Visualising and quantifying rhizosphere processes: root-soil contact and water uptake

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Abstract
Root-soil contact is vital for water transport via liquid films. X-ray microtomography potentially makes it possible to study the root-soil interface non-invasively. This paper presents first attempts to quantify the contact areas of roots with the three phases of soil systems using X-ray microtomography. Root-particle contact was investigated producing 3D volumetric images of maize in soil and vermiculite wetted to matric potentials of -0.03MPa and -1.6MPa. Root-soil contact was calculated to be greater in soil than in vermiculite. Water volumes in artificial porous media, formed by cellulose acetate beads, were determined by weighing samples before and after wetting and by image analysis of 3D volumetric images. Image analysis underestimated the water volume by about 10%. Contact between an artificial root (porous glass fibre tube) and the solid, liquid and gaseous phases in sand were calculated from 3D volumetric images. The total sum of contact areas was about 8% greater than the estimated surface area of the root. Results of repeated image analysis of root-particle contact revealed that changes in threshold values influenced the final results by up to 12%. Thus, whilst this method has clear potential for investigating the root-soil interface, there remain several limitations that are being addressed.

Key Words
X-ray microtomography, 3-D visualisation, root-soil interface, root-contact surface area

Introduction
Knowledge of root contact with the soil solution is essential for understanding water and nutrient adsorption by plants. The contact is influenced by soil and root properties, such as particle size, soil bulk density, root diameter, soil matric potential and volumetric water content (Tinker 1976; Nye 1994). In water saturated and heavily compacted soils, problems with gas exchange can occur (Veen *et al.,* 1992). Conversely, incomplete root-soil contact due to soil structure or root shrinkage can reduce the uptake of water and nutrients (Veen *et al.,* 1992). A thin section technique was used by Noordwijk *et al.* (1992) to derive the degree of root-soil contact in 2D slices. An increase in root-soil contact with increasing bulk density was investigated by Kooistra *et al.* (1992). A decrease in water and nitrate uptake per unit root length was associated with smaller root-soil contact (Veen *et al.,* 1992). Neutron and X-ray computed tomography have been used to provide a non-invasive methodology to visualise and quantify roots growing in opaque soils (Heeraman *et al.,* 1997; Lontoc-Roy *et al*., 2006; Tumlinson *et al.,* 2007; Oswald *et al.,* 2008; Moradi *et al.,* 2009; Carminati *et al.,* 2009) and establish an approach to examine root-soil contact. Root-soil contact dynamics of lupin plants under drying and wetting cycles were investigated by Carminati *et al.* (2009). Changes of water contents around roots were detected with neutron radiography. A non-uniform water uptake around the root was observed (Oswald *et al.,* 2008). Quantification of the spatial distribution of solid, liquid and gaseous phases using X-ray computed tomography was achieved by a cluster analysed segmentation method (Wildenschild *et al.,* 2002).
In this paper, the use of X-ray microtomography to study the root-soil interface will be discussed and preliminary data on root-soil contact and water distribution will be presented. Processes for quantifying the solid, liquid and gaseous phases around the root surface will be reviewed with both artificial and natural systems.

Methods
X-ray microtomography
3D volumetric images were obtained using a Metris X-Tek HMX CT scanner with a Varian Paxscan 2520V detector with a 225kV X-ray source and a molybdenum target. 3D volumetric images were reconstructed with the Metris software CT-Pro v2.0. VGStudioMAX v2.0 was used to analyse the 3D volumetric images.

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Water visualisation
3D volumetric images of water in packed cellulose acetate beads of 1mm and 3mm diameter were obtained. A 0.75g/100g sodium iodide-spiked solution was used as a wetting fluid to obtain a greater contrast between the water and solid phases. A high absorption was achieved through the sodium iodide rendering the water and solid phase separable. Samples were scanned before and after wetting, using a beam with energy of 65kV and a current of 486µA. 2855 projections were acquired. A resolution of 21µm (isotropic voxel size) was attained. Typically, scans took approximately 150 minutes.

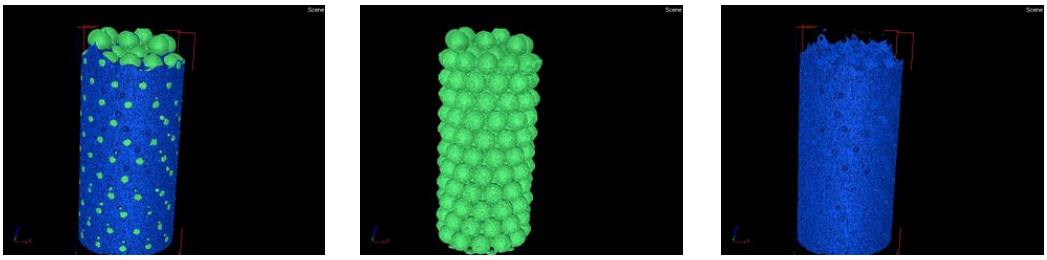

Figure 1. False coloured 3D volumetric images of wetted cellulose acetate beads (3 mm diameter; left), extracted beads (middle) and extracted water (right).

The volume of water was segmented using 3D volume segmentation tools, which identify and extract voxels belonging to calculated ranges of greyscale values (representing water phase in the sample). As a control the sample was weighed before and after wetting.

Contact areas of the root with the water, air and solid phases of the growth medium
Root-particle contact was estimated for 3D volumetric images of maize seeds growing in soil and vermiculite at matric potentials of -0.03MPa and -1.6MPa. The pre-germinated seeds were grown for 2 days at 20ºC in darkness. The samples were scanned using a beam with energy of 145kV and a current of 140µA. 2987 projections were acquired. Typically, scans took 180 minutes. Three replications of each treatment were analysed twice by the same operator. The sample size was 3 cm in diameter and 10 cm high. To differentiate the contact areas of the root with air, water and solid phase, an artificial root (porous glass fibre tube of 1.5 mm in diameter) in sand was scanned. The sand was wetted with 0.75% sodium iodide solution (see above) and drained by applying suction to the artificial root. The sample was scanned with energy of 85kV and a current of 296µA with 2855 projections acquired. The sample was 1.5 cm in diameter and 10 cm high. Resolutions of 35µm for the samples in soil and vermiculite and 18µm for the sample in sand were attained.

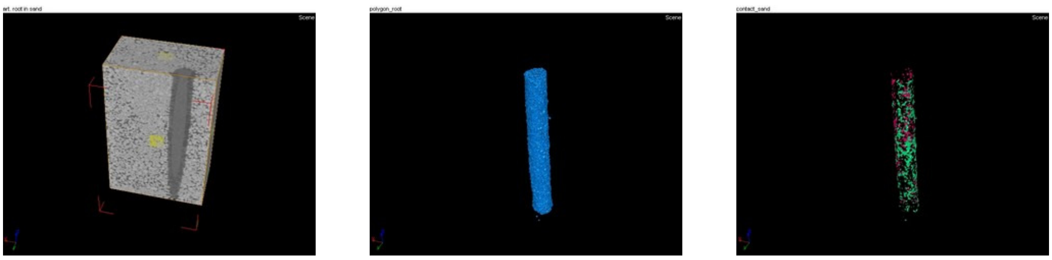

Figure 2. Region of interest with artificial root in sand (partly drained; left), polygonal mesh of root volume for calculating the root surface area (middle) and polygonal mesh of root sand contact surface area (right).

Image analysis was performed to determine the contact areas of liquid, air and solid phases with the root. For calculating the root surface and contact surface area, the root volume was segmented. Advanced calibration and 3D volume segmentation tools, which identify and extract voxels belonging to calculated ranges of greyscale values (representing root, water, air and solid particle) were used to segment the root volume. The surface area of the root was determined for the extracted root volume. The contact surface area of root with the three different phases of the sample were investigated, by subtracting voxels representing each phase which were adjacent to voxels of the root volume from the volumetric region of the root.

Results

Water visualisation
In Figure 3 the volumes of water added to the packed cellulose acetate beads (1 mm and 3 mm diam.) and calculated from 3D images are shown. Image analysis underestimated the volume of water by 11% for 1 mm beads and 8% for 3 mm beads.

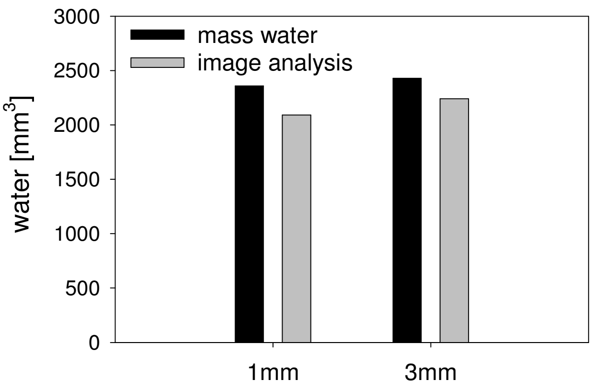

Figure 3. Water volume determined by weighing samples before and after adding water to cellulose acetate beads of 1mm and 3mm size and by image analysis with VGStudioMAX v2.0.

Root-soil interface contact areas

The results for root-particle contact of maize in soil and vermiculite at -0.03MPa and -1.6MPa are shown in Figure 4. The determination of root-particle contact in VGStudioMAX v2.0 was done twice. The second time (VGStudio MAX (2)) the root-soil contact for all samples except maize in vermiculite at -1.6MPa was greater than that determined in the first attempt (VGStudio MAX (1)). The results for both investigations showed greater root-particle contact in soil than in vermiculite.

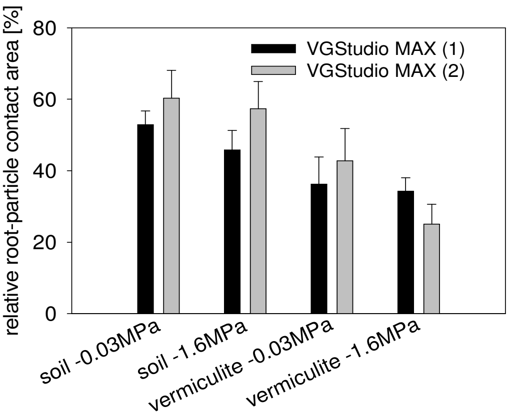

Figure 4. Root-particle contact of maize in soil and vermiculite at matric potentials of -0.03MPa and -1.6MPa analysed in VG Studio MAX v.2.0 at two time points, average differences between the 1st and 2nd attempt are ca. 6-11% (left).

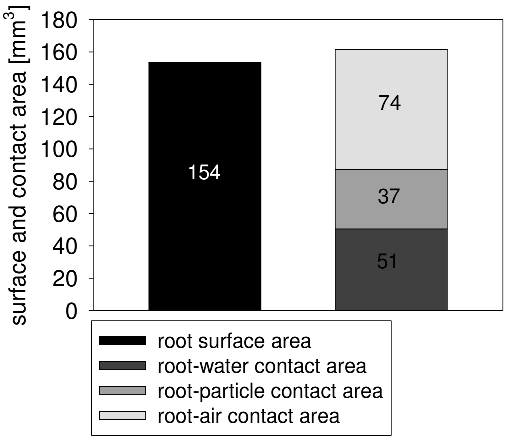

Figure 5. Surface area of artificial root (black) and contact surface areas of artificial root in contact with water, solid and air (grey; right).

The contact surface areas of an artificial root with air, water and sand particle are shown in Figure 5. The total root surface area of the root volume was 154 mm^2. The total contact surface area was 162 mm^2 according to the image analysis in VGStudioMAX, where the water phase contributed the greatest part at 74 mm^2 and the solid phase the smallest part at 37 mm^2.

Discussion and Future Work

These studies investigated the use of X-ray microtomography for quantifying contact areas of roots in a three phase system. The water, air and solid phases could be clearly distinguished. It was possible to segment voxels containing water in a bead system using a contrast enhancer (NaI). The concentration of the contrast enhancer was kept as small as practicable, resulting in relatively small contrast between solid and liquid phase, making discrimination between water and solid voxels difficult. A small proportion of voxels containing water might have been counted as voxels representing beads. However, the water volume was underestimated by 8-11%. To better quantify this source of uncertainty, we intend comparing the volume of the beads in dry samples (two phase system, big contrast) with the volume estimated with water present to help specify the effects of decreasing contrast. A similar approach was used by Culligan *et al.* (2004) where

partially saturated images were registered with the dry image to create tertiary volume in which the solid, liquid, and air phase were quantified.
Image analysis of root-particle contact of maize grown in soil and vermiculite showed a greater root-soil contact than in vermiculite. This was expected because of the greater particle sizes of vermiculite and smaller bulk density (Kooistra *et al.,* 1992). Yet, repeating the image analysis on the same samples showed a variation of 6 to 12% in the relative root-particle contact. This was probably because the contrast between root and solid particles was relatively poor and partial volume effects made it difficult to specify the borders between root and solid phase. In the procedure, several parameters are changed manually to extract the different phases and define the root-particle contact areas. A sensitivity analysis of those parameters on a model system of known dimensions is underway to help improve the methodology. Extracting the three phases and calculating the contact areas with an artificial root in sand was possible but a greater cumulative contact surface area resulted – this is probably because voxels were classified non-uniquely, with certain voxels being double-counted as both root-solid and root-water interfaces. This may also have been associated with partial volume effects making the segmentation of the phases more difficult and adding to this double counting. We are currently modifying the procedure to reduce this source of error.
In conclusion, X-ray microtomography and 3D image analysis algorithms are useful tools to study the root-soil interface and determine contact areas, but require both careful optimisation of image quality and the development of rigorous image analysis protocols that minimise elements of subjectivity.

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