Visualising the effect of compaction on root architecture in soil using X-ray Computed Tomography

Saoirse Tracy, Colin Black, Jeremy Roberts and Sacha Mooney

School of Biosciences, University of Nottingham, Nottingham, NG7 2RD, UK. Email: plxsrt1@nottingham.ac.uk.

Abstract
The effect of soil compaction on the root architecture of the genetic model plant, *Arabidopsis thaliana*, was investigated using novel techniques which allow visualisation and quantification of undisturbed root architecture over time, and elucidation of responses to specific soil-based stimuli. X-ray Computed Tomography (CT) is an exciting tool for the study of root-soil interactions and offers major benefits. These include non-destructive measurements and 3-D visualisation, which are vital to advance our understanding of the dynamic nature of soil-plant interactions. Two soil types, a Newport series light sandy brown soil and a Worcester series heavy clay, were uniformly compacted to provide bulk densities of 1.1, 1.4 and 1.6 g/cm^3. Soil columns were scanned using a Nanotom® X-ray micro CT scanner at a resolution of 17 μm to enable the fine roots of *A. thaliana* to be visualised. The images were analysed and root structures were quantified. Destructive analysis of root architecture was undertaken for comparison with the X-ray CT images. Root architecture differed between treatments, and compaction had a marked effect on total root mass and root architecture. Percentage porosity and average pore size decreased as compaction increased.

Introduction
Soil compaction creates unfavourable conditions for root growth by limiting water, nutrient and oxygen supplies and increasing mechanical impedance (Cook *et al*. 1996). Lipiec *et al*. (1991) showed that roots became increasingly restricted to the upper soil horizons and decreased in length as bulk density increased. Root architecture is an important consideration as it determines the ability of root systems to acquire water and nutrients (Lynch 1995) and so influences overall productivity. As soil is a heterogeneous medium, effects on root architecture may influence the ability of plants to locate and absorb water and nutrients. Effective use of water and nutrients by plants is of vital importance as the availability of these resources may become increasingly scarce in future years.

As described by Whalley *et al.* (2000), there is an urgent need for non-invasive techniques capable of analysing the physical interactions between roots and the surrounding soil. Our understanding of how roots interact with their immediate soil environment largely remains a mystery as the opaque nature of soil has previously precluded *in situ* visualisation of undisturbed roots (Perret *et al*. 2007). New developments in non-invasive techniques such as X-ray CT provide an exciting opportunity to examine detailed root architecture in three dimensions for the first time (Tracy *et al*. 2010).

X-ray CT overcomes some of the limitations associated with previous methodologies for studying roots by providing non-invasive 3-D images (Heeraman *et al*. 1997; Pierret *et al*. 2002; Lontoc-Roy *et al*. 2006). The ability to view intact soil cores in this way enables accurate non-destructive quantification of soil parameters such as pore connectivity and tortuosity (Mooney 2002). Previous commonly employed destructive methods such as root washing cannot provide detailed information on root architecture, including branching characteristics and extension rate, which are inherently linked to conditions within the soil matrix.

Previous studies of roots using X-ray CT have often used substrates containing little organic matter (e.g. homogeneous sand or loamy sand) as growth media because the attenuation coefficient for root material is typically similar to that of other soil organic matter, making it difficult to visualise roots in the images obtained. However, as stressed by Gregory and Hinsinger (1999), our current need is for research involving complex growth media such as soil, as opposed to hydroponics, gels and sand-culture, to represent field conditions more closely. For this reason the experiment described here involved the use of field soils. *A. thaliana* was chosen because it is the genetic model plant, yet few experiments have investigated its root architecture when growing in heterogeneous substrates such as field soils.

Wild type *A. thaliana* plants and mutant lines in which root architecture was altered relative to the wild type were used. The soil was scanned at several growth stages to visualise temporal changes in root architecture and responses to a specific soil-based stimuli.

Methods

Plants were grown in columns of soil obtained from the University of Nottingham experimental farm at Bunny, Nottinghamshire, UK (52.52 ° N, 1.07 ° W). The two soil types used were a Newport series light sand (brown soil) and a Worcester series heavy clay (argillic pelosol). The soils were sieved to <2 mm before being uniformly compacted to provide bulk densities of 1.1, 1.4 and 1.6 g/cm^3. The soil columns were scanned using a Nanotom® X-ray micro computed tomography (CT) scanner at 100 kV, 210 µA to give a resolution of 17 µm. This high resolution permitted fine roots of *A. thaliana* to be identified. The image slices obtained were reconstructed to provide a 3-D visualisation of the soil column. The resultant images were then analysed to quantify soil physical parameters, including porosity, mean pore size and number of pores. Root length, tortuosity of the root path and root angle were also determined. Tortuosity of the root path can only be calculated using 3-D visualisation techniques as traditional destructive methods do not allow such information to be obtained. Manual root extraction methods were undertaken using Avizo® and VG Studio MAX®. The manual extraction methods involved selecting a specific range of grey pixel values believed to represent root material and isolating these from the rest of the sample. Destructive analysis of root architecture was undertaken using WinRHIZO® software for comparison with the X-ray CT images. Morphological characteristics including root length, diameter, area and volume were determined automatically. The results were analysed by two-way general analysis of variance (ANOVA) using Genstat 12.1.

Results

Root architecture differed between treatments and bulk density had major effects on total root mass and the 3-D architecture of the root systems. As the severity of compaction increased, percentage porosity and mean pore size decreased. Root lengths were measured using the polyline tool in VG Studio MAX® software (Figure 1). This tool can trace individual roots by following the shape of the root path, thereby providing accurate measurements of the length of roots as they extend down the soil column and encounter obstacles which may impede growth. Such measurements, made whilst roots are still encased within the soil matrix, are only possible using non-destructive approaches. Soil columns were also scanned before roots were present to visualise the original soil structure (Figure 2) and allow changes resulting from root growth to be identified. This approach enabled specific obstacles to root growth and the manner in which individual roots overcame these to be observed. For example, in Figure 3 an individual A. *thaliana* root can be seen bending round a 3.21 mm diameter mineral grain. This level of detail cannot be achieved using traditional methods for root studies.

Conclusions

In summary, X-ray CT is an exciting tool for the study of root-soil interactions which offers major benefits, and is vital to the advancement of our understanding of the dynamic nature of soil-plant interactions. X-ray CT studies provide further insights into the nature and limitations of root growth in different soil types and under contrasting edaphic conditions. The technology reported here has considerable potential to enhance our understanding of how the immediate soil environment affects root architecture. The present study has shown that compaction has a significant impact on root growth and further experiments will be undertaken to increase our knowledge of how root growth may influence soil structure over time. By exploiting innovative techniques such as X-ray CT, the below-ground impact of genotype on phenotype and, in particular, interactions between roots and their soil environment can be visualised. Such research may pave the way towards identification of novel genes with an important role in optimising the acquisition of water and nutrients to drive future crop breeding initiatives and enhance food security.

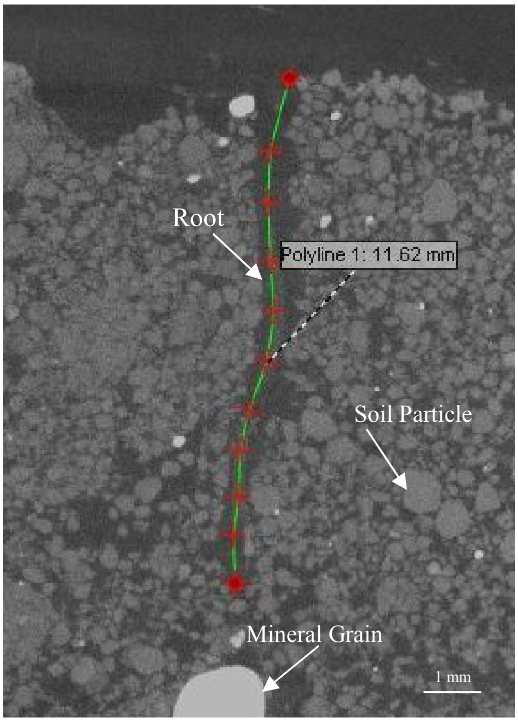

Figure 1. The root path of an individual *A. thaliana* root was traced through a column of Newport series loamy sand using the polyline tool in VG Studio MAX® software. Root length was 11.62 mm. Measuring the straight linear distance (10.08 mm) of the root provided a tortuosity value for the root path of 1.15.

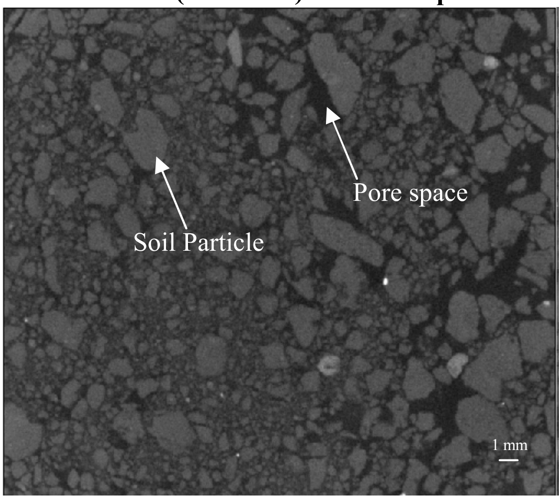

Figure 2. Image slice of a clay loam at 3-5 % moisture content. Porosity = 44.6 %, total pore count = 2183, total pore area = 107.25 mm^2, mean pore area = 0.049 mm^2.

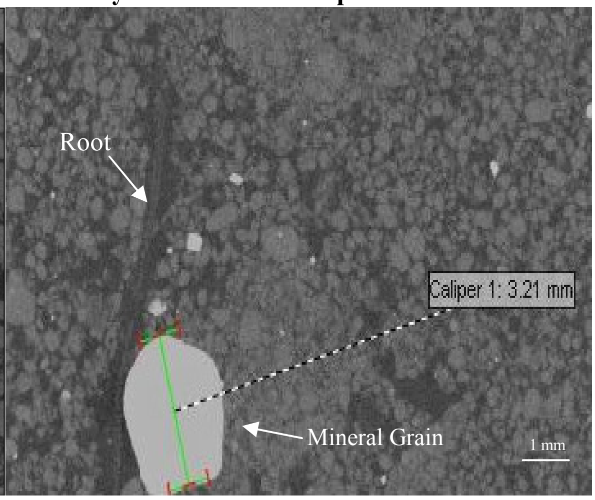

Figure 3. X-ray CT image of a root growing in a Newport series loamy sand. The root can be clearly seen growing around a 3.21 mm diameter mineral grain, measured using the caliper tool in VG Studio MAX® software.

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