X-ray CT investigations of intact soil cores with and without living crop roots

Ann McNeill[1] and Peter Kolesik[2]

[1] Soil and Land Systems University of Adelaide Roseworthy Campus SA5371, Australia. Email:ann.mcneill@adelaide.edu.au
[2]Soil and Land Systems University of Adelaide Waite Campus SA5064, Australia. Email: peter.kolesik@adelaide.edu.au

Abstract

X-ray computed tomography (CT) was used to non-destructively visualise intact cores of soil (15 cm diameter and 50 cm depth) collected from a farming region in South Australia. The texture contrast soil comprised 20-30 cm of nutrient poor sand over dense sodic clay. Cores were taken from a non-ripped paddock and from an adjacent paddock that had been ‘deep ripped’ to 50 cm. Image-reconstruction software was used to visualise in three dimensions (3D) macro-morphological features of the soil cores. Canola was grown in some of the cores and several times during plant development the cores were scanned. Roots visualised were of a diameter equal or larger than 1 mm and the volume, surface area, length and position of these ‘exploratory’ roots was quantified. At the end of the experiment destructive root sampling and two-dimensional (2D) scanning were used to measure the total length and volume of all roots. The work revealed the architecture and morphology of root systems *in situ* and tracked response to soil macro-structures such as layers of organic matter in the sand, the clay domes at the interface with the sand, old root channels, calcium deposits, stones, and areas of soil that were naturally more loose. Root penetration was much slower in cores from the non-ripped paddock than in cores from the ripped paddock, indicating a restriction to growth even in the sand. Superimposition of root and non-ripped soil images showed that the roots grew preferentially in looser soil matrix, organic deposits and old root channels. However, roots did not necessarily seek sand-filled cracks between the clay domes as was expected from anecdotal evidence. Root growth in the ripped soil was not influenced by the redistributed macro-morphological features with roots growing in a straight fashion through both sand and clay clumps spread throughout the soil profile by the ripping procedure.

Key Words

X-ray imaging, 3D computed tomography, soil-root systems, deep ripping

Introduction

Methods to investigate root systems range from non-destructive techniques such as rhizotrons to highly destructive approaches involving excavation in the field or washing roots from soil samples (Smit *et al.* 2000). Research into the architecture and function of root systems *in situ* has been severely hampered by the fact that they grow in an opaque, heterogeneous, porous, semi-compressible medium containing solid, liquid and gaseous phases, collectively known as soil (Robinson 1991). Stimulated by pioneering work in the 1980s (Hainsworth and Aylmore 1983, Hainsworth 1986; Hainsworth and Aylmore 1989) the use of non-invasive imaging, together with computed tomography (CT) has been used to provide three-dimensional information on root and soil water distribution for intact root-soil systems (Heeraman *et al.* 1997; Pierret *et al.* 1999; Asseng *et al.* 2000; Hamza *et al.* 2001; Gregory *et al.* 2003) as well as for spatial characterisation of soil macropores (Pierret *et al.* 2002). However, most reported 3D root or soil studies generally restrict field of view in order to maximise resolution, and thus often do not report on larger more mature root systems. One study, focussing on tree rooting spatial distributions, utilised a larger field of view but employed resin impregnation and sectioning techniques, thus effectively restricting measurements to a single point in time (Pierret *et al.* 1999). The ability to visualise, non-invasively, relatively large intact soil cores at successive points in time offers the potential to provide hitherto unseen insights into the interaction of plant root systems with the surrounding soil, although the resolution may limit the proportion of the root system investigated. This paper reports on a study using medical X-ray-imaging with computed tomography to monitor the development of canola root systems to maturity in a texture contrast duplex soil.

Materials and Methods

Intact cores of soil (15 cm diameter and 50 cm depth) were collected from a farming region in South Australia. The texture contrast soil comprised 20-30 cm of nutrient poor sand over a dense sodic clay. In 2003 cores were taken from a non-ripped paddock and in 2004 from an adjacent paddock that had been ‘deep ripped’ to 50 cm. Sample cores underwent medical X-ray scanning and 3D images were rendered using computer tomography. Individual canola (*Brassica napus*) plants were grown in each core under glasshouse conditions and all cores were supplied with adequate water and nutrients (N, P, S, Cu and Zn). At intervals during plant development cores were scanned in a medical X-ray CT scanner (Aquilion, Toshiba, Japan). 3D images were rendered with image analysis software (Amira, Template Graphics Software, USA) and analysed for root length, surface area and volume using an integrated purpose-written skeletonization algorithm (Kolesik *et al.* 2004). Destructive sampling was also carried out on some of the cores used for 3D analysis and involved root washing and 2D scanning to measure the total length and volume of all roots.

Results

Macro-morphology of a texture-contrast soil with or without deep ripping
Observation of the 3D images enabled several distinct features to be identified in the relatively undisturbed soil core (Fig. 1A), including 'domes' characteristic of massive sodic clay surfaces, layers of organic material in the sand plus cracks and old root channels in the clay, as well as some organic matter at depth. A drastic impact of ripping on the soil macro-morphology was also clearly evident with a complete disruption of the sand-clay interface, 'clods' of clay present throughout the 50 cm profile (Fig. 1B) and large areas of loose soil and air-filled spaces.

Architecture of exploratory canola root penetration in a texture contrast soil with or without deep ripping
Considering the extent of the visualised main taproot (Fig. 2A) as an indicator of root system penetration depth it was evident that root growth rate was relatively slow and tortuous in a non-ripped situation, even in the coarse sand. Thus, 16 weeks after sowing the root system was not visible at the base of the core. This contrasted to the ripped situation where visible canola roots had penetrated to 50 cm within 12 weeks after sowing (Fig. 3), although this is only shown as an X-ray image and not a 3D reconstruction due to difficulties in rendering root images in the extremely heterogenous ripped soil. The architectural behaviour of roots in response to soil macro-morphological features in the undisturbed soil was visualised (Fig. 2B-E). Roots in the non-ripped core followed layers of organic matter in the sand and appeared to directly penetrate into clay layers. In contrast, the path of the canola taproot in the ripped soil was less tortuous and did not appear to deviate in response to the clay 'clods' present throughout the profile after ripping. Roots visualised were of a diameter equal or larger than 1 mm and the volume, surface area, length and position of these 'exploratory' roots were quantified (Fig. 4). The rate of increase in depth of visible taproot was faster during early growth, between 5 and 7 weeks, than later in the cycle between 9 and 12 weeks where lateral root extension appeared more dominant (Fig. 4). Some of the lateral exploratory roots were visualised taking the path of least resistance and growing rapidly down the side of the PVC container; examples of this can be seen in the time-course 3D reconstructions (Fig. 4d) where there is an observed sudden change in direction of growth for two of the widest-extending lateral roots.

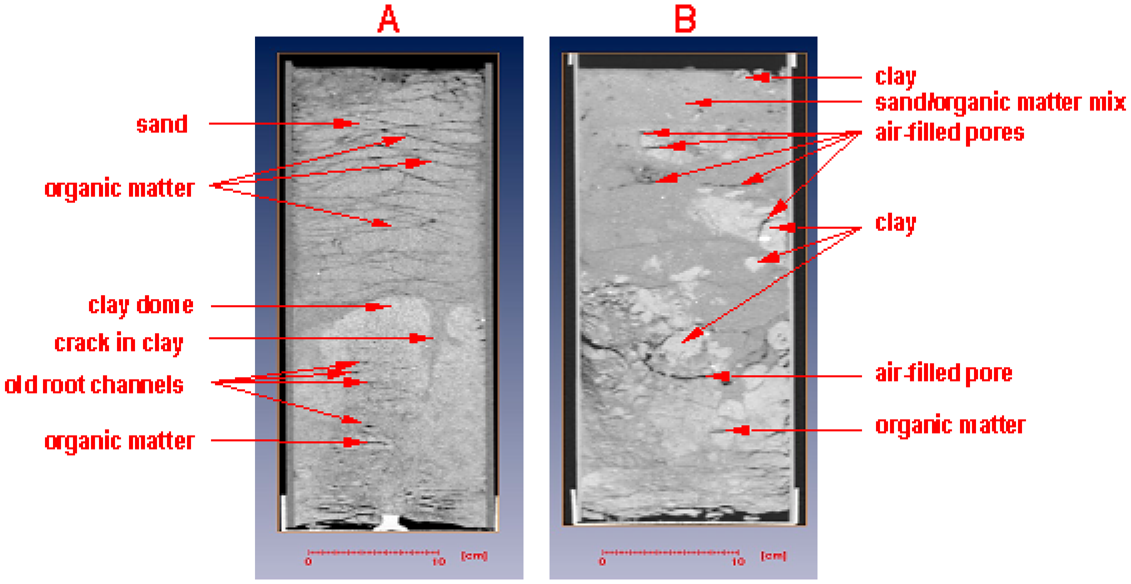

Figure 1. Annotated vertical section through an X-ray CT scan image of a soil core sampled from (A) a non-ripped paddock and (B) a deep ripped paddock.

Published on CDROM. Website www.regional.org.au/au/asssi/

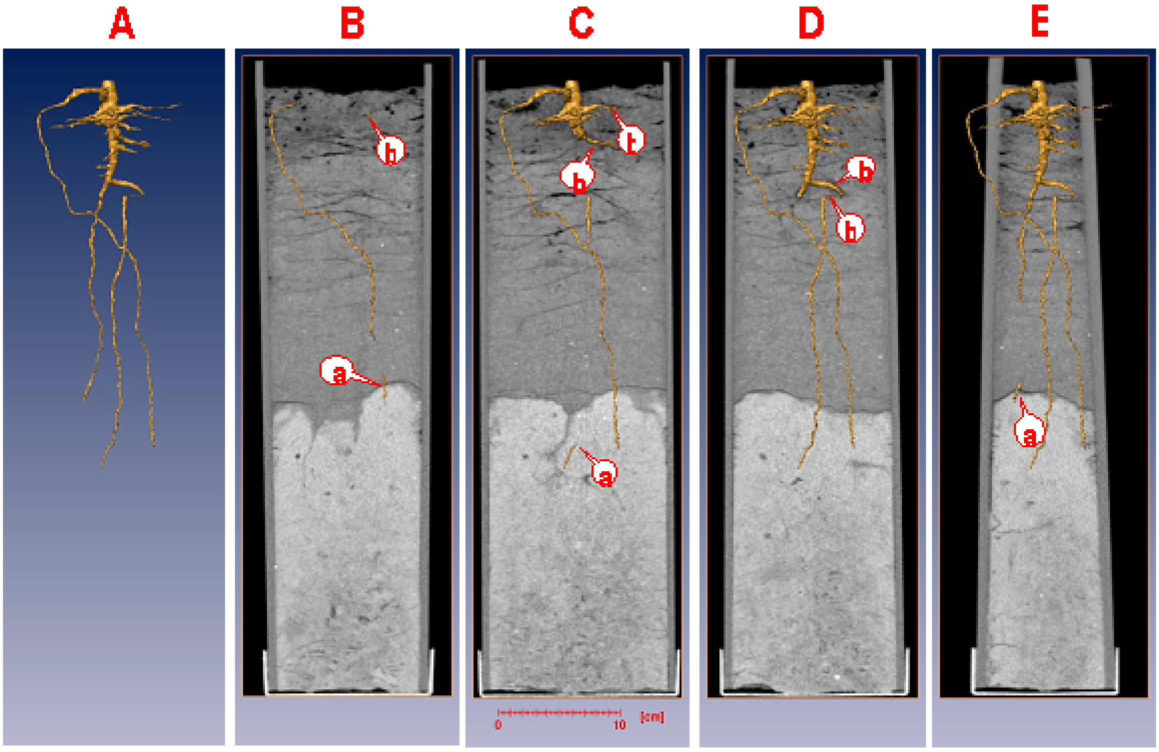

Figure 2. Canola root system behaviour in non-ripped soil visualised with CT 16 weeks after sowing. 3D image of root (A) superimposed on longitudinal slices through the soil (B – E). Root penetration into clay horizon denoted with an (a) and roots following layer of organic matter denoted by (b).

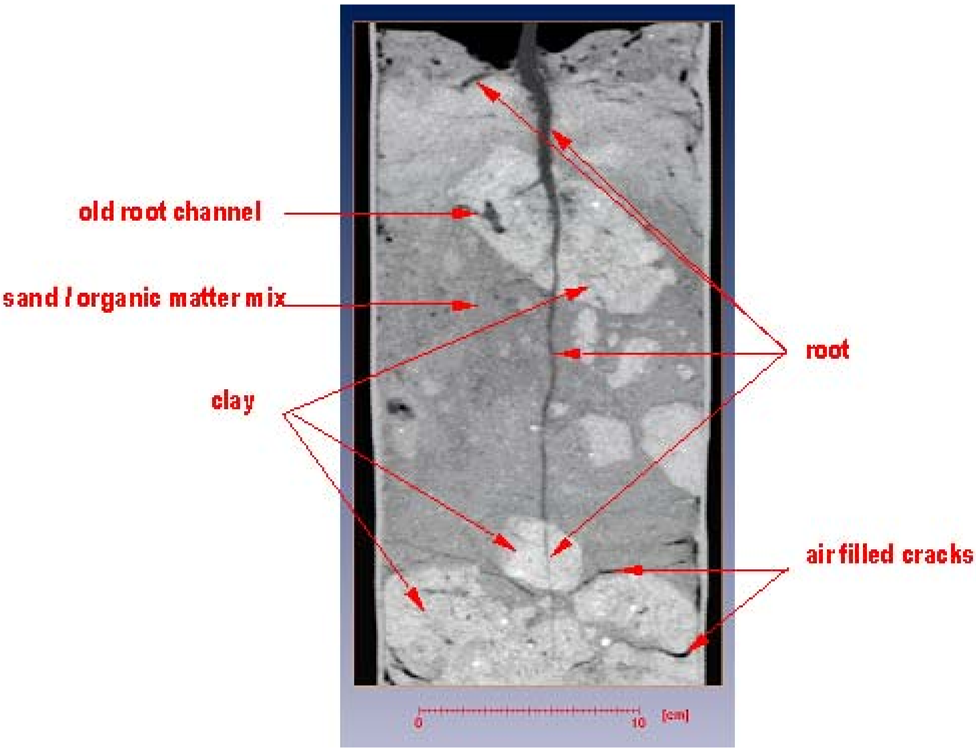

Figure 3. Canola taproot penetration in ripped soil visualised with CT (curved plane projection) 12 weeks after sowing.

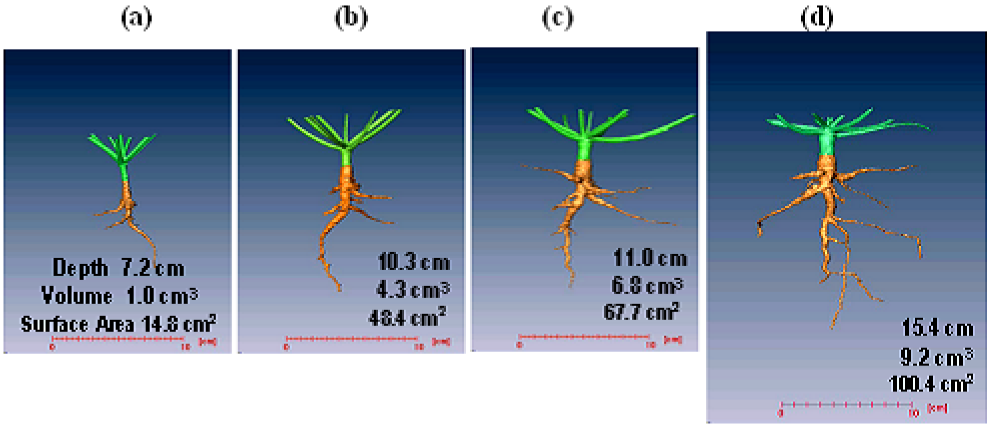

Figure 4. CT visible canola root volume (cm^3), surface area (cm^2) and visible taproot depth (cm) 'in situ' in an undisturbed soil core for a time sequence (a) 5, (b) 7, (c) 9 and (d) 12 weeks after sowing.

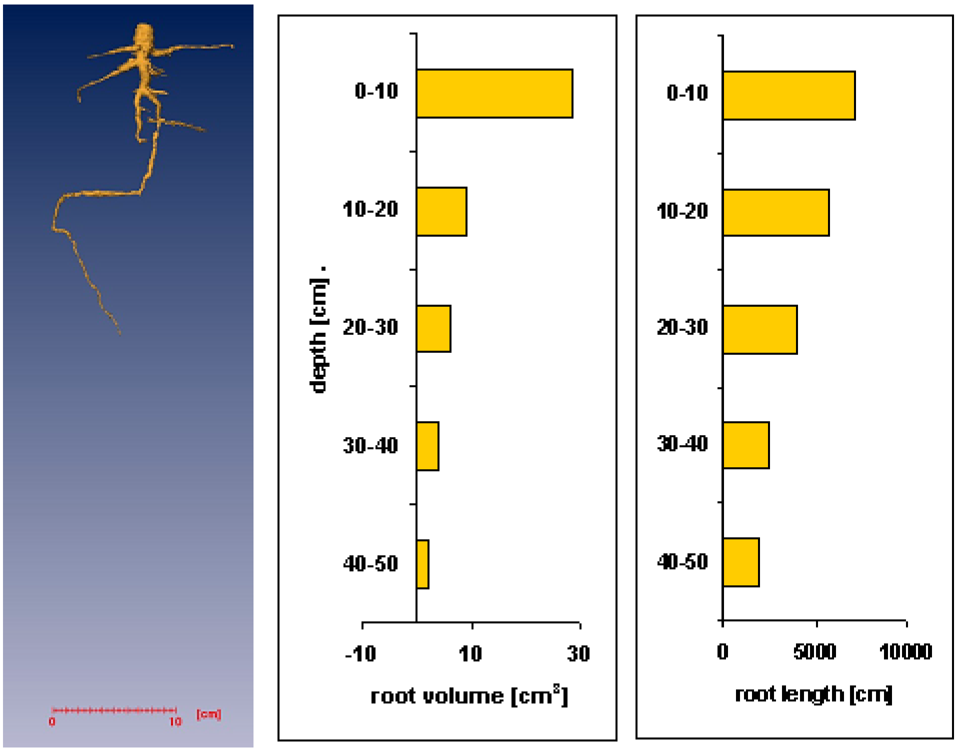

Figure 5. Canola root (week 16 after sowing) in non-ripped soil (N fertiliser injected at 20 cm depth) visualised with CT (left) and vertical total volume and length distribution measured with destructive 2D sampling.

Vertical root distribution (total root length and volume)

The total length and volume of the root system was evaluated with the non-destructive 3D method and the destructive 2D scanning method (Fig. 5). In this particular core the non-destructive 3D method visualised 13.1 cm^3 of root volume representing 26% of the total volume (49.9 cm^3). Less than 1 % of the total length of the canola root system was visualised penetrating to about 30 cm depth with only fine roots less than 1 mm diameter detected from 30-50 cm (Fig. 5).

Discussion

Since the surrounding soil structure clearly influences root growth (Atkinson and Mackie-Dawson 1991; Passioura 1991; Tardieu 1994) the ability to study roots *in situ* is imperative if our understanding of the inter-play between plant roots and soil structure is to be enhanced. The X-ray CT technique clearly provides geometrical and structural information on undisturbed root systems and responses to the soil in which they are growing, that cannot be obtained by any of the more traditional or widely used methodologies for investigating roots. Thus, in the study reported by this paper it was possible to 'see' exploratory roots of a canola plant over-ride the natural driver of geotropism and grow laterally for some distance in response to layers of organic matter in the soil. Such 3D images of the relatively mature root system of a dicotyledenous crop plant growing undisturbed in soil have, as far as the authors are aware, not been reported before. It was also possible to 'see' these exploratory roots penetrating directly into the domes of a dense sodic clay layer with a measured penetration resistance of 2 MPa, although it should be noted that the clay was quite wet in these cores. The roots also penetrated macro-aggregates of the sodic clay that were mixed into the coarse sand layer after deep ripping, and did not avoid them by following coarser channels in the sand as was anticipated. By scanning plants grown in cores it was possible to clearly see the vexatious problem of the 'edge' effect where, once lateral roots encountered the path of least resistance offered by the smooth side of the PVC core, the downward growth rate increased markedly. The impact of rapid growth of these roots at the edge on total resource capture by the plant is unclear, but the imaging serves to highlight a major problem inherent in all container-based studies of plant growth.

X-ray CT has the advantage that sequential imaging through time of the same root system can be carried out to obtain 'in situ' growth rates as well as other parameters such as diameter, volume, length and angle of direction of root growth. Due to the extreme soil heterogeneity induced by ripping it was extremely difficult to render 3D images of roots for this treatment, although the rapid root growth engendered by deep ripping in this study could still be analysed in terms of downward growth rate as well as numbers of laterals per unit length. The morphometric measurements of roots in the undisturbed situation can be used as input data for root architecture mapping software programs such as AMAPMod (Danjon *et al.* 1999) and can be used to test output from 3D root architectural models such as Rootmap (Diggle 1988) or SARAH (Pages *et al.* 1989). More generally the information could be widely applicable for testing root depth and root length density models (listed by (Pages *et al.* 2000) that underpin most dynamic plant/crop or ecosystem models. The information could possibly also be used to assess what the most appropriate strategy might be for root sampling at a field scale (Pages and Pellerin 1996).

A major drawback of this medical CT imaging technique is that fine roots cannot currently be visualised, and thus only a very small proportion of total root length was detected in 3D, although due to the substantial taproot and primary laterals in canola a larger proportion of the root volume was visualised. The apparent 'restriction' to root proliferation at the sand-clay interface observed from the destructive sampling data, and consistent with reports from imaging of the A-B interface by other workers (Moran *et al.* 2000), was not detected in 3D. The construction of a dedicated scanner for research purposes in the UK has improved resolution to 0.1 mm but in doing so has reduced the sample size to 25 mm diameter and 25 mm height (Jenneson *et al*. 2003). Although the fine roots are the primary water and nutrient uptake organs, the 3D spatial configuration of the coarser 'exploratory' or structural roots may give information on fine root distribution if relationships between these can be established.

Although the technology for producing 3D CT scans at a spatial resolution sufficient to image fine roots is currently complex, expensive and relatively difficult to access (Pierret *et al.* 2003), the situation is rapidly changing (Jenneson *et al.* 2003; Hargreaves *et al.* 2004, McNeill *et al.* 2004). The 3D architectural nature of the outputs from scanning also heralds the possibility of studying inter-plant root competition *in situ*. It is our belief that the next few years will see major advances in the understanding of soil-root interactions based on further application of X-ray CT.

Summary

X-ray CT was used to quantitatively describe 3D morphology of the exploratory root system (>1 mm diameter) of a canola plant growing in soil, and to determine the response of that root system to soil macro-structural properties. The power of the technique rests with the ability to be able to image, without disruption, intact soil cores from field situations and thus to combine these soil macro-morphological observations with the response of the root systems in 'real time'. Thus, both qualitative and quantitative information on 'in situ' root growth can be generated and used to increase the understanding of root system form and function in natural and agricultural ecosystems.

Acknowledgements

The authors are grateful to Julie Olsen and Geoff West, Queen Elizabeth Hospital Adelaide, for access to the medical scanner and CT image collecting. The technical assistance of Colin Rivers and Penny Day was invaluable and the enthusiasm of Honours students Kieran Wauchope and Carly Buttrose was appreciated. The work was funded by DEST under the Systemic Infrastructure Initiative for Backing Australia's Ability to undertake innovative research with additional support from Adelaide University.

References

Asseng S, Aylmore LAG, MacFall JS, Hopmans JW, Gregory PJ (2000) Computer-assisted tomography and magnetic resonance imaging. In 'Root Methods A Handbook'. Ed. AL Smit pp. 343-363. Springer-Verlag: Berlin

Atkinson D, Mackie-Dawson LA (1991) Root growth: methods of measurement. In 'Soil analysis:physical methods'. Eds KA Smith and CA Mullins pp. 447-509. Marcel Dekker: New York

Danjon F, Sinoquet H, Godin C, Colin F, Drexhage M (1999) Characterisation of structural tree root architecture using 3D digitising and AMAPmod software. *Plant and Soil* **211**, 241-258.

Diggle AJ (1988) ROOTMAP- a model in three-dimensional coordinates of the growth and structure of fibrous root systems. *Plant and Soil* **105**, 169-178.

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

Hainsworth JM, Aylmore LAG (1983) The use of computer assisted tomography to determine spatial distribution of soil water content. *Australian Journal of Soil Research* **21**, 435-443.

Hainsworth JM, Aylmore LAG (1989) Non-uniform soil water extraction by plant roots. *Plant and Soil* **113**, 121-124.

Hamza MA, Anderson SH, Aylmore LAG (2001) Studies of soil water drawdown by single radish roots at decreasing soil water content using computer-assisted tomography. *Australian Journal of Soil Research* **39**, 1387-1396.

Hargreaves C, Gregory PJ, McNeill AM, Kolesik P, Read D (2004) Non-invasive imaging: Root response to physical and chemical properties of the rhizosphere Paper presented at The International Rhizosphere Congress Munich September 2004

Heeraman DA, Hopmans JW, Clausnitzer V (1997) Three dimensional imaging of plant roots in situ with X-ray Computed Tomography. *Plant and Soil* **189**, 167-179.

Jenneson PM, Gilboy WB, Morton EJ, Gregory PJ (2003) An X-ray micro-tomography system optimised for the low-dose study of living organisms. *Applied Radiation and Isotopes* **58**, 177-181.

Kolesik P, Fouard C, Prohaska S, McNeill A (2004) Automated method for non-destructive 3D visualisation of plant root architecture using X-ray tomography. *4th International Workshop on Functional-Structural Plant models*, CIRAD Montpellier, France, 7-11 June 2004, p. 27.

McNeill AM, Kolesik P, Wauchope K, Coventry, DC (2004) Root response to localised nitrogen supply: destructive sampling and non-destructive 3D imaging. Paper presented at The International Rhizosphere Congress Munich September 2004

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 and Soil* **223**, 99-115.

Pages L, Asseng S, Pellerin S, Diggle A (2000) Modelling root system growth and architecture. In 'Root Methods A Handbook'. Eds AL Smit, AG Bengough, C Engels, M Van Noordwijk, S Pellerin and SC Van De Geijn pp. 112-146. Springer-Verlag: Berlin.

Pages L, Jordan MO, Picard D (1989) A simulation model of the three dimensional architecture of the maize root system. *Plant and Soil* **119**, 147-154.

Pages L, Pellerin S (1996) Study of differences between vertical root maps observed in a maize crop and simulated maps obtained using a model for the three-dimensional architecture of the root system. *Plant and Soil* **182**, 329-337.

Passioura J (1991) Soil structure and plant growth. *Australian Journal of Soil Research* **29**, 717-28.

Pierret A, Capowiez Y, Belzunces L, Moran CJ (2002) 3D reconstruction and quantification of macropores using X-ray computed tomography and image analysis. *Geoderma* **106**, 247-271.

Pierret A, Capowiez Y, Moran C, Kretzschmar A (1999) X-ray computed tomography to quantify tree rooting spatial distributions. *Geoderma* **90**, 307-326.

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

Robinson D (1991) Roots and resource fluxes in plant communities. In 'Plant Root Growth'. Ed. D Atkinson pp. 103-130. Blackwell: Oxford.

Smit AL, Bengough AG, Engles C, Van Nordwijk M, Pellerin S, Van De Geijn SC (2000) 'Root Methods A Handbook.' Springer-Verlag: Germany.

Tardieu F (1994) Growth and functioning of roots and of root systems subjected to soil compaction - towards a system with multiple signaling. *Soil and Tillage Research* **30**, 217-243.