X-ray Computed Micro-Tomography Indices of Soil Microstructure within a

Tree-Based Intercropping System

by

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ABSTRACT

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X-ray computed micro-tomography surface soil microstructural indices associated with a 25-year-old tree-based intercropping (TBI) system and an adjacent sole-cropped conventional agricultural system at the University of Guelph’s Agroforestry Research Station were investigated spatially and temporally. At the level of observation of this analysis, tree species have little effect on X-ray μCT measured void characteristics. Void phase parameters between the TBI and solecrop systems were similar due to the same agricultural management adopted in both cropping areas. However, there was a very strong indication of void phase parameters change with time. Ultimately, void phase parameters and soil matrix radiodensity variability appear to be dynamic and difficult to understand at a system level as they’re influenced by many variables.
Acknowledgements

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<tr>
<td>TBI</td>
<td>Tree-based intercropping</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>SOM</td>
<td>Soil Organic Matter</td>
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<tr>
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<td>GHG</td>
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<td>COV</td>
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1 Chapter 1: Introduction & Literature Review

1.1 Introduction

1.1.1 Agroforestry
Over the past 30 years, agroforestry management has become a viable method of food and bio-energy production. Today, agroforestry is considered an integrated applied science that can potentially address many land management and environmental problems in both developing and industrialized nations (Ramachandran Nair et al. 2009). Tree-based intercropping (TBI) is a form of alley cropping which grows trees and annual crops together (Lelle and Gold 1994). These systems are usually designed with rows of trees that are perpendicular to the predominant wind direction, and which have rows that are spaced sufficiently wide enough apart to allow traditionally managed row crops to grow in between them and to be accessed by farm machinery. This form of management allows for increased efficiency in nutrient management (Nair and Graetz 2004), reduced greenhouse gas emissions (Schoeneberger 2009), increased soil organic matter (SOM) (Gupta et al. 2009) and increased soil microbial biomass (Rivest et al. 2010).

Investigations that have been conducted over the past 20 years in a TBI system in southern Ontario, Canada, have revealed several complementary biophysical interactions for this type of temperate agroforestry system (Thevathasan et al. 2004). These observations include the transfer of nitrogen (N) to adjacent crops, increased soil organic carbon (SOC), greater earthworm abundances close to trees, all of which occur largely as a result of tree litterfall inputs and fine root turnover (Thevathasan et al. 2004). It has been speculated that these observations indicate changes in the flow of energy among trophic groups within TBI systems.

1.1.2 Soil Micromorphology
Soil micromorphology is the observation, and recognition of micro-scale attributes within soil. Some of these attributes include porosity, mineralogy, and texture, which provide insight into soil genesis, and
soil-plant interactions. The discipline of micromorphology was developed in Austria during the mid-1930s studying soil thin-sections by use of microscopes. Traditionally micromorphology, based on thin sections, was a support for other morphological, physical, chemical, mineralogical, and biological methods to explain soil processes and properties (Wilding and Flach 1985). Even in the 1990s, micromorphology had been seen as a procedure to obtain essential extra information on aspects of the soil which was being studied (Kooistra 1990). More recently X-ray Computed Micro-Tomography (X-ray μCT) has been used effectively for studies in soil micromorphology. Micromorphology has become a robust discipline not only acting as a support, but used to directly explain soil processes and properties (Wilding and Lin 2006).

### 1.1.3 Context for Studies in TBI Systems

The ability of TBI to sequester carbon (C) above ground is well understood. There is still a knowledge gap in accurately predicting above and below ground C sequestration in TBI systems, especially with different tree species, age and climatic conditions. The greenhouse gas (GHG) dynamics in TBI systems are influenced by tree species, crop species, climate, manure and fertilizer application rates and schedules, soil type and the age of the system. No study to-date has linked C sequestration and GHG mitigation, in TBI, to these varying but important parameters. The mechanisms behind these systems and resulting environmental services derived from these systems are to a certain extent unknown, especially within soils. Ball (2013) in a review of soil structure effects on greenhouse gas emissions highlighted many studies agreeing with the findings of Mangalassery et al. (2013). One of the main concepts discussed were an increase in nitrous oxide (N₂O) flux with soil compaction and wetness, indicated by positive correlation of bulk density with N₂O emission. The review further indicated that carbon dioxide (CO₂) flux was greatest in the ‘best’ structured soils that provided good aeration due to loose, well-aggregated structure. Similarly to conclusions made by Mangalassery et al. 2013, Ball (2013) indicated that the small intra-aggregate pores influence the size of anaerobic zones (N₂O emissions), and
the larger inter-aggregate macropores influenced the exchange of oxygen at the boundaries of aggregates and exchange of CO$_2$ with the atmosphere.

This research is part of a multi-partnership project that addressed and identified mechanisms by which TBI systems mitigate GHG emissions and enhance C sequestration. Combinations of conventional and novel experimental approaches were adopted to understand these mechanisms. The project’s results have made contributions towards enhanced understanding and accessibility of TBI that can be adopted by farmers in Canada and other temperate climate countries to mitigate GHGs.

### 1.1.4 The Importance of Soil Structure Studies

Agroforestry management can influence soil aggregation and pore characteristics. Research has shown that production, consumption, and transport of GHG can be linked to soil structure (Mangalassery et al. 2013). Specifically, results have demonstrated the importance of pore characteristics on emission of CO$_2$ and methane (CH$_4$) (Ball 2013). Although N$_2$O emission do not show a direct link to soil pore characteristics (Mangalassery et al. 2013), it is responsive to the soil aeration status. Soil compaction and ponding, effected by porosity, are known to be major factors in increasing N$_2$O emission (Ball 2013).

To fully understand the benefits of tree-based intercropping, it is important to identify measurable indices of soil structure, such as void space, that influence potential for GHG emissions or mitigation. X-ray μCT analysis allows the objective and quantitative measurement of soil microstructure indices and their variability. It was hypothesize that the use of this technique would enhance the understanding of mechanisms by which TBI systems can mitigate GHG emissions and enhance C sequestration.

### 1.2 Thesis approach

The thesis, had two main objectives: 1) Quantify selected soil microstructure indices in the surface soil (3.5cm) using high-resolution X-ray μCT imagery, and penetrometer resistance measurements, and 2)
Within the context of multi-disciplinary research, to identify X-ray µCT based microstructural indicators linked to overall soil quality in TBI systems. Chapter 1 of the thesis provides a general introduction to the topics within the thesis paper, and provides a detailed literature review of relevant work related to this study. Chapter 1 also outlines the hypotheses and objectives of this work. Chapter 2 outlines general methods that were used in the thesis work. The remaining Chapters of the thesis have been organized by the type of sampling that was completed, and the final chapter evaluating penetrometer resistance. Chapter 3 focuses on the soil samples that were collected to collaborate with fellow researchers *excavations* of entire tree root masses; within this chapter the X-ray µCT evaluated soil structure indices were calculated and discussed. Chapter 4 focuses on samples collected to evaluate *temporal* changes in X-ray µCT evaluated soil structure indices. Chapter 5 evaluates X-ray µCT derived indices with reference to soil penetrometer resistance measurements.

The approach to methodological applications was similar among these chapters for the X-ray µCT derived soil structure indices, but each contained distinct data analysis applications. As the study site, tree species, and X-ray µCT data collection protocol are common to all chapters, these components are described first in Chapter 3 under General Methods.

The research questions guiding the work reported in Chapter 3 were:

\[ a. \quad \text{Do soil X-ray µCT soil microstructure indices in surface 3.5cm differ between tree species?} \]
\[ b. \quad \text{Do soil X-ray µCT soil microstructure indices in surface 3.5cm differ between crop alleys and tree rows?} \]

And for Chapter 4:

\[ a. \quad \text{Do soil X-ray µCT soil microstructure indices in surface 3.5cm differ seasonally?} \]
\[ b. \quad \text{Do soil X-ray µCT soil microstructure indices in surface 3.5cm differ between TBI and solecrop systems?} \]
And for Chapter 5:

a. Do soil X-ray µCT soil microstructure indices in surface 3.5 cm correlate with traditionally measured penetrometer resistance?

b. Can these X-ray µCT soil microstructure indices in surface 3.5 cm link to work completed within the multi-disciplinary research?

1.3 Literature Review

1.3.1 Tree Influences on Soil

Tree species have varying characteristics which may result in differences in the soils beneath them. One important characteristic is the variability in tree leaf litter quantity, and chemical inputs to surrounding soils (Binkley 1996). It has been suggested that decomposition of leaves may be more rapid for poplar than for other tree species; Chander et al. (1995) found that soils amended with leaves from Populus deltoides exhibited greater microbial decomposition rates of SOM than did soils that had been amended with Eucalyptus tereticorni leaf litter. Further, Thevathasan et al. (1999) found significantly lesser quantities of SOC in soils under poplar trees compared to walnut trees. However, leaf litter decomposition was more rapid, and litter biomass inputs were greater under poplar trees than under walnut (Thevathasan & Gordon 1997). These relationships suggested that there were greater rates of SOM decomposition under poplar trees compared to walnut trees. Additionally, black walnut trees are known to produce high concentrations of juglone close to trees (< 2m), which also could inhibit the microbial community (Dawson & Seymour 1983). However, Thevathasan et al. (1999) found that juglone had no inhibition effects of juglone on nitrification rates in a 60-year-old black walnut plantation.

Additionally, Peichl et al. (2006) observed that the concentration of soil carbon within the upper 20 cm increased in a poplar intercropping system versus sole cropping or spruce intercropping systems.
This was explained by the greater height, crown diameter and litterfall of poplar trees, resulting in a much greater C input to soil.

Due to its light weight and large surface area, hardwood leaf litter is easily redistributed by wind both during and after it falls (Welbourn et al. 1981). However, Thevathasan & Gordon (1997) found that most leaf litter accumulates 2 meters into the row cropping alley immediately adjacent to the grass buffer where the tree trunk is located. Peichl et al. (2006) further suggest that greater C input in the form of litterfall closer to the tree rows creates favourable conditions for soil micro-organisms leading to enhanced microbial activity and CO₂ evolution (Matteucci et al., 2000). Peichl et al. (2006) recorded soil CO₂ respiration at a poplar tree intercropping site to be the greatest closer to the tree rows. They suggested this may be due to greater tree root respiration and/or greater soil microbial respiration.

Rates of litter decomposition in ecosystems, among many important drivers, can be affected by differences in the composition of decomposer communities and their trophic interactions (Beare et al. 1992; Šnajdr 2013). Beare et al. (1992) also concluded that the trophic relationships may be strongly influenced by altered microclimatic conditions, such as those induced by tillage (surface available versus burial of litter). Similarly, TBI systems may cause microclimates as different tree species may have different canopy architectures, and leaf traits including litter quantity, shape, size, and chemical composition (Binkley 1996). However, Mungai et al. (2005) highlighted the greater role of litter quality and quantity in influencing microbial function in contrast to small variations in microclimate that were observed close to trees in an alley-cropping system. At the University of Guelph agroforestry site, Price and Gordon (1999) also found that, across various seasons, earthworm densities were almost always greatest closest to the poplar treatment compared to silver maple (Acer saccharinum L.) and white ash (Fraxinus americana L.). This trend was attributed to the greater concentrations of N and SOM that originated from poplar leaf litter.
1.3.2 Soil Structure

Soil structure, as defined by Brewer (1976), is

“… expressed by the size, shape and arrangement of the solid particles and voids, including both the primary particles to form compound particles, and the compound particles themselves. “

This concept of primary particles and compound particles is referred to as ‘aggregate hierarchy’, which exists in soils where aggregate stability is controlled by organic materials (Six et al. 2004; Oades & Waters 1991). In clay microaggregates (< 2 µm diameter), the clay-organic matter complexes are stabilized by humic acids and inorganic ions (Tisdall & Oades 1982). Larger microaggregates (2-250 µm diameters) are stabilized by microbial materials such as polysaccharides, hyphal fragments and bacterial cells (Kooistra & van Noordwijk 1996; Tisdall & Oades 1982). Soil aggregation occurs as a hierarchical process in which microaggregates are located at the center of macroaggregates.

Elliott and Coleman (1988) suggested a simultaneous hierarchical void formation model that would mirror aggregate hierarchy (Figure 1.1). Void space represents the volume of a soil that is not occupied by solid particles. The authors further suggested the formation of four hierarchical classes of soil void space: (1) macrovoid, (2) inter-macroaggregate, (3) inter-microaggregate (4) intra-microaggregate space. Macrovoids, as defined by Elliott and Coleman (1988), are usually created by roots or earthworms (channels) or through shrink/swell processes. The inter-macroaggregate voids are where water is retained when the soil is at field capacity and are sufficiently large to be inhabited by nematodes. Inter-microaggregate voids are large enough to accommodate small nematodes, protozoans, and fungi. Intra-microaggregate voids are extremely small and may be inhabited mostly by bacteria.
1.4 Application of X-ray Computed Micro-Tomography in Soil Assessment

1.4.1 Overview of X-ray µCT

X-ray µCT is a non-destructive, non-invasive technique that has been successfully used for 3-dimensional (3D) studies of soil over the last two decades; the theory has been presented by numerous researchers in soil science and geology (Clausnitzer and Hopmans 2000; Cnudde et al., 2006; Taina et al., 2008). There are many types of computed tomography acquisition systems, but they all contain common elements; an X-ray source, an object, and detector system to measure extent that the X-rays have been attenuated by the object, representing the radiodensity of the material. Computed tomography’s fundamental objective is to acquire multiple sets of views of an object over a range of angular orientations, allowing for 3D visualization (Ketcham and Carlson 2001).
1.4.2 Void Evaluation

By using the X-ray μCT method, the spatial arrangement of soil components can be quantified. Thus far, much of the use of X-ray μCT for soil investigation has been focused on the evaluation of soil microstructure, essentially represented by the quantitative characterization of the porosity and void networks (Taina et al., 2008). A wide range of void sizes exist in soils, both between and within aggregates. The void space, size and amount can influence SOC and its turnover, conversely SOC and soil texture influence porosity (Bronick and Lal 2005). The void space of soils (size, shape, and continuity) is also of great importance in describing water movement, solute transport, aeration capacity, biological diversity, and root proliferation (Wilding and Lin 2006). The structural degradation of void space results in loss of voids, decreasing gas diffusivity and water availability, and soil compaction causing soil crusting and decreased water infiltration (Wilding and Lin 2006). Continuity of macrovoids induces preferential flow especially near water saturation compared to the more tortuous void system within aggregates (Beven and Germann, 1982). Various properties of soil macrovoids and macrovoid network have been estimated using μCT imagery (Grevers et al., 1989; Anderson et al., 1990; Čislerová and Votrubová 2002; Pierret et al., 2002; Rachman et al., 2005; Piñuela et al., 2010). Soil macrovoid space is a reflection of the spatial arrangement of mineral particles, aggregates, and biological activity. There has also been recognition of soil processes (hydro-physical, shrink-swelling, root systems) and management (tillage, crop rotation) being reflected in void/soil microstructure (Gantzer and Anderson 2002; Rachman et al., 2005; Sander et al., 2008; Luo et al., 2010).

1.4.3 Semivariance Analysis

The spatial variability of X-ray attenuation can be evaluated using semivariance of values in three orthogonal directions. A semivariogram is a plot of semivariance versus distance and reflects the degree of dissimilarity for pairs of points selected at increased separation distances (termed lags). These methods of studying heterogeneous porosity and void morphology have demonstrated that variograms are good indicators for the distribution and orientation of voids in the soil void system. Čislerova and Votrubová
10

(2002) concluded that high variance of attenuation of individual voxels in the CT imagery of two soil samples was attributed to a combination of dense regions of clay aggregates or stones, and more porous regions. Further, Taina et al. (2013) demonstrated anisotropy between vertical and horizontal semivariograms as a good indicator of platy structure in their analyzed soil cores. However, DeGryze et al. (2006) did not find the semivariogram parameters (nugget, range, and sill) of imagery with 13.4µm voxel size to be good indicators for observing the process of changes in soil porosity as affected by organic residue decomposition.

1.4.4 Agroforestry

Within recent years, several studies have demonstrated the applicability of X-ray μCT to examining soil microstructure within agroforestry systems (Udawatta et al., 2006; Udawatta and Anderson 2008; Udawatta et al., 2008). These authors have compared the agroforestry system’s cropping alleys with the adjacent grass buffers, and determined greater quantity and more connected voids within buffer areas compared to cropping areas. There is still a need, however, to understand how soil porosity varies at further distances from trees into crop rows, as well as the annual dynamics of soil microstructure in agroforestry systems, especially those involving inter-cropping. This will create a better understanding of detailed differences in soil microstructure due to agroforestry land management that cannot be quantified using traditional methods.

1.5 Penetrometer Resistance

Penetrometer resistance is affected by numerous soil factors: bulk density, particle size distribution, organic matter content, water content (Aggarwal et al., 2006; Whalley et al., 2007; Whitmore et al., 2011; Gao et al., 2012). These soil factors can generally be inferred directly from measured penetrometer resistance results (De Vos et al., 2005). Penetrometer resistance has been used to assess the degree of soil compaction, as it represents an index of the mechanical impedance that roots experience growing in either dry or compacted soils (Whalley et al., 2007).
1.6 Hypotheses and Objectives

1.6.1 Hypotheses
The following hypotheses have been formatted as null hypotheses to be capable of being proven false.

H1: There are no significant differences in the microstructure of soils, between tree-based intercropping and conventional management systems

H2: There are no significant differences in the microstructure of soils in tree rows, and cropping alleys within tree-based intercropping management

H3: There are no seasonal differences in soil microstructure within tree-based intercropping alleys

1.6.2 Objectives
I. Quantify selected soil microstructure indices in surface 3.5cm (void[volume, shape], soil matrix microstructural variability in x-ray attenuation, and connectivity ) using high-resolution X-ray µCT imagery, and penetrometer resistance measurements
   a. within established tree-based inter-cropping (tree rows and alleys), and conventional agricultural systems in Ontario (H2)
   b. seasonally to evaluate annual dynamics within established tree-based inter-cropping systems in Ontario (H1 & H3)

II. Evaluating X-ray µCT density parameters with reference to penetrometer resistance measurements (H1 & H3)

III. Within the context of multi-disciplinary research, considering C and N dynamics, microbial activity and root zone mapping, identify X-ray µCT based microstructural indicators in relation to overall soil quality for tree-based inter-cropping systems. (H1, H2 & H3)

IV. Provide recommendation towards establishing a framework for on-going monitoring of the impact of climatic fluctuations and changing inter-cropping management regimes, on the nature and dynamics of soil microstructure.
2 General Methods

2.1 Site Description

Research was conducted at the site of a long-term tree-based intercropping research project, initiated in 1987 on a 30-ha field (Figure 2.1) at the University of Guelph Agroforestry Research Station, Ontario, Canada (43°32’28” N latitude, 80°12’32” W longitude). Ten tree species are planted and annually intercropped with maize (*Zea mays*), soybean (*Glycine max*), and winter wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) in rotation. Alleys are spaced at 12.5 or 15 m wide with a within tree row spacing of trees at 6 m apart, except for cedar trees at 3 m within row spacing. Crops are planted within alleys every year following conventional agricultural practices. To the south-east of the tree-based intercropping is an adjacent plot under conventional agricultural practices, and follows the same crop management within the agroforestry site. The soil classification according to the Canadian System of soil classification is Grey-Brown Luvisol (mapped as Guelph Sandy Loam), situated on top of morainal deposits. The following Table 2.1 outlines the rotation and tillage practices at the Agroforestry Research Station:

*Table 2.1: Crop grown and tillage practices for respective growing seasons (Years).*

<table>
<thead>
<tr>
<th>Year</th>
<th>Crop</th>
<th>Tillage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>Corn</td>
<td>Spring Light disc</td>
</tr>
<tr>
<td>2009</td>
<td>Corn</td>
<td>Spring Disc ripper</td>
</tr>
<tr>
<td>2010</td>
<td>Soybeans</td>
<td>Spring Light disc</td>
</tr>
<tr>
<td>2011</td>
<td>Corn</td>
<td>Spring Light disc</td>
</tr>
<tr>
<td>2012</td>
<td>Soybeans</td>
<td>Spring Light disc</td>
</tr>
<tr>
<td>2013</td>
<td>Spring Barley</td>
<td>Spring Light Disc</td>
</tr>
</tbody>
</table>
2.2 Sampling Design

In order to meet the objectives, as outlined in section 1.6.2, and to fit within the context of the overall project, four sampling periods were adopted: 1) undisturbed soil sampling coordinating with belowground root excavation of colleagues, 2) temporal sampling [during growing season, after harvest, pre-planting]. The top 15cm soil near 5 tree species were sampled using acrylic tubes, according to a technique developed by the Soil Imaging Laboratory at the University of Guelph (Taina et al. 2013). This technique involves cores taken vertically from surface by slowly carving a soil column within a small pit (1’ x 1’ x 6”) to minimize disturbance during collection (Figure 2.2). Progressively, with even pressure, a tube is pushed (with a beveled -edge ring attachment) down. As the tube is pushed lower, the excess soil is carefully craved away.
To meet Objective 3, as outlined in Section 1.6.2, excavation sampling occurred during May-June 2012 coordinating with fellow researchers. The excavations included the entire tree root system within a 16 m² area. Consequently the X-ray µCT sampling occurred prior to the excavations at the same sites, sampling at 3 locations; 2 m east and west into the adjacent crop alleys (Figure 2.3), and 2.0 m north along the tree row, from the tree stem. Three replicates of each Deciduous: *(Juglans nigra* (black walnut), *Populus* sp. (poplar –hybrid), *Quercus rubra* (red oak)), and Evergreen: *(Picea abies* (Norway spruce), *Thuja occidentalis* (white cedar)) were sampled.

To fulfill Objectives 1 and 4, the temporal sampling occurred on three occasions: June 2012, October 2012, and April 2013). These three sample times were chosen to coordinate with the traditional agricultural practices as: pre-plant, during growing season, and after harvest. Soil cores were collected 2 and 6 m east and west from the tree trunk into cropping alleys within the agroforestry management (Figure 2.3). Three replicates of each Deciduous: (black walnut, hybrid poplar, red oak), Evergreen: (Norway spruce), and Solecrop Conventional Agriculture: (Soybean) were sampled. All tree replicates were chosen by random selection. Sampling in different directions allowed determination of east-west variations attributable to wind-directed litterfall. In addition, the solecrop conventional management were sampled at 4 locations across a slope transect (only in October 2012, and April 2013).
2.3 X-ray $\mu$CT Imaging and Analysis

2.3.1 Image Capturing
After the soil core was collected from the field, the cores were dried slowly at 40°C to obtain a constant weight. This was done to reduce the water phase to a negligible level vis-à-vis X-ray $\mu$CT imagery. The soil X-ray $\mu$CT imagery was obtained using an MS8x-130 Micro $\mu$CT Scanner (EVS Corp., Toronto, ON) at 120 kV and 155 $\mu$A, with an acquisition pixel size of 20 x 20 $\mu$m. Subvolumes of the surface 3.5cm of cores were subsequently reconstructed as voxels (volumetric picture elements) with a resolution of 60 $\mu$m (voxel size: 60 x 60 x 60 $\mu$m), using the eXplore Reconstruction Utility of GE Healthcare software (2005), to maximize resolution, and manageable file size. The acquisition of microscale imagery allowed addressing Objectives 1-4.

2.3.2 Image Preprocessing
Low-pass Gaussian smoothing with a radius of 1 was subsequently applied to the imagery in MicroView (GE Healthcare 2005) to decrease noise. Image cropping was performed to extract the centre...
portion of the soil core and to eliminate from the analysis the compacted edge areas of the core that were incurred during sampling, together with beam-hardening artifacts on the edge of the soil core. The individual cropped images were imported into ImageJ (Rasband 2007), after converting the images from a vff to tiff file format.

Image segmentation was done using a special purpose plugin for ImageJ. This plugin is a modification of the technique that was outlined by Schlüter et al. (2010), for a proposed bimodal histogram described by two Gaussian distributions, with a lesser grey-scale value distribution representing void space, and a greater grey-scale value distribution representing the soil matrix (Figure 2.4a). The first step in segmentation was to identify voxels in the image that were low variance regions (pure voxel extraction), as described by Elliott and Heck (2007), which were then used to refine the image histogram and allow easier differentiation of peaks. The low variance bimodal histogram is `clamped' at a lesser threshold, $T_{\text{min}}$, where all voxels with grey-scale values that are less than $T_{\text{min}}$ are assumed definitively to belong to the void space; at an upper threshold, $T_{\text{max}}$, all voxels with grey-scale values that are greater than $T_{\text{max}}$ are assumed to represent the solid soil matrix. However, this bimodal histogram is not always as pronounced in soils which contain very little void space. The grey-scale attenuation distribution is predominantly uni-modal with a void phase that is represented by a long left tail on the histogram (i.e., left-skewed distribution) (Figure 2.4b). When there is only one peak, it is assumed that this peak predominantly represents the `solid soil matrix’, because all samples have a greater proportion of solid versus void. By finding the mean point of the single peak and subtracting one standard deviation from this value, it can be assumed that any grey-scale values that are greater than this threshold can be considered `solid soil matrix’ with a high level of certainty. Further, to identify areas that are definitively ‘not soil solid phase’, all values that are less than 4 standard deviations from the peak mean were considered ‘not soil solid phase’ and, therefore, considered to constitute void space. The interval of 4 standard deviations was chosen as on a normal curve, 99.99% of the observations will fall within this
interval. The image was ‘clamped’ by having the lowest grey-scale value set to ‘mean – 4 standard deviations’, while the highest grey-scale value was set to ‘mean - 1 standard deviation.’

Immediately following the clamping, a 3D Laplacian operator was used to identify regions in the clamped image with zero-crossings (i.e., areas in the image where the change in value is from positive to negative, or vice versa). These voxels are what would be considered mixed voxels, as they were zones of transition, and it is unclear if they should be classified as solid or void phase. Consequently, it was unclear whether they should be classified as void space or solid phase. Subsequently, the areas that were previously identified through histogram clamping as having a high probability of being either voids or solid areas were used as ‘seeds’ for growing a seeded region that would aid in classifying the unknown zero crossing regions. Seeded region growing enlarges the `seeds` until all unclassified areas are either classified as void or soil solid phase. The plugin produces a binary image of void areas (= 0) and solid (= 255) as output. Void areas that were identified represent ‘resolvable voids’, which are defined as those voids that are larger than the image resolution of 60µm. The solid areas that were identified represent ‘aggregate’ or ‘soil matrix’, which may contain a combination of mineral material, organic material, and perhaps voids smaller than our scale of observation. Segmentation allows for the structure to be evaluated as an entire entity, the void phase alone, or the solid phase alone. The various contributions to soil microstructure allow for quantification of the microstructural indices meeting all the objectives, and providing evidence to prove hypotheses false.
Figure 2.4: a) bimodal histogram described by two Gaussian distributions (centred on $T_{\text{min}}$ and $T_{\text{max}}$); $T_{\text{min}}$ distribution representing soil void phase; $T_{\text{max}}$ distribution representing soil solid phase b) uni-modal histogram described by a single Gaussian distribution (centred on $T_{\text{max}}$) with a long left tail; $T_{\text{max}}$ distribution representing predominantly soil solid phase; long left tail representing soil void phase

2.3.2.1 Image Analysis: 3D Semivariance Analysis

The spatial variability of the grey-scale imagery was performed using a specially designed semivariance plugin, for ImageJ, which was based on the equation:

$$\gamma(h) = \frac{1}{2n} \sum_{i=1}^{n} (Z_i - Z_{i+h})^2$$

where $h$ is the distance (lag) between a pair of points; each pair of points is denoted with $(Z_i, Z_{i+h})$ for a total of $n$ pairs that can be counted in a given direction (Taina et al. 2013). This algorithm and its design are described in further detail by Taina et al. (2013). As is common practice in semivariance analysis the calculation was limited to 25% of the maximum distance in the shortest dimension of the imagery (8.16mm). The semivariance data that are presented were normalized, i.e., the total semivariance was divided by total sample variance, thereby expressing semivariance values as a ratio of the whole variance. The normalization allowed for evaluation of anisotropy of the structure of the semivariogram. The semivariograms were also generated from an image in which the void phase was masked. This means that the ‘total resolvable void’, as identified in the segmentation phase, was set to equal Not a Number (NAN). Consequently, the void areas were not included in the semivariance calculations. This was done in order to evaluate the soil structure, as evidenced in the spatial variability of radiodensity, which exists within the ‘aggregate’ portion of the imagery.
To calculate directional anisotropy the three orthogonal semivariograms were parameterized to represent a point in three-dimensional Cartesian space (X, Y, Z) for each lag distances. The x-value was the semivariance in the x-direction at that specific lag distance, with the corresponding y-value representing the y-direction semivariance, and the same for the z-value. The shortest distance from the point at each lag distance to a Euclidian vector with all components having a slope of 1 was calculated to generate a new curve. If there is isotropy between all orthogonal semivariograms this curve would be a straight line with values equal to 0 at all lag distances. This parameterization allowed for comparison of anisotropy in grey-scale variability between samples. The mean anisotropy is calculated as the mean of the anisotropy values for all lag distances. These semivariance parameters with the ‘void’ phase masked provide indices of how the soil varies with space within the solid matrix (aggregate) itself potentially indicating unobservable microporosity smaller than the image resolution. These indices provide object indications of soil microstructure variation with space, and allow for successful completion of objective 1.
2.3.3 Image Analysis: Morphometric Analysis of Voids

To fulfill Objective 1, the ImageJ plug-in, Analyze Particles (Doube et al. 2010), was used, and followed by calculations within Microsoft Excel (2010) to determine void sizes. Voids were segregated into large inter-macroaggregate (> 150,000 voxels or 32.4 mm$^3$), medium intra-aggregate (8-150,000 voxels or 0.002-32.4 mm$^3$), and small intra-aggregate voids (< 8 voxels or < 0.002 mm$^3$) to facilitate processing (Figure 2.5).

![Image](image.png)

Figure 2.5: Example imagery identifying voids of various size classes used in void phase analysis

The Analyze Particles plugin assigns a unique number to each individual void space, producing a 16-bit image with a maximum of 65,536 unique numbers. In some cases there are greater than this number of unique voids in an image, consequently, voids were divided in categories; 8 voxels was chosen for small voids because it is the smallest cube of voxels that you can still generate a morphometric shape. Anything smaller than only 2 voxels in any direction, does not have morphometric characteristics. The
large inter-aggregate void was evaluated as their proportion of the total sample volume, whereas the small and medium intra-aggregate voids were evaluated for their proportion of the total soil solid ‘aggregate’ or ‘matrix’ volume. Further, for the medium voids, a void size distribution that was evaluated on a logarithmic scale was determined. The distributions were compared using the same parameters (span, coefficient of curvature, coefficient of uniformity) as particle size analysis described by Gee & Bauder (1986). Additionally, the medium voids were evaluated for their shape, and classified according to Bullock et al. (1985) (Figure 2.6). The Analyze Particles plugin fits ellipsoids to a unique void space. To determine void shape (circularity, elongation) ratios of long, intermediate, and short axes of the ellipsoids can be calculated. The large voids category was almost always represented by one single branching void, but in some instances contained two unique void spaces. To produce metrics representing how connected this large void was, the ImageJ plug-in, BoneJ Connectivity (Doube et al. 2010) was used to quantify the connectivity of ‘aggregate’ phase (solid = 255).

Figure 2.6: Schematic showing void phase shapes used in void phase analysis as classified by Bullock et al. (1985)
2.4 Penetrometer Resistance
Penetration resistance was measured using an ELE Digital Tritest 50, instrumented with load cells for measurements of compressive force (ELE International Ltd., Hemel Hempstead, England). A cone penetrometer with a 30° cone angle and a basal diameter of 4 mm was used to make penetrometer resistance measurements. Resistance was measured in each sample at a constant rate of penetration (3.6 mm/min) in the centre of each core. Readings were obtained to a depth of 3.5cm, gathering a data point every 1 second or 0.06mm, corresponding to the X-ray image resolution of 60µm. The penetrometer probe was penetration resistance measurements were used both as a tool to validate X-ray µCT density measurements with depth to meet objective 2, and as soil microstructural index indicating soil compaction to meet objective 1.

2.5 Statistical Analysis
A Kruskal-Wallis nonparametric analysis of variance, followed by a Dunn-Nemenyi post hoc analysis was used to compare means across the 4 species (groups). The Kruskal-Wallis analysis was implemented in SAS (SAS v. 9.4, SAS Institute 2013) using a macro developed by Elliott & Hynan, 2011. The nonparametric analysis was chosen because the data did not meet the parametric ANOVA assumptions of normality; however, the data was continuous, independent and variances were homogeneous. The model was used to investigate covariance structure of the data (total percentage void; small, medium, large proportions; medium void size distribution and shapes, soil matrix phase connectivity, and soil matrix phase spatial variability). The Dunn-Nemenyi analysis, as described by Elliott & Hynan (2011) was done to identify pairwise differences among species. Using the macro, significance was determined at p =0.05.

The Friedman non-parametric repeated measures test, followed by a Wilcoxon Signed-Rank post hoc test was used to compare means across 3 sampling times. The analysis was implemented in SAS (SAS v. 9.4, SAS Institute 2013). The model was used to investigate significant differences with time for the data (total percentage void; small, medium, large proportions; medium void size distribution and
shapes, soil matrix phase connectivity, and soil matrix phase spatial variability). Using the Wilcoxon signed-rank test, significant differences between paired sampling times were determined at a Bonferroni corrected p-value = 0.0167. Bonferroni adjustment was done on the results of the test because we were making multiple comparisons, and were more likely to have a Type I error.

A Wilcoxon two-sample test was used to compare means between the two management systems (TBI, and solecrop), and separately the two different sampling distances from the tree rows (2m, and 6m). The analysis was also completed in SAS (SAS v. 9.4, SAS Institute 2013), for the same data as the previous tests. Significance was again determined at a Bonferroni corrected p-value = 0.0167.

The SAS code and interpretation was completed with the assistance of the textbook titled ‘Discovering Statistics Using SAS’ (Field & Miles 2010). Example code can be found in Appendix 1: Example SAS Code.
Chapter 3: Characterizing surface soil structure in a temperate tree-based intercropping system using X-ray computed tomography

3.1 Introduction

Tree-based intercropping (TBI) is one of many temperate agroforestry systems where trees and annual crops are grown together (Lelle and Gold 1994). These systems are usually designed with rows spaced sufficiently wide enough to allow row crops to grow in between them and to be accessed by farm machinery. Today, agroforestry is considered an integrated applied science that can potentially address many land management and environmental problems, in both developing and industrialized nations (Nair et al. 2009). Investigations conducted over the past 20 years, in a TBI system in southern Ontario, Canada, have revealed several complementary biophysical interactions between trees and soil. These observations include the transfer of nitrogen to adjacent crops, increased soil organic carbon, and higher earthworm abundances close to trees, all of which occur largely as a result of tree litterfall inputs and fine root turnover (Thevathasan and Gordon, 2004).

X-ray computed micro-tomography (X-ray μCT) is a non-destructive, non-invasive technique that has been successfully used for more than two decades in 3-dimensional (3D) studies of soil structure (Crestana et al. 1986, Taina et al. 2008), which also offers a solution to the limitations posed by traditional thin-section analysis (Heck 2009). There are many types of X-ray μCT acquisition systems, but they all contain common elements: an X-ray source, an object, and a detector system to measure the extent to which X-rays have been attenuated by the object. Computed tomography’s fundamental principle is to acquire multiple views of an object over a range of angular orientations, thereby allowing for 3D reconstruction and visualization (Ketcham and Carlson 2001). By using the X-ray μCT imagery, soil can be analyzed spatially and quantitatively.

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Most applications of X-ray µCT for soil investigations have focused on evaluating soil microstructure, which is represented by the quantitative characterization of porosity and void networks (Taina et al. 2008). Recently, Udawatta et al. (2008) demonstrated the applicability of X-ray µCT for examining soil structure within an agroforestry system, comparing agroforestry systems with grass buffers. However, there is still a need to understand variation in soil structure within agroforestry systems, especially those involving intercropping with various tree species. In this study, we hypothesize that there would be significant differences in surface soil (uppermost 3.5 cm) microstructure between tree species, soil organic carbon, and bulk density. Further, it was also hypothesized that in the soil surface, soil matrix radiodensity would have significantly different variability structures in tree rows compared to willow biomass, and annual cropping alleys.

3.2 Methods

3.2.1 Sample Site and Collection

Soil samples were collected at the site of a long-term TBI research project, which was initiated in 1987, on a 30-ha field at the University of Guelph Agroforestry Research Station, Ontario, Canada (43°32′22″ N, 80°12′32″ W). In the alleys of ten tree species, maize (Zea mays L.), soybean (Glycine max (L.) Merr.), and winter wheat (Triticum aestivum L.) or barley (Hordeum vulgare L.) are planted in a maize-bean-grass rotation. Alleys are spaced with 12.5 or 15 m widths between tree rows, which contained individual trees that are spaced 3 or 6 m apart. The soil is locally mapped as a Guelph Sandy Loam, a Grey-Brown Luvisol under the Canadian System of Soil Classification (SCWG 1998). A more detailed description of the site and its management history can be found in Bainard et al. (2012).

Soil sampling for this study was during May and June of 2012, in coordination with excavations performed by fellow researchers to expose entire tree root systems within a 16 m² area. Consequently, sampling for X-ray µCT analysis occurred prior to excavations. The top 15 cm of soil near four species of trees were sampled using acrylic tubes, according to a technique that was developed in the Soil Imaging Laboratory, University of Guelph (Taina et al. 2013). Cores were collected at 3 locations relative to the
tree trunk, i.e., 2 m eastward and westward into the crop alleys (tilled soils and willow biomass), and 2.0 m north along the tree row (perennially vegetated soils). Three replicates of the soils were taken from the system under Norway spruce (*Picea abies* L.) and each of the following hardwoods: black walnut (*Juglans nigra* L.), hybrid poplar (*Populus* spp., clone DN 177), and northern red oak (*Quercus rubra* L.) all within the same field.

### 3.2.2 X-ray µCT Image Acquisition and Preprocessing

Prior to CT imaging, soil cores were dried to a constant weight at 40°C, in order to eliminate as much of the water phase as possible from the sample. X-ray µCT imagery of the soil was obtained using a MS8x-130 Micro µCT Scanner (EVS Corp., Toronto, ON) at 120 kV and 155 µA, with an acquisition pixel size of 20 x 20 µm. Volumes of the surface 3.5cm of the 15 cm cores were subsequently reconstructed with volumetric picture elements (voxels) at a spatial resolution of 60 µm (voxel size: 60 x 60 x 60 µm; slice thickness 60 µm), using the eXplore Reconstruction Utility of GE Healthcare software (2005). Low-pass Gaussian smoothing with a radius of 1 was applied to the data in MicroView (GE Healthcare 2005) to decrease image noise. Image cropping was performed to extract the centre portion of the soil core and to eliminate, from the analysis, the compacted edge areas of the core that were incurred during sampling, together with beam-hardening artifacts on the edge of the soil core. The individual cropped images were then imported into ImageJ 1.38V (Rasband 2007), after converting the images from a vff to tiff file format. The decision to focus the analysis on the surface 3.5cm of mineral soil was made to ease the image acquisition and time limitations of processing, but also because the surface of the soil is under the most degradation pressure. Soil surface degradation causing crusting or slumping from raindrop impact, or more intense wetting and drying in an unconfined condition can have consequences agriculturally (seedling emergence), and environmentally (flooding). Consequently, if there were differences in soil structures within the TBI system as affected by tree species, at our scale of observation, they would be beneficial to quantify at the surface.
Image segmentation was done using a special purpose plugin for ImageJ. This plugin was a modification of the technique that was outlined by Schlüter et al. (2010), for a proposed bimodal histogram described by two Gaussian distributions, with a lower grey-scale value distribution representing void space, and a higher grey-scale value distribution representing the soil matrix (Figure 3.1a). The first step in segmentation was to identify voxels in the image that were low variance regions (pure voxel extraction), as described by Elliott and Heck (2007), which were used to refine the image histogram and allow easier differentiation of peaks. The low variance bimodal histogram is ‘clamped’ at a lower threshold, $T_{\text{min}}$, where all voxels with a grey-scale values that are less than $T_{\text{min}}$ are assumed definitively to belong to the void space; at an upper threshold, $T_{\text{max}}$, all voxels with grey-scale values that are greater than $T_{\text{max}}$ are assumed to represent the solid soil matrix. However, this bimodal histogram does not always occur in soils which contain very little void space. The grey-scale attenuation distribution becomes predominantly uni-modal with a void phase that is represented by a long left tail on the histogram (i.e., left-skewed distribution) (Figure 3.1b). When there is only one peak, we must assume that this peak predominantly represents the soil solid phase, because all samples have a greater proportion of solid versus void. By finding the mean point of the single peak and subtracting one standard deviation from this value, it can safely be assumed that any grey-scale values that are higher than this threshold can be considered ‘soil solid phase’ with a high level of certainty. Further, to identify areas that are definitively ‘not soil solid phase’, all values that are less than 4 standard deviations from the peak mean were considered ‘not soil solid phase’ and, therefore, considered to constitute void space. The image was ‘clamped’ by having the lowest grey-scale value set to ‘mean – 4 standard deviations’, while the highest grey-scale value was set to ‘mean - 1 standard deviation.’
Immediately following this rescaling, a 3D Laplacian operator was used to identify regions in the clamped image with zero-crossings (i.e., areas in the image where the change in value is from positive to negative, or vice versa). These voxels are what would be considered problem areas, as they were zones of transition. Consequently, it was unclear whether they should be classified as void space or solid phase. Subsequently, the areas that were previously identified through histogram clamping as having a high probability of being either voids or solid areas were used as ‘seeds’ for growing a seeded region that would aid in classifying the unknown zero crossing regions. Seeded region growing enlarges the ‘seeds’ until all unclassified areas are either classified as void or soil solid phase. The plugin produces a binary image of void areas (= 0) and solid (= 255) as output. Void areas that were identified represent ‘resolvable voids’, which are defined as those voids that are larger than the image resolution of 60µm. The solid areas that were identified represent ‘aggregate’, which may contain a combination of mineral material, organic material, and perhaps voids smaller than our scale of observation.

### 3.2.3 Void Phase Analysis

The ImageJ plugin, Analyze Particles (Doube et al. 2010), was used, and followed by calculations within Microsoft Excel (2010) to determine void sizes. Voids were segregated into large inter-macroaggregate (> 150,000 voxels or 32.4 mm³), medium intra-aggregate (8-150,000 voxels or 0.002-32.4 mm³), and small intra-aggregate voids (< 8 voxels or < 0.002 mm³) to facilitate processing. The
Analyze Particles plugin assigns a unique number to each individual void space, producing a 16-bit image with a maximum of 65,536 unique numbers. In some cases there are greater than this number of unique voids in an image, consequently voids were divided. 8 voxels was chosen for small voids because it is the smallest cube of voxels that cannot provide any morphometric data due to only having 2 voxels in all directions. The large inter-aggregate void was evaluated as their proportion of the total sample volume, whereas the small and medium intra-aggregate voids were evaluated for their proportion of the total soil solid ‘aggregate’ volume. Further, for the medium voids, a void size distribution that was evaluated on a logarithmic scale was determined. The distributions were compared using the same comparisons as particle size analysis described by (Gee and Bauder 1986). Additionally, the medium voids were evaluated for their shape, and classified according to Bullock et al. (1985). The large voids category was almost always represented by one single branching void, but in some instances contained two unique void spaces. To produce metrics representing how connected this large void was, the ImageJ plugin, BoneJ Connectivity (Doube et al. 2010) was used to quantify the connectivity of solid phase (solid = 255).

A Kruskal-Wallis nonparametric analysis of variance, followed by a Dunn-Nemenyi post hoc analysis was used to compare means across the 4 species (groups) for samples collected in the perennial vegetated area. The Kruskal-Wallis analysis was implemented in SAS (SAS v. 9.4, SAS Institute 2013) using a macro developed by Elliott and Hynan, 2011. The nonparametric analysis was chosen because the data did not meet the parametric ANOVA assumptions of normality, however, the data were continuous, independent and variances were homogeneous. The model was used to investigate covariance structure of the data (total percentage void; small, medium, large proportions; medium void size distribution and shapes). The Dunn-Nemenyi analysis as described by Elliott and Hynan, 2011, was done to identify pairwise differences among species. Using the macro, significance was determined at p =0.05.
3.2.4 Soil Matrix Phase Analysis

Mean X-ray bulk radiodensity was determined by averaging the entire soil volume to obtain the mean Hounsfield value, to test for correlations with traditionally measured soil bulk density. Further, the intra-aggregate X-ray radiodensity was determined by averaging the entire soil volume with the void space masked to obtain the mean Hounsfield value, to test for correlations with soil organic carbon. Last, the spatial variability of the grey-scale imagery was performed using a specially designed semivariance plugin, for ImageJ, which was based on the equation:

$$\gamma(h) = \frac{1}{2n} \sum_{i=1}^{n} (Z_i - Z_{i+h})^2$$

where $h$ is the distance (lag) between a pair of points; each pair of points is denoted with $(Z_i, Z_{i+h})$ for a total of $n$ pairs that can be counted in a given direction (Taina et al. 2013). This algorithm and its design are described in further detail by Taina et al. (2013). As per common practice in semivariance analysis the calculation was limited to 25% of the maximum distance in the shortest dimension of the imagery (8.16mm). The semivariance data that are presented were normalized, i.e., the total semivariance was divided by total sample variance, thereby converting semivariance values to proportions of the whole variance. The normalization allowed for better comparison of the structure of the semivariogram identifying anisotropy between directions. The semivariograms were also generated to evaluate only the aggregate x-ray radiodensity, i.e. ‘Void’ was set to equal Not a Number.

To calculate directional anisotropy the three orthogonal semivariograms were parameterized to represent a point in three-dimensional Cartesian space (X, Y, Z) for each lag distances. The x-value was the semivariance in the x-direction at that specific lag distance, with the corresponding y-value representing the y-direction semivariance, and the same for the z-value. The shortest distance from the point at each lag distance to a Euclidian vector with all components having a slope of 1 were calculated to generate a new curve. If there is isotropy between all orthogonal semivariograms this curve would be a straight line with values equal to 0 at all lag distances. This parameterization will allow for comparison of
anisotropy in grey-scale variability between samples. The mean anisotropy is calculated as the mean of the anisotropy values for all lag distances.

Again the Kruskal-Wallis nonparametric analysis of variance, followed by a Dunn-Nemenyi post hoc analysis was used to compare means across the 3 sample locations for soil matrix parameters; mean semivariance anisotropy, and coefficient of variation. Further, Spearman correlation analysis was implemented in SAS to test for relationships between X-ray μCT measured radiodensity, and traditionally measured bulk density. Significance was determined at $p = 0.05$ for both types of analysis.

### 3.3 Results and Discussion

#### 3.3.1 Soil Void Phase Analysis

Nonparametric analysis of variance of the total resolvable voids revealed that, in the perennially vegetated soils (within the tree rows) there were no differences among tree species ($H(3) = 2.74, p=0.43$). There were also no significant differences in the quantity of total void space that was represented by one large void ($H(3) = 2.13, p=0.55$) in the perennially vegetated soils under different tree species. When investigating the proportion of intra-aggregate void in perennially-vegetated soils there was also no significant effect of species on medium voids ($H(3) = 2.13, p=0.55$), but small voids were significantly affected by species ($H(3) = 7.82, p=0.05$) (Figure 3.2). Soils under poplar had a significantly greater proportion of small intra-aggregate voids than soils under spruce ($q=3.69$). However, the medium-void size distributions showed no statistical differences in the coefficient of uniformity ($H(3)=1.87, p=0.60$), coefficient of curvature ($H(3)=3.05, p=0.38$), or span ($H(3)=2.65, p=0.45$). Further, soils under different tree species did not exhibit a significant difference in medium sized void shapes (equant $p=0.23$, prolate $p=0.23$, oblate $p=0.19$, triaxial $p=0.99$, planar $p=0.46$, acicular $p=0.93$, and acicular-planar $p=0.32$). Last, soil solid phase connectivity determined that the perennial vegetated soils did not have a significant interaction with tree species type ($H(3) = 7.00, p=0.07$).
Figure 3.2: Small Intra-aggregate void space as a percentage of solid ‘aggregate’ volume identified by X-ray \( \mu \text{CT} \) for four tree species. Bars show max and min, box represents 25th -75th percentile, and middle line represents median value (n=3). Bars labelled with the same letters were not significantly different according to Kruskal-Wallis test and Nemenyi-Dunn Post hoc test.

Tree species affect several variables which may result in changes to the void space properties of surface soils. However, it is difficult to quantify the significance of these effects, as there is an inadequate perception of inherent soil heterogeneity within the systems (Stone, 1975). Stone (1975) further concluded that the effect of introduced trees on soils (profile genesis and morphology) is slow or very limited. Further compounding the argument, Augusto et al. (2002) stated the long-term effects of spruce on soil structure and porosity are still unclear and site factors (e.g., parent material, texture) seem to play an important role. Probably the most cited tree species effect on soils is the variability in tree leaf litter quantity, and chemical inputs to surrounding soils (Binkley 1996). Wotherspoon (2014) studying the same TBI system determined by \textit{in situ} measurements that highest litterfall was found beneath poplar trees, followed by walnut, spruce, and oak with 3.79, 3.48, 2.97, and 2.50 t ha\(^{-1}\) y\(^{-1}\), respectively. In relation to C inputs to soil, the same study indicated that poplar, walnut, oak and spruce added 1.63, 1.50,
1.07, and 1.49 t C ha\(^{-1}\) y\(^{-1}\), respectively. It was also determined that the rate of litter decomposition varied between tree species with walnut being significantly higher and spruce being the lowest due to the presence of varying amounts of lignin present in the litter.

Fundamentally, the litterfall differences in quality and quantity should have an effect on soil properties (Binkley, 1995); however, within a mixed species intercropping system these effects may be less distinct (Rothe and Binkley, 2001). Ferrari and Sugit (1996) examined spatial patterns in litterfall within a mixed forest of hardwood and conifer species. The average radius within which 90% of the leaf litter fell to a trap originated within 17.1 m ± 1.8m (crown radius ranging from 2.8-5.1 m from the trunk). Moreover, the proportion of litter that fell directly beneath the canopies of the isolated trees ranged from 12 to 50%. This reported radius, of litterfall, is greater than the width of the alleys within the studied TBI system, meaning that the tree species influence on adjacent soil could have been in other areas of the field and not just under the tree canopy where soils were sampled. This was confirmed by work completed by Wotherspoon (2014), showing no significant differences between litterfall accumulations at 1m compared to 4m into cropping alleys. Furthermore, the samples collected for our analysis, were collected from within the tree row. The tree row qualitatively contained very similar amounts, and species of perennial vegetation as it was not annually tilled. Perennial grasslands have been shown to have significant effects on soil organic carbon, nutrients, and microbial activity (Culman 2010), as well as in the formation and stabilization of soil structure (Oades 1993). Consequently, since tree litter accumulation from a single species is unlikely to be isolated under the canopy of that tree, and the tree buffers contain similar perennial vegetation, we can, with some confidence, understand the similarity in measured soil void characteristics between tree species.

Rates of litter decomposition in ecosystems, among many important drivers, can be influenced by differences in the composition of decomposer communities and their trophic interactions (Beare et al. 1992; Šnajdr et al. 2013). At the University of Guelph Agroforestry Site, Price and Gordon (1999) found
that across various seasons, earthworm densities were almost always highest closest to the poplar treatment compared to silver maple \((Acer saccharinum \text{ L.})\) and white ash \((Fraxinus americana \text{ L.})\). This trend was attributed to the higher concentrations of organic matter that originated from poplar leaf litter. Tree species effects on faunal and microbial community are important, as the soil microbial community has long been recognized as a vital component in the process of soil aggregate formation and, consequently, effect both soil void quantity and complexity. This interaction between void characteristics, organic matter and microbial community could be an interesting relationship to investigate in the future.

### 3.3.2 Soil Matrix Phase Analysis

Differential leaf litter distribution could also cause differences in soil organic carbon. In the early establishment stages of TBI system, Thevathasan et al. (1999), at a different study site also located in southern Ontario, found significantly lower quantities of soil organic carbon (SOC) under poplar trees compared to walnut trees. However, Wotherspoon (2014) evaluating the same tree species at the same site as this study did not detect any significant differences in SOC between tree species at a depth of 0-10cm. Again this change can be related to leaf litter distribution. As indicated by Thevathasan and Gordon (2004), as trees age, their litterfall is more evenly distributed across the crop alley, reducing isolated effects of tree species. In the early 1990s, when the trees were smaller, litterfall was distributed close to the tree row, 2-3m (Thevathasan and Gordon 2004). By 2002, poplar trees were 14m tall, and the leaf litter was being spread across the cropping alley at distances up to 15m (Thevathasan and Gordon 2004). Although there were no differences by tree species, there was still heterogeneity in the sampled SOC values. However, when trying to correlate the measured X-ray μCT void characteristics to SOC, no significant relationships were seen. There was, however, a significant positive correlation between X-ray bulk radiodensity and traditionally measured bulk density \((r_s=0.53, p<0.01)\) (Figure 3.3). There was also a significantly negative correlation identified between mean intra-aggregate X-ray radiodensity and SOC \((r_s=-0.48, p=0.03)\) (Figure 3.4). This relationship suggests, as expected, that the X-ray CT method is identifying relationships related to SOC and bulk density. Consequently, since there are no significant
differences between the amount of SOC and bulk density in soils under specific tree species, no relationship is seen either between tree species and measured X-ray µCT void parameters. This suggests, at the scale of observation of the void-phase analysis, there are alternate controlling factors than tree species which are affecting void parameters, and SOC accumulation. Stone (1975) discussed the effects of tree species on soils from three viewpoints: nutrient cycling, soil genesis and classification, and short term changes in soil properties. As previously mentioned, the summary by Stone (1975) suggests that it is difficult to determine changes due to species because of the slow or limited effect, and also the lack of understanding of existing soil heterogeneity in the systems. Further, Stone (1975) indicates that maximum changes in soil, due to choice of species, seem to be minor in comparison with the mechanical and chemical impacts associated with the agricultural management, which could be evident in this particular study, especially in the surface soil as investigated.

![Figure 3.3: Correlation of bulk density (y-axis), with X-ray µCT bulk radiodensity (x-axis); Insert shows Spearman correlation coefficient and p-value (n=24)](image-url)

- $r_s = 0.53$, $p<0.01$
Mean anisotropy between directional semivariograms for different ground cover types revealed significant differences \((H(2)=10.5671, \ p=0.0051)\) (Figure 3.5). Soils collected within the tree row had significantly less mean directional anisotropy than soils in annually cropped areas, with biomass-willow soils falling in-between. To get an understanding of the magnitude of variability in soil matrix radiodensity among soils of different ground cover, the COV was determined. The soils collected within the tree row had a significantly higher COV than the cropped, and willow biomass areas \((H(2)=13.07, \ p=<0.001)\) (Figure 3.6). No significant differences were determined between cropped areas, and those soils collected in willow biomass plots, although there seems to be a slight trend showing increased COV in biomass-willow soils. The correlation between mean anisotropy and COV was insignificant at \(p=0.05\), however an apparent inverse relationship was significant at \(p=0.1\) \((r_s=-0.26, \ p=0.12)\). These relationships suggest that, if the soil matrix is more random, the amount of anisotropy seen with direction will be less (ie. trends of radiodensity in specific directions are eliminated).
Figure 3.5: Mean directional anisotropy of soil matrix radiodensity identified by X-ray µCT semivariogram analysis for three types of ground cover. Bars show max and min, box represents 25th -75th percentile, and middle line represents median value (n=11 for cropping alley, n=13 for willow, and n=12 for tree row). Bars labelled with the same letters were not significantly different according to Kruskal-Wallis test and Nemyeni-Dunn Post hoc test.
Figure 3.6: Coefficient of variability of soil matrix radiodensity identified by X-ray μCT for three types of ground cover. Bars show max and min, box represents 25th -75th percentile, and middle line represents median value (n=11 for cropping alley, n=13 for willow, and n=12 for tree row). Bars labelled with the same letters were not significantly different according to Kruskal-Wallis test and Nemyeni-Dunn Post hoc test.

The tree rows, which were uncultivated and contained perennial grasses and weeds, as expected showed differences for semivariance parameters. Biological activity of both grass roots and soil microorganisms tend to be greater under perennial vegetation than under row-crop management (Culman 2010). Paudel et al. (2011) determined that higher enzyme activities in perennial vegetation treatments were correlated with increased organic carbon accumulation and higher root activity. Paudel et al. (2011) further indicated that there was a feedback, in that the greater organic carbon supplied by roots, by perennial vegetation, led to greater microbial activity and biomass accumulation. In this study, the greater
randomness of X-ray radiodensity, correlated with less directional anisotropy observed within the tree row was interpreted as a more homogeneous structure (no layering) as a result of the aforementioned soil processes. The higher organic carbon, higher root activity, and higher microorganism activity are known to be factors which increase aggregation, and promote the creation of hierarchical aggregation and improved soil structure (Oades, 1993). The row crop soils have been managed using tillage practices, and it is well-known that tillage disrupts soil aggregates, compacts soil, and disturbs soil organisms that contribute to aggregation (Bronick and Lal 2005). Consequently, there is likely more destruction of soil structure at the surface in the row crop soils either by crusting, or by compaction. In the case of destruction of soil structure, it is more likely that directional anisotropy may arise where there are trends in X-ray radiodensity in a particular direction, usually the z-direction (vertical). In almost all samples, where anisotropy was observed, it was primarily caused by differences between the ‘vertical-direction’ (z) compared to the ‘horizontal-directions’ (x,y) which tended to be more isotropic. In cases where there was a greater amount of anisotropy, the ‘z-direction’ usually showed an exponential dependence, evidenced by a rapid increase in semivariance with distance. As discussed by Yang et al. (2001), when interpreting semivariograms, this indicates strong spatial dependence and is usually caused by a continuous increase or decrease of the variable of interest; in this case X-ray radiodensity with distance. The different type of anisotropic relationships can be seen in a portion of the collected samples shown in Figure 3.7.
Representative example semivariograms, for intra-aggregate radiodensity, from soils adjacent to poplar, oak, walnut, and spruce trees can be seen in Figure 3.8. There were no distinct or consistent anisotropic structures evident for the various species (Kruskal-Wallis of mean anisotropy $H(3) = 0.29$, $p=0.96$). There also was no significant difference among treatments in COV of radiodensity ($H(3)=3.33$, $p=0.34$), suggesting the distribution of intra-aggregate radiodensity was similar among treatments. Relationships were also investigated for relation to SOC. The correlation between SOC and mean
anisotropy proved to be insignificant \( r_s=-0.071, p=0.7950 \). As suggested by Nunan et al. (2006), at the fine resolution of this imagery (60µm), the degree of structural variability seen in the data, as revealed by the geostatistical parameters, suggests that the physical framework of the soil at the scale of observation is highly heterogeneous. The lack of significant differences between tree species may reflect that the soils were collected from different locations within a field, and at this scale of observation inherent soil particle radiodensity (especially mineralogy and texture) has a greater effect on variability than potential carbon inputs from the TBI management system tree species.

### 3.4 Summary and Conclusion

Our goal was to examine X-ray µCT-measured voids, and quantify spatial variability in X-ray radiodensity as influenced by tree species. X-ray µCT measured void characteristics were not found to be significantly affected by the tree species. We did not find that tree species had an effect on void characteristics at this level of observation (top 3.5 cm); this being attributed to mixed leaf litter in the system, and soil samples being collected under perennial (grass) vegetation. Further, correlations between soil organic carbon and intra-aggregate X-ray radiodensity, and between soil bulk density and bulk X-ray radiodensity were detected. Through the use of semivariograms, it showed that there was greater variability, correlated with less directional anisotropy observed within the tree row as compared to soils in the cropping alley. It was interpreted that tree row soils had a higher degree of structure, and that the structure of row-cropped soils had been disturbed, leading to more directional anisotropy. Additionally, it was determined, through the use of geostatistics, that there were no distinct or consistent anisotropic structures in directional semivariograms evident for the various tree species. The distribution of intra-aggregate radiodensity was similar among species. This study supports the theoretical point of view, proposed by Augusto et al. (2002): The impact of overstory species on soil fertility (or as in this case the structure component) is not significant as along as the processes of the system being modified do not limit the functioning of the trees or other parts of the soil system (factors affecting aggregation).
Figure 3.8: Example semivariograms for soils from under (a) poplar, (b) oak, (c) spruce, (d) walnut
4 Chapter 4: Characterizing soil surface structure in tree-based intercropping and solecrop systems

4.1 Introduction

Tree-based intercropping (TBI) is one of many temperate agroforestry systems where trees and annual crops are grown together (Lelle & Gold 1994). These systems are usually designed with rows spaced sufficiently wide enough to allow row crops to grow in between and to be accessed efficiently by farm machinery. Today, agroforestry is considered an integrated applied science that can potentially address many land management and environmental problems, in both developing and industrialized nations (Nair et al. 2009). Investigations conducted over the past 20 years, in a TBI system in southern Ontario, Canada, have revealed several complementary biophysical interactions between trees and soil. These observations include the transfer of nitrogen to adjacent crops, increased soil organic carbon, and higher earthworm abundances close to trees, all of which occur largely as a result of tree litterfall inputs and fine root turnover (Thevathasan and Gordon, 2004).

X-ray computed micro-tomography (X-ray μCT) is a non-destructive, non-invasive technique that has been successfully used for more than two decades in 3-dimensional (3D) studies of soil structure (Crestana et al. 1986, Taina et al. 2008), which also offers a solution to the limitations posed by traditional thin-section analysis (Heck 2009). There are many types of X-ray μCT acquisition systems, but they all contain common elements: an X-ray source, an object, and a detector system to measure the extent to which X-rays have been attenuated by the object. Computed tomography’s fundamental principle is to acquire multiple views of an object over a range of angular orientations, thereby allowing for 3D reconstruction and visualization (Ketcham & Carlson 2001). By using the measured X-ray μCT radiodensity imagery, the soil’s microstructure can be analyzed spatially and quantitatively.
Most applications of X-ray μCT for soil investigations have focused on evaluating soil microstructure, which is represented by the quantitative characterization of porosity and void networks (Taina et al. 2008). Recently, Udawatta et al. (2008) demonstrated the applicability of X-ray μCT for examining soil structure within an agroforestry system, comparing agroforestry systems with grass buffers. However, there is still a need to understand variation in soil structure within agroforestry systems, especially those involving inter-cropping with various tree species. Further, there is a need to determine the effect of tree-based intercropping compared to a conventional solecrop agricultural system, and the temporal effects on soil structure within tree-based intercropping (tree species and distance from trees). In this study, we hypothesize that there would be significant temporal variations (over the growing season) in surface soil (uppermost 3.5 cm) microstructure.

4.2 Methods

4.2.1 Sample Site & Collection

Soil samples were collected at the site of a long-term TBI research project, which was initiated in 1987, on a 30-ha field at the University of Guelph Agroforestry Research Station, Ontario, Canada (43°32’28” N, 80°12’32” W) (Figure 4.2). In the alleys of ten tree species, maize (Zea mays L.), soybean (Glycine max (L.) Merr.), and winter wheat (Triticum aestivum L.) or barley (Hordeum vulgare L.) are planted in a maize-bean-wheat/barley rotation. Alleys are spaced with 12.5 or 15 m widths between tree rows, which contained individual trees that are spaced 3 or 6 m apart. Immediately adjacent to the TBI system is a small field plot which is under the same crop rotation, but is managed as a conventional solecrop system. The soil is locally mapped as a Guelph Sandy Loam (Hoffman et al. 1963), a Grey-Brown Luvisol under the Canadian System of Soil Classification (SCWG 1998). A more detailed description of the site and its management history can be found in Bainard et al. (2012).

The temporal sampling occurred on three occasions (June 2012, October 2012, and April 2013), sampling 2 and 6 meters East and West from the tree trunk into cropping alleys within the agroforestry
management area (Figure 4.1). Three replicates of each *Deciduous*: (black walnut, hybrid poplar, red oak), *Evergreen*: (Norway spruce), and *Conventional Agriculture*: (Soybean) [only in October & April] were sampled (Figure 4.2). All tree replicates were chosen by random selection, and sampling in different directions allowed determination of an east-west variation from wind-directed litterfall. In addition, the solecrop conventional management was sampled at 4 locations across a slope transect (only October 2012, and April 2013).

Figure 4.1: Schematic of Sampling Locations in Reference to Tree

4.2.1 X-ray µCT Image Acquisition and Preprocessing

The acquisition and preprocessing procedure has been described in detail in Section 3.2.2.

4.2.2 Void Phase Analysis

The void phase analysis procedure has been described in detail in Section 3.2.3.

Connectivity is defined as the degree to which a structure is multiply connected (Odgaard and Gundersen 1993). Connectivity was run on the soil solid matrix phase to interpret the complexity of the soil void structure. Connectivity was interpreted to change under two scenarios:
a) fragmentation: a decrease in void volume caused by fragmentation of larger voids, increasing the number of void objects, and consequently increasing soil matrix phase connectivity;

b) extinction: a decrease in void volume caused by extinction of voids, decreasing the number of void objects, and decreasing soil matrix phase connectivity.

Figure 4.2: Agroforestry Research Site. Google Satellite imagery viewing from South to North, highlighting sampling locations.

The relationship of increasing number of void objects (greater efficiency packing) was confirmed by a small test completed in ImageJ, comparing spherical packing of simple cubic packing, and hexagonal close packing (Figure 4.3). Connectivity decreased from 13 to 8 between a single layer of cubic packing to hexagonal close packing, with the same volume.

### 4.2.3 Soil Matrix Phase Analysis

The soil matrix phase analysis procedure has been described in detail in Section 3.2.4.
4.3 Results & Discussion

4.3.1 Void Phase Analysis

4.3.1.1 Void Phase Parameters Change with Time – Pooled Samples

To measure the seasonal affect on soil void phase parameters, the data collected from all samples during each sampling month were pooled together. Almost all measured void phase parameters showed significant differences with time of sampling (Table 4.1). Most of the differences occurred with the month of April 2013, however many parameters had differences between June 2012 and October 2012 (Figure 4.4). Total void showed a significant decreasing trend from June 2012 (17.6%±0.89) to October 2012 (12.6%±0.88) to April 2013 (8.9%±0.49). Void volume classified as large inter-aggregate void showed a similar decline as total void from June 2012 (95.1%±0.85) to October 2012 (93.6%±0.95) to April 2013 (88.3%±0.67). This relationship was expected as the total void of a sample has usually greater than 90% of its proportion represented by large voids.
Table 4.1: A comparison of measured soil void phase parameters between time of sampling. All samples collected for each month were pooled for analysis. To test for temporal changes a Friedman anova ($\chi^2$) was used, with a Wilcoxon Signed-Rank (S) test as a post-hoc test. Table shows the calculated test statistics of the respective tests, and the significance values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$X^2$</th>
<th>df</th>
<th>p-value</th>
<th>June vs October S</th>
<th>p-value</th>
<th>June vs April S</th>
<th>p-value</th>
<th>October vs April S</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Void</td>
<td>26.8</td>
<td>2</td>
<td>&lt;0.01</td>
<td>240</td>
<td>0.01</td>
<td>442</td>
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<td>-506</td>
<td>&lt;0.01</td>
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<td>-142</td>
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<td>175</td>
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<td>&lt;0.01</td>
<td>593</td>
<td>&lt;0.01</td>
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<tr>
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<td>-177.5</td>
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<td>-15.5</td>
<td>0.86</td>
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<tr>
<td>Cc</td>
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<td>-185</td>
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<td>-147.5</td>
<td>0.09</td>
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<td>0.41</td>
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<td>&lt;0.01</td>
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<td>0.60</td>
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<tr>
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<td>0.06</td>
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<td>0.17</td>
<td>317</td>
<td>&lt;0.01</td>
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<td>0.27</td>
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<tr>
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<td>0.12</td>
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<td>195</td>
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<td>0.37</td>
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<td>Triaxial</td>
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<td>0.55</td>
</tr>
<tr>
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<td>-206</td>
<td>0.03</td>
<td>-290</td>
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</tr>
<tr>
<td>Acicular-Planar</td>
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<td>-246</td>
<td>0.01</td>
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</tr>
<tr>
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<td>&lt;0.01</td>
<td>232</td>
<td>0.02</td>
<td>-3</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Note: June (N=48), October (N=52), and April (N=52). Cu = Coefficient of Uniformity, Cc= coefficient of curvature, and Span of the medium void size distribution.

The trend of decreasing large inter-aggregate voids was paralleled by a decrease in connectivity of the solid matrix phase. Connectivity represented as number of connections per total image volume decreased from June 2012 to October 2012 to April 2013, 67290±8266, 61949±7015, 21789±1753. The decrease in connectivity could be interpreted as extinction of soil voids. The voids classified as small and medium intra-aggregate void did not show as strong of a trend, but were still significant. The trend shown was as expected opposite of the large inter-aggregate void. The small intra-aggregate void increased from 0.012%±0.001 in June 2012 to 0.021%±0.001 in October 2012, to 0.024%±0.001 in April 2013. Medium intra-aggregate voids also showed an increasing trend from June 2012 (0.95%±0.08) to April 2013 (1.13%±0.02) but October 2012 (0.92%±0.04) accounted for a smaller proportion of intra-aggregate volume.
The temporal changes in soil void volume (Figure 4.4) observed in this study, agree with those reported in the Or et al. (2000) model of post-tillage void space evolution. These researchers outlined two principal processes: 1) aggregate disintegration during wetting and drying cycles, leading to smaller interaggregate voids, and 2) viscoplastic deformation and combining of wet aggregates leading to void deformation. Or and Ghezzehei (2002) illustrated this process, showing that after successive wetting and drying cycles aggregates became welded at their contacts, reducing inter-aggregate porosity. It is also possible that there is reduction in the inter-aggregate voids located at the soil surface as a result of ponding and soil crusting that likely occurred during snow melt and spring rainfall (Or and Ghezzehei 2002). However, the extent to which soil crusting occurred is unknown, and also likely would not account for the majority of the void space changes in the 3.5 cm depth of soil imaged, as many other factors including tillage, biological activity, or frost lensing are at work (VandenBygaart et al. 1999a).

The size distribution of the intra-aggregate voids was consistent, as evidenced by consistency in the span, coefficient of uniformity, and coefficient of curvature (Figure 4.5). However, the void shape of medium intra-aggregate voids does show a trend with time (Figure 4.6). Intuitively the dynamic nature of void shape makes sense. Soil texture differences can cause a change in soil void shape, due to its influence on processes such as packing and shrinking/swelling (Chun et al. 2008, Or et al. 2000). Chun et al. (2008) observed that an increase in clay content caused a significant increase in voids with irregular and rounded shape. Since the samples collected over time were collected from various locations

Figure 4.4: A comparison of measured soil void phase parameters between time of sampling. All samples collected for each month were pooled for analysis. Note June (N=48), October (N=52), and April (N=52). (a) Total resolvable void proportion of volume; (b) large void as a proportion of total resolvable; (c) medium void as a proportion of total resolvable; (d) small void as a proportion of total resolvable; (e) connectivity of solid phase
Figure 4.5: A comparison of measured medium intra-aggregate void size distribution parameters between time of sampling. All samples collected for each month were pooled for analysis. Note June (N=48), October (N=52), and April (N=52), and all proportions are as those of classified voids. (a) span; (b) coefficient of curvature (Cc); (c) coefficient of uniformity (Cu);

Figure 4.6: A comparison of measured soil void phase shape parameters between time of sampling. All samples collected for each month were pooled for analysis. Note June (N=48), October (N=52), and April (N=52), and all proportions are as those of classified voids.
there is likely some variance in soil texture depending on the sampling location. Consequently, it is not surprising that the proportion of the various void shapes seem to change with time. The planar, acicular, acicular-planar, and triaxial void shape increased significantly with time, from June 2012 to April 2013, whereas the proportion of equant and prolate shapes decreased from June 2012 to April 2013. This may be evidence of frost lensing, causing platy structure as evidenced by the elongated void shapes. Freeze-thaw processes may cause wide, lateral ice lens features in many soils, that when thawed leave voids intact with the horizontal orientation, and elongated shape (VandenBygaart 1999b). It is possible that there may have been an effect of moisture content at sampling, however, the effect of wetting and drying on void characteristics is more prevalent in the inter-aggregate porosity versus the intra-aggregate porosity (Or and Ghezzehei 2002).

### 4.3.1.2 Solecrop System vs. Tree-Based Intercropping System

Very few void phase parameters showed differences between the solecrop system (SC) and the tree-based intercropping system (TBI), with results from all soil cores of all tree species pooled (Table 4.2). The proportion of total resolvable voids classified as medium intra-aggregate voids was significantly greater in the TBI system compared to the solecrop during the sampling month of June (Figure 4.7). However, the connectivity of the soil matrix phase (Figure 4.7), and the span of the medium intra-aggregate void (Figure 4.8) were significantly greater under solecrop management compared to the TBI system. During the sampling month of April 2013, significant differences between the void shapes between the two systems were determined (Figure 4.9). Consequently, at this scale of observation, observing the surface 3.5cm it can be argued that there are no consistent differences between a SC and TBI system void phase structures that would indicate a system effect.
Table 4.2: A comparison of measured soil void phase parameters between TBI and solecrop systems. All samples collected for the TBI system were pooled for analysis. To test for differences a Wilcoxon two sample test was used. Table shows the calculated test statistics ($W_s$), the Z-score, the significance values, and the effect size ($r$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>October</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$W_s$</td>
<td>$Z$</td>
</tr>
<tr>
<td>Total Void</td>
<td>118</td>
<td>0.41</td>
</tr>
<tr>
<td>Large Void</td>
<td>151</td>
<td>1.54</td>
</tr>
<tr>
<td>Small Void</td>
<td>156</td>
<td>1.72</td>
</tr>
<tr>
<td>Medium Void</td>
<td>42</td>
<td>-2.20</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
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<td></td>
</tr>
<tr>
<td>C_U</td>
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</tr>
<tr>
<td>C_C</td>
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<td><strong>Size Distribution</strong></td>
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<td></td>
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<tr>
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<td><strong>Shape</strong></td>
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</tr>
<tr>
<td>Equant</td>
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<td>0.14</td>
</tr>
<tr>
<td>Prolate</td>
<td>119</td>
<td>0.45</td>
</tr>
<tr>
<td>Oblate</td>
<td>31</td>
<td>-2.57</td>
</tr>
<tr>
<td>Triaxial</td>
<td>111</td>
<td>0.17</td>
</tr>
<tr>
<td>Planar</td>
<td>100</td>
<td>-0.21</td>
</tr>
<tr>
<td>Acicular</td>
<td>100</td>
<td>-0.20</td>
</tr>
<tr>
<td>Acicular-Planar</td>
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</tr>
<tr>
<td>Connectivity</td>
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<td>2.09</td>
</tr>
<tr>
<td>Mean Anisotropy</td>
<td>82</td>
<td>-0.82</td>
</tr>
</tbody>
</table>

Note: solecrop (N=4), and TBI (N=48). C_U = Coefficient of Uniformity, C_C = coefficient of curvature, and Span of the medium void size distribution.

There are a number of reasons why there could be a lack of difference between these systems. One explanation is the common reoccurring disturbance of the soil structure from agricultural management occurring in both TBI and SC. The row crop soils have been managed using tillage practices (on occasion disc ripping, but frequently light disc), it is known that tillage disrupts soil aggregates, compacts soil, and disturbs soil organisms that contribute to aggregation (Bronick & Lal 2005). Consequently, within both systems there will be a consistent destruction of soil structure at the surface in the row crop soils either by crusting, or by compaction. In the case of destruction of soil structure, it is more likely that random variability may arise where changes and trends we see, cannot be directly related to a known cause.
Figure 4.7: A comparison of measured soil void phase parameters between solecrop and TBI systems. All samples collected for the TBI system were pooled for analysis. Note TBI (N=48), solecrop (N=4). (a) Total resolvable void proportion of volume; (b) large void as a proportion of total resolvable; (c) medium void as a proportion of total resolvable; (d) small void as a proportion of total resolvable; (e) connectivity of solid phase e
Figure 4.8: A comparison of measured medium intra-aggregate void size distribution parameters between solecrop and TBI systems. All samples collected for the TBI system were pooled for analysis. Note TBI (N=48), solecrop (N=4), between time of sampling, and all proportions are as those of classified voids. (a) span; (b) coefficient of curvature (Cc); (c) coefficient of uniformity (Cu);
Figure 4.9: A comparison of measured soil void phase shape parameters between solecrop and TBI systems. All samples collected for the TBI system were pooled for analysis. Note TBI (N=48), solecrop (N=4), and all proportions are as those of classified voids.
The fundamental difference between the TBI and SC systems is the presence of trees. Tree species affect several variables which may result in changes to the soil surface. Probably the most cited tree species effect on soils is the variability in tree leaf litter quantity, and chemical inputs to surrounding soils (Binkley 1996), one of the main inputs being organic carbon. Void phase structure of soil is strongly affected by soil organic carbon (SOC) (Kay 1988). Higher organic carbon, higher root activity, and higher microorganism activity are known to be factors which increase aggregation, and promote the creation of hierarchical aggregation and improved soil structure (Oades, 1993). The effect of higher organic carbon levels do not appear to be affecting our data, as evidenced by the inconsistent statistical differences between the two systems. One likely cause may be that the SOC in fact may not be different between the systems because of the consistent disturbance from row crop agricultural practices. Oelbermann et al. (2006), studying the same site, as in our work, determined to a depth of 20cm, the SOC pool between the TBI and SC system was not significantly different. The authors indicated that perhaps the alley cropping system had not been established long enough to detect any significant differences in the SOC pool. However, Wotherspoon (2014) investigated again the SOC pools of the same systems 27 years after establishment. Within the top 10cm of soil, no significant differences were determined between SOC for the SC and TBI management of 5 different species. However, Wotherspoon (2014) did find significant differences with depth >30cm in the soil profile. It is possible that trees may be influencing soil structure at these lower depths more so than at the soil surface. Voroney and Angers (1995) suggested that short-term management effects on SOC in temperate systems are usually not easily detected because of high variability of existing SOC in surface soils within the field. Additionally, changes in SOC are difficult to predict due the complexity of SOC constituents and their relative importance in various soils, climates and the type of crop grown (Voroney and Angers, 1995).
4.3.1.3  Distance from Tree Row

The TBI agroforestry system has a setup, with rows of trees spaced sufficiently wide enough to allow row crops to grow in between them and to be accessed by farm machinery. Due to the setup of the system there are possible differences in leaf litter distribution and organic carbon accumulation with distance from the tree row into the cropping alley. A study completed on the same TBI system, in a different portion of the field, found higher total soil organic carbon close to the tree row, possibly as a result of leaf biomass input (Thevathasan and Gordon, 1997). In the study poplar trees were 7-9 years old, and 80% of the total poplar leaf biomass deposited was within 2.5m of the tree row, with the rest in the 2.5-6.0m distance. This corresponded to a 1% higher SOC value <2.5m vs. 4-11m from the tree row. The authors mentioned that as the tree canopy develops they expected the area of increased SOC from litterfall to extend further into the cropping alley. This comment was supported by research completed by Wotherspoon (2014), showing no significant differences between litterfall accumulations, or SOC (surface 10cm) at 1m compared to 4m into cropping alleys in the same system as our study.

Very few void phase parameters had significant differences with distance from tree rows (Table 4.3). Differences occurred in some void shapes; in the month of June 2012, acicular-planar; in the month of October 2012, prolate and planar; and in the month of April 2013, oblate, triaxial, and acicular. The underlying factors causing these changes in void shape are unknown, as there are many variables that could cause these seemingly random changes.
Table 4.3: A comparison of measured soil void phase parameters by distance from tree row. All samples collected for all tree species at the specific distance were pooled for analysis. To test for differences a Wilcoxon two sample test was used. Table shows the calculated test statistics ($W_s$, the Z-score, the significance values, and the effect size ($r$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>June</th>
<th>October</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$W_s$</td>
<td>$Z$</td>
<td>p-value</td>
</tr>
<tr>
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</tr>
<tr>
<td>Volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large Void</td>
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</tr>
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<tr>
<td>Medium Void</td>
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<td>-0.87</td>
<td>0.40</td>
</tr>
<tr>
<td>C_U</td>
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<td>0.46</td>
</tr>
<tr>
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</tr>
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<td>Equant</td>
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<td>0.09</td>
</tr>
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</tr>
<tr>
<td>Oblate</td>
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<td>0.77</td>
</tr>
<tr>
<td>Triaxial</td>
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</tr>
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<td>0.57</td>
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</tr>
<tr>
<td>Mean Anisotropy</td>
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<td>-1.40</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Note: 2 meters (N=24), and 6 meters (N=24). Cu = Coefficient of Uniformity, Cc= coefficient of curvature, and Span of the medium void size distribution.

4.3.1.4 Effect of Tree Species

The effects of tree species on void phase characteristics were inconsistent, however, some showed significant differences (Table 4.4 and Table 4.5). For the sampling month of June 2012, oak trees appeared to show the most differences when compared to all other tree species. For the sampling period in October 2012, the solecrop area showed differences, mostly from the soils under walnut but not with other tested tree species. In the spring sampling in April 2013, soils from under the spruce trees were different from those under poplar trees.

During the sampling month of June 2012, the soils sampled under oak have a greater total void space than spruce, and walnut trees, with 23.0±1.30, 15.4±2.02, 12.5±1.28 percent, respectively (Figure 4.10). Consequently, soils under oak also have a greater proportion of large inter-aggregate voids than...
those under walnut, with 97.4±0.72 and 91.0±1.62 percent respectively. Oak soils also exhibited significantly less small intra-aggregate void than those of spruce, 0.012±0.0004, 0.018±0.0028 percent respectively. There also were differences between the quantities of certain void shapes during this sampling period (Figure 4.11), and the coefficient of uniformity was significantly greater in soils under spruce versus oak (Figure 4.12). It is difficult to suggest reasons for why these trends have occurred, considering that Wotherspoon (2014) observed less litter fall, and organic carbon input under oak tree species. It is possible that due to there being less organic carbon under oak trees, there is greater ability for cracking caused by wetting and drying. At our scale of observation the volume changes in larger inter-aggregate voids would be most affected by large cracks, as opposed to bio-void creation.

During the spring sampling, in April, the soils associated with the spruce were significantly different than those with poplar in both categories of intra-aggregate voids. Poplar had a greater proportion of medium intra-aggregate voids (1.35±0.09 vs. 0.89±0.06), and a lower proportion of small intra-aggregate void (0.020±0.0013 vs. 0.039±0.0041) when compared to spruce. This is a difficult relationship to interpret as, there is no clear trend. There were distinct differences visible in snow melt patterns between spruce trees and the deciduous trees (Figure 4.13). Snow melt was much faster under spruce trees compared to the deciduous trees, as observed visually in April 2014. However, it is difficult to provide a direct link between these two observations.

Rates of litter decomposition in ecosystems, among many important drivers, can be influenced by differences in the composition of decomposer communities and their trophic interactions (Beare et al. 1992; Šnajdr 2013). At the University of Guelph Agroforestry Site, Price and Gordon (1999) found that across various seasons, earthworm densities were almost always highest closest to the poplar treatment
Table 4.4: A comparison of measured soil void phase parameters tree species. All samples collected for both distances into cropping alleys (2 & 6m) for each tree species were pooled for analysis. A Kruskal-Wallis nonparametric analysis of variance was used to test for significant differences. Table shows the calculated test statistics (H), the degrees of freedom (df), and the significance values (p-value).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>June</th>
<th>October</th>
<th>April</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>df</td>
<td>p-value</td>
</tr>
<tr>
<td>Total Void</td>
<td>12.3</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Large Void</td>
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<td>0.03</td>
</tr>
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<td>14.2</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Medium Void</td>
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<td>3</td>
<td>0.21</td>
</tr>
<tr>
<td>Volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_U</td>
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<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C_C</td>
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</tr>
<tr>
<td>Span</td>
<td>2.2</td>
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<td>0.53</td>
</tr>
<tr>
<td>Size Distribution</td>
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</tr>
<tr>
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<td>&lt;0.01</td>
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<tr>
<td>Acicular-Planar</td>
<td>6.87</td>
<td>3</td>
<td>0.08</td>
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<tr>
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<td>0.47</td>
</tr>
<tr>
<td>Mean Anisotropy</td>
<td>6.3</td>
<td>3</td>
<td>0.10</td>
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compared to silver maple (*Acer saccharinum* L.) and white ash (*Fraxinus americana* L.). This trend was attributed to the higher concentrations of organic matter inputs that originated from poplar leaf litter. Tree species effects on faunal and microbial community are important, as the soil microbial community has long been recognized as a vital component in the process of soil aggregate formation and, consequently, soil void quantity and complexity. This interaction between void characteristics, organic matter and microbial community could be an interesting relationship to investigate in the future.

As previously mentioned, tree species affect several variables which may result in changes to the soil surface. Probably the most cited tree species effect on soils is the variability in organic carbon inputs from tree litter (Binkley 1996). Wotherspoon (2014) studied the same TBI system and determined that greater litterfall was found beneath poplar trees, followed by walnut, spruce, and oak. It was also determined that the rates of leaf litter decomposition varied between tree species with walnut being
significantly faster and spruce being the slowest, which was suggested to be related to the amounts of lignin present in the litter (Wotherspoon 2014). Consequently, in relation to actual annual C inputs to soil, the same study indicated that poplar contributed the most, followed by spruce, walnut, oak, and soybean solecrop.

Table 4.5: Results of Dunn-Nemenyi post-hoc analysis following Kruskal-Wallis analysis comparing soil void parameters for different tree species. All samples collected for both distances into cropping alleys (2 & 6m) for each tree species were pooled for analysis. Tree species pairs listed for each measured parameter showed significant differences at p=0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>June</th>
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<td>Total Void</td>
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<td>Large Void</td>
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<td>Small Void</td>
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<td>Oak</td>
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<td>Medium Void</td>
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<td><strong>Size Distribution</strong></td>
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<td>C_U</td>
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<tr>
<td>Acicular-Planar</td>
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<td>Connectivity</td>
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<td>Solecrop</td>
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<td><strong>Mean Anisotropy</strong></td>
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Fundamentally, the litterfall differences in quality and quantity should have an effect on soil properties, chemically, biologically and physically (Binkley, 1995); however, within a mixed species intercropping system these effects may be less distinct (Rothe and Binkley, 2001). Ferrari and Sugit (1996) examined spatial patterns in litterfall within a mixed forest of hardwood and conifer species. The average radius within which 90% of the leaf litter fell to a trap originated within 17.1m±1.8m (crown radius ranging from 2.8-5.1m) from the trunk. Moreover, the proportion of litter that fell directly beneath the canopies of the isolated trees ranged from 12 to 50%. This reported radius, of litterfall, is greater than the width of the alleys within our studied TBI system, suggesting that the tree species influence on soils could extend to areas of the field outside of just under the tree canopy where soils were sampled. This was confirmed by work completed by Wotherspoon (2014), showing no significant differences between litterfall accumulations at 1m compared to 4m into cropping alleys. Consequently, since tree litter accumulation from a single species is unlikely to be isolated under the canopy of that tree, we can, with some confidence, understand the similarity in measured soil void characteristics between tree species.
Figure 4.10: A comparison of measured soil void phase parameters for different tree species, at three sampling times (June, October, and April). All samples collected for each species were pooled for analysis. Note solecrop (N=4), oak (N=12), poplar (N=12), spruce (N=12), and walnut (N=12). (a) Total resolvable void proportion of volume; (b) large void as a proportion of total resolvable; (c) medium void as a proportion of total resolvable; (d) small void as a proportion of total resolvable; (e) connectivity of solid phase
Figure 4.11: A comparison of measured soil void phase shape parameters for different tree species, at three sampling times (June, October, and April). All samples collected for each species were pooled for analysis. Note solecrop (N=4), oak (N=12), poplar (N=12), spruce (N=12), and walnut (N=12), and all proportions are as those of classified voids.
Figure 4.12: A comparison of measured medium intra-aggregate void size distribution parameters for different tree species, at three sampling times (June, October, and April). All samples collected for each species were pooled for analysis. Note solecrop (N=4), oak (N=12), poplar (N=12), spruce (N=12), and walnut (N=12), and all proportions are as those of classified voids. (a) span; (b) coefficient of curvature (Cc); (c) coefficient of uniformity (Cu);
4.3.2 Soil Matrix Phase Analysis

The X-ray µCT technique has a minimum void size which it is capable of resolving, that is defined by the resolution of the imagery. In the case of this study, the imagery was reconstructed at 60µm resolution meaning that the smallest void that can be resolved would be 0.06 x0.06 x0.06mm or $2.16 \times 10^{-4}$ mm$^3$. Research in the past has demonstrated that there are voids smaller than this, and that these voids are an important component of soil structure, especially for water retention. To evaluate the structure at scales smaller than the image resolution, a technique was used evaluating the semivariogram structure of the greyscale imagery. Those areas identified as void were masked to equal not a number, creating an image representing the soil ‘matrix’ average radiodensity.

Figure 4.13: Snow Melt Compared between spruce trees and poplar trees. Note Photos taken April 2014, similar patterns expected in April 2013.

When observing the coefficient of variability of the greyscale image as a whole (Figure 4.14), there were no significant changes as a result of time ($H(2)=3.28$, $p=0.19$), or as a result of tree species, distance from tree, or management system (Table 4.6). This inherently means that the materials of all of the samples were relatively similar, and likely have similar densities. If the densities differed significantly, we would see an increase in the variability of the greyscale images.
Table 4.6: A comparison of measured coefficient of variation of soil matrix greyscale attenuation. For testing significant difference among tree species (A) a Kruskal-Wallis nonparametric analysis of variance was used. Table shows the calculated test statistics (H), the degrees of freedom (df), and the significance values (p-value). For testing significant difference for distance from the tree row, and between systems (B & C) a Wilcoxon two sample test was used. Table shows the calculated test statistics (Wₜ), the Z-score, and the significance values(p-value).

<table>
<thead>
<tr>
<th></th>
<th>June H</th>
<th>df</th>
<th>p-value</th>
<th>October H</th>
<th>df</th>
<th>p-value</th>
<th>April H</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Species</td>
<td>2.89</td>
<td>3</td>
<td>0.41</td>
<td>5.24</td>
<td>4</td>
<td>0.26</td>
<td>4.60</td>
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</tr>
<tr>
<td></td>
<td>Wₛ</td>
<td>Z</td>
<td>p-value</td>
<td>Wₛ</td>
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<td>p-value</td>
<td>Wₛ</td>
<td>Z</td>
<td>p-value</td>
</tr>
<tr>
<td>B</td>
<td>Distance</td>
<td>683</td>
<td>1.96</td>
<td>0.06</td>
<td>608</td>
<td>0.41</td>
<td>0.68</td>
<td>593</td>
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</tr>
<tr>
<td>C</td>
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<td>157</td>
<td>1.75</td>
<td>0.09</td>
<td>55</td>
<td>-1.75</td>
<td>0.09</td>
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</tr>
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</table>

Although there were no differences in the soil matrix greyscale variability, there were differences in the structure of that variability. The structure of the variability was investigated by measuring anisotropy between directional semivariograms of greyscale attenuation. A decrease in mean anisotropy was determined with time (H(2)=10.36, p=<0.01). The mean anisotropy decreased significantly from June 2012 to October 2012 (S=266, p<0.01), and from June 2012 to April 2013 (S=232, p=0.02) sampling times (Figure 4.15). This decrease in anisotropy can be interpreted as a decrease of randomness of soil structure, as the greyscale variability of the soil matrix becomes more isotropic with direction. The pattern of increased efficiency was evidenced by the reduction in connectivity when comparing cubic close packing to hexagonal close packing of spheres, as described in Section 4.2.2. The decreasing trend of connectivity of the solid matrix phase paralleled the decrease in mean anisotropy. Connectivity represented as number of connections per total image volume decreased from June to October to April, 67290±8266, 61949±7015, 21789±1753. These observed patterns are attributed to the likely greater level of soil moisture during the sampling months of October 2012, and April 2013, and the effect that this is known to have on shrink-swell creation of aggregates. During the wet months, the soils are swollen, and consequently are more isotropic with less intra-matrix porosity, leading to more efficient packing, whereas in the drier months the soils will have shrunk, and some of those intra-matrix voids will have opened up. These intra-matrix voids will show lower density regions within the soil matrix, observed as an increase in anisotropy.
Figure 4.14: A comparison of measured coefficient of variation of soil matrix greyscale attenuation for a) sampling time b) distance from the tree row, c) agricultural management system, and d) tree species.
The mean anisotropy of the greyscale directional semivariograms did not show any significant differences (Table 4.7) for tree species (Figure 4.16), between the TBI and solecrop systems (Figure 4.17), or distance from tree trunk (Figure 4.18). This is likely a result of the soil matrix material being similar in density amongst all samples as evidenced by the prior analysis. Further, due to the distinctively lower proportion of less dense voxel regions within the soil solid matrix, it is likely that a change in low density regions (void) would not have much effect on semivariogram properties. A soil rich in iron oxides, or other high density anomalies likely would have a greater effect on anisotropy in semivariograms.

Table 4.7: A comparison of measured mean anisotropy of orthogonal semivariograms of soil matrix greyscale attenuation. For testing significant difference among tree species (A) a Kruskal-Wallis nonparametric analysis of variance was used. Table shows the calculated test statistics (H), the degrees of freedom (df), and the significance values (p-value). For testing significant difference for distance from the tree row, and between systems (B & C) a Wilcoxon two sample test was used. Table shows the calculated test statistics (W_s), the Z-score, and the significance values(p-value).

<table>
<thead>
<tr>
<th></th>
<th>June</th>
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<tr>
<td></td>
<td>H</td>
<td>df</td>
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<tr>
<td>A</td>
<td>Species</td>
<td>2.89</td>
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<td>W_s</td>
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<td>B</td>
<td>Distance</td>
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<td>C</td>
<td>System</td>
<td>157</td>
<td>1.75</td>
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4.4 Summary & Conclusion

It was hypothesized that there would be significant differences in soil surface microstructure between tree species, and the distance from tree trunk into cropping alley. Further, differences in soil surface microstructure between TBI and a solecrop system, and differences with time were expected.

There was a very strong indication of seasonal effects on void phase parameters. Almost all measured void phase parameters showed significant differences and trend with time of sampling. Most of the differences occurred with the month of April 2013, however, some differences occurred between June
2012 and October 2012. The distinct differences in April were attributed to the increased moisture at that time of year, which caused soil swelling.

When evaluating void phase parameters between the TBI and solecrop systems there were very few differences. Consequently, it may be argued that at this scale of observation there were no consistent differences in void phase parameters because of the continual similarities in agricultural management of both cropping areas. Some differences in void shape, especially in the April 2013 sampling, were seen with distance from the tree trunk into cropping alleys. The effect of tree species on soil void phase parameters was inconsistent. For June 2012 samples, significant differences were determined for oak trees and all other species. It was suggested that this difference may be due to more cracking during drying because of these soils having less organic carbon. During April 2013, soils from under spruce had fewer medium, and more small intra-aggregate voids than poplar tree soils. It was unclear why this relationship exists, but was observed that snow melt occurred faster under spruce trees than deciduous trees.

No differences were found in soil matrix greyscale variability between samples collected. However, differences in anisotropy of directional semivariograms were observed. A decrease in anisotropy was seen from June 2012 to October 2012 and June 2012 to April 2013, indicating a breakdown in soil structure during the fall, and over the winter.

This study found difficulties in understanding changes in void phase parameters and soil matrix variability in X-ray attenuation. These parameters appear to be very dynamic and difficult to understand at a system level. There are many complex chemical, physical and biological interactions which may affect the measured parameters. As discussed by Letey (1991) the arrangement of soil particles at any time is a result of many complex interactions of physical, chemical, mineralogical and biological factors. Due to these complex interactions the amount of spatiotemporal heterogeneity can limit the ability to relate structure to other gathered information. The agroforestry system under study has many potential
factors which could cause direct effects on the system; factors including mixed tree species, agricultural management (tillage, planting, harvest), and inherent soil and landscape properties. Determining which factors are most critical is a large task, and may be a good avenue for future research.
Figure 4.15: A comparison of measured mean anisotropy of orthogonal semivariograms of soil matrix greyscale attenuation between time of sampling. Semivariograms were calculated at a lag interval of 0.06mm, and for 25% of the total lags in the sample. All samples collected for each month were pooled for analysis. Note June (N=48), October (N=52), and April (N=52), and all proportions are as those of classified voids.

Figure 4.16: A comparison of measured mean anisotropy of orthogonal semivariograms of soil matrix greyscale attenuation for 4 tree species, 1 solecrop management, with 3 sampling times. Semivariograms were calculated at a lag interval of 0.06mm, and for 25% of the total lags in the sample. All samples collected for each species during each sampling month were pooled for analysis. Note solecrop (N=4), oak (N=12), poplar (N=12), spruce (N=12), and walnut (N=12) for each sampling time.
Figure 4.17: A comparison of measured mean anisotropy of orthogonal semivariograms of soil matrix greyscale attenuation for cropping systems. Semivariograms were calculated at a lag interval of 0.06mm, and for 25% of the total lags in the sample. All samples collected for each species during each sampling month were pooled for analysis. Note solecrop (N=4), TBI (N=48) for each sampling time.

Figure 4.18: A comparison of measured mean anisotropy of orthogonal semivariograms of soil matrix greyscale attenuation for distance from tree row. Semivariograms were calculated at a lag interval of 0.06mm, and for 25% of the total lags in the sample. All samples collected for each species during each sampling month were pooled for analysis. Note 2m (N=24), 6m (N=24) for each sampling time.
Chapter 5 - Soil Micro-Penetrometer Correlation with X-ray Micro CT Z-axis Profile

5.1 Introduction

Bulk density measurements are used to determine and describe soil compaction (Nawaz et al. 2013). However, traditional bulk density measurements have limitations in detecting small zones of spatial variability of bulk density within soil (Petrovic et al. 1982). Consequently, researchers have been experimenting with alternative techniques which allow for measurement and quantification of spatial heterogeneity in soil - one of these techniques is X-ray computed micro-tomography (X-ray μCT). Some of the first results of X-ray μCT showed a linear relationship between soil bulk density and X-ray attenuation (Petrovic et al. 1982). More recently, much of the use of X-ray μCT for soil investigation has been focused on the evaluation of soil microstructure, essentially represented by the quantitative characterization of the porosity and void networks (Taina et al. 2008). A wide range of void sizes exist in soils, both intra and inter-aggregate. These void spaces can have differing effects on emission of GHG from soils. The structural degradation of soil usually results in loss of biopores, decreasing gas diffusivity and water availability, and compaction causing crusting, decreased water infiltration (Wilding and Lin 2006), ultimately effecting soil GHG emissions.

Another proven method of assessing soil bulk density effects on plant growth is through penetrometer resistance. Penetrometer resistance has been used as an index of the mechanical impedance that roots experience in either dry or compacted soils (Whalley et al. 2007). Penetrometer resistance is affected by numerous soil factors: bulk density, particle size distribution, organic matter content, and soil water content (Aggarwal et al 2006, Whalley et al. 2007, Whitmore et al. 2012). These soil factors can also be inferred directly from measured penetrometer resistance results (DeVos et al. 2005).
Quantifying information on bulk density at the X-ray µCT scale of observation is important for understanding gas exchange properties of soil, as even a thin layer of dense soil can alter the gas flows (Petrovic et al. 1982). In terms of soil methane emissions specifically, there is extremely high amounts of spatial variability at the 1cm scale (Wachinger et al. 2000). Wachinger et al. (2000) showed that soil cores with undisturbed structure showed high spatial variability with standard deviations exceeding 100% even if taken from the same horizon within 1m² of each other. These researchers found no differences in soil matrix of high and low productivity cores, but the highly productive cores did contain inclusions of particulate organic material. Further, Mangalassery et al. (2013), demonstrated that soil texture and aggregate size affect the soil’s porous architecture, and can have implications on GHG emissions. Average CO₂ and N₂O emissions were greater from clay loam versus sandy loam soil. The sandy loam soils were found to have higher average macroporosity than clay loam soil. It was discussed that the soil macroporosity plays a key role in driving the CO₂ produced by microbial respiration to the surface. A negative relationship was determined between average pore size and CH₄ flux, indicating the decreasing size of soil pore may provide a more anaerobic environment. Soil porosity did not appear to significantly affect N₂O fluxes, therefore, soil management that has effects on pore characteristics may influence GHG emissions.

Ball (2013) in a review of soil structure effects on greenhouse gas emissions highlighted many studies. One concept discussed as N₂O flux increased with soil compaction and wetness, indicated by positive correlation of bulk density with N₂O emission. The review further indicated that CO₂ flux was greatest in the soils that provided good aeration due to loose, well-aggregated structure. Similarly to conclusions made by Mangalassery et al. (2013), Ball (2013) indicated that the small intra-aggregate pores influence the size of anaerobic zones (N₂O emissions), and the larger inter-aggregate macropores influenced the exchange of oxygen at the boundaries of aggregates and exchange of CO₂ with the atmosphere.
The purpose of this study was to 1) validate X-ray µCT density parameters with reference to penetrometer resistance measurements; 2) relate measures of compaction to specific tree species and management types; and 3) Propose X-ray µCT based microstructural parameters as indicators of greenhouse gas emissions.

5.2 Methods

5.2.1 X-ray µCT Analysis
See Methods in Introduction Section 3.2.3 for the methods of void phase analysis.

ImageJ was used to measure Z-axis attenuation of the greyscale imagery. The Radial Profile plugin was used to select a circular area of similar size to the penetrometer diameter. The circle shaped ‘Region of Interest’ was placed in the centre of the image to roughly align with the placement of the penetrometer probe. To obtain the Z-axis attenuation profile for the entire depth of the sample, the Plot Z-axis Profile plugin of ImageJ was used. This plugin provided output of the average attenuation within the selected region of interest for each depth increment (image slice) of 0.06mm, corresponding to the image resolution.

5.2.2 Penetrometer Resistance
Penetration resistance was measured using an ELE Digital Tritest 50, instrumented with load cells for measurements of compressive force (ELE International Ltd., Hemel Hempstead, England). Since the soil cores were dried to a constant weight at 40°C to perform X-ray µCT analysis, the cores had to be rewetted in order to perform this penetration resistance analysis. The cores were gradually sprayed with a hand spritz bottle to reach field capacity (when excess water had drained away). A cone penetrometer with a 30° cone angle and a basal diameter of 4 mm was used to make penetrometer resistance measurements. Resistance was measured in each sample at a constant rate of penetration (3.6 mm/min) in the centre of each core. The centre of each core was chosen so that roughly the same area could be selected for the z-axis attenuation profile in the greyscale imagery. Readings were obtained to a depth of
3.5cm, gathering a data point every 1 second or 0.06mm, corresponding to the X-ray image resolution of 60µm. Penetration resistance measurements were used both as a tool to validate X-ray µCT density measurements with depth, and as soil microstructural index of soil compaction.

5.3 Results

5.3.1 Correlation of X-ray µCT Z-Axis Density Parameters to Penetrometer Resistance

For all sample cores (n=152), significant correlations were determined between measured penetrometer resistance and X-ray µCT derived Z-axis density profiles. However, there was little consistency in the degree of the correlation, as measured by Spearman rho (Figure 5.1). For all tree species, at almost all sampling times (exception oak in April) the median Spearman’s rho exceeded a value of 0.60 which is considered a strong positive correlation. The strong correlation between these measurements was expected, as a relationship between soil bulk density and X-ray attenuation has long been recognized (Petrovic et al. 1982, Crestana et al. 1985, Anderson et al. 1988). Interestingly, the median Spearman rho for the soil cores from the solecrop system, samples never exceeded the 0.60 value, with medians of 0.52 (moderate) in October, and 0.16 (very weak) in April (Figure 5.2).

The lower strength of correlation, upon inspection of the imagery and profile plots, appears to be due to a consistently high attenuation with depth, with little deviation, as evidenced by the histograms from the regions of interest in each sample (Figure 5.3). This is the case in three of the solecrop samples from April (M1, M3, M4), where the correlation coefficient is low. This observation was confirmed in the imagery of these samples (Figure 5.4), where there are very few void spaces within the region of interest with depth, meaning there is a consistently high attenuation. The low degree of porosity within the region of interest also will cause the penetrometer resistance to increase steadily with depth. Generally this trend occurs as illustrated in Figure 5.2. The penetrometer pushes soil out of the way as it penetrates into the
soil. If there is no void space, or places for it to push the soil to, gradually it greater force will be needed to through the soil.

Figure 5.1: Correlation Coefficient (Spearman Rho) between X-ray CT measured Z-axis attenuation, and soil micropenetrometer measurements. All samples for each sampling time were pooled (N=12 for Tree Species, N= 4 for solecrop).
Figure 5.2: Comparison of penetrometer resistance and X-ray µCT attenuation for solecrop System samples for April sampling. Penetrometer curves and Z-axis attenuation curves were unity-based normalized in order to range between values of 0-1, to allow for Spearman Rho to be calculated between the two curves. Spearman Rho values ($r_s$) can be seen in the lower right corner of the figures.
Figure 5.3: Histograms of greyscale radiodensity within X-ray µCT imagery from the solecrop samples collected in April. Proportion of void in sample can be seen in the top right corner.
Figure 5.4: Greyscale, and binarized imagery from middle slice of each sample, showing the greater proportion of solid phase within the region of interest.
5.3.2 Void Size, Compaction and Potential Greenhouse Gas Emissions

Within the collected data, those samples with higher maximum resistance and X-ray attenuation showed a negative correlation with total void and large inter-aggregate void (Table 5.1). This relationship suggests, as expected, a more compacted soil surface as measured by penetrometer resistance, in soils with less total void.

Table 5.1: Correlation coefficients between measured soil void phase parameters, measured soil matrix Z-axis x-ray attenuation, and maximum penetration resistance. The table shows void phase parameters on left side of table, penetration resistance and x-ray attenuation on top of table. There are three columns representing each sampling period (June, October, April). All samples collected for each month were pooled for analysis. To test for correlation a Spearman correlation was used. For each correlation Spearman’s Rho and the p-value for the correlation are presented.

<table>
<thead>
<tr>
<th></th>
<th>Maximum Penetration Resistance</th>
<th>Maximum X-ray Attenuation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>October</td>
</tr>
<tr>
<td>Total Void</td>
<td>-0.45</td>
<td>-0.21</td>
</tr>
<tr>
<td>Large Inter-aggregate Void</td>
<td>-0.29</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

The most consistent temporal trend identified in the X-ray CT, and penetrometer measured data was a decrease in total porosity and large inter-aggregate voids, and an increase in penetrometer resistance with time from June 2012 to October 2012 to April 2013. Wotherspoon (2014) measured the efflux of CO₂ within the same TBI system throughout the year determined that the highest respiration occurred during the warmest months (June 2012, July 2012, August 2012). This relationship generally correlates with the measured X-ray µCT parameters of greater void space, and less compaction. This indicates that there is likely a relationship between the structure and gas emissions in this system, similar to what has been referenced in the literature (Ball 2013, Mangalassery et al. 2013) that could be investigated in future.

Only soils under walnut trees, within TBI management, showed differences in void characteristics when compared to conventional soybean solecrop (Table 5.2). In October 2012 after soybean harvest, the walnut species had a smaller proportion of large inter-aggregate void versus the solecrop (H(4)=10.82,
p=0.03). Further, the medium intra-aggregate void was greater in soils collected adjacent to walnut trees versus the solecrop (H(4)=9.24, p<0.01). However, the proportion of small intra-aggregate void was significantly greater in solecrop (H(4)=9.82, p=0.04). The maximum penetrometer resistance differed, with walnut trees having significantly greater resistance (H(4)=14.47, p<0.01). This same relationship existed as a general trend in the samples collected after harvest (October 2012), the solecrop treatment has a lower maximum penetration resistance compared to poplar, walnut, and spruce trees (Figure 5.5).

Table 5.2: Medians and standard deviations of measured soil void phase parameters which showed significant differences between walnut species, and solecrop system.

<table>
<thead>
<tr>
<th></th>
<th>Large Inter-aggregate</th>
<th>Medium Intra-aggregate</th>
<th>Small Intra-aggregate</th>
<th>Max Penetrometer Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>walnut</td>
<td>87.2±2.53</td>
<td>1.10±0.08</td>
<td>0.015±0.0014</td>
<td>22.14±9.99</td>
</tr>
<tr>
<td>solecrop</td>
<td>95.7±0.39</td>
<td>0.65±0.03</td>
<td>0.028±0.0013</td>
<td>5.15±1.42</td>
</tr>
</tbody>
</table>

Thevathasan and Gordon (2004), summarizing research completed at the same site as this study, have estimated that a hybrid-poplar TBI system could potentially reduce N₂O emissions by 0.69 kg N₂O ha⁻¹ compared to a conventional solecrop system by facilitating reductions in leached nitrate of which 2.5% becomes denitrified and lost as N₂O gas. Ball (2013) discussed the importance of soil structure in affecting greenhouse gas emissions. The author outlined that the small intra-aggregate voids influence the amount of anaerobic zones which cause greater N₂O emissions. A similar negative relationship between average void size and CH₄ flux exists (Mangalassery et al. 2013). The larger inter-aggregate macrovoids influence the exchange of oxygen at the boundaries of aggregates and exchange of CO₂ with the atmosphere. Consequently, in future it would be of great interest to have research conducted linking soil structure as investigated through X-ray CT analysis to GHG emissions from the TBI system.
5.4 Summary & Conclusions

This study identified that soils under different species have different structural characteristics which potentially act as mechanisms affecting GHG emissions. There are differences between solecrop system and TBI after harvest (October 2012), showing more surface soil compaction in TBI. Across both systems there is a significant effect of time on void characteristics and penetrometer resistance. This indicates that the micro-scale soil structural effects on GHG emissions could be very dynamic. Consequently, quantifying the benefits of one system on the total reductions in GHG emissions may be very difficult. These conclusions are not meant to imply that soil void as determined by X-ray μCT should be investigated as an absolute predictor of GHG emission potential, but rather that the method is able to quantify an important mechanism affecting GHG emissions. It would be valuable to evaluate this concept in more detail with the direct measurement of GHG emissions from the cores being collected for X-ray CT analysis.
6 Chapter 6: Summary & Final Thoughts

One of our goals was to examine X-ray μCT-resolvable voids, and quantify spatial variability in X-ray radiodensity as influenced by tree species. X-ray μCT measured void characteristics were not found to be significantly affected by the tree species within samples collected prior to the excavation of entire tree root systems. This was because samples were collected within perennial vegetated tree rows. However, the effect of tree species on soil void phase parameters was also conflicting within the samples collected for the temporal analysis. Tree species appear to have little effect on void characteristics at the level of observation of this analysis. This relationship can likely be attributed to the mixed leaf litter, caused by the numerous tree species in the TBI system. It was also determined, through the use of geostatistics, that there were no distinct or consistent anisotropic structures in directional semivariograms evident for the soils of various tree species. Through the use of semivariograms, greater variability was shown, correlated with less directional anisotropy observed within the tree row as compared to cropping alley soils. It was interpreted that tree row soils had a higher degree of structure, and that row crop soils had destruction of surface structure leading to more directional anisotropy. We can conclude that there are inconsistent significant differences between the microstructure of soils in tree rows, and cropping alleys within tree-based intercropping management.

When evaluating void phase parameters between the TBI and solecrop systems there were very few differences. Again, it may be argued that at this scale of observation there were no consistent differences in void phase parameters because of the continual similarities in agricultural management of both cropping areas. There are differences between solecrop system and TBI after harvest (October 2012), showing more soil surface compaction in TBI as measured by the soil penetrometer. Across both systems there is a significant effect of time on void characteristics and penetrometer resistance. It is possible that future work could be completed investigating soil structural changes with depth within the TBI system as
compared to the solecrop system. Many tree species are known to contribute a significant amount of below ground biomass in the form of organic carbon. *We can conclude there are some significant differences between the microstructure of soils, under tree-based intercropping and conventional management systems.*

There was a very strong indication of void phase parameters change with time. Almost all measured void phase parameters showed significant differences and trend with time of sampling. Most of the differences occurred with the month of April 2013; however, some differences occurred between June 2012 and October 2012. The distinct differences in April were attributed to the increased moisture at that time of year, which caused swelling. Differences in anisotropy of directional semivariograms were observed. A decrease in anisotropy was seen from June 2012 to October 2012 and June 2012 to April 2013, indicating a breakdown in soil structure. *We can conclude that there are seasonal differences in soil microstructure within tree-based intercropping alleys.*

Ultimately, this study found difficulties in understanding changes in void phase parameters and soil matrix radiodensity variability. These parameters appear to be very dynamic and difficult to understand at a system level. There are many complex chemical, physical and biological interactions which may affect the measured parameters. Determining which are most critical is a large task, and may be a good avenue for future research. The research completed indicates that the micro-scale soil structural effects on GHG emissions could be very dynamic. Consequently, quantifying the benefits of one system on the total reductions in GHG emissions may be very difficult.

My research provides direct quantification of four tree species’, and the tree-based intercropping effects on the surface 3.5cm soil structure beneath them, which can be used towards tree species selection and management prescriptions. Moreover, the quantified parameters can help in establishing key indicators
for on-going monitoring of the impact of changing inter-cropping management regimes, on the nature and dynamics of soil microstructure.
7 References


# Appendix 1: Example SAS Code

title "Friedman Analysis";

data friedman;
input Species$ Rep ID$ June Oct April;
cards;
M 1 M1 . 95.04865398 75.70560114
M 2 M2 . 96.38312991 96.31838065
M 3 M3 . 96.26952625 90.04288229
M 4 M4 . 94.55426617 67.12205278
O 1 O1_2.0E 98.27686745 95.16552434 92.14343354
O 1 O1_2.0W 98.27563568 87.19708913 78.28492013
O 1 O1_6.0E 98.70311497 81.26323325 97.39634871
O 1 O1_6.0W 98.76247257 98.19138077 92.47874559
O 2 O2_2.0E 94.17453406 93.49845902 91.41521024
O 2 O2_2.0W 95.29783485 95.44180353 74.99677432
O 2 O2_6.0E 96.82448668 92.16296515 93.05642024
O 2 O2_6.0W 98.34519881 97.76323325 97.39634871
O 3 O3_2.0E 98.70311497 81.26323325 97.39634871
O 3 O3_2.0W 98.76247257 98.19138077 92.47874559
O 3 O3_6.0E 98.70311497 81.26323325 97.39634871
O 3 O3_6.0W 98.76247257 98.19138077 92.47874559
P 1 P1_2.0E 94.87533001 98.53893934 96.03811188
P 1 P1_2.0W 95.45759751 84.37825676 77.62636363
P 1 P1_6.0E 98.47124303 83.63391794 75.46207053
P 1 P1_6.0W 95.29783485 95.44180353 74.99677432
P 2 P2_2.0E 92.47138252 93.0822678
P 2 P2_2.0W 98.20712437 94.26584111 95.04340056
P 2 P2_6.0E 93.64481989 94.17843124 87.97660362
P 2 P2_6.0W 99.88353326 99.16787899 77.67017665
P 3 P3_2.0E 97.96317805 90.62348662 91.77180114
P 3 P3_2.0W 93.95580048 84.11022712 91.19826986
P 3 P3_6.0E 87.93147783 95.71868025 85.95022086
P 3 P3_6.0W 93.75874911 92.49613773 96.53846012
P 3 P3_6.0W 93.75874911 92.49613773 96.53846012
S 1 S1_2.0E 96.51559846 98.9105976 95.56886825
S 1 S1_2.0W 97.93871958 92.79660805 85.01825118
S 1 S1_6.0E 75.43102167 94.40682732 81.89565092
S 1 S1_6.0W 98.21714942 93.01134248 90.79058071
S 2 S2_2.0E 90.63391794 84.95015006
S 2 S2_2.0W 96.88888878 98.03791326
S 2 S2_6.0E 83.26469884 85.95022086
S 2 S2_6.0W 83.21577741 96.53846012
S 3 S3_2.0E 97.71826333 93.87054742 91.03211154
S 3 S3_2.0W 97.71826333 93.87054742 91.03211154
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S 3 S3_6.0W 93.93147783 93.41675447 79.51891172
S 3 S3_6.0W 93.75874911 88.91181661 68.69548258
W 1 W1_2.0E 97.6310261 91.35394848 75.6373738
W 1 W1_2.0W 90.42744149 96.86085856 77.9704592
W 1 W1_6.0E 86.28500506 80.13368652 81.43326872
W 1 W1_6.0W 91.43447875 88.2378389 85.47285871
W 2 W2_2.0E 97.16519776 69.2223591 80.13690903
W 2 W2_2.0W 96.06534105 79.0285735 88.57737584
W 2 W2_6.0E 89.47633185 86.523897 84.98620991
W 2 W2_6.0W 93.2585483 94.33455686 63.55627399
W 3 W3_2.0E 90.50281515 87.80989371 86.87343768
W 3 W3_2.0W 96.53724624 71.60877123 94.36697185
W 3 W3_6.0E 82.35787245 93.92654551 91.06789723
W 3 W3_6.0W 79.40840537 76.46218787 91.8006236
;
run;

proc univariate data=friedman normal;
var june oct april;

QQplot june oct april/normal (MU=EST SIGMA=EST);
run;

proc transpose data=friedman out=friedman2 (rename=(col1=Measurement)) name=Month;
var june oct april;
by species rep ID;
run;

proc print data=friedman2;
run;

proc freq data=friedman2;
by species;
tables species*Month*Measurement/CMH2 scores=rank exact no print;
run;

proc rank data = friedman2 out=friedmanrank;
var measurement;
by species;
run;

proc means data=friedmanrank mean;
class Month;
var Measurement;
by species;
run;

/*Wilcoxon Sign-Rank Post Hoc Tests - Oct-April*/
data diff;
set friedman;
OA = oct-april;
by species;
run;

proc univariate data=diff;
by species;
var OA;
run;

/*Wilcoxon Sign-Rank Post Hoc Tests - June-Oct*/
data diff2;
set friedman;
JO = June-oct;
by species;
run;

proc univariate data=diff2;
by species;
var JO;
run;

/*Wilcoxon Sign-Rank Post Hoc Tests - June-April*/
data diff3;
set friedman;
JA = June-april;
by species;
run;

proc univariate data=diff3;
by species;
var JA;
run;
title "Wilcoxon Two Sample Test";

data wilcoxon;
input distance$ Rep ID$ June Oct April;
cards;
M 1 M1 . 0.09737647 0.040677431
M 2 M2 . 0.0898776 0.059357047
M 3 M3 . 0.014011658 0.055825638
M 4 M4 . 0.039529119 0.076998086
TBI 1 O1_2.0E 0.095319814 0.035759383 0.093445

TBI 1 O1_2.0W 0.086106394 0.07910948 0.05976919
TBI 1 O1_6.0E 0.014011658 0.04106023 0.024838387
TBI 1 O1_6.0W 0.057752111 0.03153972 0.126475216
TBI 3 O3_2.0E 0.122849741 0.03208058 0.195970543
TBI 3 O3_2.0W 0.048216185 0.047101185 0.039968013
TBI 3 O3_6.0E 0.17452933 0.030847025 0.089079382
TBI 3 O3_6.0W 0.072712887 0.102156628 0.029842485
TBI 1 P1_2.0E 0.131665488 0.060267256 0.074775083
TBI 1 P1_2.0W 0.134176951 0.031363249 0.084100393
TBI 1 P1_6.0E 0.124223112 0.06814704 0.054086669
TBI 1 P1_6.0W 0.190910945 0.067522973 0.054331562
TBI 2 P2_2.0E 0.058086599 0.107348876 0.046646561
TBI 2 P2_2.0W 0.069981008 0.054160626 0.186724756
TBI 2 P2_6.0E 0.167498464 0.078054816 0.103380364
TBI 2 P2_6.0W 0.193087806 0.149936289 0.070412962
TBI 3 P3_2.0E 0.083832427 0.0497355 0.058039555
TBI 3 P3_2.0W 0.056161225 0.070587127 0.05427514
TBI 3 P3_6.0E 0.063845492 0.06957361 0.039881667
TBI 3 P3_6.0W 0.115280253 0.160174516 0.039881667
TBI 1 S1_2.0E 0.061239658 0.07964818 0.106253222
TBI 1 S1_2.0W 0.089926762 0.041749957 0.039968013
TBI 1 S1_6.0E 0.046150361 0.099440419 0.062953111
TBI 1 S1_6.0W 0.224629997 0.07269307 0.06018639
TBI 2 S2_2.0E 0.036726016 0.055979626 0.054046614
TBI 2 S2_2.0W 0.046326226 0.06421468 0.109705851
TBI 2 S2_6.0E 0.120157055 0.088195923 0.04724161
TBI 2 S2_6.0W 0.052751853 0.04960944 0.036870728
TBI 3 S3_2.0E 0.04084792 0.016109611 0.083627248
TBI 3 S3_2.0W 0.077175951 0.080653691 0.041746015
TBI 3 S3_6.0E 0.072156775 0.106410955 0.193776812
TBI 3 S3_6.0W 0.072919594 0.082080609 0.097950306
TBI 1 W1_2.0E 0.127345745 0.099112164 0.062020137
TBI 1 W1_2.0W 0.031174519 0.10760026 0.050042839
TBI 1 W1_6.0E 0.063645896 0.05034088 0.041601004
TBI 1 W1_6.0W 0.089472373 0.071029221 0.028484694
TBI 2 W2_2.0E 0.013794042 0.031179405 0.009295093
TBI 2 W2_2.0W 0.112743993 0.067713577 0.046507906
TBI 2 W2_6.0E 0.078484592 0.050365955 0.069529824
TBI 2 W2_6.0W 0.105922092 0.037591957 0.059475741
TBI 3 W3_2.0E 0.070323084 0.095922707 0.173617382
TBI 3 W3_2.0W 0.081712715 0.064774083 0.083519686
TBI 3 W3_6.0E 0.054770541 0.108909869 0.072464946
TBI 3 W3_6.0W 0.077225643 0.038913303 0.060699893

; run;

proc univariate data=wilcoxon normal;
var june oct april;
QQplot june oct april/normal (MU=EST SIGMA=EST);
run;
proc NPAR1WAY data=wilcoxon wilcoxon correct=no;
class distance;
var june oct april;
run;
exact wilcoxon;
run;
title "Kruskal-Wallis Analysis";

%include 'c:sasmacrosKW_MC.sas';
data KW;
input species$ Rep id june oct april;
cards;
M  1      M1      .      97064.38      15421.25
M  2      M2      .      111788.25      72109
M  3      M3      .      111691.5      68955.75
M  4      M4      .      109576.75      10525.25
O  1      O1_2.0E      .      81343.12      85579.25      29320.5
O  1      O1_2.0W      .      37744.62      102920.62      89215.38
O  1      O1_6.0E      .      143234.5      100199      35734.75
O  1      O1_6.0W      .      50499.25      152942.75      30390.25
O  2      O2_2.0E      .      56365.88      69901      19437
O  2      O2_6.0E      .      106133.25      54748.12      13597.75
O  2      O2_6.0W      .      83760.25      72825.88      91935.25
O  3      O3_2.0E      .      98230.75      20640.75      52468.62
O  3      O3_2.0W      .      63551.5      163896.5      30416
O  3      O3_6.0E      .      101567.875      32640.5      45301.88
O  3      O3_6.0W      .      91055      135231.25      16166
P  1      P1_2.0E      .      53089.75      105910.62      73533.38
P  1      P1_6.0E      .      174564.5      59870.12      14972.5
P  1      P1_6.0W      .      108807.75      254466.5      7549.12
P  2      P2_2.0E      .      61087.75      90742.88      37437.75
P  2      P2_6.0E      .      122017.38      82350.75      65974.75
P  2      P2_6.0W      .      61498.125      64102.12      25284.75
P  2      P2_6.0W      .      175798.25      130831.25      6277.88
P  3      P3_2.0E      .      40192.5      61410.12      34466.88
P  3      P3_6.0E      .      54756.62      100102.75      22711.38
S  1      S1_2.0E      .      65695      46737.88      33457.38
S  1      S1_6.0E      .      192133.12      42258.5      11086
S  1      S1_6.0W      .      54756.62      100102.75      22711.38
S  1      S1_6.0W      .      93293      55664.25      18791.88
S  2      S2_2.0E      .      70013.62      122312      18343.38
S  2      S2_6.0E      .      68855.88      27428.25      15459.75
S  2      S2_6.0W      .      49555.25      28977.75      39580
S  3      S3_2.0E      .      31462      52129.88      18155.88
S  3      S3_6.0E      .      42135.25      72006.25      18628
S  3      S3_6.0W      .      87770.5      217431.375      35537
W  1      W1_2.0E      .      84021.25      62488.25      11986.25
W  1      W1_2.0W      .      161972.75      110425.88      7629.5
W  1      W1_6.0E      .      21158      15623.5      11350.5
W  1      W1_6.0W      .      55157.75      49774.12      7287.25
W  2      W2_2.0E      .      60777.88      18561.5      9696
W  2      W2_2.0W      .      63578.62      15154.75      25404.88
W  2      W2_6.0E      .      64580.25      23508.25      21169
W  2      W2_6.0W      .      53817.125      42011.38      8438.75
W  3      W3_2.0E      .      59263.8    14745.12      4927.38
W  3      W3_6.0E      .      53817.125      42011.38      8438.75
W  3      W3_6.0W      .      31421      27049.62      33133.25
;
run;

proc univariate data=friedman normal;
var june oct april;
QQplot june oct april/normal (MU=EST SIGMA=EST);
run;
/* Define Required Variables for the Macro*/
%LET NUMGROUPS=5;
%LET DATANAME= KW;
%LET OBSVAR= june oct april;
%LET GROUP=species;
%LET ALPHA=0.05;

Title "Kruskall-Wallis of Size Distribution Curves";
Title2 "with equal sample sizes for each group";

/* invoke the KW_MC macro*/

ODS HTML Style = statistical;
%KW_MC(source=&DATANAME, groups=&NUMGROUPS, obsname=&OBSVAR, gpname=&GROUP, sig=&alpha);
run;

ODS HTML close;
9 Appendix 2: Agroforestry Research Site Imagery

Figure 9.1: Google Earth snapshot showing spatial distribution of where each replicate of soils under various tree species, and solecrop system samples were extracted. The image shows the TBI system further north, and the solecrop system in the southern corner.
Figure 9.2: Image showing view from within the tree based intercropping system. Tree rows are spaced 12m, and soybeans are being grown in between the rows. Image was taken in June of 2012 during soil sampling.
Figure 9.3: Image showing the solecrop system from ground level. Photo was taken in October of 2012.