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MARSCHNER REVIEW

Developing X-ray Computed Tomography to non-invasively image 3-D root systems architecture in soil

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Abstract
Background The need to observe roots in their natural undisturbed state within soil, both spatially and temporally, is a challenge that continues to occupy researchers studying the rhizosphere.
Scope This paper reviews how over the last 30 years the application of X-ray Computed Tomography (CT) has demonstrated considerable promise for root visualisation studies. We describe how early CT work demonstrated that roots could be visualised within soils, but was limited by resolution (ca. 1 mm). Subsequent work, utilising newer micro CT scanners, has been able to achieve higher resolutions (ca. 50 μm) and enhance imaging capability in terms of detecting finer root material. However the overlap in the attenuation density of root material and soil pore space has been a major impediment to the uptake of the technology. We then outline how sophisticated image processing techniques, frequently based on object tracking methods, have demonstrated great promise in overcoming these obstacles. This, along with the concurrent advances in scan and reconstruction times, image quality and resolution (ca. 0.5 μm) have opened up new opportunities for the application of X-ray CT in experimental studies of root and soil interactions.
Conclusions We conclude that CT is well placed to contribute significantly to unravelling the complex interactions between roots and soil.

Keywords Rhizosphere · Roots · Soil · X-ray Computed Tomography · Image analysis

Introduction

Roots are crucial for the delivery of water and nutrients to plants. Their activity impacts on both the physiochemical and biological status of the surrounding soil, with root development and the structure of the soil pore space closely interlinked. It is well established that many processes occurring at the root-soil interface are a direct consequence of a plant's water and nutrient demand and they rely on a favourable environment enabling microbial proliferation. In fact the term 'rhizosphere' dates back to Lorenz Hiltner in 1904 (see Hartmann et al. (2008) for further details). Since then many researchers have realised that roots may alter

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Marschner Review: Developing X-ray CT to image root architecture in soil

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their immediate environment to assist resource capture at their surface (reviewed extensively in Hinsinger et al. 2006; Gregory 2006a; Hinsinger et al. 2009; Hodge et al. 2009).

The visualisation and quantification of plant rooting structure is essential for a better understanding of growth dynamics and productivity (Tollner et al. 1994). However, our knowledge of how soil structure forms and subsequently interacts with roots is fragmented. Whilst some research has focused on root system complexity, the majority has been performed in two-dimensional (2-D) space (Fitter and Stickland 1992; Costa et al. 2003). Heeraman et al. (1997) reported that rooting systems are difficult to quantify due to both their complex three dimensional (3-D) morphology, and because they naturally grow in soil, an opaque medium that prevents their direct visualisation. Root analysis often leads to a disturbance of the soil matrix, inevitably impacting the resultant root architecture (Taylor et al. 1991). In order to discover the extent to which the soil environment controls spatial rooting and water content distributions in space and time, and to enable a more precise prediction of plant performance, efficient techniques to simultaneously analyse root and soil structure are required (Whalley et al. 2000; Heijs et al. 1995). Gregory and Hinsinger (1999) stated in a literature review that previous research on root-soil interactions had been based more on qualitative observations than quantitative determinations, and that there is a need to develop new techniques for studying the chemical and physical processes that take place in the rhizosphere.

This review focuses on one such technique; the use of X-ray Computed Tomography (CT) to visualise roots in situ (i.e. within soil columns) and to illustrate how it may offer new opportunities in the future to monitor their growth and development temporally and spatially in undisturbed environments. We provide a brief overview of alternative approaches for visualising root systems architecture before detailing some of the key principles of operation of X-ray CT systems. The main body of the paper reviews the work over the last 30 years that has helped develop X-ray CT as a system for observing undisturbed root-soil interactions before discussing some of the most recent developments and key experimental considerations. Finally, the relative merits and constraints imposed by other non-destructive approaches for visualising root systems architecture are briefly discussed.

Traditional methods of observing root structure

One of the most common requirements of root research is knowledge regarding the size of the root system, its distribution at depth and how these change with time. It is rarely possible to extract intact root systems directly from the soil so, typically, intact samples are collected in the field and the roots washed free of soil prior to the measurement of root properties (Pierret et al. 2005). Various root-washing devices have been used, ranging from a bucket and hosepipe to a combination of water and compressed air (Pallant et al. 1993). Due to the spatial variation of rooting systems, a large number of replicates are generally required for the accurate determination of rooting parameters. Gregory (2006b) indicated that 15–20 samples of 10 cm diameter are needed for structured soils to have a 90% chance of detecting significant rooting variations at the 10% confidence level, suggesting that root washing is unlikely to detect small differences in rooting parameters under different treatments. Nevertheless, Oliviera et al. (2000) defend the technique of root washing, highlighting that it still gives the best quantitative information regarding rooting mass and length. Trachsel et al. (2011) recently illustrated how 'shovelomics', a method of rapid root architecture visualisation and assessment in the field, can be used to rank root architecture traits, discriminate between populations and tailor a crop system to a specific soil environment.

In addition to limited root recovery from the soil, washing and storage processes can also lead to incorrect measurement of root mass. Van Noordwijk and Floris (1979) observed 20–40% loss in dry weight of wheat roots grown in solution, dependent upon the washing and storage conditions utilised. Furthermore, difficulties in distinguishing live from dead roots can also cause problems, but again no universal characterisation standard is apparent. Separation is usually based on colour, but this creates the tedious task of manually, physically separating roots, using forceps, with samples spread in shallow dishes of water—a process which can often take several hours per plant.

Rhizotrons enable direct monitoring of root growth and provide insights into short-term root growth dynamics (Neumann et al. 2009). They also offer the opportunity to quantify spatial and temporal variations of rhizosphere chemistry and microbial populations (Smit et al. 2000), in non-disturbed soils along rooting profiles. The main limitation is that the viewing window is static, providing only a limited, 2-D visualisation that is unrepresentative of a rooting system's total extension. Although such set-ups allow for non-destructive sampling along single roots, mechanical impedance, moisture distributions, redox conditions and solute concentrations along the 2-D observation plane may be dramatically different from those in in situ field soils (Neumann et al. 2009). Whilst rhizotron systems allow for repeated and non-destructive visualisations of rooting growth, the system still suffers from problems of altered root-zone temperatures, disturbed soil structures and a restricted rooting volume at the window interface. Furthermore, processes occurring at a 3-D level are limited to analysis in 2-D.

Hydroponic, aeroponic and gel-based culture systems allow for the easy monitoring of root growth and morphology without background interference. Combined with rapid imaging systems it is possible to undertake high-throughput quantification of root growth in minutes. French et al. (2009) describe techniques which allow root growth and curvature to be extracted from sequences of images of plated *Arabidopsis thaliana* roots growing on agar. Image sequences are captured by a purpose-built robotic system, making the system truly high-throughput. Yazdanbakhsh and Fisahn (2009) also used a robot, though in their system the plates move rather than the cameras for measuring root growth by tip tracking. Achieving a similar effect with different methods, Hund et al. (2009) grew seedlings on the surface of blotting paper sheets held in sealed pouches. After image acquisition by an A4 scanner, image analysis software distinguishes and measures axial and lateral roots of maize plants. SmartRoot (International Atomic Energy Agency 2006) has also been developed as a method of analyzing root architecture from scanned images of plant roots. The system provides a powerful vector representation of the root architecture, allowing comparison of architectures across plants and time points. However, it is designed with a relatively large amount of interaction from the user in mind; as a result it is not currently suited to high-throughput analysis. Iyer-Pascuzzi et al. (2010) employ gellan gum in their 2-D root imaging system, while Fang et al. (2009) and Clark et al. (2011) recovered 3-D root descriptions using a laser scanner and multiple cameras respectively. While these methods allow much useful data to be gathered, root growth patterns can be dramatically different when grown in the absence of a solid substrate (Hargreaves et al. 2009; Wojciechowski et al. 2009).

Basics of X-ray Computed Tomography (CT)

The ideal scenario is to visualise roots directly in soil, their natural environment. Mooney et al. (2007) successfully used soil thin sections to look at undisturbed root-soil interactions and explore the mechanisms of cereal root lodging. This technique is, however, limited to 2-D (unless many serial sections are taken) and extremely laborious as one thin section can take several weeks to prepare. X-ray Computed Tomography (CT) can overcome these limitations. Tomography (tomo = 'slice' and graph = 'to write') enables the visualisation of the interior of solid objects. Several energy sources can be used for tomographic imaging (some alternatives are discussed towards the end of this review) but X-rays have been more readily adopted for the examination of root-soil interactions.

The development of X-ray Computed Tomography (CT) is largely attributed to Hounsfield (1973), for which he (and Allan Cormack) received the Nobel Prize in 1979. Essentially, CT is a non-destructive, non-invasive technique that can be used to visualise the interior of objects in 2-D and 3-D based on the principle of attenuation of an electromagnetic wave. X-ray CT has been repeatedly shown to be an efficient and non-invasive tool for imaging and studying soil sytems. The early pioneers of CT in the plant and soil sciences were Crestana et al. (1986) and Aylmore (1993). However Pierret et al. (2003a, b) stated that it was Hainsworth and Aylmore work throughout the 1980's e.g. (1983) that highlighted the potential use of X-ray CT to visualise root systems. Tollner (1991) demonstrated that both biotic and abiotic components can be detected successfully using

X-ray CT. Since the introduction of X-ray CT scanners several decades ago they have been continuously improved and, while they can be categorized into different generations based on their functionality, the underlying principles are still the same.

Here we provide a brief commentary on the principles of X-ray CT operation. For further details we refer the reader to the excellent reviews by Wildenschild et al. (2002)(hydrological sciences), Stock (2008)(material sciences), Taina et al. (2008) and Pires et al. (2010)(soil sciences). During CT acquisition, X-rays are produced in a highly evacuated tube which contains two electrodes: an anode, usually platinum or tungsten, and a cathode (Wildenschild et al. 2002). When a high voltage is applied across these electrodes, accelerated electrons produce X-rays as they strike the anode. As the X-ray beams pass through a sample, the object itself becomes a secondary source of X-rays and electrons. A portion of the primary incident beam is therefore absorbed or scattered. For monochromatic radiation of incident intensity I_0, the beam produces an attenuated intensity I after passing through a sample of thickness D as described by Lambert-Beer's Law:

$$I = I_0 \exp(-\mu \mathrm{D}) \tag{1}$$

where μ is the linear attenuation coefficient, which depends on the energy of the radiation, the bulk density of the sample and the electron density of the material (Wildenschild et al. 2002). This reduction in intensity of the X-ray as it passes through the object in question is called attenuation. The beam is projected onto the detector, which measures the change in energy intensity. In medical CT scanners both the source and detector rotate around the object, taking several projections from different angles. However in most industrial scanners it is the sample that rotates between a fixed X-ray source and a detector. Each non-invasive 'slice' is comprised of an array of pixels, describing the X-ray attenuation coefficient of volume elements (voxels, a 3-D pixel) of the scanned object. These projections are used to construct a cross-sectional image representing a 2-D slice through the 3-D object. This process is called reconstruction, which implies the calculation of CT numbers (CTN). These CTNs are frequently expressed in Hounsfield units (HU) (Duliu 1999) which reflect the density of the sample material and are calibrated in a way such that 0 HU corresponds to water and −1,000 HU to air. From this it follows that bright image pixels correspond to high attenuation of the X-ray beam and hence to a high density scanned object. Equation 2 describes the normalisation with respect to water and air of an attenuation coefficient value

$$\mathrm{CTN(HU)} = \left[\left(\mu_{\mathrm{subject}} - \mu_{\mathrm{water}}\right) / \left(\mu_{\mathrm{water}} - \mu_{\mathrm{air}}\right)\right] {}^{*}k \tag{2}$$

where $\mu_{\mathrm{air}} = 0$ and $k = 1000$.

Medical scanners have been used for investigating specific macroscopic features in soils such as transport effects associated with macropores (Perret et al. 1999; Mooney 2002) and roots (Heeraman et al. 1997). Medical scanners are advantageous because several scans can be obtained over a relatively short time period and larger samples imaged. However their resolution is typically limited to a voxel slice thickness of 0.5 mm, so if research objectives require resolution of finer root systems then an industrial X-ray or synchrotron scanner is required.

Synchrotron scanners use electromagnetic radiation emitted by high-speed electrons spiralling in a magnetic field of a particle accelerator (Wildenschild et al. 2002). Large electromagnets are used to focus the beam, causing synchrotron radiation emissions as the electrons decelerate. The emissions are of such high energy that the polychromatic (white) radiation produced can be made monochromatic (single energy) and yet still have sufficient photon flux for tomographic purposes. The source provides a fan-beam of high intensity radiation, which is collimated to a vertical size of approximately 5 mm. A monochromator splits the incident white synchrotron light into different wavelengths, giving a customisation option for radiation of the desired monochromatic level. Through this ability, it is possible to manipulate the resultant image contrast by adding an artificial dopant to one phase (e.g. iodide) and scanning at its peak adsorption energy. Whilst synchrotron images can provide information about pore space geometry, Wildenschild et al. (2002) observed a poor phase contrast without the addition of chemical dopants, which has implications for root-based studies. Further limitations include restrictions on access and typically only being able to accommodate very small sample sizes e.g. few centimetres in diameter.

Using X-ray computed tomography (CT) to visualise roots in soil

X-ray CT offers great potential for examining undisturbed root systems architecture in soils, and is less affected by soil paramagnetic elements than other non-destructive techniques like Magnetic Resonance Imaging (MRI) (Tollner et al. 1994). During the last two decades, X-ray CT has been applied in many different studies exploring the structure and function of roots and soils e.g. Heeraman et al. (1997), Gregory et al. (2003). Its application has expanded rapidly, covering the characterisation of pore space and bulk density (Anderson et al. 1990), spatial correlation of tortuosity and porosity (Heijs et al. 1995), permeability and volumetric water content (Hopmans et al. 1992) and solute breakthrough (Clausnitzer and Hopmans 2000). Fewer studies have examined root-soil interactions (<30 papers in 20 years). This is due to limitations of resolution, restrictions on access and a poor ability to distinguish between different media phases, since the attenuation density of roots and soil are similar (Fig. 1) and highly dependent on soil water and organic matter content (Kaestner et al. 2006). Very recent advances in spatial resolution, image quality and computing hardware, however, promise to lead to a significant increase in the utilisation of CT in root-soil studies (Tracy et al. 2010). New instruments such as the one demonstrated by Tracy et al. (2010) are able to detect microscopic changes in soil structure (to 0.5 μm), root structures and water-filled pores, facilitating the direct measurement of soil structural changes both spatially and temporally through a root's full growth cycle. A summary of the key papers in this field over the last 20 years is provided in Table 1.

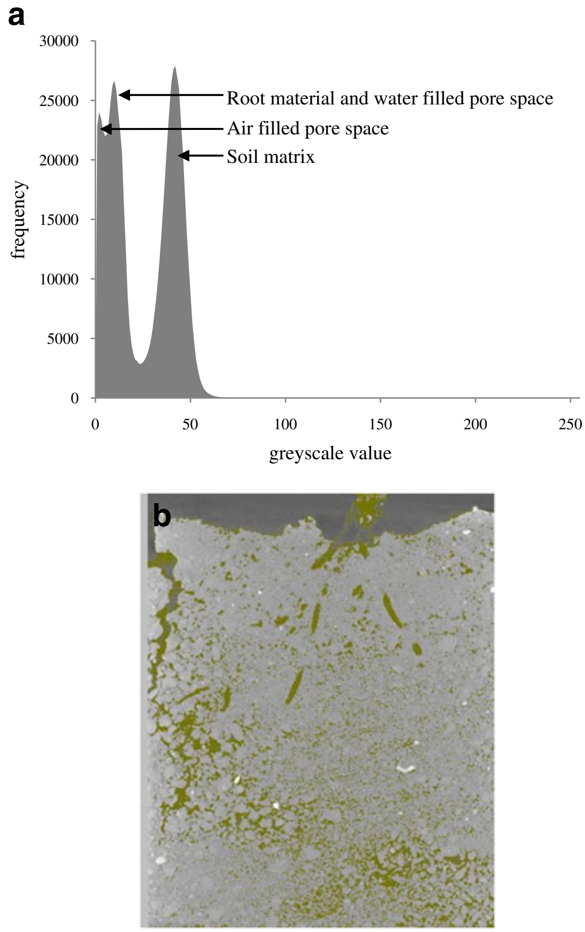

Fig. 1 Typical distribution X-ray attenuation values in soil. **a** Histogram showing the separate and overlapping phases from an X-ray CT image of a sandy loam soil core at field capacity with a wheat roots and **b** the accompanying vertical cross section showing the failure of a global threshold to discriminate roots and soil pores. Resolution=30 μm. Sample dimensions 50×60 mm

Wantanabe et al. (1992) were amongst the first to utilise X-ray CT to study plant roots. They visualised the coarse storage roots of the Chinese Yam (*Dioscorea oppositifolia*) at a resolution of 2–5 mm, although the full root morphology could not be clearly observed. Tollner et al. (1994) investigated the finer rooting systems of Soybean (*Glycine max* L.) and Bahiagrass (*Pasoalum notatum* Fluegge). Weekly scans of repacked sand cores (460×150 mm diameter) were conducted for a period of 84 days. The taproot was identifiable at the near surface after 3 weeks, but other Soybean and Bahiagrass roots were not visible in any scans, suggesting the resolution was inadequate. At a similar resolution Heeraman et al. (1997) used a high-energy industrial scanner to image a small core (100×50 mm) of bush bean (*Phaseolus vulgaris* L.), collecting 40 consecutive scans over a depth range of 8 mm and successfully detecting root diameters around 0.35 mm, although the authors acknowledged that the application of CT to soil and plant systems was not well developed at the time. They also noted that the ability to segment root voxels was limited by the water content of the soil. Grose et al. (1996) used a medical scanner to explore the water content in sand:clay mixtures with wheat, cotton and radish roots, although they were unable to resolve roots

Table 1 Summary of selected key research papers in which X-ray Computed Tomography (CT) has been used to visualise roots in soil columns

Author	Year	X-ray CT type	Resolution (μm)	Plant Species	Soil Type	Sample dimensions (diameter x height, mm)	Results/Conclusions
Wantanabe et al.	1992	Conventional	2,000–5,000	Chinese Yam (*Dioscorea oppositifolia* L.)	Not specified	165×300	The root-zone could be visualised so that biophysical interactions could be non-destructively studied.
Tollner et al.	1994	Conventional	500	Soybean (*Glycine max* L. Merr) and Bahiagrass (*Paspalum notatum* Fluegge)	Lakeland sand	152×1,000	Quantitative measures of plant root development.
Heeraman et al.	1997	Conventional	~160	Bush bean (*Phaseolus vulgaris* L.)	Sand	50×100	Fine roots could not be detected due to volume averaging effects and poor spatial resolution.
Pierret et al.	1999	Conventional	~500	Chestnut tree (*Aesculus hippocastanum* L.) and Maple (*Acer pseudoplatanus* L.)	Sandy Loam	200×500	Tree rooting patterns analysed by the application of new imaging method.
Jenneson et al.	1999	Conventional	~160	Wheat (*Triticum aestivum* L.)	Not specified	10 and 25 mm dia.	Root length and volume were determined from 3-D time-lapse images of growing wheat roots.
Gregory et al.	2003	High Resolution	100	Wheat (*Triticum aestivum* cv. Charger) and Rape (Brassica napus cv. Shannon)	Sandy loam	25×80	Development and growth of both root axes and some first-order laterals were observed.
Pierret et al.	2003a, b	High Resolution	~50	Pea (*Pisum sativum* L.)	Artifical potting mix	250×500	Simultaneous observation of root growth and plant water uptake.
Pierret et al.	2005	High Resolution	~50	Canola (*Brassica napus* L. var Rainbow)	Red Kandosol	70×70	Fine roots significantly contribute to the overall root system.
Mooney et al.	2006	Conventional	~500	Wheat *(Triticum aestivum L)*	Sandy loam	120×120	Visualised root and subterranean stem lodging.
Kaestner et al.	2006	High Resolution	36	Alder (*Alnus incana* (L.) Moench)	Quartz sand	36.9×70	Presented new image processing approach.
Lontoc-Roy et al.	2006	Conventional	~ 120	Maize (*Zea mays* L.)	Sand and loamy sand	100 mm dia.	Concluded mineral soils with minimal organic matter currently give the best visualisation of root systems in CT images.
Hamza et al.	2007	Conventional	~500	Lupin (*Lupinus angustifolius* L.) and Radish (*Raphanus sativus* L.)	Sand kaolinite mix	45×120	Two plant species showed different response to selected levels of osmotic potential.
Perret et al.	2007	Conventional	~275	Chickpea (*Cicer arietinum* L.)	Sand	230×140	Coarse resolution caused an underestimation of fine roots and root tips.
Han et al.	2008	Conventional	~350	Potato (*Solanum tuberosum*)	Sand	190×200	Root systems of diseased plants were less complex than those of healthy plants.
Hargreaves et al.	2009	High Resolution	100	Barley (*Hordeum vulgare* ssp. Vulgare and ssp. Spontaneum)	Sandy loam	25×50	Roots grown in gels different in comparison to soil grown plants.
Seignez et al.	2010	High Resolution	10	*Arabidopsis halleri*	Contaminated soils	10×80	Needed 10 mm diameter cores to visualise roots, otherwise metal contmainated soil caused artefacts.
Aravena et al.	2011	High Resolution	4.4	Sweet pea (*Lathyrus odoratus*) and Sunflower (*Helianthus annuus*)	Clay loam	aggregates (0.5–0.8)	Measured compaction and new insights into soil-water uptake.

<0.4 mm. Despite the low resolution, they were successful in creating water distribution contour plots which illustrated changing matric potential over time and in response to root growth. Similarly Mooney et al. (2006) used medical X-ray CT to successfully examine the root response to soil failure i.e. lodging, however this work was also limited by coarse resolution (0.4 mm) and the inherent difficulties in segmenting roots from soil images due to similarities in attenuation.

The first reported results from a microtomography system were by Jenneson et al. (1999). Wheat seeds (*Triticum aestivum*) were grown and excellent 3-D time lapse images of growing roots were obtained at a 100 μm voxel resolution. Gregory et al. (2003) also used a microtomography system to visualise roots of pre-germinated wheat (*Tricium aestivum*) and rape (*Brassica napus*) seeds that were grown in a sandy loam soil at the same resolution as Jenneson et al. (1999). The low energy source resulted in some impressive images (Fig. 2), however this investigation was made on roots embedded in a supporting media with a finer pore space than could be registered at the given resolution, so conclusions regarding the influence of the root on the physical soil environment were limited.

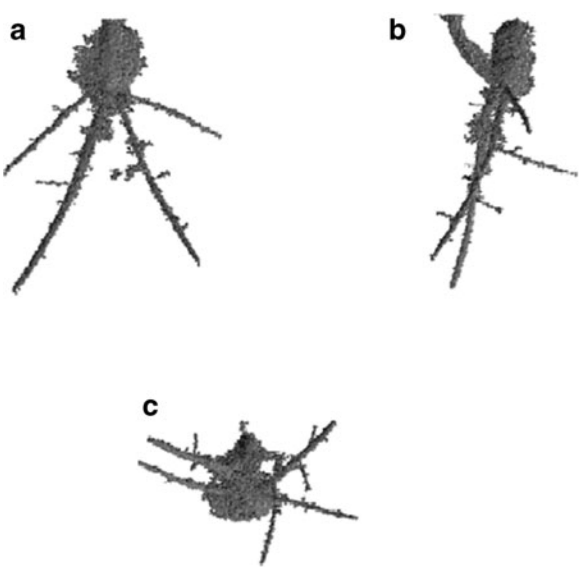

Fig. 2 3-D visualisation of a wheat seedling segmented from sand viewed from different angles at 8 days after germination. Spatial resolution=100 μm. Sample dimensions=25×25 mm. Taken from Gregory et al. (2003)

As already highlighted, the imaging of plant roots in soil using X-ray CT relies on sufficient contrast in X-ray attenuation between growth medium solids, air filled pores, soil water, plant material and organic matter. The attenuation of these materials varies with several factors including soil type (Pálsdóttir et al. 2008), soil moisture content (Pálsdóttir et al. 2008), proximity of roots to organic matter or air filled pores (Perret et al. 2007) and root water status (Hamza et al. 2007). Root diameter can be overestimated during image analysis due to the proximity of water and air within the soil (Perret et al. 2007). Root length and number of lateral roots is often underestimated because the root material cannot be easily distinguished from other soil components such as air or water. To minimise the effects of similar attenuation between the soil and plant fractions, researchers have focused on plants with coarse roots (Hamza et al. 2007; Perret et al. 2007; Hargreaves et al. 2009), artificial soil systems (Kaestner et al. 2006; Perret et al. 2007), manipulating the water content of the sample (Pálsdóttir et al. 2008) and undertaken convoluted image processing to enhance contrast. Nevertheless the boundaries between adjacent structures is never clearly defined, which makes segmentation by thresholding very problematic (Coleman and Colbert 2007). The continuum of greyscale values means that at any threshold level some voxels will be misclassified (Coleman and Colbert 2007). One boundary that can cause difficulties is at the top of the container between the sample and air. Gregory et al. (2003) explain that this interface gave attenuation coefficients that were similar to the plant seed and so to combat this issue a thin layer of fine silica was added to the top of the sample.

Segmentation of roots in soil images

Segmentation of an input image into regions corresponding to distinct phases is a longstanding problem in image analysis and computer vision. A wide variety of approaches have been proposed (Pal and Pal 1993; Cheng et al. 2001; Wirjadi 2007), based on differing formulations of the segmentation task. Some treat segmentation as a clustering problem, aiming to divide the set of input data items (pixels or voxels) into subsets, each of which satisfies or maximises some shared property. Clustering methods often take pixel or voxel position into account, but may not. Region-growing approaches cluster from a seed point, adding nearby data items which satisfy some pre-determined similarity criterion. Other methods, most notably those based on the Expectation-Maximisation algorithm (Dempster et al. 1977) cluster by fitting models of the expected distribution of data values to the input image. Edge-based techniques seek the sharp changes in data properties which typically mark the boundaries between objects. Methods viewing region boundaries as watersheds of some surface (Vincent and Soille 1991) have been particularly successful in pattern recognition studies, as have those modelling the image as a graph structure (Shi and Malik 2000).

In order to segment roots from images of soil, two approaches have generally been adopted: clustering by global thresholding based on an estimate from the histogram of the image (Pierret et al. 1999) or region growing by adaptive local thresholding, starting from seed points containing specified properties, usually the greyscale value of the local minima (Gregory et al. 2003). Thresholding defines clusters as containing voxels whose values lie within a fixed range and usually generates an over-segmentation of the image. This requires cleaning to remove structures that have been mis-classified as root and to connect structures that belong to each other (Kaestner et al. 2006). Image over-segmentation is solved by rejecting items with volumes smaller than a given size, as they are unlikely to represent roots.

Pierret et al. (1999) used low energy X-ray CT to collect images of the root-network of chestnut (*Aesculus hippocastanum*) and maple (*Acer pseudoplatanus*) trees growing in sandy and sandy clay soil. In this study the large roots permitted the use of a global thresholding approach as the root and soil materials were apparent as distinctive attenuation peaks. Interestingly, Pierret et al. (1999) were among the first to identify one of the major imaging challenges concerned with segmentation where roots touch. In this instance the authors assumed they were part of the same object, although they acknowledge that this leads to misclassification during the segmentation phase and creation of a discontinuous representation of root segments. Kaestner et al. (2006) attempted to solve the problem by applying a non-linear diffusion filter to smooth the image followed by assigning a threshold value derived from Rosin's (2001) algorithm and concluded by a morphological dilation operation, to remove misclassified objects. The approach is based on the assumption that an object (i.e. the root) must be tube shaped and exceed a given volume threshold value. They illustrated that the method is capable of detecting not only the primary root, but some of the fine lateral roots as well. However, crucially, the research was undertaken on plant grown in a homogeneous sandy substrate with a low water content which would facilitate the imaging but is not well representative of field conditions.

Lontoc-Roy et al. (2006) examined maize (*Zea mays*) roots grown in a homogeneous sand and a sieved loamy sand in both a dry and water-saturated state. Their image analysis approach was based on an initial identification of root material achieved by global thresholding using manually selected values. Different thresholds were used on different samples, because CT responses differ depending on the type and condition (e.g. moisture content) of roots under study. This simple thresholding method resulted in a model composed of a larger primary root surrounded by 'clouds' of labelled voxels which per se contained parts of finer lateral roots. In a second step these clouds are analysed by iteratively increasing the threshold boundaries, thus finding similar voxels connected to the primary root. The final step involves skeletonising the region identified as root. In this iterative phase, the outermost voxels are successively removed until a single voxel wide model of the structure of the root remains. This illustrates how water content effects the overall density of the root when examined using X-ray CT. In non optimal situations, the attenuation values of the soil and plant roots overlap, making the final result inaccurate.

Perret et al. (2007) developed a range of methods to quantify and calculate parameters, such as volume, surface area, length, orientation and number of roots, using high resolution X-ray CT. Using chickpea (*Cicer arietinum*) roots grown in very fine sand they were able to isolate roots from their surrounding environment with a binary thresholding technique that labelled root material, but also air and water filled pores. The authors then used a geometric filter method to detect and eliminate disconnected voxels by starting from the seed and using a flood-fill algorithm to extract the root in 3-D. However, they reported that their method suffers from the presence of air spaces near root objects, because of the similarity in observed attenuation values between roots and air. Since these air spaces are close to root segments and therefore not disconnected, they are not eliminated by the filter. Another drawback of the presented top down process is the loss of root material that is not connected to the seed.

Very recently some researchers have begun reporting overcoming a number of the previous limitations. An impressive study by Aravena et al. (2011) utilised a synchrotron facility to, amongst other measures, visualise root induced compaction of aggregates at a 5–10 μm resolution which was then used to model water flow towards the root. The aim of this research was not to segment the root architecture from the soil images, but to illustrate their impact on soil structure. As shown in Fig. 3 the quality of the images is impressive in terms of definition and contrast. Ferriera et al. (2010) utilised successive CT scanning of the same sample to illustrate tuber volume expansion in potatoes using a ca. 0.1 mm resolution. These values were highly correlated (0.986) with the actual volumes of excavated samples, although no information was provided regarding the type of soil used in this experiment. Tracy et al. (2010) and Lucas et al. (2010) have also recently demonstrated that new X-ray CT systems can also visualise fine roots, such as those of *Arabidopsis thaliana* (Fig. 4), down to a few micrometers in diameter.

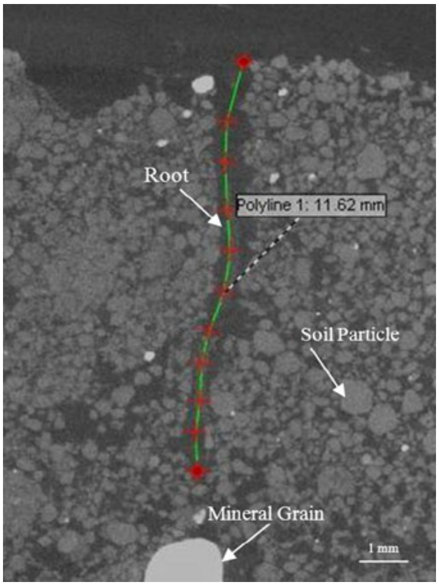

Fig. 4 2-D X-ray CT image slice in vertical orientation of Arabidopsis roots in a sandy loam soil at a spatial resolution of ca. 10 μm with example of root tracing

Root tracking approaches to segment 3-D root systems architecture

Whilst many studies have successfully visualised roots in situ fewer have been able to extract the volumetric descriptions of rooting material needed to produce a 3-D model of root architecture. This was recently highlighted by Seignez et al. (2010) in a study to explore the root development of *Arabidopsis*

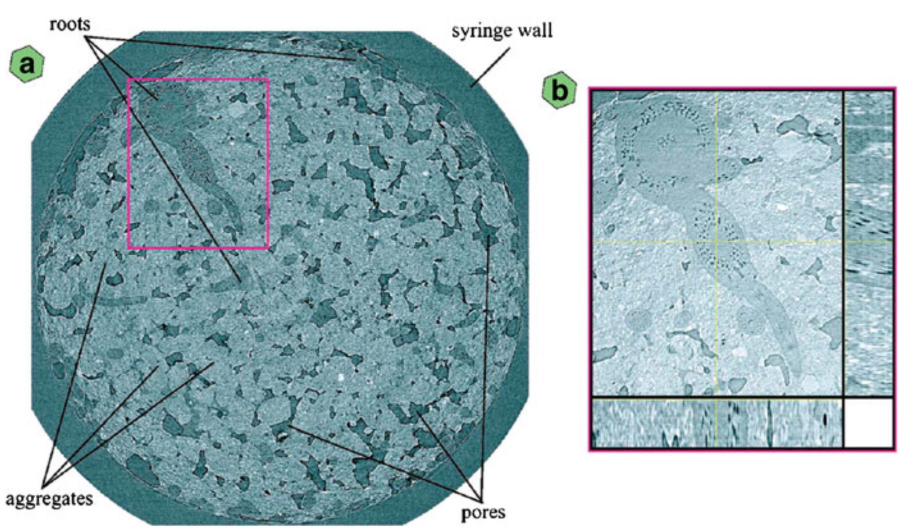

Fig. 3 2-D X-ray CT slice image of **a** sample of *L. odoratus* in soil aggregates; **b** zoom-in to the root area and orthogonal views. Spatial resolution=ca. 10 μm. Sample diameter=14 mm.Taken from Aravena et al. (2011)

halleri growing in polluted soils. The soil cores were impregnated with resin to avoid sample deformation, but despite being able to observe root architecture, Seignez et al. (2010) were unable to successfully segment the roots by image analysis due to similarities in grey scale values.

The overlapping attenuation values produced by root material and the soil environment are at the heart of the root/soil segmentation problem: approaches which consider only attenuation values struggle to identify root material accurately. Further knowledge of or assumptions about the appearance of plant roots in CT data are required to make the problem tractable. One way to introduce a priori knowledge of roots into the segmentation process is to consider CT data not as a volume to be segmented but as a sequence of images through which roots appear to move. Consider a CT data set showing a root system extending through a soil core. This voxel data can easily be separated into horizontal, planar slices, mimicking the physical slicing of soil cores e.g. Mooney et al. (2007). If a given slice is displayed as a 2-D grey level image, the root system will appear as a set of (usually) elliptical regions. If the slices comprising the CT volume are ordered from top to bottom and displayed as a video, these root regions will appear to move, their size, shape and apparent motion reflecting the geometry of the root. As roots are generally smooth, continuous objects, the motion of the elliptical regions they produce should also be smooth and continuous. This raises the possibility of extracting root system architectures from CT data by tracking the motion of 2-D regions through an image sequence representation of the 3-D attenuation values.

Methods of tracking moving objects through video data have attracted considerable interest within the computer vision community (Yilmaz et al. 2006). Tracking algorithms typically contain three components. The appearance model captures the image features likely to be associated with the target object; often its colour or shape. The motion model describes the target's expected change in position, and sometimes likely changes in its motion, between frames. The appearance and motion models are linked by a tracking engine or framework which applies them to the input image data to recover estimates of the target's state in each image. Predictive filters (e.g. Kalman 1960; Isard and Blake 1998) are the most common type of tracking engine. These use the tracker's estimate of target state at time t to predict its state at time $t+1$. A restricted search is then performed in the neighbourhood of that prediction, producing an estimate of actual state. Differences between the predicted and measured target properties can be used to update the appearance and/or motion models. Predictive filter tracking algorithms have previously been used to extract descriptions of the roots of *Arabidopsis thaliana* seedlings (French et al. 2009) and promise to enable us to visualise isolated root networks and quantify their effects on the surrounding soil structure in the near future. The key advantage of the tracking approach is the ability to deploy explicit models of the expected appearance and shape (motion) of root system architectures, which can develop as segmentation proceeds.

One approach was to process the first slice as a 2-D image, assign a greyscale attenuation value to a known root structure, and perform a top down slice by slice connectivity search. The initial greyscale value constitutes a simple appearance model, while the connectivity search corresponds to a motion model. Whilst this technique detects both thick and thin roots growing vertically, any disconnected roots, or those growing upwards are not registered. Kaestner et al. (2006) proposed a dilation operation to avoid a disconnection of the root networks and the suppression of thin root structures. The underlying principle is that voxels in a mask image that are connected to those in a marker image are labelled. As the algorithm iteratively expands, the region connected to the marker voxel continues to be labelled until no more voxels containing greyscale attenuations in the given threshold range remain. Using these techniques Kaestner et al. (2006) successfully classified the root architecture of alder (*Alnus incana* (L.) Moench), with identical root growth directions and number of branches extracted by the algorithm and observed in the growing plant (Fig. 5).

Pierret et al. (1999) used an approach based on the tracking of elliptical shaped objects through 2-D slices of the sample and connected them to make a final 3-D image. Pierret et al. (1999)'s appearance model was therefore based on shape, rather than attenuation value. A measure of the degree of overlap was used to reconnect the ellipses, which implies that an object identified in one slice is connected to an object in the following slice only if there is face-to-

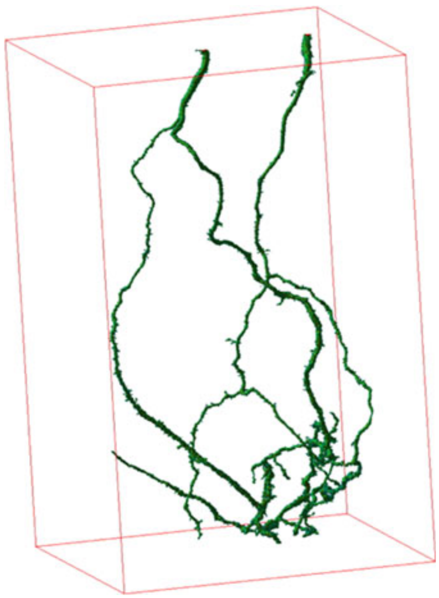

Fig. 5 3-D visualisation of Alder root obtained by a tracking style approach at 36 μm resolution. Spatial sample dimensions =36.9×36.9×59.15 mm^3 Taken from Kaestner et al. (2006)

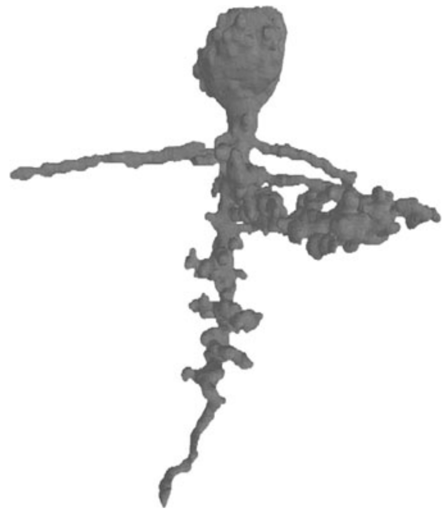

Fig. 6 A Three-dimensional visualisation of early growth stage chick pea root system grown in sand. Spatial resolution= 0.275 mm. Sample dimensions=0.23×0.14 m diameter. Taken from Perret et al. (2007)

face voxel contact. As with the top down slice by slice connectivity search, this approach will disregard any roots growing upwards and may miss fine rooting regions, but will require considerably less computational power than methods suggested by others. Gregory et al. (2003) was also able to track roots from one tomographic slice to another and discriminate them from the surrounding soil. The appearance model used here was a fixed range of attenuation values, determined by the operator after examination of the first image. This was modified by the addition of connectivity conditions constraining the spatial distribution of these values, though no further details are given.

Another example of an image created by 3-D root tracing (Fig. 6) was produced by Perret et al. (2007). The authors admit that some root laterals are missing from this figure as the visualisation algorithm volume averages the CT matrices to generate a list of vertices and polygons, which form the 3-D image. The resolution of the CT scanner (set at 0.275 mm in this study) also meant that some fine root segments could not be detected. Once again, the appearance model used relied on specified ranges of attenuation values and the motion model was a straightforward connectivity requirement.

Recently, Han et al. (2008) successfully extracted the architecture of first order potato (*Solanum tuberosum*) roots from CT data. No automatic image analysis was involved. Instead, an interactive system allowed a human operator to track the root through a stack of CT slices by marking the centre of the root region. This approach was successful in detecting root architectural differences in plants inoculated with a scab-inducing bacterium (Sc. Scabies EF-35) which illustrates the enormous potential for future studies to examine the impact of biological control agents and management strategies. However it should be noted that the plants were grown in a homogeneous sand medium which maximises the contrast of roots at the image analysis stage. In addition the resolution was limited to 0.2–0.35 mm, so only relatively coarse roots were imaged.

Very recently, two more automated tracking approaches, one based on assigning probability functions called RootViz (Jassogne 2009; Tracy et al. 2010; Tracy et al. 2011) and one based on level set methods called Rootrak (Mairhofer et al. 2011) have demonstrated great potential in overcoming the limitations associated with overlap of phases in the attenuation histogram. RootViz, developed by Davidson (www.rootviz3d.org), assigns a probability function to determine whether specific pixels within images represent root material and can be used to provide 3-D visualisations of root distribution in undisturbed soil columns. There is strong evidence that this automated and rapid methodology for root detection can remove the subjectivity of identifying 'root' pixels in noisy images and enable detailed visualisation of root architecture in situ (Tracy et al. 2010). Jassogne (2009) used RootViz to segment the 3-D root architecture of saltbush (*Atriplex hortensis)*, lucerne (*Medicago sativa*) and canola *(Brassica napus L.),* albeit at a coarse resolution as the samples were large (15×50 cm) and they were scanned in a medical scanner. Tracy et al. (2011) recently demonstrated the application of RootViz to segment 3-D wheat *(Triticum aestivum)* root architecture at early growth stages at a 15 µm resolution (Fig. 7).

The technique (Rootrak) developed by Mairhofer et al. (2011) uses local models of the distribution of attenuation values observed within root sections to identify root material in the subsequent frame. Distinct appearance models may be used to track different root branches, and the appearance model used may vary as tracking of a given root segment proceeds. The user initialises tracking by indicating, in the first image, the top of the root system. A level set (Osher and Sethian 1988) method identifies the marked root section (or sections) and an initial appearance model is constructed. A simple motion model, as in previous systems reflecting a connectivity requirement, provides a point in the next image that is expected to lie within the tracked root. The level set method is applied to this point, seeking a region around the starting point which contains a similar distribution of attenuation values to that represented by the current appearance model. Once this is done, the model is updated to describe the new root section and tracking continues. A danger when applying this type of combined segmentation and tracking algorithm is that inaccurate segmentation may introduce attenuation values into the appearance model that do not arise from root material. Subsequent segmentations may then suffer greater inaccuracy, leading to more pollution of the appearance model and eventually tracker failure. To avoid this, the shapes of the root sections extracted from adjacent images are compared; if they differ by a statistically significant amount, model updating is discontinued. Shape is therefore not a part of the tracker's motion model as in Pierret et al. (1999), but is used to verify tracking results. The method has been successfully applied to CT scans of maize (Fig. 8), wheat and tomato grown in a range of contrasting soil textures.

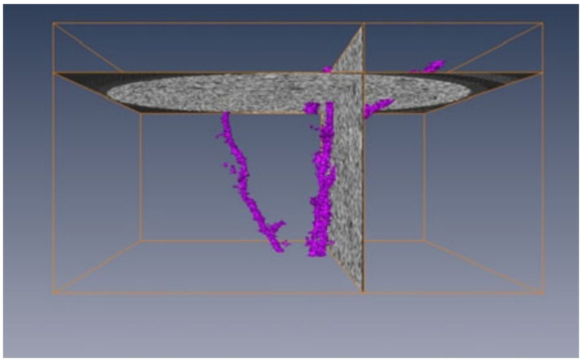

Fig. 7 An example of 3-D visualisation of wheat roots grown in a sandy loam soil at 18 µm resolution segmented using RootViz. Sample dimensions=91 mm height×29 mm diameter. Image courtesy of Rob Davidson

Optimisation of root imaging in soil using X-ray CT

A key consideration in evaluating the potential of X-ray CT as a tool for root-soil interaction studies is the rapid development in X-ray technology, primarily driven from other disciplines such as medicine and engineering. There are several areas in X-ray CT where artefacts can be introduced. Some of these errors cannot be easily corrected for, such as changes in the X-ray beam intensity (Pierret et al. 2003a, b).

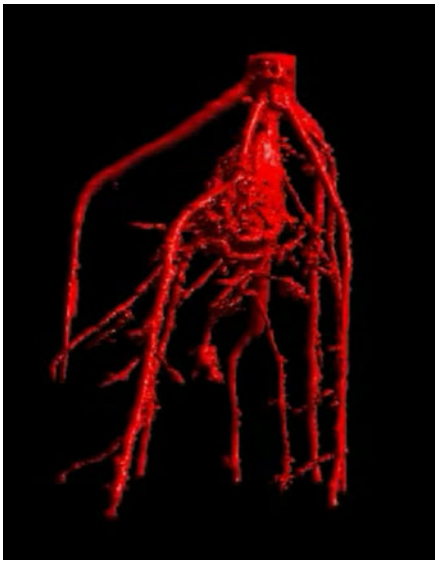

Fig. 8 3-D visualisation of maize roots grown in a sandy loam soil at a resolution of 30 µm obtained by Rootrak Sample dimensions=50×120 mm

The X-ray beam source intensity can drift as a result of over-heating, which can introduce errors, however most modern CT systems now have a cooling system to prevent this occurring. Degradation of target materials and movement of the focal spot can also lead to fluctuations in X-ray intensity which impacts on image quality although new target materials that resist breakdown for longer are subject of research by most CT scanner manufacturers. Another problem that can arise is scattered X-rays directly hitting the detector causing anomalously bright pixels, which result in ring artefacts (Wildenschild et al. 2002) although again many new systems have image tools to remove or minimise these effects.

Spatial resolution

Spatial resolution explains the level by which features in images can be resolved. It is generally defined as the smallest separation distance for which attenuation values of two known points can be perceived as separate entities and thereby accurately measured (Wildenschild et al. 2002). The spatial resolution of CT reconstructions depends not only on the magnification (ratio of the distance between the source and detector divided by the distance between the source and sample), but also on the type of X-ray source and detector, the signal to noise ratio and the focal spot size of the X-ray tube (Sleutel et al. 2008; Stock 2008). Kinney and Nichols (1992) explain that the ability to collimate the source will determine spatial resolution capability, as source divergence will lead to image blur. However, as collimation reduces X-ray intensity, problems of contrast sensitivity and spatial resolution become inter-related (Wildenschild et al. 2002). Typically undertaking a scan at a higher resolution i.e. decreasing voxel size leads to increased scan time to compensate for increased noise although this effect is highly variable between different CT systems. Spatial resolution therefore requires larger incident photon intensity or longer integration times (Wildenschild et al. 2002). As X-ray CT sources emit only a small proportion of their power as X-rays, high resolution images are often obtained at the cost of the ability to distinguish low-contrast object features e.g. fine roots. Kaestner et al. (2006) state that to detect an object, the scanner's resolution must be two to four times that of the object diameter. The resolution may even need to be greater if the background is heterogeneous or if the image is noisy.

Perret et al. (1997) reported that the spatial resolution of CT scanners at the time caused difficulty in the detection of fine tips in root laterals. Differences in the number and length of root segments obtained through X-ray CT and destructive root analysis were confirmed by Gregory et al. (2003), who reported that CT methods systematically underestimate root length. However, Heeraman et al. (1997) reported that the root length per unit volume of soil was overestimated in CT approaches. A large proportion of a rooting system is represented by root diameters smaller than 0.5 mm (Pierret et al. 2005), so a lack of information available on the fine root environment limits understanding of essential root and soil structural processes e.g. microbial interactions, aggregate formation and water and nutrient uptake (Paul and Clark 1996).

A key impediment to the uptake of X-ray CT for root studies is the trade-off between sample size and scan resolution (Pierret et al. 2003a, b). This is because for high quality images the sample should remain in view during CT scanning, at least when imaging the whole sample (Sleutel et al. 2008), and X-ray attenuation increases with sample diameter. The sample size is also restricted by the physical ability of the scanner sample stage to accommodate it. A thicker sample needs either higher photon intensity or energy to achieve adequate X-ray penetration. The problem with this is that higher energy photons have less interaction with the material and therefore contrast is lower. In addition, image artefacts caused by highly attenuating materials irrespective of sample size, can obscure aspects of interest in the image or skew the greyscale, minimising the actual contrast of the overall image. To overcome these problems, some researchers have favoured a relatively small sample size (e.g. 25 mm diameter) with a higher resolution (e.g. voxel size 100 μm) to ensure that fine roots can be imaged (Jenneson et al. 2003). When coarser root architecture characteristics are visualised, a larger sample (e.g. 150 mm diameter; 500 mm high) can be used with a relatively lower resolution (e.g. >1 mm) (Johnson et al. 2004). However a larger sample size can mean that fine roots are not detected (McNeill and Kolesik 2004). While many systems allow region of interest scans i.e. the ability to zoom in at a higher resolution, the link between sample size and spatial resolution remains a limitation.

Image contrast and artefacts

Contrast is the ability to distinguish a feature from its surrounding background, often defined by the differences in attenuation between the feature and background, divided by the background attenuation (Wildenschild et al. 2002). The application of a filter on the acquired images can offer many benefits. For example it can reduce the effect of beam hardening, enhance the roots and smooth the texture of the background elements (Kaestner et al. 2006). Sleutel et al. (2008) also found that using a filter had a significant effect on discriminating objects and were able to reduce CT-artefacts. Pierret et al. (2003a) used an averaging filter to eliminate the majority of overlaps on the histogram, whereas Feeney et al. (2006) used a sigma filter that reduces noise yet preserves fine details. The beam hardening effect occurs because low energy (strongly attenuated) photons of a polychromatic beam are emitted faster than the high energy (weakly attenuated) photons. Therefore the longer the X-ray path, the more low energy photons that are absorbed, which gives an apparent higher attenuation at the boundaries of objects, as the beams pass from a dense material to a less dense material. Ketcham and Carlson (2001) made three suggestions to prevent beam hardening; i) pre-hardening of the X-ray beam using an attenuating filter (aluminium, copper or brass); ii) smaller sample sizes and iii) correction during image reconstruction. The effect can be removed altogether if a monochromatic beam is used (Wildenschild et al. 2002). However as accessibility to synchrotron systems is frequently limited, this is not an easily applied option.

For low energy scans (<100 kV), X-rays interact predominantly through photoelectric adsorption, a factor strongly dependent on the atomic number of the matter in question. At higher energies Compton scattering prevails, with resultant attenuations being controlled by electron densities. Differences in the attenuation values of two materials with different electron densities and atomic numbers can be small, if a difference in atomic number of one material is compensated by a similar difference in electron density of the other (Wildenschild et al. 2002). In such an instance, it can be very difficult to distinguish different materials in a CT image. Dual-energy imaging has been proposed to enhance the contrast between two materials by utilising differences in the effects of photoelectric adsorption and Compton Scattering (Rogasik et al. 1999; Kaneyasu and Uesaka 2005) as it allows for contrast to be obtained between two materials that would be otherwise undetectable in single energy scans.

Difficulties in effectively assigning changes in attenuation coefficients to a limited number of classes, for example bulk density, water content and root structures, cause problems with CT image analysis. Whilst filters can be applied to decrease ambiguity between the relevant classes, such difficulties can cause the mis-characterisation and therefore incorrect evaluation of CT results. It is important to select an appropriate X-ray source for the material in question. Whilst sufficient X-ray energy is required to penetrate a sample effectively, it should not be too powerful otherwise the relative attenuation will be low and no contrast between the various phases visible. A fine balance of incident X-ray energy to image contrast is needed to clearly visualise the features of a sample in question. Most research based CT scanners now come with interchangeable targets, typically tungsten or molybdenum which enables the CT operator to fine tune the spectrum of the polychromatic beam to achieve the highest quality images.

Ring artefacts can be caused by local defects in the detection device, causing incorrect low or high beam intensities to be recorded and appear as rings on the reconstructed image (Wildenschild et al. 2002). Likewise, scattered X-rays hitting the detector chip directly can create erroneous bright pixels, showing as rings on a CT image. However, like beam hardening, many new CT scanners have built in image processing tools that can cope with such artefacts, both pre and post scanning, such that the operator might not even notice the artefact existed.

Photon starvation artefacts are caused by a lack of photons in certain areas of the scan. These typically occur in the centre of large samples or around high density objects where too few X-ray photons reach the detector characterised as region with a lack of CT information or the presence of streaks in the CT image (Mahesh and Hevezi 2009). Optimisation of X-ray conditions (e.g. X-ray energy, number of projections etc.) and sample size should therefore be considered to reduce or avoid these artefacts. Root detection can be difficult if the root is close to highly attenuating mineral grains in soil.

The most recent scanners have the ability to undertake scanning in much shorter time periods than previously possible especially via new ‘fast scan’ protocols, however this is usually at the expense of increased image noise (due to lower image averaging) and artefacts (e.g. rings). Depending on the objectives of the study this can often be overcome by post scanning image correction which leads to a frequent question of how long does a scan need to be? Other factors are important here such as the resolution and sample size, however now scans can be undertaken during minutes rather than hours changes in water content during the scan, which can be attributable to drainage, redistribution and evaporation, can impact on the image quality and therefore need to be considered.

The radiation dose is related to the amount of energy that X-ray photons deliver during a CT scan, which depends on the total number of photons and their individual energies (Primak et al. 2006). As dose is inversely proportional to noise therefore it is common to try to seek to increase the dose / X-ray energy to reduce the noise. However this can have implications for image quality plus this is unlikely to be desirable for biological specimens (further comment on the effect of radiation on plants is provided below). The X-ray energy required to adequately penetrate a sample to ensure a high image quality is frequently linearly related to the sample diameter in that more energy is needed for larger samples, which in turn can have a negative impact on image quality through increased noise. Therefore it is important to optimise the sample size in that the focus should be on using the smallest sample necessary for the given study, although when the objective is to looking at growing roots in soil columns this is problematic as if the sample is too small the plants become pot bound very quickly.

Sample preparation

In general, during X-ray CT acquisition the sample should remain in the field of view, implying that image resolution is determined by the sample size. The exception to this is during region of interest scanning where one effectively zooms in to a sample to achieve visualisation at a higher resolution (see Carminati et al. 2009 for example). Obtaining a given resolution may be at the cost of a smaller, and often impractical object size. The quality of a CT image is highly influenced by slice thickness, both in region of interest single scans and in volumetric reconstructions. The slice thickness is a trade-off between the higher spatial resolutions of thin slices and the improved contrast to noise ratios of thicker slices (Heijs et al. 1995). A low image contrast and high noise levels makes it difficult to accurately detect image objects. Whilst a thin image slice may produce an image of high resolution, fewer photons will reach the detector causing image noise to increase. Sleutel et al. (2008) found that poor CT image quality caused by noise at lower energies could be overcome by an increase in scan time. However thermal expansion of the X-ray tube filaments over time may cause artefacts although it may be that new target materials can minimise this impact. For long scans, a software based correction may be possible to overcome such problems.

Using an appropriate sample container material is an important consideration prior to scanning plant and soil samples in CT systems. Heijs et al. (1995) used Perspex to avoid scattered radiation from metal objects. Likewise, Pierret et al. (2003a, b) used Perspex containers due to their density being half that of glass, reducing X-ray attenuations in the container walls. Mooney (2002) observed increased image artefacts when polyvinyl chloride (PVC) containers were used. Lontoc-Roy et al. (2006) used standard plastic pots in their investigation due to their low X-ray absorption levels, allowing the production of better quality CT scan data than pots of higher density. Container material is therefore an important consideration to maximise X-ray transmission into a sample. Sample containers composed of low density plastics with a small width (<3 mm) are preferable to metal based cylinders.

Other considerations

The soil water content of the sample is a key consideration when seeking to segment the root architecture from X-ray CT images. Previous research has shown that to optimise image quality coarse textured soils at low water contents are preferable (Table 1). This is due to the attenuation overlap with pore space and root material attributed to water however more recently, as imaging protocols have become more refined, some researchers have undertaken scanning on clay textured soils e.g. Aravena et al. (2011). Although it has yet to be adequately researched it is likely that imaging soil cores in water conditions below field capacity are likely to yield images of higher quality than those closer to saturation due to the moisture content of the root.

A further area that has yet to receive the appropriate attention is the potential deleterious effects on plants of repeated exposition to X-rays. This will become more important as the rapid scanning functions on new CT instruments have opened up the possibility of more accurate '4-D' scanning i.e. exploring root growth characteristics over time. Previous research has identified changes in the above ground elements of the plant system associated with radiation exposure (e.g. Johnson 1936) however no research to date has addressed the impact on a plant's roots to the repeated scanning on an individual sample which needs to be considered as scan times have reduced to the extent that a single plant could be scanned several times within a few hours. A further consideration of repetitive (4-D) scanning is the careful positioning of the sample within the scanner in the exact same position. Although cross referencing the morphological properties of the same sample scanned on different occasions is possible after scanning by image analysis, this can be a tiresome process especially if the sample has not be located on the exact z axis. As such using some highly attenuating material attached to the sample to orientate it during positioning can be very useful.

Recent advances in X-ray micro CT technology

X-ray micro CT theory and technology are rapidly evolving areas which have generally been pushed forwards by medical, engineering and material sciences. Stock (2008) provides an excellent commentary on recent developments from a materials perspective which is highly relevant for the plant and soil community. One previous limitation in the application of X-ray CT in root-soil studies has been the lack of rigorous experimental design specifically regarding the use of replicate samples. To an extent this has been a result of difficulties in access to CT instruments but is also a function of scan speed, which has been recorded at several hours per sample, in some cases up to 8 h per sample (Kaestner et al. 2006). Most modern micro CT scanners have the ability to scan a sample within ca. 60–90 min although with varying image quality, however some of the latest micro CT systems have the facility to undertake a 'fast scan' as previously mentioned which can in some cases result in image acquisition in a matter of a few minutes. Generally fast scans are achieved by the samples undertaking a continuous rotation during the scan process rather than stopping to allow X-ray penetration for each projection, which facilitates much faster scan speeds. The disadvantage of this approach is that image quality is usually significantly reduced through increased noise compared to conventional scanning operations but in some instances this can be improved considerably with post scanning image processing.

There have been several further advances in recent years that have significantly enhanced the potential of X-ray CT as a tool for the spatio-temporal examination of root in situ. One of the most important which appears to have been adopted by most new CT manufacturers has been the transfer of the reconstruction algorithm processing operation to a Graphics Processing Unit (GPU) card from a computer's central processor. This has increased reconstruction speeds by up to 100 times (generally from hours to minutes) (Yan et al. 2008). Higher resolution has been achieved in some systems using nanofocus (achieved by an extra electromagnetic lens), with resolutions as high as 200 nm reported by some manufacturers.

A commonly reported problem arises when X-rays are generated at high power; the focal spot has to be wider in order to prevent the target material from melting. Therefore, the inspection of small features with high absorbing materials is limited either by power (where reduced X-ray penetration results in noisy images or very long image acquisition time) or by resolution (increasing focal spot with power can result in blurred images). To solve this CT manufacturers have begun successfully developing new target materials that have a higher thermal conductivity which allows higher power on a smaller focal spot ensuring high resolution even at a high output.

As the technology advances so the use of micro CT by researchers is able to adjust focus towards understanding the complex interactions between plant roots and soil. Where as many papers over the last 20 years have concentrated on method development from visualisation of roots in soil (e.g. Gregory et al. 2003) to automated segmentation of the whole root system architecture (e.g. Tracy et al. 2011), some of the most recent research is now using X-ray CT to address some of the fundamental questions regarding the function of the rhizosphere. Carminati et al. (2009) used X-ray CT to observe the dynamics of air gaps at a 90 μm resolution in lupin (*Lupinus albus L.*) in response to wetting and drying cycles. Although this work was only conducted on one soil type, sand, and undertaken at a relatively coarse resolution in comparison to the current state of the art, it is interesting that the technology was used to observe the different response to the wetting and drying cycles by tap roots (larger gaps) in comparison to lateral roots. A similar study by Moradi et al. (2011) has recently shown how water gradients around roots differed in comparison to the bulk soil demonstrating roots can retain significant moisture even at high matric potentials. Although this work was undertaken using neutron tomography (described below), similar work using X-ray CT might be possible in the near future as the contrast and sensitivity of detectors improves.

3-D visualisation of rooting systems using other non-destructive methods

A number of other imaging techniques have been developed to visualise and quantify root properties in situ, including Nuclear Magnetic Resonance (NMR) (Rogers and Bottomley 1987; Jennette et al. 2001), Magnetic Resonance Imaging (MRI) (Pohlmeier et al. 2008), Thermal Neutron Tomography (Tumlinson et al. 2008) and Neutron Radiography (Carminati et al. 2010). Like X-ray CT, each approach has a number of merits and some limitations when used to visualise root systems architecture, and particularly root systems architecture in soil.

By using magnetic field gradients NMR can produce images of the distribution of protons in an object (Hemminga and Buurman 1997). NMR allows the direct visualisation of a sample placed in a strong magnetic field (Perret et al. 2007), and has attracted much attention due to its use in the medical and petroleum industries. NMR has been used in the soil sciences to gain information regarding the pore size distributions of soils (Hemminga and Buurman 1997). MRI is one of several techniques under the umbrella of NMR technology. It utilises a gradient of the magnetic field to create differential behaviour of spins enabling imaging (Zhou and Luo 2009). The non-zero spin properties of these nuclei cause them to resonate at a particular frequency proportional to a local magnetic field. Differences in nuclei resonations as the magnetic field is manipulated form a visual model of different media. MRI can be used to visualise root morphology and volumes, and is particularly sensitive to the water phase of samples. Previously this technique has been limited to investigations in soil for root diameters >1 mm due to the presence of paramagnetic ions (Cu^{2+}, Fe^{2+}, Fe^{3+} and Mn^{2+})(Box 1996). Recently Jahnke et al. (2009) elegantly combined the use of MRI with Positron Emission Tomography (PET) to quantify carbon allocation and storage in sugar beet (*Beta vulgaris*) and maize (*Zea mays*). However, unlike X-ray CT, where bench top systems have become widely available, access to NMR facilities limits its use in root-soil interaction investigations. NMR and MRI have one other major disadvantage when compared to X-ray CT for imaging the root-soil interface, which is that most soil contains iron or manganese ions. These are paramagnetic and have a negative influence on image quality (Heeraman et al. 1997).

Single Photon Emission Computed Tomography (SPECT) scanning, a technique that uses a radioactive tracer detected by a gamma camera, has shown promise as it produces emission images. X-ray CT in contrast relies on the attenuation of materials, and so produces transmission images (Perret et al. 2000). Whilst SPECT imaging has a relatively poor resolution (ca. 1 cm), the process is much quicker (Young et al. 2001). Another alternative was presented by Moran et al. (2000) who combined X-ray absorption with phase contrast imaging (PCI) and quantified root radius, root length density and root branching intensity. This promising work illustrated plant root systems contain more biomass (based on calculations of root radius and root length density), than is generally accepted, although to date there has not been any further publications that have used this technique. A further technique that has been utilised in recent years is neutron radiography (or tomography) to reveal the water distribution in a plant and soil sample. The volumetric water content of plant roots typically varies between 70 and 95%, whereas a soil at field capacity usually ranges between 5 and 30% (Menon et al. 2007) which permits sufficient contrast to separate roots from the surrounding soil. Menon et al. (2007) successfully used this technique to isolate the root system architecture of lupin *(Lupinus albus L.)* in soils contaminated with boron and zinc, although they noted image quality was considerably reduced when plants were imaged in soil as opposed to a quartz sand media. More recently Esser et al. (2010) combined neutron radiography and tomography to visualise the water distribution at daily intervals at the root-soil interface for lupin *(Lupinus albus L.)* and maize (*Zea mays*) grown in a sandy substrate. However, as with MRI and NMR, access to such facilities is generally limited globally, which is a major obstacle to their uptake in this research field.

Conclusions

Throughout this review we have demonstrated the impressive progress and enormous potential of X-ray CT as a tool to observe and quantify in situ root-soil interactions. Progress has been slow, primarily because access to CT instruments for plant and soil scientists has been restricted, and where it has been possible, researchers have had to contend with systems not configured for their specific needs. The advent of industrial micro CT systems represented a significant advance, since instrument availability has increased rapidly and the micro/nano scale resolutions now available have brought visualisation of the whole root system architecture i.e. fine roots within reach. Equally important has been the recent application of root tracking approaches to overcome difficulties in segmenting roots from soils where their attenuation values overlap. The plant and soil community also stands to benefit from recent rapid advancements in X-ray CT technology, which have included reduction in scan and reconstruction times by at least an order of magnitude, automated algorithms to remove artefacts and more sophisticated detectors that have significantly increased the raw scan image quality. Such has been the recent progress that it was only in 2009 that Gregory et al. wrote that the main limitations to using micro-tomography to visualise roots systems in soils is that typically <10 samples can be analysed in day, the large datasets are difficult to handle and the limited resolution means that fine roots remain unresolved. Yet 2 years later it might be argued that none of the above would be considered major limitations and all have to an extent been addressed, or at the least, significantly improved.

The next stage is to utilise the technique to address some of the fundamental research questions in the rhizosphere area to validate its potential, and progress past the demonstrations of the tool that have been the focus of most studies to date. For example, improvements in X-ray CT technology and image analysis algorithms will soon make it possible for this approach to be extended from low throughput root imaging studies to higher throughput applications such as root phenotyping. Until recently, high throughput phenotyping of root architecture has only been possible employing soil-free approaches like aeroponics and hydroponics or invasive procedures such as 'shovelomics' (Trachsel et al. 2011). New X-ray CT-based root imaging approaches promise to complement and extend root screening approaches, potentially providing breeders with a 'deep phenotyping' capability. For example, crop root systems could be studied at high resolutions and in 3-D to reveal which architectural features might be most readily associated with water and nutrient uptake. Whilst the resolution-sample size trade off still exists, new CT systems are becoming available that can visualise much larger soil columns (e.g. 30 cm diameter× 100 m length), effectively scaling up on what has previously been possible and removing some of the previous/current limitations associated with plants becoming pot bound. The quality of 'region of interest' scans i.e. zooming into to a large sample and scanning a smaller volume at a higher resolution has also recently improved. With the addition of sample automation, much greater throughput and/or 4-D resolution will also be possible. The increase in throughput raises important issues relating to experimental replication. It will be equally important to bring together researchers from around the world, as shown by Baveye et al. (2010), to ensure careful optimisation of image quality and the development of new, rigorous image analysis protocols and tools to minimise elements of subjectivity. In summary, X-ray CT looks set to become an important tool for plant and soil scientists in the coming years.

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References

Anderson SH, Peyton RL, Gantzer CJ (1990) Evaluation of constructed and natural soil macropores using X-ray computed tomography. Geoderma 46:13–29

Aravena JE, Berli M, Ghezzehei TA, Tyler SW (2011) Effects of root-induced compaction on rhizosphere hydraulic properties —X-ray microtomography imaging and numerical simulations. Env Sci & Tech 45:425–431

Aylmore LAG (1993) Use of computer-assisted tomography in studying the water movement around roots. Adv Agron 49:1–54

Baveye PC, Laba M, Otten W, Grinev D, Bouckaert L, Goswami RR, Hu Y, Liu J, Mooney SJ, Pajor R, Dello Sterpaio P, Tarquis A, Wei W, Sezgin M (2010) Observer-dependent variability of the thresholding step in the quantitative analysis of soil images and X-ray microtomography data. Geoder 157:51–63

Box JE (1996) Modern methods for root investigations. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant Roots: The Hidden Half. Marcel Dekker, New York, pp 193–237

Carminati A, Vetterlein D, Weller U, Vogel HJ and Oswald SE (2009) When roots lose contact Vadose Zone Journal 8: 805–809

Carminati A, Moradi AB, Vetterlein D, Vontobel P, Lehmann E, Weller U, Vogel HJ, Oswald SE (2010) Dynamics of soil water content in the rhizosphere. Plant Soil 332:163–176

Cheng HD, Jiang XH, Sun Y, Wang J (2001) Color image segmentation: advances and prospects. Pattern Recognition 34:2259–2281

Clark R, MacCurdy R, Jung J, Shaff J, McCouch SR, Aneshansley D and Kochian L (2011) 3-Dimensional Root Phenotyping with a Novel Imaging and Software Platform Plant Phys. March 2011. doi:10.1104/pp.110.169102

Clausnitzer V, Hopmans JW (2000) Pore-scale measurements of solute breakthrough using microfocus X-ray tomography. Water Resour Res 36:2067–2076

Coleman MN, Colbert MW (2007) Technical note: CT thresholding protocols for taking measurements on three-dimensional models. Am J Phys Anthropol 133:723–725

Costa C, Dwyer LM, Dutilleul P, Foroutan-pour K, Liu A, Hames C, Smith DL (2003) Morphology and fractal dimension of root systems of maize hybrids bearing the leafy trait. Can J Bot 81:706–713

Crestana S, Cesaero R, Mascarenhas S (1986) Using a computer assisted tomography miniscanner in soil science. Soil Sci 142:56–61

Dempster A, Laird N, Rubin D (1977) Maximum likelihood from incomplete data via the EM algorithm. Journal of the Royal Statistical Society, Series B 39:1–38

Duliu OG (1999) Computer axial tomography in geosciences: an overview. Earth Sci Rev 48:265–281

Esser HG, Carminati A, Vontobel P, Lehmann EH, Oswald SE (2010) Neutron radiography and tomography of water distribution in the root zone. J Plant Nut & Soil Sci 173:757–764

Fang S, Yan X, Liao H (2009) 3-D reconstruction and dynamic modeling of root architecture in situ and its application to crop phosphorus research. The Plant J 60:1096–1108

Feeney DS, Crawford JW, Daniell T, Hallett PD, Nunan N, Ritz K, Rivers M, Young IM (2006) Three-dimensional micro-organization of the soil-root-microbe system. Microbial Ecol 52:151–158

Ferriera SJ, Senning M, Sonnewald S, Keßling PM, Goldstein R, Sonnewald U (2010) Comparative transcriptome analysis coupled to X-ray CT reveals sucrose supply and growth velocity as major determinants of potato tuber starch biosynthesis. BMC Journal Genomics 11:93–110

Fitter AH, Stickland TS (1992) Architectural analysis of plant root systems. 3. Studies on plants under field conditions. New Phyt 121:243–248

French A, Ubeda-Tomas M, Holman TJ, Bennett MJ, Pridmore T (2009) High-throughput quantification of root growth using novel image analysis tool. Plant Phys 150:1784–1795

Gregory PJ (2006a) Roots, rhizosphere and soil: the route to a better understanding of soil science? Euro J Soil Sci 57:2–12

Gregory PJ (2006b) Plant Roots: Growth, activity and interaction with soils. Blackwell Publishing, Oxford

Gregory PJ, Hinsinger P (1999) New approaches to studying chemical and physical changes in the rhizosphere: an overview. Plant Soil 211:1–9

Gregory PJ, Hutchinson DJ, Read DB, Jenneson PM, Gilboy WB, Morton EJ (2003) Non-invasive imaging of roots with high resolution X-Ray micro-tomography. Plant Soil 255:351–359

Grose MJ, Gilligan CA, Spencer D, Goddard BVD (1996) Spatial heterogeneity of soil water around single roots: use of CT-scanning to predict fungal growth in the rhizosphere. New Phytol 133:261–272

Hainsworth JM, Aylmore LAG (1983) The use of computer-assisted tomography to determine spatial-distribution of soil-water content. Aust J Soil Res 21:435–443

Han L, Dutilleul P, Prasher SO, Beaulieu C, Smith DL (2008) Assessment of common scab-induced pathogen effects on potato underground organs via computed tomography scanning. Phytopathology 98:1118–1125

Hamza MA, Anderson SH, Aylmore LAG (2007) Computed tomographic evaluation of osmotic on shrinkage and recovery of lupin (*Lupinus angustifolius* L.) and radish (*Raphanus sativus* L.) roots. Env and Exp Bot 59:334–339

Hargreaves CE, Gregory PJ, Bengough AG (2009) Measuring root traits in barley (hordeum vulgare ssp. Vulgare and ssp. Spontaneum) seedlings using gel chambers, soil sacs and X-ray microtomography. Plant Soil 316:285–297

Hartmann A, Rothballer M, Schmid M (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant Soil 312:7–14

Heeraman DA, Hopkins JW, Clausnitzer V (1997) Three dimensional imaging of plant roots in situ with X-Ray computed tomography. Plant Soil 189:167–179

Heijs AWJ, de Lange J, Schoute JF, Bouma J (1995) Computed tomography as a tool for non-destructive analysis of flow patterns in macroporous clay soils. Geoderma 64:183–196

Hemminga MA, Buurman P (1997) NMR in soil science. Geoder 80:221–224
Hinsinger P, Plassard C, Jaillard B (2006) Rhizosphere: A new frontier for soil biochemistry. J Geochem Explor 88:210–213
Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152
Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil 321:153–187
Hopmans JW, Vogel T, Koblik PD (1992) X-ray tomography of soil water distribution in one-step outflow experiments. Soil Sci Soc Am J 56:355–362
Hounsfield GN (1973) Computed transverse axial scanning (tomography). Part I.Description of system. Brit J Radiol 46:1016–1022
Hund A, Trachsel S, Stamp P (2009) Growth of axile and lateral roots of maize: I development of a phenotying platform. Plant Soil 325:335–349
International Atomic Energy Agency (2006) Mutational Analysis of Root Characters in Food Plants. International Atomic Energy Agency, Vienna
Isard M, Blake A (1998) CONDENSATION—conditional density propagation for visual tracking. Int J Comp Vis 29:5–28
Iyer-Pascuzzi AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, Weitz JS, Benfey PN (2010) Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. Plant Phys 152:1148–1157
Jahnke S, Menzel MI, Dusschoten D, Roeb GW, Buhler J, Minwuyelet S, Blumer P, Temperton VM, Hombach T, Streun M, Beer S, Khodaverdi M, Ziemons K, Coenen HH, Schurr U (2009) Combined MRI-PET dissects dynamic changes in plant structures and functions. Plant J 59:634–644
Jassogne L (2009) Characterisation of porosity and root growth in a sodic texture- contrast soil. PhD Thesis. The University of Western Australia
Jenneson PM, Gilboy WB, Morton EJ, Luggar RD, Gregory PJ and Hutchinson D (1999) Optimisation of X-ray micro-tomography for the in situ study of the development of plant roots. 1999 Ieee Nuclear Science Symposium—Conference Record, Vols 1–3, 429–432
Jenneson PM, Gilboy WB, Morton EJ, Gregory PJ (2003) An X-ray micro-tomography system optimised for the low dose study of living organisms. App Rad Isotopes 58:177–181
Jennette MW, Rufty JR. TW and MacFall JS (2001) Visualization of soybean root morphology using magnetic resonance imaging. In: Zhou, X and Luo X (2009). Advances in Non-destructive measurement and 3D visualization methods for plant root based on machine vision. Key Laboratory of Key Technology on Agricultural Machine and Equipment, Ministry of Education, South China Agricultural University
Johnson EL (1936) Susceptability of seventy species of flowering plants to X irradiation. Plant Physiol 11:319–342
Johnson SN, Read DB, Gregory PJ (2004) Tracking larval insect movement within soil using high resolution X-ray microtomography. Ecol Entom 29:117–122
Kaestner A, Schneebeli M, Graf F (2006) Visualising three-dimensional root networks using computed tomography. Geoderma 136:459–469
Kaneyasu T and Uesaka M (2005) Dual-Energy X-ray CT bu Compton Scattering. Hard X-ray Source. *Particle Accelerator Conference*, pp 1291–1293. Knoxville, Tennessee
Kalman RE (1960) A new approach to linear filtering and prediction problems. Transactions of the ASME. Journal of Basic Engineering 82:35–35
Ketcham RA, Carlson WD (2001) Acquisition, optimization and interpretation of X-ray computed tomographic imagery: applications to the geosciences. Comp & Geosci 27:381–400
Kinney JH, Nichols MC (1992) X-ray tomographic microscopy (xtm) using synchrotron radiation. Ann Rev Mat Sci 22:121–152
Lontoc-Roy M, Dutilleul P, Prasher SO, Han L, Brouillet T, Smith DL (2006) Advances in the acquisition and analysis of CT scan data to isolate a crop root system from the soil medium and quantify root system complexity in 3-D space. Geoderma 137:231–241
Lucas M, Swarup R, Paponov IA, Swarup K, Casimiro I, Lake P, Peret B, Zappala S, Mairhofer S, Whitworth M, Wang J, Ljung K, Marchant A, Sandberg G, Holdsworth M, Palme K, Pridmore T, Mooney S, Bennett MJ (2010) SHORT-ROOT regulates primary, lateral and adventitious root development in *Arabidopsis*. Plant Phys 155:384–398
Mairhofer S, Zappala S, Tracy SR, Sturrock C, Bennett MJ, Mooney SJ and Pridmore TP (2011) Automated recovery of 3D plant root architecture in soil from X-ray Micro Computed Tomography using object tracking. Plant Physiology (accepted)
Mahesh M, Hevezi JM (2009) Slice wars versus dose wars in multiple row detector CT. J Am Coll Rad 6:201–203
McNeill A and Kolesik P (2004) X-ray CT investigations of intact soil cores with and without living crop roots. Proceedings of the 3rd Australian New Zealand Soils Conference, University of Sydney, 5–9 December 2004
Menon M, Robinson B, Oswald SE, Kaestner K, Abbaspour KC, Lehmann E (2007) Visualisation of root growth in heterogeneously contaminated soil using neutron radiography. Eur J Soil Sci 58:802–810
Moran CJ, Pierret A, Stevenson AW (2000) X-ray absorption and phase contrast imaging to study the interplay between plant roots and soil structure. Plant Soil 223:99–115
Mooney SJ, Morris C, Craigon J, Berry PM (2007) Quantification of soil structural changes induced by cereal anchorage failure: Image analysis of thin sections. J Plant Nut & Soil Sci 170:1–10
Mooney SJ (2002) Three-dimensional visualization and quantification of soil macroporosity and water flow patterns using computed tomography. Soil Use & Manage 18:142–151
Mooney SJ, Morris C, Berry PM (2006) Visualization and quantification of the effects of cereal root lodging on three-dimensional soil macrostructure using X-ray Computed Tomography. Soil Sci 171:706–718
Moradi AB, Carminati A, Vetterlein D, Vontobel P, Lehmann E, Weller U, Hopmans JW, Vogel HJ, Oswald SE (2011) Three-dimensional visualisation and quanitification of water content in the rhizosphere. New Phytol. doi:10.1111/j.1469-8137.2011.03826.x

Neumann G, George TS, Plassard C (2009) Strategies and methods for studying the rhizosphere—the plant science toolbox. Plant Soil 321:431–456

Oliviera MDORG, van Noorwijk M, Gaze SR, Brouwer G, Bona S, Mosca G, Hairiah K (2000) Auger sampling, ingrowth cores and pinboard methods. In: Smit AL, Sobotik M (eds) Root methods. Springer, Berlin Heidelberg, pp 175–210

Osher S, Sethian JA (1988) Fronts propagating with curvature-dependent speed: Algorithms based on hamilton-jacobi formulations. J Comp Phys 79:12–49

Paul EA, Clark FE (1996) Soil Microbiology and Biochemistry, 2nd edn. Academic, San Diego

Pal NR, Pal SK (1993) A review on image segmentation techniques. Pattern Recognition 26:1277–1294

Pallant E, Holmgren RA, Schuler GE, McCracken KL, Drbal B (1993) Using a fine root extraction device to quantify small diameter corn roots (≥0.025 mm) in field soils. Plant Soil 153:273–279

Pálsdóttir AM, Alsanius BM, Johannesson V, Ask A (2008) Prospect of Non-Destructive Analysis of Root Growth and Geometry Using Computerised Tomography (CT X-Ray). Proc. IS on Growing Media. Editor Michel, J.C. Acta Horticulture 779:155–159

Perret JS, Prasher SO, Kantzas A, Langford C (1997) 3-D visualization of soil macroporosity using x-ray CAT scanning. Can Agric Eng 39:249–261

Perret JS, Prasher SO, Kantzas A, Langford C (1999) Three-dimensional quantification of macropore networks in undisturbed soil cores. Soil Sci Soc Am J 63:1530–1543

Perret JS, Prasher SO, Kantzas A, Hamilton K, Langford C (2000) Preferential solute flow in intact soil columns measured by SPECT scanning. Soil Sci Soc Am J 64:469–477

Perret JS, Al-Belushi ME, Deadman N (2007) Non-Destructive visualization and quantification of roots using computed tomography. Soil Biol Biochem 39:391–399

Pierret A, Capowiez Y, Moran C, Kretzschmar A (1999) X-Ray computed tomography to quantify tree rooting spatial distributions. Geoderma 160:307–326

Pierret A, Kirby M, Moran M (2003a) Simultaneous X-ray imaging of plant root growth and water uptake in thin-slab systems. Plant Soil 255:361–373

Pierret A, Doussan C, Garrigues E, McKirby J (2003b) Observing plant roots in their environment: current imaging options and specific contribution of two-dimensional approaches. Agronomie 23:471–479

Pierret A, Moran C, Doussan C (2005) Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytol 166:967–980

Pires LF, Borges JAR, Bacchi OOS, Reichardt K (2010) Twenty five years of computed tomography in soil physics: A literature review of the Brazilian contribution. Soil & Till Res 110:197–210

Pohlmeier A, Oros-Peusquens A, Javaux M, Menzel MI, Vanderborght J, Kaffanke J, Romanzetti S, Lindenmair J, Vereecken H, Shah NJ (2008) Changes in soil water content resulting from Ricinus root uptake monitored by magnetic resonance Imaging. Vad Zone J 7:1010–1017

Primak AN, McCollough CH, Bruesewitz MR, Zhang JZ, Flecther JG (2006) Relationship between Noise, Dose, and Pitch in Cardiac Multi–Detector Row CT. RadioGraphics 26:1785–1794

Rogasik H, Crawford JW, Wendroth O, Young IM, Joschko M, Ritz K (1999) Discrimination of soil phases by dual energy X-ray tomography. Soil Sci Soc Am J 63:741–751

Rogers HH, Bottomley PA (1987) In situ nuclear magnetic resonance imaging of roots: influence of soil type, ferromagnetic particle content and soil water. Agron J 79:957–965

Rosin PL (2001) Unimodal thresholding. Patt Recog 34:2083–2096

Seignez N, Gauthier A, Mess F, Brunel C, Dubios M, Potdevin JL (2010) Development of plant root networks in polluted soils: an X-ray Computed Microtomography investigation. Water, Air & Soil Poll 209:199–207

Shi J and Malik J (2000) Normalized Cuts and Image Segmentation IEEE Trans. on Pattern Matching and Machine Intelligence, 22: 888–905

Sleutel S, Cnudde V, Masschaele B, Vlassenbroes J, Dierick M, Van Hoorebeke L, Jacobs P, De Neve S (2008) Comparison of different nono- and micro-focus X-ray computed tomography set-ups for the visualization of the soil microstructure and soil organic matter. Comp & Geosci 34:931–938

Smit AL, George E and Groenwold J (2000) Root observations and measurements at (transparent) interfaces with soil. *In* Root growth and function: a handbook of methods. *Edited by* AL Smit, C Engels, G Bengough, M van Noordwijk, S Pellerin and S van de Geijn. Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 235–272

Stock SR (2008) Recent advances in X-ray microtomography applied to materials. Int Mat Rev 53:129–180

Taina IA, Heck RJ, Elliot TR (2008) Application of X-ray Computed Tomography to soil science: A literature review. Can J Soil Sci 88:1–19

Taylor HM, Upchurch DR, Brown JM and Rogers HH (1991) Some methods of root investigations. In: McMichael BL and Persson H (eds), Plants Roots and Their Environment. Elsevier Science Publisher B.V., 1991; 553–564

Tollner EW (1991) X-ray computed tomography applications in soil ecology studies. Agric Ecosys & Env 34:251–260

Tollner EW, Rasmseur EL and Murphy C (1994) Techniques and approaches for documenting plant root development with X-ray computed tomography. *In* Tomography of soil-water-root processes. *Edited by* S.H. Anderson and J.W. Hopmans. SSSA Special Publication No. 36, Madison, Wis. pp. 115–133

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. Plant Soil 341:75–87

Tracy SR, Roberts J, Black CR, McNeill A, Davidson R, Mooney SJ (2010) The X-factor: visualizing undisturbed root architecture in soils using X-ray computed tomography. J Exp Bot 61:311–313

Tracy SR, Black CR, Roberts JR, McNeill A, Davidson R, Tester M, Samec M, Korošak D, Sturrock C and Mooney SJ (2011) Quantifying the effect of soil compaction on three varieties of wheat (*Triticum aestivum* L.) with differing root architecture using X-ray Micro Computed Tomography (CT) Plant and Soil

Tumlinson LG, Liu HY, Silk WK, Hopmans JW (2008) Thermal neutron computed tomography of soil water and plant roots. Soil Sci Soc Am J 72:1234–1242

van Noordwijk M, Floris J (1979) Loss of dry weight during washing and storage of root samples. Plant Soil 53:239–243

Vincent L, Soille P (1991) Watersheds in digital spaces: an efficient algorithm based on immersion simulations. IEEE Trans on Pattern Matching and Machine Intelligence 13:583–598

Wantanabe K, Mandang T, Tojo S, Ai F and Huang BK (1992) Non-destructive root-zone analysis with X-ray CT scanner. Paper 923018. American Society of Agricultural Engineers. St Joseph, MI, USA

Wojciechowski T, Gooding MJ, Ramsay L, Gregory PJ (2009) The effect of dwarfing genes on seedling root growth of wheat. J Exp Bot 60:2565–2573

Whalley WR, Lipiec J, Stepniewski W, Tardieu F (2000) Control and measurement of the physical environment in root growth experiments. In: Smit AL, Bengough AG, Engels C, Noordwijk M, Pellerin S, van de Geihn S (eds) Root methods: A handbook. Springer, Berlin, pp 75–112

Wildenschild D, Hopmans JW, Vaz CMP, Rivers ML, Rikard D, Christensen BSB (2002) Using X-ray computed tomography in hydrology: systems, resolutions and limitations. J Hydrol 267:285–297

Wirjadi O (2007) Survey of 3-D image segmentation methods. Technical Report 123, Fraunhofer ITWM, Kaiserslautern

Yazdanbakhsh N, Fisahn J (2009) High throughput phenotyping of root growth dynamics, lateral root formation, root architecture and root hair development enabled by PlaRoM. Funct Plant Biol 36:938–946

Yan GR, Tian J, Zhu SP, Dai YK, Qin CH (2008) Fast cone-beam CT image reconstruction using GPU hardware. J X-Ray Sci Tech 16:225–234

Yilmaz A, Javed O and Shah M (2006) Object tracking: a survey ACM Computing Surveys 38: 4–12

Young IM, Crawford JW, Rappoldt C (2001) New methods and models for characterising structural heterogeneity of soil. Soil & Till Res 61:33–45

Zhou X and Luo X (2009) Advances in Non-destructive measurement and 3D visualization methods for plant root based on machine vision. Key Laboratory of Key Technology on Agricultural Machine and Equipment, Ministry of Education, South China Agricultural University