3-Dimensional characterization of substrates with X-ray microtomography

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Abstract

X-ray tomography is an imaging technique pioneered by the medical and petroleum industries to spatially characterize materials. Advances in X-ray technology, software, and commercially available instruments have opened the door for its utilization in horticultural substrate research. Tomographic reconstruction of rooting matrix could provide 3-D, in situ characterization of substrate particles, pore structure, and root architecture. An X-ray microtomography instrument was used to scan pine bark, sphagnum peat, coconut coir, and wood fiber substrates. Each material was scanned at a resolution of 50, 35, and 25 µm and evaluated qualitatively. At all resolutions, materials were effectively scanned with clear distinctions between pores, substrate particles, and roots. Increasing the scan resolution resulted in more definable internal pore structures observed most notably in pine bark. Three dimensional surfaces were successfully rendered at each resolution and for each material. These results demonstrate that horticultural substrates could be visualized during root development, opening new opportunities for the application of X-ray microtomography in experimental studies concerning substrate characteristics and plant interactions.

Keywords: image analysis, particle size, particle shape, rootball matrix, soilless substrate

INTRODUCTION

The need to observe undisturbed roots within a container, both spatially and temporally, is a challenge for researchers studying plant growth and substrate interactions in containers. The visualization and quantification of plant root and soil structure are essential for a better understanding of growth dynamics and productivity. However, our knowledge of how substrates interact with plant growth is fragmented. This fragmentation is due to the limited dimensionality and destructive harvesting necessitated by current root and substrate analysis methods.

X-ray computed tomography (CT) allows researchers the unconstrained accessibility to visualize and quantify objects and materials of interest both spatially and temporally. CT is a non-destructive, non-invasive technique that can be used to visualize the interior of objects in 2-D and 3-D based on the principles of X-ray attenuation and detection. Detailed explanations on the processes of X-ray tomography and reconstruction have been reported in (Ketcham and Carlson, 2001; Wildenschild et al., 2002). To describe briefly, as an X-ray passes through an object, the density of the object will attenuate (alter) the X-ray wave and the degree of attenuation is detected by a panel. When a series of X-rays are taken at different angles around an object and combined, the reconstruction of these images results in a 3-D rendering of the object.

To date, little research has been published in the area of plant-substrate (organic) research utilizing CT (Brown et al., 1987; Pálsdóttir et al., 2008). The work conducted by Brown et al. (1987) utilized a medical-grade CT scanner at Duke Hospital (Durham, NC). The objective of the work was to evaluate CT as a method of non-destructive monitoring of water distribution in phenolic foam media. The authors deemed the method successful in spatially detecting water content, but this work has very little modern-day implications due to the

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advancements made in tomographic imaging and computational power. Pálsdóttir et al. (2008) provided similar qualitative results by manipulating water content of a variety of substrate components such as peat, rockwool, sand, and pumice.

Apart from horticulture, soil and agronomic researchers have continuously utilized tomography throughout the development of the technology (Crestana et al., 1986; Clausnitzer and Hopmans, 2000; Aravena et al., 2011). Recent advances in spatial resolution, image quality, and computing power have led to a significant increase in the utilization of CT, specifically computerized microtomography (MCT), in root-soil studies (Mooney et al., 2012). These advances have allowed soil researchers to evaluate soil pore structure (Iassonov et al., 2009), characterize soil-root interactions (Aravena et al., 2011), and visualize genetic variability in root architecture (Rogers et al., 2016).

Though similar to soil science in many respects, horticultural substrate science offers the unique capability to completely design the root environment based on a plant's needs and specific growing requirement. Additionally, the components utilized as substrates in container production differ markedly from soils with respect to size, material composition (i.e., organic), internal porosity, hydrological characteristics, and density. Our aim in this research is to evaluate the viability of horticultural substrates and plant root in situ characterization utilizing MCT.

MATERIALS AND METHODS

Sample preparation

The substrate components analyzed in this study were sphagnum peat (BPP; Berger, Saint-Modeste, QC, Canada), pine bark (*Pinus palustris*; 0.32 cm screened aged bark; T.H. Blue, Eagle Springs, NC), wood fiber, and coconut coir. All materials were air-dried to contain 50% moisture by weight. Peat and coir substrates were packed into cores at a dry bulk density of 0.1 g cm^{-3}. Wood fiber was packed at 0.2 g cm^{-3} and pine bark at 0.3 g cm^{-3}. Each material was packed into three polyacrylic cores of decreasing internal diameter, 7.60, 6.35, and 3.81 cm. Once packed, the open ends of the containers were sealed with PVC sheets held by rubber bands to prevent desiccation. These container sizes allowed us to visualize the materials at increased resolutions while maintaining the entire sample in view. In addition to analyzing substrate components, geranium cuttings rooted in a Fertiss® (Oasis Grower Solutions, Kent, OH) 100 plug with a peat-vermiculite substrate were analyzed in order to evaluate the potential of plant root characterization in situ.

X-ray imaging

For this study, a high-resolution X-ray computed tomography scanner (XTH 225 ST, Nikon, Melville, NY) housed in the Shared Material and Instruments Facility at Duke University (Durham, NC) was utilized. The instrument utilizes a tungsten target, white beam, conical beam with a detection panel containing 2000×2000 pixels to achieve a maximum resolution ~3 µm. The 7.6 cm diameter cores were imaged at 105 kV, 120 µA, 708 ms exposure, acquiring 2,200 radiographs by rotating the sample by 0.164 degrees (stacked 2× to reduce image noise). The kV was adjusted to 95 and 85 kV to acquire images for the 6.35- and 3.81-cm cores, respectively. These settings produced quality images at a voxel resolution of 50 µm (7.6 cm dia. cores), 35 µm (6.35 cm dia. cores), and 25 µm (3.81 cm dia. cores). A Feldkamp cone-based CT algorithm was applied to convert the X-ray radiographs into 2-D reconstructed slices. Two-dimensional horizontal cross sections of the samples were visualized using ImageJ, a public, java-based image processing program. Three-dimensional images were reconstructed using Avizo Lite 9.2 (Visualization Science Group, Inc., Burlington, MA).

RESULTS AND DISCUSSION

Since the aim of the study was evaluate the viability of MCT analysis for future work, much of the data and results are qualitative. Each material was successfully visualized in 2-D (Figure 1). Qualitative differences in the inherent physical structures of each material were apparent. Peat may be best described as a heterogeneous mixture of aggregates, fibrous particles, and partially decomposed stems. Coir, often thought to be similar to peat in texture, appeared more homogenous and consisted of granular shaped, sponge-like particles. Pine bark appeared the coarsest and contains particles consisting of two layers, dense periderm layers and less dense layers comprised of crushed phloem and expanded parenchyma cells. The elongated, fibrous network of the wood fiber substrate visually distinguished it from other materials.

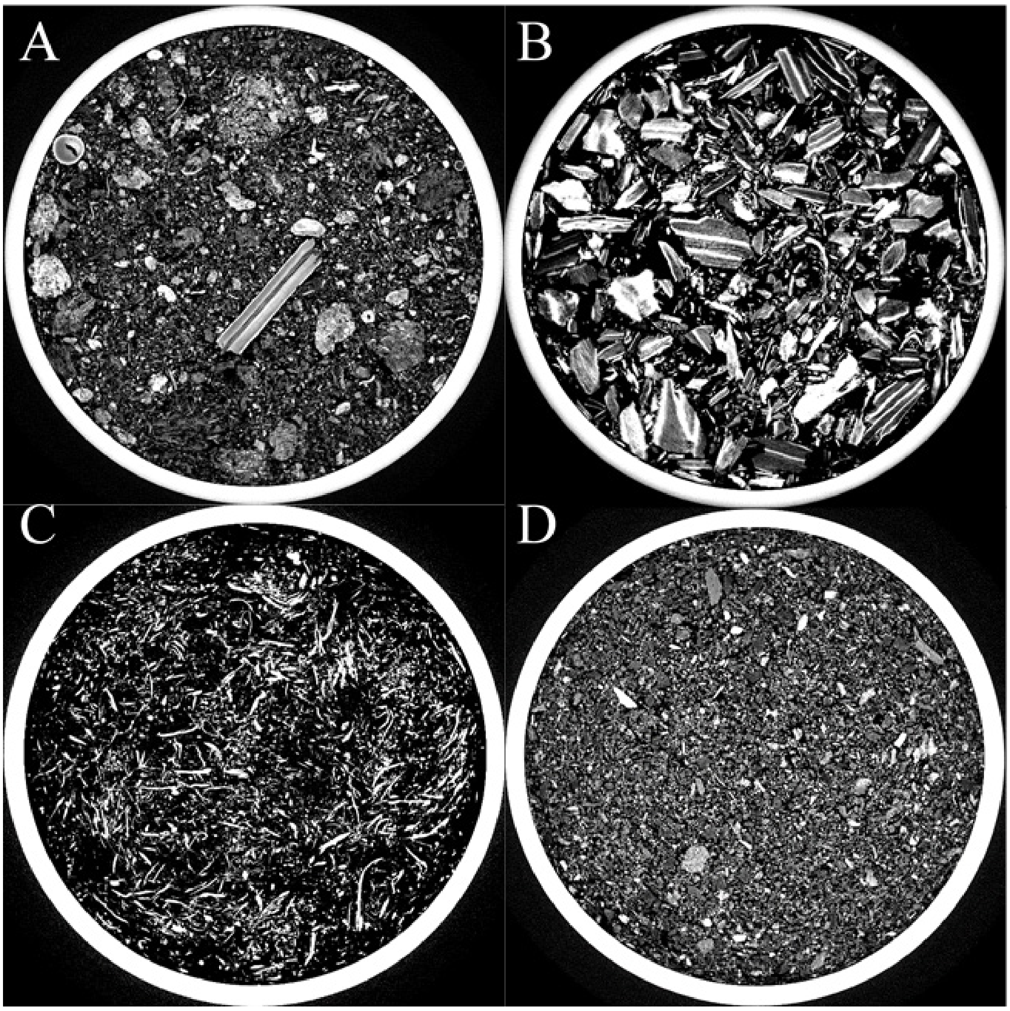

Figure 1. Two-dimensional horizontal slices of peat (A), pine bark (B), wood fiber (C), and coconut coir (D) substrate components packed in 7.6 cm diameter polyacrylic cores. Images obtained from an X-ray microtomography scanner at a pixel resolution of 50 µm.

Data obtained from MCT does not limit the researcher to qualitative data. Utilizing software like Avizo Lite, quantitative data can be obtained after properly applying segmentation and threshold algorithms. Subsamples of each material (16.4 cm^3) were segmented to isolate the solids from the air for three-dimensionally rendering. Once rendered, the solids and pores can be visualized, labeled, and analyzed to obtain data such as the volume, surface area, or number of pores (Figure 2). Utilizing other algorithms to separate touching particles or pore spaces, may allow the researcher to obtain particle size and pore size distribution in-situ. However, the quality of the data may be determined by the resolution of the scan.

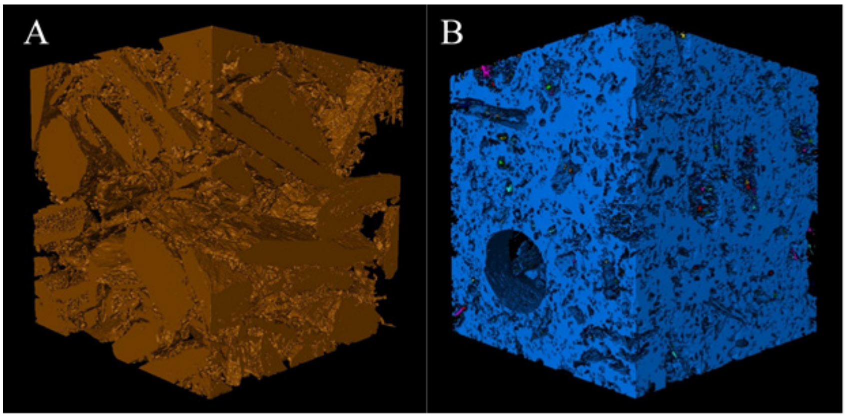

Figure 2. Subsamples can be acquired to reduce computation time for 3-D quantitative analysis of tomographic images. Subsamples are 16.4 cm^3 with a voxel resolution of 50 µm. Once segmented, the solids (A, pine bark) and pores (B, peat) can be analyzed.

The internal structure and surface detail of each material were resolved as resolution increased. This was best observed with the images acquired from pine bark (Figure 3A-C). In order to best visualize the internal structure of pine bark, a separate image was acquired at a resolution of 17 µm. Rendering the surfaces of the image revealed the internal structure and surface characteristics of the pine bark particle (Figure 3D). Future work on substrate characterization should consider the resolution of the images with respect to the objective of the study. To best characterize the entirety of the pore matrix, a multi-scale approach should be considered. If the objective of the work is to characterize the macroporosity, the resolutions obtained in this study (50-25 µm) would be adequate.

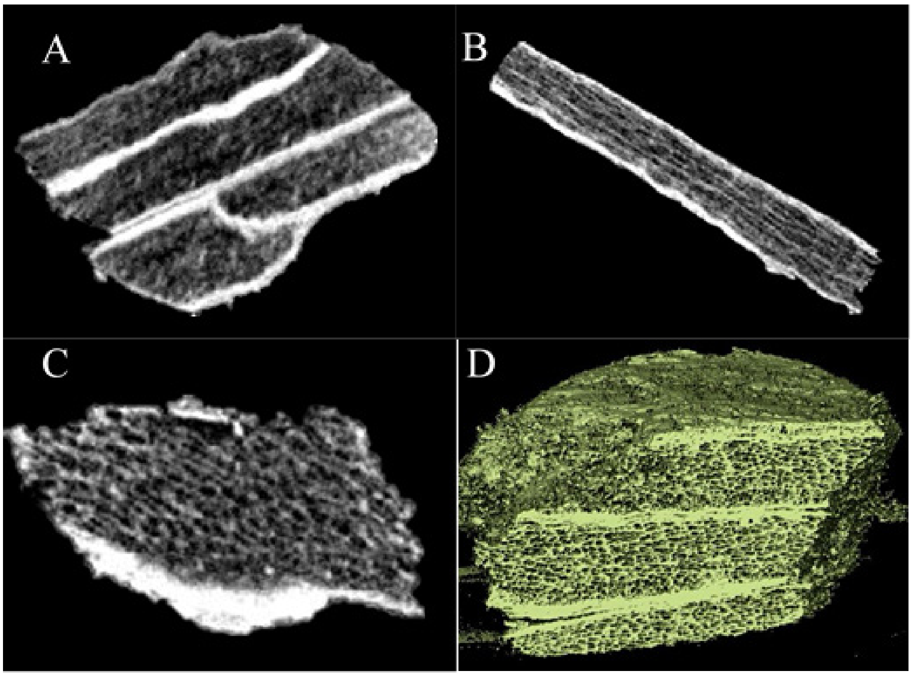

Figure 3. Two-dimensional cross sections of pine bark particles imaged at 50 µm (A), 35 µm (B), and 25 µm (C). A 3-D rendered particle was imaged at a resolution of 17 µm and clipped to reveal in the internal structure (D). Increasing the resolution of the scans allowed more internal structure/porosity to be resolved.

The characterization of plant roots utilizing MCT has been challenging for soil scientist (Mooney et al., 2012). In order for different materials to be segmented, there must be sufficient contrast in the gray values between materials. In soils, the densest and most easily distinguished material is the soil (particle density (ρd) = 2.65 g cm^{-3}). The similarities in the density of water, organic matter, and roots (ρd = ~1-1.5 g cm^{-3}) are more difficult to distinguish and isolate for analysis (Mooney et al., 2012). However, in the image acquired rooted geranium and substrate, there was sufficient contrast between the substrate and plant roots to isolate the roots from the sample (Figure 4). Additional research is required in order to fully understand the variables which led to these results. Nevertheless, these results show that the characterization of roots in situ, both spatially and temporally, in horticultural substrates is possible.

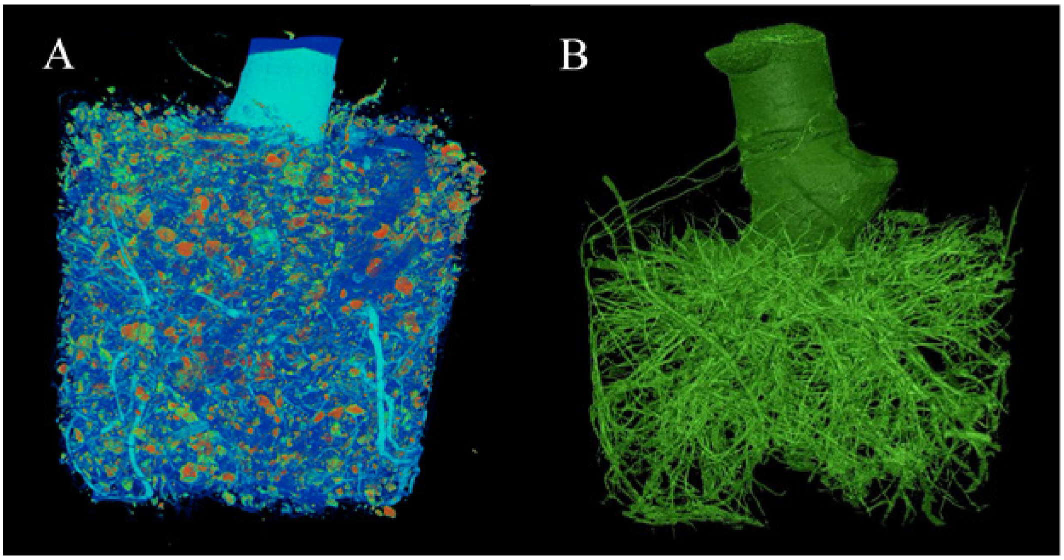

Figure 4. X-ray microtomography image acquired of a rooted geranium cutting at a voxel resolution of 30 µm. The image was first rendered and displayed using a color panel to differentiate materials by their gray value (A). The root system of the geranium cutting was isolated from the substrate (B).

These results, though qualitative, demonstrate that the spatial and temporal characterization of horticultural substrates and plant roots is possible despite their lack of transparency. The next stage is to utilize this technique to address fundamental research questions concerning plant-substrate interactions and abiotic/biotic factors which may affect both. As technological advancements continue to be made, the availability and applications of tomographic research in plant sciences will continue to broaden.

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