished image contrast in wet conditions, the lateral roots could not be fully captured and segmented with either method. In parts NCL outperformed NT because it detected a significantly

greater number of horizontally oriented lateral roots. Helfen et al. (2011a) already demonstrated through comparative X-ray synchrotron scans that the resolution power is higher for structures aligned perpendicular to the tomography axis when using the laminographic mode. They were able to reconstruct filigree wires of an electronic chip that could not be resolved in the conventional tomographic mode.

However, applied to our maize sample in wet conditions, almost all of the lateral roots with a vertical orientation could only be segmented from the NT image, though not from the NCL image (Fig. 8b). This suggests that the superior horizontal resolution of the NCL is compromised by a lower sensitivity to vertical structures. Ultimately, neither method demonstrated a clear superiority in fully reproducing the lateral roots.

Potential applications of NCL vs. NT

An important motivation to scan rhizotrons in 3D and capture the plant’s RSA is to link root position with biogeochemical patterns within the same experimental system. When co-registering the three-dimensional root system information with concentration gradients or patterns obtained at the rhizotron plane (e.g., via planar optodes), it is important to prioritize image quality in regions proximate to the rhizotron windows. In our set-up, this concerns the roots growing along the front (or back or both sides) of the rhizotron. Because plant roots grow laterally as foraging for water and nutrients they tend to accumulate at the container walls, even if the rhizotron is not tilted during cultivation. This effect can be amplified when a hydrophilic sensor material is placed within the rhizotron during the plant growth phase ((Rudolph-Mohr et al. 2021). Consequently, it is crucial to ensure the successful capture of roots growing along the front and back windows. Both NCL and NT have demonstrated comparable efficacy in capturing roots growing in proximity to the optode. However, missing wedge artifacts as evident in conventional NT at higher soil moisture levels can lead to a loss of information at the outer edges of the front/back plane, which slightly reduces the area for which the root position can be accurately co-registered with biogeochemical gradients.

Conventional tomography offers several important practical advantages for plant root studies due

to the upright orientation of the sample during the scan. This configuration prevents gravimetrically driven redistribution of soil water and preserves existing biogeochemical gradients. Moreover, it allows repeated 3D imaging of the root system between successive chemical imaging sessions, thereby facilitating the documentation of root growth and associated changes of biogeochemical concentration patterns over an extended time period. In principle, the sample can remain within the same sample holder and be automatically transferred between the neutron beam modalities (e.g., from neutron tomography to fluorescence imaging setup), which is not possible using NCL with the tilted sample. Another advantage of NT is that it can be used alongside X-CT to obtain complementary information on the soil structure, such as macropores, cracks or voids between soil and rhizotron plate (Tötze et al. 2021b). Additionally, the mechanical stress experienced by the plant during the 3D scan is minimal.

In the comparative rhizotron measurements, NCL was expected to demonstrate its advantages particularly at higher soil moisture levels, where the image quality of NT is limited by reduced transmission. However, even at the upper end of the investigated moisture range, both methods yielded comparable image quality, indicating no discernible advantage of NCL for such plant imaging applications. Nevertheless, NCL holds considerable potential for future use as promising developments aim to enhance imaging resolution for NCL measurements by aligning the detector parallel to the plane of rotation of the sample (Lin et al. 2025). This configuration would enable targeted imaging of regions of interest of the root system at very high resolution, as the size and position of the field of view becomes very flexible.

In general, the quality of the 3D images obtained from NT and NCL applied to maize plants cultivated in slab-shaped rhizotrons is lower than that achieved when scanning plants in small, cylindrical containers. However, the slab-shaped design allows the investigation of larger and more mature plants as lateral root growth is less constrained. Despite the limited image quality, both NT and NCL are suitable for three-dimensional acquisition of the RSA. Moreover, the high contrast of water-rich tissues in neutron imaging allows clear visualization of roots, including lateral roots, under conditions where X-ray CT would provide insufficient contrast, making NT and NCL

particularly effective for studying root architecture in situ.

When the objective is a precise assessment of local soil moisture, particularly within the rhizosphere, artifacts surrounding the roots pose a major challenge as they obscure gray-scale values at the root-soil interface. Consequently, direct quantitative analysis of rhizosphere soil water distribution in three dimensions from NT of slab-shaped rhizotrons is strongly limited. However, qualitative evaluation of water distribution remains feasible and should be complemented by quantitative, two-dimensional neutron radiography acquired with the rhizotron oriented parallel to the detector. This combined approach can provide insights that extend beyond those accessible from two-dimensional measurements alone.

Due to the required tilting of the sample, however, it is not feasible to obtain undisturbed three-dimensional moisture distributions in soils exhibiting relevant water percolation fluxes within a time scale of hours using NCL.

Limitations of the study

The time offset between the NT and NCL scans, which was required for the change in imaging setup, led to slight changes in the sample structure, such as root growth and minor differences in local soil water distribution. These changes precluded a strictly quantitative comparison between the two measurements. Future studies could address this limitation by employing temporally invariant sample structures. Rather than measuring a living root system, a 3D-printed root model could be used to eliminate the effects of root growth and evapotranspiration. In addition, future measurements should be complemented by test or calibration patterns to ensure quantitative comparability (Xu et al. 2012).

Although the present analysis was limited to a qualitative comparison of NT and NCL, it provides valuable practical insights into the applicability of both methods for 3D imaging plant roots in rhizotrons, a widely used sample geometry.

Conclusion

Our comparative imaging experiments indicate that, under the tested conditions and across the investigated

moisture range, NCL is not required to resolve the three-dimensional root architecture of maize grown in flat, slab-shaped rhizotrons. When a high-flux neutron beam is available, such as at the NeXT instrument in Grenoble, conventional NT provides image quality comparable to NCL within the tested soil moisture range, allowing reliable segmentation of main roots and a substantial portion of the lateral root system. Scanning rhizotrons in an upright orientation simplifies sample handling, reduces plant stress, and preserves the in situ distribution of soil moisture.

We therefore recommend the use of NT in combination with fluorescence-based planar optode imaging (e.g., for pH or oxygen), as this enables precise spatial co-localization of root structures with rhizosphere chemical gradients. In contrast to NCL, NT also allows simultaneous monitoring of soil water redistribution via 2D neutron radiography. This multimodal approach facilitates detailed, non-destructive analysis of root–soil interactions under realistic growth conditions, capturing both structural and functional characteristics of the root zone.

Despite certain limitations, this combined methodology provides a robust platform for investigating root system architecture and its coupling with dynamic processes in the rhizosphere. It enables spatially and temporally resolved quantitative measurements that are difficult to obtain with other techniques.

Moreover, the resulting datasets are highly suitable for integration with transport modelling, particularly for studying the interplay between water, pH, oxygen, and root activity. As such, this approach holds significant potential for advancing mechanistic understanding and predictive modelling of plant–soil interactions in complex, vegetated systems.

Abbreviations CNR: Contrast-to-Noise Ratio; NCL: Neutron Computed Laminography; NT: Neutron Tomography; NeXT: Neutron and X-ray Tomograph; ROI: Region Of Interest; RSA: Root System Architecture; SNR: Signal-to-Noise Ratio

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Author contributions All authors contributed to the study conception and design. Plant cultivation was performed by S.B., N.L. and C.C. The neutron imaging experiment was performed by L.H., N.L. and C.C. Image processing and analysis of results was performed by C.T., L.H., N.K., O.S-P. and S.E.O.

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Data availability The datasets used in this study were generated as part of a measurement campaign on the neutron imaging instrument NeXT at the ILL and are available on the ILL Data Portal at <https://doi.org/10.5291/ILL-DATA.UGA-111>.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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