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X-Ray Computed Tomography for Root Quantification

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Abstract

Soil cores from a field growing barley and barley mutants without root hairs under conventional and minimum tillage were sampled. They were X-ray scanned to produce a 3D image and then the roots were washed out and weight and length were determined by conventional means. Root volume and surface area were then calculated from the 3D images using state of the art software and methodology, and the measured and calculated measures were correlated. The only strong and significant correlation was between measured weight and calculated volume for mutants without root hairs. It is concluded that the software cannot segment out very small roots, but segmentation accuracy also depends on root structure in some unknown way. Any study using X-ray computed tomography to quantify roots as they grow *in situ* should start with a calibration for the conditions in question.

Keywords

Roots, 3D Image, X-Ray Computed Tomography

1. Introduction

Whilst aboveground plant development and productivity can usually be easily observed and quantified, at least on the plot scale, quantification of roots still pose significant challenges. None of the methods available can quantify root biomass or turnover reliably. Furthermore, most methods are destructive, and there are few methods available to observe roots as they grow.

X-ray CT was first developed for medical applications, and was later used for a variety of industrial applications. The development scanners that could scan a variety of size classes, including small ones (micro-tomography) made it useful also for geological applications, including soils [1]. X-ray CT has had a variety of useful applications in studies of soil physics (e.g. [2]-[6]), but studies of biological properties have turned out to be dif-

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ficult as organic matter has a similar X-ray attenuation as air [7].

Although imaging roots using X-ray CT can be challenging because roots are difficult to distinguish from pores, progress has been made [8]. The power of the technology lies in that the method is non-destructive, so that roots can be observed repeatedly over time as they grow. Some progress has been made in segmenting soil from pore space [9]-[11]. Segmentation of roots has also been attempted, but has so far succeeded for very young roots or small parts of roots [12] [13].

In 3D scans of roots in soil the roots can be segmented out and volume and surface area can be calculated using dedicated software. However, it is not known how accurate these measures are. The purpose of this paper is to compare surface and volume calculated by software on 3D scans to weight the length of roots measured by traditional destructive techniques on roots washed out from the samples after scanning.

2. Materials and Methods

2.1. Field Experiment

The field experiment was established in Invergowrie, Dundee, Scotland, UK (56˚27'N, 3˚W) in 2003 (see [14] for details) to compare tillage treatments, among them conventional and minimum tillage. The field was situated in a mainly agricultural area in eastern Scotland close to the sea. The soil was Dystric-Fluvic Cambisol (FAO) with a sandy-loam texture. It had a pH (1 part soil to 2.5 parts 1M $CaCl_2$) of 5.7, was freely drained and underlain by colluvial sand at 60 cm depth. In 2012 the fields were planted to barley with different rooting pattern, among them wild type and a mutant lacking root hairs. The mutants and their origin are described in [15].

2.2. Sampling and Direct Root Measurements

Soil cores were sampled in plastic rings (4 cm height, 4 cm diameter). Before sampling, aboveground plant material and top soil were removed in the area to be sampled, so that the sampling depth was 4 - 8 cm. To make sure all samples were fresh when scanned and processed, each replicate (n = 3) were sampled on different days. Sampling days were 21, 23, 25 May. The samples were stored in a cold store (4˚C) until scanning (after 1 day) and further sample processing (after 2 days). The treatments sampled were barley mutants without root hairs and the wild type (with root hairs) at minimum and conventional tillage.

Roots were washed out, and total roots length was determined by scanning the roots and using the software WinRhizo. The roots were then dried at 70˚C overnight and dry weight determined.

2.2.1. Scanning Specifications and Root Segmentation Method

3D volumetric images in this study were obtained using a Metris X-Tek HMX CT scanner with a Varian Paxscan 2520 V detector and a 225 kV X-ray source (Nikon Metrology X-Tek Systems Ltd, Tring, UK) giving a resolution of up to 5 μm. Samples were scanned at 160 kV and 201 mA using a 0.1 mm Al filter to obtain 3003 angular projections (based on a 360˚ rotation).

VGStudio MAX 2.2 (Volume Graphics, Germany) was used for root segmentation. Roots were identified by eye, and segmented using "region grower". This was repeated several times in each sample until no more roots could be found. Erode/dilate (radius 2) was then used. The total volume and surface of the root region was calculated by the software. The procedure was repeated twice for each sample, to assess repeatability of the procedure.

2.2.2. Statistics

Minitab v15 was used. Correlation analyses between root weight (measured by weighing) and root volume (calculated by VGStudioMAX) and between root length (measured by WinRhizo) and root surface area (calculated by VGStudioMAX) and between root surface area measured by WinRhizo and calculated by VGstudioMax were performed. Varieties with and without root hairs were correlated separately.

3. Results and Discussion

It was expected that weight and volume of roots would be strongly correlated, and that testing if the volume measured in the 3D image correlates with the weight should be a good test of the calculation from the 3D image. Root length and surface area were also expected to be correlated, but this correlation was expected to be weaker.

Overall there was little correlation between measurements done on the roots and the parameters calculated from the 3D image (**Table 1**; **Figure 1**). The only strong and significant correlation was between measured weight and calculated volume for the varieties without root hairs showed (**Table 1**; **Figure 1**). Tillage treatment did not affect the relationship between measured and calculated parameters, but variety (with or without root hairs) did (**Figure 1**). How well roots can be quantified from 3D images therefore depends on the type or structure of the roots. Hirano *et al*. [16] found that detection frequency of roots below 1 cm in diameter was poor using root penetrating radar. Also in this study it was noted that very thin roots were not picked up by the software even when they were seen by the experimenter. Large roots or high resolution would help, but that means that with current technology only a small volume of soil can be examined. Any use of 3D imagery in following root development should start with a calibration like this for the type of roots and resolution to be used. Using a homogenous soil may also help, but the results here suggest that the quality of the roots are more important. It is not known why the roots without root hairs were easier to quantify. Although root hair are too small to be seen on a 3D image, they are also lost in root washing, so they would also not be included in the weight measurements. It is possible that varieties without root hairs compensate by being thicker or in other ways more distinct, and therefore easier for the software to follow.

4. Conclusion

Larger roots can be reliably quantified in 3D X-ray images, but the quantification is less reliable for smaller roots. However, reliability also depends on root structure in a way that is not fully understood, and any study of *in situ* root growth using X-ray tomography should start with a calibration of reliability for the roots and resolution to be used.

Table 1. Pearson's correlation coefficient and p-values for correlations between various parameters measured and calculated from the X-ray CT scans.

	Overall		Varieties with root hair		Varieties without root hair	
	R	p	R	p	R	p
Weight vs. volume	0.454	0.138	0.362	0.481	0.823	0.044
Surface vs. length	0.159	0.622	0.052	0.923	0.388	0.447
Surface, measured vs. calculated	0.210	0.512	-0.442	0.380	0.441	0.381

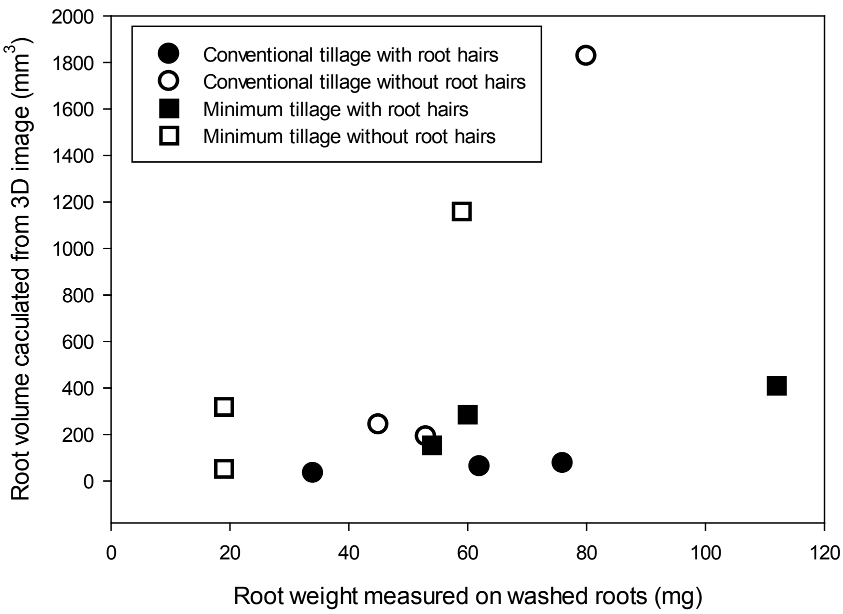

Figure 1. Root weight measured plotted against root volume calculated from the 3D image for the two tillage treatments and two varieties.

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