Non-destructive quantification of cereal roots in soil using high-resolution X-ray tomography

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Abstract

One key constraint to further understanding plant root development is the inability to observe root growth in situ due to the opaque nature of soil. Of the present non-destructive techniques, computed tomography (CT) is best able to capture the complexities of the edaphic environment. This study compared the accuracy and impact of X-ray CT measurement of in situ root systems with standard technology (soil core washing and WinRhizo analysis) in the context of treatments that differed in the vertical placement of phosphorus fertilizers within the soil profile. Although root lengths quantified using WinRhizo were 8% higher than that observed in the same plants using CT, measurements of root length by the two methodologies were highly correlated. Comparison of scanned and unscanned plants revealed no effect of repeated scanning on plant growth and CT was not able to detect any changes in roots between phosphorus treatments that was observed using WinRhizo. Overall, the CT technique was found to be fast, safe, and able to detect roots at high spatial resolutions. The potential drawbacks of CT relate to the software to digitally segment roots from soil and air, which will improve significantly as automated segmentation algorithms are developed. The combination of very fast scans and automated segmentation will allow CT methodology to realize its potential as a high-throughput technique for the quantification of roots in soils.

Key words: non-destructive, phosphorus, plant nutrition, root analysis, root growth, tomography.

Introduction

A key restriction in the development of a better understanding of plant growth in soil is the inability to examine plant roots in situ over relevant spatial and temporal scales. Because roots grow in a wide variety of opaque media and thus comprise a hidden half (Waisel et al., 1996), there are not only peculiar challenges to their visualization but also considerable obstacles to the development of methods for the accurate and rapid assessment of root system characteristics (Gregory et al., 2003; Perret et al., 2007).

Current standard methods generally employ destructive and time-consuming techniques, such as washing cores and sampling soil pits and rhizotrons. All have limited appeal and accuracy. None are able to reflect the three-dimensional (3D) growth of roots in spatially heterogeneous soil (Lontoc-Roy et al., 2006), a key characteristic of field conditions which impacts on all soil-root processes and functions. As comprehensively reviewed by Lynch (1995) and Hodge (2004), plant roots respond morphologically to their edaphic environment and genotype, making 3D analysis of the root phenotype a valuable tool in assessing the competitive advantage of different plants. Recently, Iyer-Pascuzzi et al. (2010) and Clark et al. (2011) have developed an interesting methodology using a gellan gum system as a growth medium for developing a 3D root phenotyping system. The key to this impressive technique is that the growth medium has to be transparent and thus is not of use in soil-plant systems.

Over the past decade or so, non-destructive methodologies, including ultrasound, magnetic resonance imaging, and X-rays, have been used in soil systems in an attempt to capture the inner space of soil and its contents in three
dimensions (North, 1976; Amin et al., 1993; Young et al., 2008; Gregory et al., 2009; Tracy et al., 2010). Gregory et al. (2003) neatly summarize several methodologies for measuring root development.

Of all possible non-destructive techniques, computed tomography (CT) has been recognized as the one that is able to deal adequately with the complex geophysical and geochemical complexities of soil across wide range of edaphic environments (Lontoc-Roy et al., 2006). While nuclear magnetic resonance (NMR) imaging is capable of producing high-resolution images, the use of the technology in soils is limited by the abundance of paramagnetic ions (Fe, Mn, Cu) (Gregory et al., 2003; Perret et al., 2007).

X-ray CT does not suffer the same interference and is able to spatially and digitally segment substances based on their differential attenuation of X-rays: material segmentation is a function of photon density. The technique has been applied to spatially and digitally segment substances based on their X-ray CT does not suffer the same interference and is able to deal adequately with the complex geophysical and geochemical complexities of soil across wide range of edaphic environments (Lontoc-Roy et al., 2006). While nuclear magnetic resonance (NMR) imaging is capable of producing high-resolution images, the use of the technology in soils is limited by the abundance of paramagnetic ions (Fe, Mn, Cu) (Gregory et al., 2003; Perret et al., 2007).

Recent developments in CT have led to improvements in scan resolution, scan quality, acquisition time, and sample size. The initial application of the technique to root systems predominantly used medical CT scanners with voxel resolutions of ~1–1.5 mm (Watanabe et al., 1992).

While this was sufficient to identify coarse root systems such as those for Chinese yam (Dioscorea oppositifolia L.) or soybean (Glycine max L.), it was insufficient to observe fibrous root systems such as that of Bahia grass (Paspalum notatum Fluegge) (Watanabe et al., 1992; Perret et al., 2007). Early CT work was therefore restricted to plants with coarse root systems (Tracy et al., 2010). Smaller voxel size, and hence higher resolutions, have subsequently been obtained using industrial and custom-built CT scanners: 160 μm voxels by Heeraman et al. (1997) and 100 μm voxels by Gregory et al. (2003), respectively. With greater resolution, these researchers were able to isolate roots of diameters >350 and 200 μm, respectively. More recent technological advances have seen researchers achieve resolutions of 36 μm and less (Kaestner et al., 2006) and contemporary equipment can now achieve resolutions less than 500 nm (Tracy et al., 2010). Such fine resolutions typically require small pot sizes (i.e. less than 1 cm diameter), with the resolution ultimately limited by the diameter of the sample (Wildenschild et al., 2002). Given this relationship, it is unsurprising that much of the research has been carried out with small pots and on early root development (Gregory, 2006; Perret et al., 2007).

The objective of this study was to determine the accuracy of root-length measurement by high-resolution CT (micro-CT) by comparison with results from a standard technology – soil core washing, root extraction, and length and diameter analysis using a flattened scanner and the image analysis software WinRhizo. Secondary aims were to examine the feasibility of using CT technology for high-throughput analysis of root growth parameters and to ascertain the impact of X-ray exposure on root growth.

Materials and methods

Plant material

Seeds of spring wheat (Triticum aestivum L. cv. Gregory) were imbibed in aerated 0.012 M CaSO₄ for 2 h (Kopittke and Menzies, 2004) and then weighed to select the most uniform seeds. Seeds were then distributed randomly across the treatments and planted 20 mm below the surface with the embryo oriented downward.

Growth conditions

The plants were grown in a composite porous growth medium, consisting of (w/w) 90% washed granite sand and 10% red ferrosol (Isbell, 1996). The red ferrosol was sourced from Dorrigo, NSW, Australia. Both soils were sieved to select the particle size fraction 0.5–1 mm before mixing (Perret et al., 2007). The medium was used because of its suitability for micro-CT scanning and its capacity to adsorb and buffer soluble P, with a P buffer index of 980 and a native available P concentration of 8 mg kg⁻¹, as determined colorimetrically using malachite green (Colwell, 1963; Motomizu et al., 1983; Burkit et al., 2002).

Nutrient application and phosphorus treatments

All nutrients were applied evenly as a liquid to a thin layer of the composite soil to ensure an even spatial distribution. The soil was dried at 40 °C and the pots were packed to a bulk density of 1.37 g cm⁻³. Basal nutrients (kg⁻¹ soil) of N (50 mg), S (10 mg), and K (20 mg) were added to soil before P application.

Three different P treatments were used to encourage root systems with variation in both size and complexity (Drew, 1975; Lynch and Brown, 2001; Lopez-Bucio et al., 2003). The banded-P treatment consisted of 75 mg Ca(H₂PO₄)₂.H₂O per pot concentrated into 3% of the soil volume (Caldwell et al., 1992); this concentration of P coincided with the sorption capacity of the composite soil. The diffuse-P treatment consisted of the same total quantity of P but distributed throughout the soil volume. The control treatment had no added P. The banded region was ~20 mm in depth and was centred ~50 mm below the soil surface. This region was marked with three 2-mm glass beads above and below so it could be identified in tomographs for all three treatments.

The pots were watered from the surface regularly until 5 days post germination to ensure even establishment. Thereafter, the pots were maintained at a constant volumetric water content of 20% in the banded region using a tension table. The 20% volumetric water content represented the inflection of the moisture release curve and was achieved using a 175-mm negative head. Each pot was placed on a bed of fine sand that was connected to a manifold and reservoir by 3 mm clear tubing. The reservoir water surface was maintained at constant capacity and 175 mm below the centre of the banded region with the use of a float valve and header tank. De-aired water was supplied from the header tank, which was designed to have limited air–water surface area to restrict gas diffusion into the water. The tension table allowed precise control over the water content of the soil at any one region of the pot and consequently the size of water-filled pores. There was, however, a strong gradient in water content due to the texture of the substrate and the height of the pots. The plants were disconnected from the tension table for scanning using air-tight Luer fittings. All fluid lines were flushed to remove air bubbles prior to reattachment. The base of the tension table was specifically machined from PVC to form
Experimental design and statistical analysis

Five replicates of each P treatment (15 pots) were regularly scanned while five replicates of the banded-P and diffuse-P treatments (10 pots) remained unscanned. The plant treatment positions were arranged in a randomized block design. Data were analysed using the statistical package R version 2.10.1 (R Development Core Team, 2010). Correlations between micro-CT and WinRhizo outputs were evaluated using linear models. Root data were analysed using one-way analysis of variance.

Micro-computed tomography

Soil cores were placed individually in a Vtomexs scanner (GE Phoneix) (Fig. 1A) and scanned at 100 kV and 270 µA. A set of digital radiographs (Fig. 1B) was created at a voxel resolution of 68.23 µm while the core was rotated about its vertical axis. The total scan time was 4 min 10 s per scan. Multiple laterally adjoining scans

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**Fig. 1.** Micro-computed tomography scanning to segmentation of roots from soil. (A) Plant column in scanner; the X-ray gun is on the right side of the pot. (B) Resultant two-dimensional radiograph of core with roots: grey circles indicate glass beads (see text). (C) Reconstructed core in three dimensions: grey curvilinear shapes represent plant roots. (D) Two-dimensional section of roots segmented using ImageJ. (E) Reconstructed section of soil with roots.
were used to observe growth zones greater than the scan height. Three such adjoining scans were taken to observe the banded region 11, 15, 19, and 22 days after sowing (DAS). Scanning at final harvest (29 DAS) used six adjoining scans to capture the entire root system. This means that any one part of the root system was exposed to X-ray radiation for a maximum of 20 min 50 sec over a period of 18 days. Reconstruction of the radiographs was carried out using the X-ray proprietary reconstruction software Phoenix and a graphical processing unit cluster (computational time ~30 s).

**Image analysis**

Individual tomographs from the same treatment were imported into Volume Graphics StudioMax version 2.1, where the greyscale histogram boundaries were adjusted to enhance contrast. Scans from the same replicate were adjusted consistently to maintain continuous attenuation values. Tomographs were then oriented in space using the micro-CT manipulator position data to create a virtual pot with seamless transitions between the tomographs and were finally consolidated into a single volume (Fig. 1C). The micro-CT technique to this stage took ~50 min per sample.

Root systems were segmented from the large volumes manually using the StudioMax version 2.1 Region Growing tool, which uses an adaptive local threshold from manually determined root material (Fig. 2B) as the seed point for the start of the algorithm. The dynamic mode was used to select continuous structures in 3D space and the tolerance was determined relative to the surrounding material to ensure that only the root volume was selected as a region of interest. This segmentation method was selected because a series of global thresholding techniques are confounded by volume-averaging effects, which is typical of soils where the aggregate size is greater than the resolution of the scanner and the partial volumes occupy the same attenuation values as the root systems (Kaestner et al., 2006).

The root volume was then imported into ImageJ version A 1.43h using a .tiff image stack and a mask was generated from the first image of each pot. The calibrated 8-bit images were then filtered using a Gaussian function with $\sigma = 0.1 \text{ mm}$ (equivalent to ~1.5 pixels) to reconnect root lengths with small discontinuities. A global threshold was applied to the volume with upper and lower pixel values fixed at 255 and 30, respectively, at which the root diameter was comparable to the original image.

The Find Connected Regions plug-in in ImageJ was used to isolate the largest 100 connected regions of root volume, which were then manually assessed to determine whether they represented root volume or noise. The root volumes were then recombined using the Image Calculator function in ImageJ to yield the final segmented volume (Fig. 1D, E). This process allowed the removal of fine unconnected artefacts of the StudioMax Region Growing algorithm, which at times included small unconnected voxels with similar greyscale values due to the volume-averaging effect. This was a resource-intensive process, consuming a large amount of memory, because of the upper limit of the available random access memory (96 GB), the 100 largest connected regions were analysed.

Segmenting roots at the bottom of the pot was made increasingly difficult by the abundance of water in pores and resulted in greater misclassification of root voxels, as can be seen in the lower third of Fig. 2C: the segmented volumes appeared ‘hairier’, thicker, and coarser. Applying the connectivity filter removed much of the inaccuracy caused by the pore water as can be seen in Fig. 2D: however, the bottom third of the root system remained ‘lumpy’ in contrast with the smoother volumes above. This ‘lumpiness’ slightly increased the estimated segmented root volumes and consequently root length in the lower third of the image relative to WinRhizo estimates of the same part of the root system. Roots also rapidly encountered the sides of the pot and, because small volumes constrain root exploration more than field conditions, there was an artificial increase in root density. This increase did not affect the conclusions or aims of this study.

**Results**

**Root visualization**

The raw reconstructed images were of sufficient resolution and quality (high signal-to-noise ratio and contrast) to allow observation and manual segmentation of roots with diameter approximately >144 $\mu$m (Fig. 2A, B) without the need for pre-processing filters. For the young seedlings, it was possible to observe the primary roots emerged from the embryo, the first-order branches, and a small number of second-order branches that were above the 144-$\mu$m detection limit.

While this finer fraction of roots were able to be perceived and isolated in the initial stages of segmentation, it was at times difficult to maintain the continuity of these roots because of the influences of volume-averaging effects, flaring from dense aggregates, and water in lower parts of the pot. As a result, the finest fraction of roots were often fragmented (Fig. 2C) and so they were consequently removed by the connectivity filter (Fig. 2D). Much of the root system in the final volume was consequently >250 $\mu$m in diameter.

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Fig. 2. Wheat root system after banded-P treatment imaged 29 days after germination using micro-computed tomography. (A) A single slice of a raw reconstructed tomograph depicting wheat stem roots >144 μm in diameter. Bar, 5 mm. (B) The region depicted in A after segmentation (white shading) using seeded adaptive local threshold. Bar, 5 mm. (C,D) Three-dimensional visualization of the segmented volume before the cleaning process (C) and following application of the connectivity filter to remove noise and depict the root volume (D).
Comparison of micro-CT and standard methodology

Micro-CT determination of root length compares favourably with the standard method of root washing and scanning using WinRhizo (adjusted R squared = 0.68; Fig. 3). The two methodologies were compared using length for roots of diameter >280 μm and ~250 μm for WinRhizo and micro-CT, respectively. The proportion of roots >280 μm in diameter represented an average of 86% of the total root system detectable using WinRhizo. Linear regression modelling indicated the relationship between the techniques could be described by the equation:

\[ l_w = 1.08l_c + 580 \]

where \( l_w \) refers to WinRhizo root length and \( l_c \) is root length as determined by micro-CT.

In this study, micro-CT was unable to detect the differences in root length between the banded-P and control treatments that were observed by WinRhizo (Table 1). This is a result of the large variances observed within micro-CT measurements (Fig. 3) and is thought to be because of limitations in the segmentation algorithm. Despite this, micro-CT did identify the same trends between treatment means as the conventional method (Table 1).

![Fig. 3. Linear correlation between root length as determined by WinRhizo and micro-computed tomography. The equation of the linear model is \( l_w = 1.08l_c + 580 \) where \( l_w \) refers to WinRhizo root length and \( l_c \) is root length determined by micro-CT.](image)

Table 1. Comparison of root-length measurements of wheat plants obtained using micro-CT and WinRhizo

<table>
<thead>
<tr>
<th>P treatment</th>
<th>Root length (mm)</th>
<th>Tortuosity (micro-CT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-CT (diameter &gt;250 μm)</td>
<td>WinRhizo (diameter &gt;280 μm)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2860&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3120&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2960&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4230&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Banded</td>
<td>3580&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4570&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td>1067</td>
<td>1152</td>
</tr>
</tbody>
</table>

Tortuosity was calculated using the ratio of each branch length (between two branching voxels) to its Euclidean distance. The mean tortuosity across all treatments was 1.11. However, there was a slight decrease in the tortuosity with the diffuse-P treatment as compared with the banded-P treatment (\( P < 0.05 \)) but there was no difference detected between these and the control (\( P > 0.05 \)) (Table 1).

Effects of X-ray exposure on plant growth

There was no detectable effect on the root growth of young wheat plants due to exposure to X-rays with respect to the number of branches, total root length, or average diameter, as determined by WinRhizo (\( P > 0.05 \)) (Table 2). Also, there was no effect on the growth of plant shoots (\( P > 0.05 \)) with exposure to X-rays (Table 2).

Effect of available P on plant growth

While the banded-P treatment increased leaf tissue growth on average by 45% and 85% as compared with the diffuse and control treatments, respectively (Table 2), there were less pronounced effects on the root system. The banded-P treatment tended to result in greater total root length than the diffuse-P treatment; however, the 10% increase was not significant (\( P > 0.05 \)) (Table 2). Both P treatments resulted in a greater root length than the control (\( P < 0.05 \)) (Table 2). Similarly, root branching tended to increase with the increase in available P; however, the only detectable difference existed between the control and the banded-P treatments (\( P < 0.05 \)) (Table 2).

Conversely, average root diameter measurements were 7% higher for the control treatment as compared to the other P treatments (\( P < 0.05 \)), with no detectable differences between the diffuse-P and banded-P treatments (\( P > 0.05 \)) (Table 2).

Discussion

The linear relationship between root length measured by WinRhizo and micro-CT indicates that there was a systematic underestimation of root length by micro-CT (gradient

Table 2. Comparison of root characteristics and leaf dry weight of wheat plants obtained using WinRhizo (root length and diameter) and manual measurement (branch number)

<table>
<thead>
<tr>
<th>P and X-ray treatment</th>
<th>WinRhizo total root length (mm)</th>
<th>Average diameter (mm)</th>
<th>Branch number</th>
<th>Leaf dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control and scanned</td>
<td>3570&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diffuse and scanned</td>
<td>4900&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>410&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diffuse and unscanned</td>
<td>4870&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>410&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Banded and scanned</td>
<td>5320&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>480&lt;sup&gt;d&lt;/sup&gt;</td>
<td>98&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Banded and unscanned</td>
<td>5590&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;d&lt;/sup&gt;</td>
<td>490&lt;sup&gt;d&lt;/sup&gt;</td>
<td>102&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by the same superscript letter within a column are not significantly different (\( P > 0.05 \)). LSD, least significant difference.
of 1.08, or 8%). This is consistent with the findings of Perret et al. (2007) and Gregory et al. (2003), who indicated that CT underestimated root length by less than 10%. In contrast, Heeraman et al. (1997) observed a systematic overestimation of root length by CT; however, this discrepancy is thought to be due to the limitations of resolution, the fine root diameters, and the high root density rather than a systematic characteristic of the technique (Heeraman et al., 1997; Gregory et al., 2003). The current study recorded the measurement of skeletonized root lengths to considerably larger and more complex root systems.

The limit for the smallest observable root is a function of the quality of the image (signal-to-noise ratio) and resolution (Kaestner et al., 2006). The smallest resolvable object is widely considered to be an object with a diameter of twice the pixel/voxel resolution (Kaestner et al., 2006). In the current study, it was possible to observe the fibrous root system of a cereal species in the original images with a diameter >144 μm (Fig. 2A), which is consistent with the previous observation (current voxel resolution 68 μm). Morphologically, this represents axial roots as well as first- and second-order laterals. As a result of the segmentation methodology, it was not possible to preserve all of this data in the final segmentation and, as such, the population of segmented roots predominantly had an average diameter of ~250 μm. As it pertains to the diameter of recoverable roots, this outcome is comparable to research conducted by Lontoc-Roy et al. (2006), who achieved successful segmentation of roots >240 μm, but falls short of the method by Gregory et al. (2003), who achieved segmentation of roots approximately >200 μm. By comparison, the current technique has the ability to image roots of smaller diameter than much of the literature where, predominantly, the resolution limited the smallest resolvable root. Recently, Perret et al. (2007) and Kaestner et al. (2006) observed root diameters >500 μm and Heeraman et al. (1997) observed root diameters >350 μm. There is no documented evidence that root systems as complex as those observed in the current study have been measured in their entirety using micro-CT. This is most likely a function of the maturity of the plants observed rather than the technical capacity. On the whole, plants less than 5 days after emergence (Gregory et al., 2003; Lontoc-Roy et al., 2006) or with tap rather than fibrous root systems (Perret et al., 2007) have been studied. However, recent advances in automated segmentation show promise for roots extracted from cereals up to 12 days old (Tracy et al., 2011).

There were several sources of variation as a result of the image analysis methodology. First, voxels were misclassified as soil aggregate due to volume-averaging effects (Heeraman et al., 1997). Second, the preliminary analysis indicated that most of the data were represented in approximately the largest 250 volumes extracted by the algorithm; thereafter, connected regions represented noise (unpublished data). While the collected data represented 92% of the original total voxels, much of the smaller-diameter root fragments were lost in the remaining 8% of unused voxels. Third, as roots have a similar electron density to soil water, water-filled pores confounded the segmentation algorithm and were misclassified as roots. This is a consistent problem with CT root imaging, particularly as resolution increases. Previous researchers have avoided the issue by not watering prior to scanning or by using dry sand as a support medium (Heeraman et al., 1997; Kaestner et al., 2006); however, the current method attempted to precisely control the moisture tension to remove water from large pores. In future, the water tension applied to the pots will be increased prior to scanning to remove water from pores at the lower extremities of the pot.

The previously mentioned interference from soil moisture made the branching data from the skeletonized images meaningless and therefore that data are not presented here. The use of morphological data such as root branching from micro-CT images is a fundamental advantage of the technique and is widely published (Gregory et al., 2003; Perret et al., 2007). Therefore, it is expected that this will not be a significant limitation with improved segmentation algorithms.

While micro-CT methods were unable to detect the same significant differences between P treatments that were detected by WinRhizo, the same trends between the methods were observed. It could be that the lack of statistical difference was a result of the large variance within the segmented root data and is not a fundamental limitation of the micro-CT processes. Nonetheless, the same trend between P treatments highlighted the potential of this technique when coupled with more effective post-processing of volumes for root studies. The current algorithm may misallocate root voxels as soil voxels when roots pass through smaller soil pores and their contribution to the X-ray attenuation value becomes proportionally lower. This may create small gaps in otherwise continuous root volumes, decreasing root length and increasing the variance in measures of root length. These difficulties could be reduced or circumvented by implementing higher-order statistical algorithms and including probability functions with the potential to breach small gaps in fine root structures (Jassogne, 2008; Tracy et al., 2010). However, the concerns with developing effective mechanisms to overcome volume-averaging effects in fully automated segmentation still remain (Tracy et al., 2011).

In terms of specific root characteristics, the mean tortuosity found in this study is 1.11, indicating that, on average, each segment of root was 10% longer than the shortest possible distance. In contrast, Perret et al. (2007) recorded tortuosity values of 2.5 for chickpea lateral branches. While some of the difference could be attributed to the use of different species, it is most likely due to the different mode of calculation. Perret et al. (2007) calculated every branch from origin to tip and respective tortuosity; the method for this study located every branch point and tip and measured the length parameters between each of these connected points. Therefore, the current study’s observing a significantly lower tortuosity than reported elsewhere was not unexpected. While this method produced data comparable to that published for all terminal branches, the data for seminal or penultimate orders of roots was not calculated in the same way. Nonetheless, there was a significant increase between the diffuse-P and the banded-P treatments (Table 1). This
difference is thought to indicate increased exploration of the P-enriched soil volume in the banded-P treatment. The decrease in branch roots and the extension of the main root axis that is common to P-deficient plants (Borch et al., 1999; Lynch and Brown, 2001) is consistent with the observed positive effect on the tortuosity values.

Phosphorus was applied to induce a range of root responses with which to assess the suitability of micro-CT for measuring changes in root characteristics. Significant increases in root length and branching were detected following P application (Table 2), which is consistent with the responsive nature of this soil. The P applications did not create significantly different root lengths or branching. Despite substantial increases in leaf growth in response to improved P availability following banded-P treatment (Table 2), there were, as expected, less pronounced effects on root system characteristics (Gregory, 2006). The banded-P treatment tended to increase root length and branching; however, the result was not statistically significant, which may be because of both the inherent root system variability and the strong gradient in moisture content. The volumetric water content ranged from 0.17 cm$^3$ cm$^{-3}$ at the top of the band to 0.24 cm$^3$ cm$^{-3}$ at the bottom. Phosphorus availability was likely limited by the low volumetric water content at the top of the band because the texture of the substrate was uniformly coarse and the moisture release curve was steep. Consequently, the total amount of P available to the plant was less than expected, reducing the magnitude of root responses (Gutierrez-Boem and Thomas, 1999). Additionally, the difference between root-length measurements between the diffuse-P and banded-P treatments is likely to be masked by increased investment in root tissue to improve P uptake for the more P-limited plants (Lynch and Brown, 2001).

Similarly, a little difference in average root diameter was detected between the diffuse-P and banded-P treatments, both of which resulted in smaller average root diameters than the control treatment ($P < 0.05$). This was most likely a result of the relatively smaller contribution of laterals to the total length, as reflected in the lower root length of the control root system (Table 2) (Lynch and Brown, 2001). The control plants were in general less developed and less mature than the other treatments as a result of P limitation. Hence, a greater proportion of the root system consisted of axial roots, which usually have greater root diameters and thus the average root diameter, was inflated when compared to treatments with greater P availability.

There was no detectable effect of exposure to X-rays in any of the parameters tested (Table 2). The settings used for X-ray generation in this experiment appear to be moderate in relation to other literature. This method used 100 kV and 270 µA with a 0.5 mm Cu filter to harden the beam, reduce the photon density, and avoid detector saturation. In comparison, Gregory et al. (2003) and Kaestner et al. (2006) used lower-energy X-rays (50 kV) with currents of 500 µA and 114 µA, respectively. Perret et al. (2007) and Lontoc-Roy et al. (2006) used much-higher-energy X-rays (130 kV) and at greater intensities (100 mA and 480 mA, respectively). While it is difficult to predict exposure rates without knowing the efficiency of individual machines, an important advantage of the methodology used in this study was the substantial reduction in scan time and, hence, the lower exposure of live tissue to radiation. Each scan in this trial took 4 min 10 s to complete and included a region of 31.38 mm of the pot in height, six scans being taken sequentially to a total depth of 188 mm. Any one section of the pot was exposed to a direct beam for a cumulative maximum time of 20 min 50 s during the five temporally spaced scans over a period of 18 days. This represents a significant decrease when compared to published scan times, which ranged from 60 min to 8.7 h or more (Gregory et al., 2003; Kaestner et al., 2006; Tracy et al., 2011). However, the current study has demonstrated that considerable, although brief, X-ray exposure does not affect plant growth and has established that micro-CT has the potential to investigate complex root morphological experiments without adverse effect.

Many questions arise as to the transfer of 3D micro-CT imaging to the field. Constraints are large in terms of technology of in situ imaging and are associated with both the scale of and the placement of the equipment and the energies required to ‘see’ through soil at useful scales. However, targeted coring of field sites is a simpler extension of the method, where core sizes can be adjusted to optimize resolution and realize the advantages of the 3D visualization of roots.

**Conclusion**

This study has observed a high correlation between micro-CT-observed roots and roots observed by standard methodology. The CT technique is fast, safe, and able to detect roots at relatively high spatial resolutions. The drawback of the CT methodology lies not in the scanning technique but in the software available to digitally segment roots from soil and air, but this will improve significantly as automated segmentation algorithms are developed. The combination of very fast scans and automated segmentation will allow CT methodology to realize its potential as a high-throughput technique for the examination of roots in soils.

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**References**


