## INVESTIGATING SOIL AGGREGATE PORE STRUCTURES AND THEIR RELATIONSHIP TO BACTERIA SPATIAL DISTRIBUTION USING X-RAY COMPUTED MICROTOMOGRAPHY

By

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### ABSTRACT

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Soils are among the extremely complicated materials with intricate internal pore geometries. Understanding the complex interaction between soil aggregate structure and soil processes will greatly help us appreciate critical processes at larger scale. However, due to technical limitations on accessing aggregate interiors, it remained relatively poorly understood until recent advances of X-ray computed microtomography ( $\mu$ CT) imaging in soils. Overall, the dissertation aims to effectively utilize soil aggregate  $\mu$ CT imaging, to understand soil aggregate structure difference under contrasting land use and managements, and to investigate bacteria redistribution and transport pattern and its relation to aggregate pore characteristics.

Synchrotron  $\mu$ CT provides a noninvasive approach to record three dimension (3D) information on soil aggregates with resolution of one to several microns. Image segmentation is the first step in  $\mu$ CT image analysis; however, lack of ground-truth images poses great challenge to soil image segmentation. Proposed simulation method successfully generated soil aggregate grey scale images from pore/solid binary images, thus provided ground-truth information. Indicator Kriging method was found preferable to other methods in this study when the image histogram is clearly bimodal. Region non-uniformity measure performs sufficiently well as a segmentation criterion. The second technical difficulty involves 3D soil aggregate boundary delineation. Image closing technique is a promising tool for boundary detection, yet it requires

selecting optimal neighborhood parameter (r) so that aggregate exteriors can be properly defined – inclusion of all surface pores while keeping the surface sufficiently irregular.

After addressing these image processing issues, soil aggregates from long term contrasting soil types and land use practices were compared. The studied soil managements are conventional tillage (CT), native succession vegetation (NS), and bare soil with no vegetation (BS). Significantly greater percent of pores > 15  $\mu$ m in LTER-CT aggregates was observed as compared to those of LTER-NS. LTER-NS aggregates had more large pores (> 90  $\mu$ m) than LTER-CT aggregates, while more medium sized pores (45-90  $\mu$ m) were found in LTER-CT aggregates. Pores were larger in the aggregate interiors than in the exterior layers; while intermediate pores (45-90  $\mu$ m) were more abundant in the aggregate formation, but long term land use could alter the magnitude and intensity of involved soil processes.

The last part of the dissertation addressed *E.coli* redistribution and transport in soil aggregates. *E.coli* redistribution within soil aggregates display a significant different spatial distribution pattern under air-dry and saturated flow condition. When *E.coli* was first applied to an air-dry aggregate, its resulting spatial distribution was highly heterogeneous in aggregates of all management practices. After saturation, equilibration, and water extraction the *E.coli* redistributed within the aggregate primarily in vertical direction and became more homogeneously spread. Only relatively small percent of *E.coli* has completely left the aggregates. Redistribution was most pronounced in CT aggregates, followed by NT, and was almost negligible in NS aggregates. *E.coli* spatial distribution was related to intra-aggregate pore characteristics; however, because of high variability of the intra-aggregate pores the relationships were weak.

I dedicated this dissertation to my wife, Ming Gu, for her love and support.

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# **Chapter 1: INTRODUCTION**

Soils are among the extremely complicated materials with intricate internal pore geometries. Distribution of countless pores within soil aggregates is highly spatially heterogeneous. Soil's hierarchical structures control various physical, chemical and biological processes at different scales. At the soil aggregate scale, configuration of pore space plays an important role in determining carbon (C) sequestration, water and gas flow as well as bacteria survival and transport. Understanding the complex interaction between soil aggregate structure and these processes will greatly help us appreciate critical processes at larger scale, e.g., C spatial distribution, global C cycle, and subsurface water contamination caused by bacteria outbreaks. However, due to technical limitations on accessing aggregate interiors, it remained relatively poorly understood until recent advances of X-ray computed microtomography ( $\mu$ CT) imaging in soils.

Synchrotron  $\mu$ CT provides a noninvasive approach to record three dimension (3D) information on soil aggregates with resolution of one to several microns. Last decade witnessed fast developments in soil imaging applications (Capowiez et al., 1998; Pierret et al., 1999, 2002; Dathe et al., 2006; Peth et al., 2008; Udawatta et al., 2008; Kravchenko et al., 2009, 2011). Even though  $\mu$ CT imaging is promising and powerful tool, it is only recently being applied to soil science and there is a large number of technical difficulties that need to be resolved. Accurate image analysis techniques are indispensable to getting meaningful and reliable results. The overall goal of my research was to effectively utilizing soil  $\mu$ CT images to understand soil aggregate structures under different land use and soil managements and their relationship to soil

processes, for which I focused on bacteria distribution and transport in soil aggregates. However, prior to reaching it I had to combat a number of technical difficulties in image processing and develop techniques for solving them.

A technique of particularly importance is image segmentation and so far a number of methods have been published (Sezgin and Sankur, 2004; Iassonov et al., 2009). Soil aggregate pore characteristics, such as porosity, pore-size distribution, tortuosity and connectivity can be calculated or derived from image analysis. The accuracy of these parameters strongly depends on the goodness of segmentation methods – the technique used to identify the pore and solid space in soil aggregates. However, pronounced artifacts such as partial volume effect and noisy nature of  $\mu$ CT pose great challenges to interpreting soil image segmentation results. Application of segmentation on soil images was still at starting stage and different methods yielded highly variable results (Iassonov et al., 2009; Baveye et al., 2010). One of the reasons was that soil matrix is much more diverse in origins and densities of its solid materials than many other natural substrates. Another difficulty is that unlike other discipline that use  $\mu$ CT imaging (e.g., sandstones (Lindquist et al., 2000); hydrate hosted sediments (Jones et al., 2007)), in soil images there is no ground-truth information regarding about pore/solid classification. Therefore, new approaches are needed in order to indentify best segmentation methods in soils.

The second technical difficulty I have to conquer involves 3D soil aggregate boundary delineation. Until now, most of the aggregate image studies have been conducted by selecting a regularly shaped region of interest within the aggregate's central part or soil cores (Dathe et al., 2006; Peth et al., 2008; Udawatta et al., 2008; Baveye et al., 2010). This precluded full assessment of the aggregate's pore structure and the differences in flow and transport properties of the exterior and interior layers and interactions between them. Image closing technique is a

promising tool for boundary detection (Nunan et al., 2006), yet it requires selecting optimal neighborhood parameter so that aggregate exteriors can be properly defined – inclusion of all surface pores while keeping the surface sufficiently irregular.

After addressing these image processing issues, soil aggregates from long term contrasting soil types and land use practices were compared. Long-term differences in land use and management generate notable changes in soil physical and hydraulic properties, including changes in soil organic matter content, porosity and hydraulic conductivity (Brye and Pirani, 2005). Surface soil disturbance through tillage alters overall soil aggregation, stability, biological properties and distribution of C in aggregates (Lal et al., 1998, 2002; Six et al., 1999; Green et al., 2007). Besides soil disturbance, presence of vegetation and vegetation type can also alter soil properties through plant roots activities and their association with soil organisms (Wienhold and Tanaka, 2001; Udawatta et al., 2008). However, precise linkage between these observations and soil pore structures are still under development.

A well known challenge to researchers' perception is on soil aggregates formation theory. It should be pointed out that despite an enormous amount of work in the aggregate studies, there is no generally accepted understanding of how the aggregates are internally assembled and what the intra-aggregate pore structure characteristics are (Tisdall and Oades 1982; Oades et al, 1984; Augustin et al., 1995; Horn et al., 1994; Chenu et al., 2001; Park and Smucker, 2005). Therefore, this dissertation is aimed to fill these knowledge gaps by investigating the pore structures among aggregates and within individual aggregate layers.

Last but not least, the dissertation studied *E.coli* redistribution and transport in soil aggregates. *E.coli* has been regarded as an important indicator of environmental quality and

public health risk (CDC; Vogt and Dippold, 2005), and understanding mechanisms controlling transport and redistribution upon precipitation in soils is crucial for agricultural practices and food safety (Jamieson et al., 2002; Guber et al., 2005). Till now, it has been found that soil structure and nutrient distribution at macro and micro scale are believed to be responsible for *E.coli* survival and transport within soil matrix (Pachepsky et al., 2006), and precipitation facilitates infiltration of bacteria suspensions through flow pathways into deeper ground (Jenkins et al., 2008). Soil organic matter can hinder *E.coli* attaching to soil particles due to its negative charges (Johnson and Logan (1996)), whereas high clay content in soils may promote bacteria attachment (Huysman and Verstrate, 1993). Though findings above were mostly at field and soil column scale, it is highly possible that *E.coli* travel within aggregates and further release from the aggregates are strongly associated with pore structures they passed en route. However, lacking of appropriate tools and modeling techniques, few past studies addressed *E.coli* distribution and transport at micro scale in soils.

The dissertation is organized as follows: In Chapter 2, a simulation approach for generating soil aggregate  $\mu$ CT images based on ground-truth information is proposed to compare several segmentation methods and criteria to assess segmentation performance were discussed. Chapter 2 studies the optimal soil aggregate boundary identification and compared soil aggregate pore structures difference for aggregates from contrasting soil type and land use managements. Spatial gradient of pore characteristics within individual aggregate were also discussed. Chapter 3 discusses multivariate relationship among soil pore characteristics, *E.coli* redistribution pattern under saturated condition, and investigates the relationship between *E.coli* spatial concentration and soil aggregate pore characteristics. Chapter 5 states the major conclusions and points out future research to extend the notions proposed in this study.

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# Chapter 2: COMPARISON OF IMAGE SEGMENTATION METHODS IN SIMULATED 2D AND 3D MICROTOMOGRAPHIC IMAGES OF SOIL AGGREGATES

#### ABSTRACT

Advances in X-ray microtomography (µCT) are opening new opportunities for examining soil pore structures. However, usefulness of µCT data for pore structure characterization depends on how accurately the grayscale images are segmented into binary pore and solid components. Multiple segmentation algorithms have been developed for a variety of physical and biological substrates. However, it is not clear how applicable they are for µCT images of soils, since soil is among the most complex porous media due to diversity in origins and densities of composing solids. One of the difficulties in comparing the performance of segmentation algorithms is the lack of ground-truth information in the soil samples subjected to µCT. This means that only the criteria that do not depend on the availability of the ground-truth data can be used in assessing performance of the segmentation methods, yet the reliability of such criteria in soil images is unclear. In this study to resolve the problem of the lack of groundtruth information we simulated 2D and 3D soil images. The objectives of the study were (i) to compare several commonly used segmentation methods, namely, entropy-based method, iterative method, Otsu's method, and indicator kriging (IK) method; and (ii) to evaluate performance of the region non-uniformity measure (NU), the criterion that does not depend on presence of the ground-truth image, and compare it with the ground-truth-based misclassification error criterion. We found that though there was no single segmentation method that preserved pore

characteristics in all the cases, IK method yielded segmented images most similar to the groundtruth in most of the cases when histogram of image grayscale values had clearly distinguishable peaks. For the image with poorly distinguishable histogram peaks the IK did not perform well, while clustering methods produced acceptable segmentation results. The results indicated that selecting a segmentation method based on NU did not always produce optimal representation of pore characteristics. However, overall, the NU was found to be an acceptable criterion for segmentation method selection in  $\mu$ CT soil images.

#### **INTRODUCTION**

Image segmentation into pores and solids is a crucial step in most soil image analyses (Vogel and Kretzschmar, 1996; Capowiez et al., 1998; Lehmann et al., 2006; Mooney et al., 2006, 2007; Papadopoulos et al., 2008a, 2008b; Kaestner et al., 2008; Schapp and Tuller, 2010; Piñuela et al. 2010; Baveye et al., 2010; Vaz, et al., 2011; Vogel et al., 2010). Without accurate segmentation, results of subsequent data analyses might be misleading.

The segmentation methods that have been applied in geosciences can be broadly classified as global thresholding methods that generate a single threshold applied to the entire image; and local thresholding methods that adapt the threshold(s) value depending on local image characteristics.

Among a large number of global thresholding methods developed, the ones that appear to be most promising for soil applications are clustering methods and entropy-based methods (Sezgin and Sankur, 2004; Iassonov et al., 2009; Iassonov and Tuller, 2010). Sezgin and Sankur (2004) reported the best performance of clustering methods and entropy-based methods among 40 segmentation methods in thermal/ultrasonic/light-microscope imaging applications. In a recent study, Iassonov et al. (2009) evaluated 14 segmentation methods for application to industrial and synchrotron computed tomography images of soils, sand-bentonite mixtures, and precision glass beads, and reported Otsu's and iterative method to be the best among the tested global thresholding methods. However, global thresholding methods were often found to be inferior to the local segmentation methods (Oh and Lindquist, 1999; Peth et al. 2008; Iassonov et al., 2009).

Among many existing local segmentation methods (Pal and Pal, 1993; Oh and Lindquist, 1999; Lehmann et al., 2006; Elliot and Heck, 2007), a two-pass indicator kriging (IK) has been shown to significantly reduce the misclassification errors (Oh and Lindquist, 1999). However, one of the difficulties with IK application is that, unlike many automated segmentation methods, IK requires user-specified input on its parameter values and despite popularity of IK segmentation in soil studies (Peth et al., 2008, 2010; Udawatta et al., 2008), as of now there are no criteria or guidelines on proper selection of the IK parameter values.

A significant difficulty in comparing accuracy of different segmentation methods for soil images is the lack of the ground-truth binary pore/solid information. When ground-truth information is available, the accuracy of segmentation methods can be assessed using a variety of discrepancy based criteria, among which are: misclassification error (ME), edge mismatch, and, Rand index and its extensions (e.g., normalized probability Rand index) (Yasnoff et al., 1977; Sezgin and Sankur, 2004; Unnikrishnan et al., 2005). Zhang (1996) has shown that ground-truth based segmentation criteria, which account for misclassified voxels and specific ground-truth based features of the segmented objects, are among the most powerful tools for

selecting segmentation methods. However, none of these criteria can be applied to soil images because of the impossibility of obtaining ground-truth information.

Because of the lack of ground-truth information for selecting segmentation methods in soil images the researchers have to use criteria that do not depend on the ground-truth. A number of such criteria have been proposed, e.g., region non-uniformity (NU) measure (Levine and Nazif, 1985; Sezgin and Sankur, 2004), inter-region contrast (Levine and Nazif, 1985) and shape measure of voxels' neighborhood (Sahoo et al., 1988). Among these criteria, NU has the potential of being particularly useful for soil X-ray imaging because of its simplicity and direct measure of the homogeneity of the regions. Several color image segmentation studies (Liu and Yang, 1994; Borsotti et al., 1998) had reported good matches between NU/inter-region contrast and visual assessments. However, how well NU criterion will perform for selecting optimal image segmentation method in soils is not known.

Information from soil imagery that is critical for modeling flow and transport processes in soils includes various soil pore characteristics, e.g., pore size distributions, pore connectivities and tortuosities. When working with artificial systems, e.g., glass beads or collections of capillary tubes, some of these characteristics (porosities, solid surfaces, and pore size distributions) can be calculated based on the properties of the system. Thus the performance of segmentation methods can be assessed based on how closely they match the theoretically calculated values (Iassonov et al., 2009; Porter et al., 2010). However, in an undisturbed soil sample it is not possible to accurately assess porosities, surface areas, or distributions of the pores visible at the X-ray image resolutions used in the study (Kasteel et al. 2000). Even though the total porosity of the sample can be accurately measured (Black and Hartage, 1986), the total porosity is based not only on the pores visible at the studied X-ray resolution, but on the entire body of soil pores, including those invisible at the studied resolution. Soil pore size distributions can be obtained experimentally. However, a variety of factors, including hysteresis and experimental measurement errors, make them also unreliable as segmentation criteria.

In this study we will generate simulated 2D and 3D X-ray soil images with a wide range of soil porosities such that they mimic features of the real soil images (Zhang, 2001; Schlüter et al., 2010). Then, the ground-truth information available from the simulated images will be used (i) to explore optimal parameter selection for IK segmentation; (ii) to compare the accuracy of several commonly used segmentation methods, namely, entropy based method, iterative method, Otsu's method, and IK method; and (iii) to evaluate the performance of NU criterion in choosing the optimal segmentation method for soil images when the ground-truth information is not available.

#### **MATERIALS AND METHODS**

#### Soil sample collection

Soil aggregates were collected at the Long Term Ecological Research Site, Kellogg Biological Station in southwest Michigan (85° 24' W, 42° 24' N). The soils are well-drained Typic Hapludalfs of the Kalamazoo (fine-loamy, mixed, mesic) and Oshtemo (coarse-loamy, mixed, mesic) series, developed on glacial outwash. Soil blocks approximately 15 x 15 cm in size were extracted from 0-20 cm depth using a sharp flat spade. Air-dried soil samples were manually sieved for 30 seconds and the aggregate fractions, 4 to 6.3 mm, were retained for this study.

#### Synchrotron X-ray microtomography of soil aggregates

#### Image acquisition

Aggregate image data were collected on the monochromatic bending magnet beam line, station 13-BM-D of the GeoSoilEnvironCARS (GSECARS) at Advanced Photon Source, Argonne National Laboratory, IL. X-ray beam line of 28 keV incident energy was used to scan the aggregate samples. 3D image consisting of 520 slices with 696x696 pixels per slice were generated. The voxel size was 14.6 μm. Detailed information on image acquisition is given in Rivers et al. (1999). The scanned grayscale images were used to generate binarized ground-truth images.

#### Data processing

Raw data was processed using GSECARS tomography processing software (Details are available at <u>http://cars9.uchicago.edu/software/idl/tomography.html</u>). Data processing consisted of preprocessing and reconstruction. Preprocessing of the 2D images read the raw data from camera and wrote the data as a single 3D file. Ring artifacts were removed and a fast Fourier transform algorithm was used to reconstruct the data (Rivers, 1998). After reconstruction, the grayscale values (GV) ranged from 0 (black) to 255 (white) corresponding to low and high X-ray attenuation, respectively.

#### **Generation of simulated grayscale images**

Please note that for all the following discussions we refer to 'pixel' for 2D images, and 'voxel' for 3D images.

#### 2D cases

To assess the accuracy of different classification methods and compare different segmentation criteria, we generated eight 2D binary soil aggregate images that served as the ground-truth image information. In order to assess a wide variety of soil porosity conditions, we generated the images with porosity ranging from low (3-5%) to high (22-28%).

Generation of the simulated grayscale images consisted of combining several image layers. The first pore/solid image layer corresponded to the scale of the true aggregate images (pixels of  $14.6x14.6 \mu m$ ) and was 100x100 pixels in size (Figure 2.1(a)). The pore/solid data of this layer represented the soil pore structure visible at the studied scanning resolution.

In  $\mu$ CT images of real soil samples, the actual pore structure visible at the studied resolution is obscured by a number of influences. These influences include partial volume effects due to the presence of pores and solids in a single pixel (Ketcham and Carlson, 2001); variability in densities and X-ray attenuations among the pixels of the solid material (e.g. quartz versus organic matter); and noise generated during scanning. In generating the images we simulated contribution of these three influences.

The partial volume effect was simulated by generating three layers representing a range of pore sizes smaller than the scanned 14.6x14.6  $\mu$ m pixel size (Figure 2.1(b-d)). The smaller scale layers had pixel sizes of 7.3x7.3, 3.7x3.7, and 1.9x1.9  $\mu$ m, respectively. To ensure realistic representation of the soil pore structure we used manually thresholded  $\mu$ CT images from real soil samples to produce the binary images for these three layers. When the size of the small scale images exceeded that of the available aggregate images from the  $\mu$ CT scan, several binarized

 $\mu$ CT images were combined. For example, in order to sufficiently cover the 100x100 pixel 14.6x14.6  $\mu$ m layer, the 1.9x1.9  $\mu$ m layer had to consist of 800x800 pixels. To generate the 1.9x1.9  $\mu$ m layer, four images from real soil samples 400x400 pixels in size were joined and then scaled down in their physical size to match 100x100 14.6x14.6  $\mu$ m layer (Figure 2.1(d)). Simulation of the partial volume effect proceeded as follows: first, the binary pore information of the 1.9x1.9  $\mu$ m resolution image (Figure 2.1(d)) was overlaid with that of the 3.7x3.7  $\mu$ m image (Figure 2.1(c)). Then, corresponding 1.9x1.9  $\mu$ m values were averaged for each 3.7x3.7  $\mu$ m image pixel. Then the 3.7x3.7  $\mu$ m image was overlaid by and averaged across pixels of the 7.3x7.3  $\mu$ m image (Figure 2.1(b)) and then with 14.6x14.6  $\mu$ m image (Figure 2.1(a)). Their averaging to the actual 14.6x14.6  $\mu$ m pixel size produced the grayscale image that reflects the partial volume effect (Figure 2.1(e)).

**Figure 2.1:** Illustration of image simulation process. White represents the solid space and black (gray) represents the pore space. (a) Binary image of the "true" image pixel size (14.6x14.6  $\mu$ m); (b) Binary image of the pores visible only at pixel size 7.3x7.3  $\mu$ m;(c) Binary image of the pores visible only at pixel size 3.7x3.7  $\mu$ m; (d) Binary image of the pores visible only at pixel size 1.9x1.9  $\mu$ m; (e) Gray scale image of the pores visible at pixel size 14.6x14.6  $\mu$ m obtained by combing a), b), c), and d); (f) Final simulated grayscale image.



After simulating partial volume effect, the resulting image (Figure 2.1(e)) was used to simulate variability in atomic densities and X-ray attenuations among the pixels of the solid material. The pixels that contained no pore space (pure 'white' (GV = 255) pixels on Figure 2.1(e)) were regarded as pure solid space. We assumed that spatial correlation was present in the solid material density distribution, that is, the pixels were more similar in their grayscale values to the pixels that were closer in space than to those that were farther away. To represent the spatial correlation, simulations based on LU decomposition technique (Christakos, 1992) were

performed using PROC SIM2D (SAS Inc., 2001). LU decomposition technique was employed because it was proven to be computationally efficient in spatial simulations (Deutsch and Journel, 1998). The mean values and covariance structures for LU simulations were determined by analyzing actual  $\mu$ CT images of soil aggregates using subroutine GAM in GSLIB (Deutsch and Journel, 1998). Pixels that contained pore space ('gray' pixels on Figure 2.1(e)) were kept unchanged in the simulations.

To simulate scanning noise, after LU simulation, we added Gaussian noise. Please notice that we manually truncated the final GV within [0, 255] to ensure its validity. The resulting simulated grayscale image which was used for testing segmentation methods is shown on Figure 2.1(f).

#### A 3D case

Because of time and labor consuming process of generating simulated 3D images only one 3D image was produced. The same procedure described in detail for 2D simulation was used for 3D. The first pore/solid image layer corresponded to the scale of the true aggregate images (voxels of 14.6x14.6x14.6  $\mu$ m) and was 50x50x50 voxels in size. As in the case of 2D, the pore/solid data of this layer represented the soil pore structure visible at the studied scanning resolution. Partial volume effect was simulated by generating three image stacks with voxel sizes of 7.3x7.3x7.3, 3.7x3.7x3.7, and 1.9x1.9x1.9  $\mu$ m, respectively. These smaller scale stacks were combined in the same fashion as was done for 2D cases. The variability in densities in solid space was simulated using simulation annealing technique (Deutsch and Journel, 1998) implemented in WinGslib V1.5 (<u>http://www.statios.com/WinGslib/</u>, Accessed *December*, 08, 2010).

#### **Segmentation methods**

Global image segmentation methods used in this study were Renyi's entropy-based method (Sahoo et al, 1997), iterative method (Ridler and Calvard, 1978) and Otsu's method (Otsu, 1979). For this study we selected several of the existing methods that have received most positive feedback in the reviews, namely, we used entropy based and clustering based methods and indicator kriging approach. Entropy and clustering are global threshold procedures (Sezgin et al., 2004) that demonstrated potential for applications to soil CMT image segmentation. Indicator kriging method is a local threshold integrated approach that considers both the grayscale histogram and local spatial dependence structure, which has been successfully applied in rock and fluid research (Oh and Lindquist, 1999). Here we only provide brief descriptions of the segmentation procedures used in this study along with references to the sources where detailed method descriptions could be found.

#### Entropy based method

Originated from thermodynamics, the paradigm of entropy in information theory was first established by Shannon (1948) in order to quantify the information and uncertainty in communication networks. Many different generalizations, such as Renyi's entropy and Tsallis entropy were developed thereafter by parameterizing the Shannon entropy (Renyi 1961; Tsallis, 2001). Jaynes (1957) proposed the principle of maximum entropy which stated that the probability distribution on a proposition is the true probability distribution when it maximizes the information entropy, providing that all the possible probability distribution encode this information. This idea has been successfully applied to image segmentation field (Kapur et al., 1985; Chang et al., 1995; Sahoo et al., 1997). The image segmentation using entropy based method consists of finding a global threshold, such that maximizes the entropy of the two classes of objects, i.e., pore and solid pixels.

Renyi's entropy method (Sahoo et al., 1997) is a generalization of the maximum entropy sum method (Kapur et al., 1985) and entropic correlation method (Chang et al., 1995) by incorporating a parameter  $\alpha$  in Shannon's entropy (Shannon, 1948). The Renyi's entropies of the pore and solid space are calculated as

$$H_{\Lambda_1}^{\alpha} = \frac{1}{1-\alpha} \ln \sum_{i=0}^{t} \left(\frac{p_i}{p(\Lambda_1)}\right)^{\alpha}$$
(2.1)

$$H^{\alpha}_{\Lambda_2} = \frac{1}{1-\alpha} \ln \sum_{i=t+1}^{255} \left(\frac{p_i}{p(\Lambda_2)}\right)^{\alpha}$$
(2.2)

respectively, where  $P_i$   $(i = 0, 1, \dots, 255)$  denotes the probability distribution of image GV, t is the threshold,  $p(\Lambda_1) = \sum_{i=0}^{t} p_i$ ,  $p(\Lambda_2) = \sum_{i=t+1}^{255} p_i$  are the proportion of pore and solid pixels in the whole image, and  $\alpha > 0$  is the parameter that generalizes Shannon's entropy.

Reparameterization of  $\alpha$  (0 <  $\alpha$  < 1,  $\alpha \rightarrow$  1,  $\alpha$  > 1) in Renyi's entropy allows us to obtain three distinct thresholds where two correspond to the thresholds obtained from maximum

entropy sum method (Kapur et al., 1985) and entropic correlation method (Chang et al., 1995). Then a weighted average of the three thresholds is used as a threshold to segment the entire image. The weights are determined by the relative differences among the previously found three thresholds. This procedure was implemented using a program developed in MATLAB.

#### **Clustering methods**

This class of methods applies clustering analysis to the pore and solid pixels in order to minimize the error of classifying a pore pixel as a solid pixel or vice versa. These methods are helpful when it is difficult to identify the threshold due to the overlapping GV's range of the pore and solid pixels (Kittler and J. Illingworth, 1985 & 1986).

#### Iterative method

The iterative clustering method allows selecting the threshold automatically (Riddler et al., 1978). In this approach, an initial estimate of the threshold is selected so that the image is partitioned into "presumably" pore and solid spaces. Typically the initial estimate is the midpoint between the minimum and maximum GV of the image. Then the new threshold is calculated as

 $T = \frac{1}{2}(\mu_1 + \mu_2)$ , where  $\mu_1$  and  $\mu_2$  are the means of the two spaces. The iterations

terminate when the difference in the thresholds of the successive iteration is sufficiently small. This procedure was implemented in MATLAB.

#### The Otsu's method

The clustering method proposed by Otsu (1979) calculates the optimal threshold separating the pore and solid spaces so that their GV's intra-class variance is minimal. The GV's intra-class variance is defined as

$$\sigma_{w}^{2}(t) = \omega_{0}(t)\sigma_{0}^{2}(t) + \omega_{1}(t)\sigma_{1}^{2}(t)$$
(2.3)

Where  $\omega_0(t)$  and  $\omega_1(t)$  are the proportion of pore and solid pixels in the whole image separated by the threshold t,  $\sigma_0^2(t)$  and  $\sigma_1^2(t)$  are the variances for pore and solid pixels respectively. This procedure was implemented in MATLAB by stepping through each possible GV and finding the thresholds corresponding to the minimum  $\sigma_w^2(t)$ .

#### Indicator kriging method

Indicator kriging is the local adaptive segmentation method where each voxel is classified as either pore or solid based on its GV and local spatial correlation structure (Oh and Lindquist, 1999). The method involves selecting two thresholds such that the voxels with GV below the lower threshold,  $T_0$ , and the voxels above the upper threshold,  $T_1$ , are classified as pores and solids, respectively. Voxels with intermediate grayscale values are classified into pores or solids based on their proximity to previously classified voxels through indicator kriging system.

The two threshold values can be obtained by fitting the image GV histogram with mixed Gaussian distributions. For example, if a GV histogram has a clear bimodal pattern, then we can reasonably assume the existence of two Gaussian distributions corresponding to GV of pore and solid space respectively. The parameters of the mixed Gaussian distribution can be estimated using Expectation-Maximization algorithm which computes the maximum likelihood estimates of the distribution parameters (Dempster et al., 1977). The histogram of GV has the form (Oh and Lindquist, 1999) of

$$f(x) = \sum_{i=1}^{2} w_i \frac{1}{\sqrt{2\pi\sigma_i}} \exp^{-(x-\mu_i)^2/(2\sigma_i^2)}$$
(2.4)

Example of the fitted mixed Gaussian distribution for GV in a simulated image is shown in Figure 2.2 (a). The two thresholds,  $T_0$  and  $T_1$ , can be calculated as (Oh and Lindquist, 1999):

$$T_0 = \min(z_0, z_1), \quad T_1 = \max(z_0, z_1)$$
  
$$z_0 = \min(\mu_1 + r\sigma_1, \mu_2), \quad z_1 = \min(\mu_2 - r\sigma_2, \mu_1)$$
(2.5)

where weights  $(W_i)$ , means  $(\mu_i)$  and variances  $(\sigma^2)$  are the parameters estimated from Expectation-Maximization algorithm. The user-specified parameter r controls the distance between  $T_0$  and  $T_1$ . We explored the effect of parameter r on segmentation accuracy. For that, several r values ranging from 0 to 1.96, i.e., 0, 0.50, 1.00, 1.25, 1.50, 1.75, 1.96 were tried, where r of 0 corresponds to setting the thresholds at the histogram peaks and r of 1.96 corresponds to thresholds separating the 5% lowest and 5% highest values in solid and pore distributions, respectively.

The thresholds were calculated using Expectation-Maximization algorithm implemented in R (R Development Core Team, 2006). After threshold determination, the IK segmentation was conducted using segmentation module in 3DMA-Rock software (Lindquist, 2002). **Figure 2.2:** Examples of histograms of grayscale value distributions in two of the simulated images. (a) Image 7 with well defined pore peak; and (b) Image 1 with poorly defined pore peak. The blue curve represents the fitted Gaussian distribution corresponding to the pore space. The red curve represents the fitted Gaussian distribution corresponding to the solid space. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.




# **Majority filtering**

After segmentation the images often have a number of artificial single voxel pores or solids called 'spots', the removal of which benefits segmented image accuracy (Kaestner et al., 2008; Peth et al., 2008). Majority filtering is an effective moving-window-based tool to smooth the image by removing them. In this study we applied majority filtering following

recommendations of Oh and Lindquist (1999) with 3x3x3 filtering window for 3D and 3x3 window for 2D images and set the threshold for majority at 60%.

## Segmentation performance criteria

In this study, we used two formal segmentation criteria, namely misclassification error (ME) and region non-uniformity measure (NU). ME can be applied only when ground-truth information is available and, arguably, is the most objective criterion of the segmentation method performance (Zhang, 1996). NU can be used either with or without the ground-truth information.

In addition to using the formal criteria, we examined the effect of segmentation on several pore characteristics, including porosity, mean pore size, and pore surface area.

#### Misclassification error

Misclassification error is based on enumerating pore voxels that are incorrectly classified as solids and solid voxels incorrectly classified as pores (Yasnoff et al., 1977). It is calculated as

$$ME = 1 - \frac{P+S}{T} \tag{2.6}$$

where P is the number of common pore voxels in the ground-truth image and the segmented image, S is the number of common solid voxels in the ground-truth image and the segmented image, T is the total number of voxels in the image. Misclassification error can range from 0, corresponding to the perfect segmentation, to 1, corresponding to complete mismatch between the ground-truth image and the segmented image.

## **Region non-uniformity measure**

The region non-uniformity measure can be applied to assess segmentation quality even when the ground-truth information is not available (Zhang, 1996, 2001). This criterion is calculated as:

$$NU = \frac{P}{T} \cdot \frac{\sigma_P^2}{\sigma^2}$$
(2.7)

where *P* is the number of pore voxels in the segmented image, *T* is the total number of voxels in the image,  $\sigma_P^2$  is the variance of GV of pore space in the grayscale image,  $\sigma^2$  is the total variance of GV in the grayscale image. The NU can range from 0, corresponding to the case when the variance of the GV in voxels classified as pores is equal to zero, to 1, corresponding to the case when all voxels of the image are classified as pores and thus  $\sigma_P^2$  is equal to  $\sigma^2$ .

### **Pore characteristics**

Besides the criteria that describe general accuracy of pore/solid segmentation, i.e., ME and NU, it is of interest to assess how different the segmentation methods are in representing various characteristics of soil pore structure, such as porosity, pore size distribution and pore surface area (Iassonov et al., 2009; Baveye et al., 2010). These characteristics are of utmost importance for modeling soil water flow and solute transport, and such modeling is often considered as the main goal of soil image analysis. Using simulated images with available ground-truth information allows us to estimate how accurately segmented images obtained using different methods reflect the pore characteristics. Note that pore characteristics discussed here are those of the pores visible at the resolution of the scanned images used in the study (>14.6  $\mu$ m).

Porosity is defined as the proportion of pore voxels in total number of voxels in the image. Pore size is calculated as the maximum distance between each pore voxel and the closest solid voxel. Mean pore size is the average of all pore size data. Pore surface area refers to the contact surface area of pore voxels to adjacent classified solid voxels. All pore characteristics were calculated using SCAMP V1.2 (developed in SIMBIOS Centre, University of Abertay, Dundee, Scotland)) implemented in ImageJ (Rasband, 1997-2009).

#### **RESULTS AND DISCUSSION**

In order to compare the segmentation methods, we created 8 pore/solid binary images with porosities ranging from low (3-5%) to high (22-28%), as well as one 3D image with porosity 17% (Figure 2.3). The selected porosity values are representative of the porosities from pores >14.6  $\mu$ m typically observed in soil aggregates (Park and Smucker, 2005). Note that the porosity of >14.6  $\mu$ m pores is substantially lower than the total porosity, calculating using the bulk density method, of the 4-6 mm aggregates from the studied soil, which ranges from 30% to 40% (Ananyeva et al., 2009, personal communications). The images with low, medium and high porosities (images 1-6, Figure 2.3), contained disconnected small (~30  $\mu$ m), medium (~100  $\mu$ m) and large (~200  $\mu$ m) pores. The two images with the highest porosity (images 7-8, Figure 2.3) each contained one large macropore.

Figure 2.3: Simulated grayscale images with different porosities at 14.6x14.6 µm scale.



**Image 1** Porosity = 0.048



**Image 3** Porosity = 0.078



**Image 5** Porosity = 0.165



**Image 7** Porosity = 0.228



**Image 2** Porosity = 0.036



**Image 4** Porosity = 0.083



**Image 6** Porosity = 0.158



**Image 8** Porosity = 0.285



**Image 3D** Porosity = 0.170

#### Parameter selection for indicator kriging

The first step of the indicator kriging method (IK) application consisted of determining the two thresholds ( $T_0$  and  $T_1$ ). They were determined based on the parameters of the mixed Gaussian distributions fitted to the data and based on the user-defined parameter r (Eq. (2.5)). The mixed Gaussian distribution parameters, fitted using Expectation-Maximization algorithm for GV in the simulated images are listed in Table 2.1. The estimated means of the Gaussian distributions for the pore/solid space ( $\mu_1$  and  $\mu_2$ ) are quite consistent for images 2 to 8. However, in image 1, the pore space has greater estimated mean (67.7) and larger variance (1636.5). The main reason is that while the two peaks corresponding to pores and solids, respectively, were clearly visible in histograms of images 2-8 and of the 3D image, they were poorly distinguishable in image 1. The histogram shown on Figure 2.2(a) is typical of that observed for images 2-8 and the 3D image with identifiable two peaks, while the pore peak is not distinguishable in the histogram of image 1 (Figure 2.2(b)). The unreliable fitting of the histogram for image 1 could significantly affect the accuracy of pore/solid classification by providing too high value for  $T_0$ .

Image	$w_1$	$W_2$	$\mu_{1}$	$\mu_2$	$\sigma^2_{ m l}$	$\sigma^2_2$
1	0.131	0.869	67.7	169.2	1636.5	689.3
2	0.109	0.891	48.7	166.7	852.4	830.8
3	0.187	0.813	44.0	167.0	744.5	853.1
4	0.236	0.764	42.0	161.4	617.1	1177.5
5	0.061	0.939	36.0	149.3	630.8	1050.4
6	0.103	0.897	44.1	164.9	702.7	913.6
7	0.177	0.823	41.7	166.9	676.8	893.5
8	0.290	0.710	40.9	162.3	594.4	1133.0
3D	0.209	0.791	28.9	172.6	900.2	1700.4

**Table 2.1<sup>a</sup>:** Estimated parameters of the mixed Gaussian distribution in the simulated 2D images (images 1-8) and the 3D image.

<sup>a</sup> The symbols in this table correspond to Eq. (2.4).

We examined how selection of r affects ME and specific pore characteristics in the IK segmentation. Then we assessed how well the NU criterion performs as a tool for selecting r value in the IK segmentation. Since NU is the measure of segmentation accuracy that does not require ground-truth information, using it for r parameter selection in IK is of direct relevance to soil image segmentation tasks.

In five of the images (images 1, 3, 4, 5 and 3D) the smallest ME and NU were obtained at high r value ( $r \ge 1.25$ ) (Table 2.2 (a)). In three images (images 2, 6 and 8), selecting r value based on NU would result in ME 9-14% higher than the ME that would be obtained if r value were selected by minimizing the ME itself. In one of the images (image 7) the r selected based on NU would produce a 25% higher ME. This indicates that even though in many cases NUbased selection can be regarded as only negligibly different from the ME-based selection, the performances of the two criteria are not identical. **Table 2.2:** Assessing performance of NU in selecting parameter r for IK segmentation relative to accuracy of image segmentation.

(a)	P al	lativa	arror	in	ME
(a)	<b>N</b> U	allvc	CHOI	ш	IVIL,

Image	Minimum ME <sup>a</sup> /r	Minimum NU <sup>b</sup> /r	Relative errors in ME <sup>c</sup>
1	0.048/1.25	0.088/1.25	0
2	0.010/1.5	0.014/1.25	0.09
3	0.011/1.25	0.036/1.25	0
4	0.009/1.96	0.026/1.96	0
5	0.009/1.96	0.040/1.96	0
6	0.009/1.75	0.037/1.96	0.11
7	0.012/1.5	0.049/0.5	0.25
8	0.007/1.5	0.050/1.96	0.14
3D	0.032/1.75	0.058/1.75	0
Average			0.066

<sup>a</sup> Minimal ME is obtained by selecting r such that it minimizes ME.

<sup>b</sup> Minimal NU is obtained by selecting r such that it minimizes NU.

<sup>c</sup> Relative error for ME is calculated as the difference between the minimal ME and the ME obtained when r is selected by minimizing NU criterion. The difference is then divided by the minimal ME.

(b) Relative errors in pore characteristics from IK segmentation with parameter *r* selected based on NU and ME;

Image	Porosity		Mean pore size		Surface area	
	IK_ME	IK_NU	IK_ME	IK_NU	IK_ME	IK_NU
1 <sup>a</sup>	0.927	0.927	0.951	0.951	0.066	0.066
2	0.020	0.006	0.167	0.177	0.096	0.064
3	0.082	0.082	0.079	0.079	0.026	0.026
4	0.028	0.028	0.022	0.022	0.121	0.121
5	0.004	0.004	0.011	0.011	0.027	0.027
6	0.001	0.001	0.013	0.012	0.004	0.042
7	0.021	0	0.019	0.002	0.144	0.113
8	0.009	0.009	0.009	0.009	0.068	0.068
3D	0.019	0.019	0.103	0.103	0.068	0.068
Average	0.023	0.019	0.053	0.052	0.069	0.066

<sup>a</sup> Due to relatively high segmentation error caused by lack of bimodality in the histogram, image 1 is not included in computing the averages.

We calculated relative differences between the pore characteristics obtained from the images that were segmented using either NU or ME based r selection and those of the groundtruth images. Comparison between the pore characteristics from IK segmented images with rvalues selected based on the NU values (IK NU) and based on the ME values (IK ME) indicated that selecting r based on NU was advantageous for all the studied pore characteristics (Table 2.2(b)). In almost all cases (except for mean pore size of image 2 and pore surface area of image 6) the relative error from NU-based r value selection was less than or equal to that obtained using the ME-based selection. This indicates that r selections in IK segmentation with minimum ME would not necessarily be optimal for preserving the pore characteristics similar to those of ground-truth images. The possible explanation is that NU measures the non-uniformity over the pore space on the basis of GV variance of the pore space, which was evaluated at every pore voxel. ME, on the other hand, depends on both the misclassified pore and solid voxels, hence might not reflect the pore space properties as accurately as NU. Therefore, we recommend NU, a pore space based measure, as a segmentation criteria for selecting r value in IK segmentation.

## **Comparing performance of the segmentation methods using NU and ME criteria**

The 2D segmented images obtained using IK method (with r value selected based on ME and on NU), entropy method, iterative method, and Otsu's method after 60% majority filtering are presented in Figure 2.4 for images 1-8. The 3D images are presented in Figure 2.5. The calculated thresholds for the global methods are listed in Table 2.3. For all but one image (image 2), the entropy method consistently yielded larger threshold values than both Otsu's and iterative

method. This resulted in overestimated porosity in the images segmented using the entropy method.

Image	Entropy	Iterative	Otsu's
1	125	123	122
2	116	129	130
3	122	117	116
4	123	116	115
5	124	110	109
6	130	108	108
7	124	109	108
8	122	107	106
3D	138	120	119

**Table 2.3:** Thresholds obtained from entropy based method, iterative method and Otsu's method for the simulated 2D (images 1-8) and 3D images.

Figure 2.4: Segmented images using different segmentation methods at 14.6x14.6 µm scale.

In row: images 1-8;

In column: (a) Ground-truth binary images; (b) IK method with r selected based on ME; (c) IK method with r selected based on NU; (d) entropy method; (e) iterative method; (f) Otsu's method.



Figure 2.4 (cont'd).



**Figure 2.5:** 3D image stack with (a) ground-truth image and segmented using (b) IK method with r selected based on NU; (c) entropy method; (d) iterative method; (e) Otsu's method.



The assessment of segmentation performance using criteria ME and NU for different methods for 2D images 1-8 and for the 3D image are listed in Table 2.4. We observed that selecting best segmentation method based on ME and NU agreed for majority of the images. Both ME and NU would suggest Otsu's as the best segmentation method in image 1, and IK as the best method in images 2-4, 7, 8 and 3D. Only in two of the studied images (images 5 and 6) the two criteria disagreed. In these two images, the lowest ME was obtained from IK segmentation and the lowest NU from iterative or Otsu's methods. Visual assessment (Figure 2.4

(5), (6): (c) vs. (e)) suggested that the IK segmented images might be preferable to that of Otsu's method in these two images since they appear to better preserve similarity of pore features in segmented images to those of ground-truth images. For example, pores marked by yellow circles in Figure 2.4(5)(a) are mostly preserved in the IK segmented image (Figure 2.4(5)(a)), while they are distorted in the Otsu's segmented image (Figure 2.4(5)(a)).

**Table 2.4:** Summary of (a) ME and (b) NU using different segmentation methods for the simulated 2D (images 1-8) and 3D images. Numbers in **bold** represent best segmentation results according to the each segmentation performance criterion.

Image	IK_NU	Entropy	Iterative	Otsu's
1	0.048	0.030	0.027	0.026
2	0.011	0.042	0.112	0.122
3	0.011	0.024	0.019	0.018
4	0.009	0.031	0.027	0.027
5	0.009	0.035	0.035	0.035
6	0.010	0.034	0.029	0.029
7	0.015	0.057	0.031	0.031
8	0.008	0.032	0.018	0.018
3D	0.020	0.064	0.036	0.035

a) ME

b) NU

Image	Ground- truth	IK_NU	Entropy	Iterative	Otsu's
1	0.014	0.088	0.049	0.044	0.043
2	0.013	0.014	0.067	0.149	0.157
3	0.018	0.036	0.051	0.039	0.037
4	0.023	0.026	0.046	0.034	0.033
5	0.032	0.040	0.046	0.034	0.033
6	0.032	0.037	0.062	0.032	0.032
7	0.039	0.049	0.098	0.056	0.053
8	0.045	0.050	0.075	0.051	0.050
3D	0.031	0.039	0.116	0.060	0.056

The Otsu's and iterative methods outperformed the entropy method in terms of both ME and NU in all cases except image 2 (Table 2.4). The poor performance of iterative and Otsu's methods in image 2 can be related to the presence of a 'dark' region in the center of the image which has GV below the calculated thresholds. Note that for most of the studied cases, the iterative and Otsu's methods generated very similar segmented results because the thresholds calculated from the two methods were quite close (Table 2.3). Similar results were reported in Gonzalez et al. (2004).

The IK segmentation yielded the segmented images with lowest ME as compared to the other methods in all images except image 1 (Table 2.4). The main reason for the poor performance of IK segmentation in image 1 is the lack of bimodal distribution in the histogram of their GV (Figure 2.2(b)), which introduced substantial uncertainty in the Expectation-Maximization estimations of the mixed Gaussian distribution parameters. When histogram of GV does not have a bimodal distribution, user specified values may work better for the selection of  $T_0$  and  $T_1$  values (Oh and Lindquist, 1999). However, this introduces substantial trial and error subjectivity in the threshold selection. Good performance of IK in all but one images of this study are consistent with other reports that suggested great suitability of IK in studying the pore network characteristics in various porous media (Oh and Lindquist, 1999; Peth et al., 2008).

## Comparing performance of the segmentation methods using pore characteristics

Pore characteristics of the ground-truth images and of the segmented images obtained using different segmentation methods are plotted on Figure 2.6. For porosity, IK outperformed other methods in 7 of the 9 images (including 3D image) generating porosity values closest to that of the ground-truth image (Figure 2.6(a)). For pore surface area, IK outperformed other methods in 6 of the 9 images (Figure 2.6(b)). This result agrees well with the lowest ME values obtained from IK segmentation (Table 2.4 (a)). However, results from ME did not match the mean pore size very well (Figure 2.6(b)). IK produced the best mean pore size in images 5, 7, and 8. Iterative method produced the best mean pore size in images 1 and 2. Entropy method produced the best mean pore size in images 3, 4, and 3D. Otsu's method produced the best mean pore size only in image 6.



**Figure 2.6:** Summary of pore characteristics (a) porosity; (b) mean pore size; (c) pore surface area obtained from images segmented using different segmentation methods.

Image

Figure 2.6 (cont'd).



Image

Figure 2.6 (cont'd).



We also examined the worst cases in performance of each method for the studied images (Table 2.5). By the worst case we refer to the cases where the difference between the pore characteristics from the segmented and the ground-truth images was the largest. Based on the worst case assessment, IK and iterative methods were found to be preferable to other methods.

**Table 2.5:** Number of images (total 9 images) where the segmentation methods produced the 'worst' result in terms of pore characteristics.

	IK_NU	Entropy	Iterative	Otsu's
porosity	2	3	1	3
mean pore size	1	1	3	4
pore surface area	2	4	1	2
Total	5	8	5	9

Note that in most cases IK was among the methods that yielded the segmented images with pore characteristics similar to their ground-truth counterparts, though it was not always the best method.

To further summarize how well pore characteristics will be represented in the segmented images when the segmentation method is selected based on NU values, we conducted relative assessment of method performances (Figure 2.7). We calculated the relative errors as the differences between the pore characteristic value from the segmented image and the pore characteristic ground-truth value divided by the ground-truth value. The relative errors were calculated for two scenarios: for the segmentation method that would be selected as the best based on the NU criterion; and for the segmentation method that generated the pore characteristic value the most similar to that of the ground-truth. The results showed that, NU-based selection produced overall greater relative errors (5%-8%) than the minimal errors that were obtained using the best method for each pore characteristic (1%-4%). Notice that high variability in the relative errors is present in all pore characteristics for both scenarios (Figure 2.7). However, the differences in relative errors between NU-based and the pore characteristics-based were relatively small. This further indicates that even though inaccuracies in representation of pore characteristics in segmented images is to be expected; in the absence of ground-truth information the segmentation method selection using NU will produce acceptable results.

**Figure 2.7:** Comparing accuracy of NU-based segmentation method selection to pore characteristics-based selection. The relative errors are defined as the differences between the pore characteristic value from the segmented image and that from ground-truth value divided by the ground-truth value.



# **CONCLUSION**

Synthetic soil aggregate  $\mu$ CT images were simulated based on the 2D and 3D pore/solid binary images by taking into account the partial volume effect, differences in attenuation values of different solid materials, and noise that accompanies the scanning. Comparisons among entropy, iterative, Otsu's, and IK segmentation methods indicated that IK method was an optimal segmentation tool when the histogram of studied images had a clear bimodal pattern. However, for the images with poorly distinguishable histogram peaks the Otsu's method was preferable. In the absence of ground-truth information, choosing the value of IK parameter r using NU criteria produced acceptable segmentation results.

The segmented images with minimal ME and NU values were not always optimal in representing the pore characteristics, such as porosity, mean pore size, and pore surface area. However, since the errors in pore characteristic representation when image segmentation choices were made based on NU values were only slightly greater than the minimal errors, NU is recommended as an acceptable criterion for selecting segmentation method. BIBLIOGRAPHY

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# Chapter 3: INTRA-AGGREGATE PORE CHARACTERISTICS UNDER LONG-TERM CONVENTIONAL TILLAGE AND NATIVE SUCCESSION VEGETATION: X-RAY COMPUTED MICROTOMOGRAPHY

## ABSTRACT

X-ray computed microtomography (µCT) opened new opportunities for studying internal pore structures of individual soil aggregates. However, effective use of µCT data of irregularly shaped soil aggregates requires properly identified soil aggregate boundaries, where image closing technique is needed. Soil intra-aggregate pores of different land management practices control soil physical, chemical and biological processes differentially at micro scales. Soil aggregates from a Hapludalf soil under long-term conventional tillage (LTER-CT) and native succession vegetation (LTER-NS) in southwest Michigan, and from an Ustochrept soil under native succession vegetation (CERN-NT) and bare soil (CERN-BS) in northeast China were used in this study. LTER-CT aggregates had significantly greater image based porosity than those of LTER-NS. LTER-NS aggregates had more large pores (> 90µm) than LTER-CT aggregates, while more medium size pores (45-90µm) were found in LTER-CT aggregates. Greater abundance of medium sized pores in LTER-CT aggregates could be the cause of their reported lower stability and higher macro-aggregate turnover rate. The differences in pore size distributions between LTER-CT and LTER-NS were more pronounced in the aggregate interiors, as compared to the exterior layers. No difference in intra-aggregate pore characteristics was found between CERN-NT and CERN-BS aggregates. In aggregates from both treatments of both

studied soils pores tended to be larger in the aggregate centers while medium size pores (45-90µm) were more abundant in the aggregate exterior layers.

## **INTRODUCTION**

Soil matrix can be regarded as a hierarchical system formed by aggregates of various sizes (Dexter, 1988) and only relatively recently we started to appreciate the role that intraaggregate heterogeneity plays in numerous physical, chemical and biological processes (Ellerbrock and Gerke, 2004; Van Gestel et al., 1996). Pore arrangement within soil aggregates and their connectivity to the inter-aggregate pore space are recognized to be among the main factors controlling soil flux processes on an aggregate-scale (Revil and Cathles, 1999; Schapp and Tuller, 2011). However, the exact mechanisms by which pore structure affects soil functioning are still poorly understood due to poor accessibility of soil aggregate interiors to direct measurements and lack of efficient methodology for aggregate-scale modeling.

Long-term differences in land use and management generate notable changes in soil physical and hydraulic properties, including changes in soil organic matter content, porosity and hydraulic conductivity (Brye and Pirani, 2005). Surface soil disturbance through tillage alters overall soil aggregation, stability, biological properties and distribution of carbon (C) in aggregates (Beare et al., 1994; Lal et al., 1998, 2002; Six et al., 1999; Green et al., 2007). Besides soil disturbance, presence of vegetation and vegetation type can also alter soil properties through plant roots activities and their association with soil organisms (Wienhold and Tanaka, 2001; Udawatta et al., 2008).

Soil aggregates play an important role in C sequestration (Blanco-Canqui and Lal, 2004), and thus examining intra-aggregates pore structures in aggregates that are in land uses known for their contrasting C sequestration potentials will help us understand the mechanisms by which aggregates contribute to C sequestration. There were abundant literature on soil aggregate formation, but two contrasting models exist - hierarchy concept proposed by Tisdall and Oades (1982) and later developed by others (Oades et al, 1984) who believed soil macro-aggregates were built of micro-aggregates with organic binding agents; and soil aggregate layer concept (Augustin et al., 1995) where soil aggregates were formed around plant debris with soil materials attaching to the aggregate center. Soil aggregates often exhibit spatial gradients in intraaggregate porosities, which have been assessed in a number of studies (Sextone et al., 1985; Horn et al., 1994; Chenu et al., 2001; Park and Smucker, 2005a). These studies have demonstrated that tillage and vegetation could affect aggregates' exteriors and interiors differentially. A recent study by Urbanek et al. (2011) showed that soil aggregate formation was a result of combined hierarchical model and layer developing. It should be pointed out that despite an enormous amount of work in the aggregate studies, there is no generally accepted understanding of how the aggregates are built inside and of what the intra-aggregate pore structure characteristics are.

Recent advances in X-ray computed microtomography ( $\mu$ CT) imaging enabled us to quantify the intra-aggregate structures with a resolution of one to several microns (Gibson et al., 2006; Nunan et al., 2006; Dathe et al., 2005, 2006; Peth et al., 2008). This is a significant step in characterizing heterogeneity of soil environments at micro-scales, as it allows obtaining more precise information regarding the physical structure of the soil aggregate matrix in three dimensions (3D). Multi-scale soil solid-pore structures of intact soil matrix have been

characterized by extracting fractal dimensions or multifractal spectra (e.g., Dathe et al., 2005, 2006; Gibson et al., 2006) and by morphological characterization of soil macropore networks (Pierret et al., 1999, 2002; De Gryze et al., 2004; Mooney et al., 2006, 2007; Mooney and Morris, 2008; Peth et al., 2008; Udawatta et al., 2008). Nunan et al. (2006) applied geostatistical tools to quantify pore geometry for soil aggregates 1-3 mm in size at a scanning resolution of 4.4 $\mu$ m. De Gryze et al. (2004) used X-ray computed tomography to study the pore morphology changes in the soil aggregates incubated with fresh wheat residue and observed that porosity (pores > 27 $\mu$ m in size) increased from 3.5% in the control aggregates to 7% in the incubated ones. Peth et al. (2008) applied 3D  $\mu$ CT imaging to soil aggregates from conventional tillage and grassland at resolution of 3.2-5.4 $\mu$ m, and observed morphological differences in their pore characteristics. Papadopoulos et al. (2009) reported differences in pore shapes in 5 mm aggregates from conventional and organic management at 8  $\mu$ m resolution using  $\mu$ CT.

One of the difficulties in studying 3D images of the intact aggregates and pore characteristics of the aggregate layers lies in the need for accurate delineation of the aggregate boundaries. Until now, most of the aggregate image studies have been conducted by selecting a regularly shaped region of interest within the aggregate's central part. This precluded full assessment of the aggregate's pore structure and the differences in flow and transport properties of the exterior and interior layers and interactions between them. Nunan et al. (2006) proposed an innovative approach to analysis of CT aggregate images where the aggregate boundary is identified by a sequence of image processing steps, such as binary morphological closing and filling procedures. This is a significant advancement in soil aggregate image analysis. However, conducting image closing and filling involves specification of certain parameters, e.g., the neighborhood size parameter that defines the resulting 'smoothness' of the aggregate boundary

and the volume of the aggregate. The choice of the neighborhood size parameter value used during the image processing can greatly affect the final result. How to select the value of the neighborhood size parameter appropriate for soil aggregate image analysis remains unclear. Of particular concern is that the pore structure characterization of the aggregate exterior layers can be substantially affected. Note that the exterior layers are often of the greatest interest and importance for analyses of the aggregate functioning within the soil matrix.

The first objective of this study is to investigate the selection of the optimal values of the image processing neighborhood size parameter for defining soil aggregate boundaries using 3D soil aggregate µCT images. Once the aggregate boundaries were identified, we (i) assessed intraaggregate pore characteristics both in the whole aggregates and in the aggregate layers at different distances from the aggregate boundaries and (ii) analyze effects of land use on these characteristics in the aggregates from two different soil types, namely, Hapludalfs on glacial outwash, MI, USA and Ustochrepts on loess, Shenyang, China. The contrasting land use practices that we will compare are conventionally tilled agricultural management, bare soil without plant residue inputs, and native succession vegetation.

# **MATERIALS AND METHODS**

## Soil sample collection

Soil aggregate samples were collected from two long-term experiments located in the north central part of the U.S. and in the north east part of China.

The first experiment is the Long Term Ecological Research site (LTER), W.K. Kellogg Biological Station, established in 1988 in southwest Michigan (85° 24' W, 42° 24' N). Soils are well-drained Typic Hapludalfs of the Kalamazoo (fine-loamy, mixed, mesic) and Oshtemo (coarse-loamy, mixed, mesic) series, developed on glacial outwash. Details on site description, experimental design and research protocols of the experiment are provided on LTER site (Kellogg Biological Station, 2009). The two LTER treatments sampled for this study are conventional tillage (chisel-plowed) corn-soybean-wheat rotation with conventional chemical inputs (LTER-CT), and native succession vegetation treatment, abandoned from agricultural management after spring plowing in 1989 (LTER-NS). Prior to setting up the experiment in 1988 the entire area belonged to a uniformly conventionally tilled and managed agricultural field that has been in agricultural production for at least past 100 years. Soil samples from this site were collected in spring of 2008 from two adjacent 1-ha plots, one in LTER-CT and the other in LTER-NS. The distance between the sampling locations of these two plots was approximately 25 m. Maintaining a short distance between the samples of the two studied treatments allowed us to reduce effects of the local soil spatial variability on soil aggregate characteristics. Three sites were sampled at each of the treatments.

The second experiment is located at Shenyang Experimental Station of Ecology, Chinese Ecosystem Research Network (CERN), northeast China (41 ° 31' N, 123° 22' W). Soils are aquic brown soils (silty loam, Haplic-Udic Cambisols in Chinese taxonomy; Ustochrepts in USDA taxonomy), developed on loess. The two CERN treatments used in this study were native succession vegetation (CERN-NS) and bare soil with no vegetation (CERN-BS). The treatments were established in 2002 in the field that has been abandoned from agricultural use in 1994. The native vegetation in CERN- NS is dominated by *Artemisia lavandulaefolia*, *Cephalanoplos* 

*segetum*, *Chenopodium serotinum*, *Metaplexis japonica* and *Conyza Canadensis* (Hua et al., 2008). Soil samples were collected in summer of 2007. The distances between CERN-NS and CERN-BS sampling locations were approximately 20 m. Five sites were sampled at each treatment.

At each sampling site in both LTER and CERN experiments, a soil block approximately  $15x15 \text{ cm}^2$  in size was extracted from 0-20 cm depth using a sharp flat spade. Samples were airdried and manually sieved for 30 s. Aggregate 4-6.3 mm in size were retained for this study. We decided to use the aggregates of this size since differences in porosities among the layers of the aggregates of this size have been reported previously for a silty clay loam Alfisol (Park and Smucker, 2005a). Thus we hypothesized that differences between the layers could also be observed in  $\mu$ CT images of the LTER and CERN aggregates of this size used in this study.

Soil clay, silt, and sand contents in the aggregates were determined using Bouyoucos hydrometer method (Gee and Bauder, 1986) and total C and nitrogen (N) were measured by dry combustion method using Costech ECS 4010 Carbon-Nitrogen analyzer.

## Soil aggregate image acquisition

Aggregate image data were collected on the bending magnet beam line, station 13-BM-D of the GeoSoilEnvironCARS (GSECARS) at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Illinois, USA. Data were collected with the Si (111) channel cut monochromator tuned to 28 keV incident energy. Two dimension (2D) image slices were taken at 1° rotation angle steps and combined into a 3D image consisting of 520 slices with 696x696 pixels per slice. The voxel size was 14.6 µm. Based on our preliminary results, this resolution

was found to be optimal for the 4-6.3 mm aggregate size fraction. A snapshot of the image on the mirror was taken with the CCD camera representing a depth-integrated grayscale map of the linear attenuation of the X-ray beam as it passed through the sample. After an image was collected, the column was rotated 1°, and the process was repeated. Detailed information on the procedure is given in Rivers et al. (1999).

The data were preprocessed by removing ring artifacts (Ketcham and Carlson, 2001) and adjusting for backfield projections and reconstructed using Fast Fourier Transformation. After reconstruction, the grayscale values in the image ranged from 0 (black) to 255 (white) corresponding to low and high X-ray attenuations, respectively.

## **Defining aggregate boundaries**

In order to assess the pore structure using images of the whole aggregates and aggregate layers, we need to define the aggregate boundary. For that we used flood/seed fill algorithm (Heckbert, 1990). The algorithm begins with identifying the starting point, usually the upper left corner of the image, and then the region of the image connected to the starting point is determined by checking whether they have the same 'color' (usually black), and filled with another 'color' (usually white) serving as the background. One of the difficulties with the automatic application of this process for detection of the soil aggregate boundary is that the process may result in misclassification of the pores connected to the aggregate's surface as the outside space. Misclassification of these pores may significantly affect the subsequent pore network analysis. Figure 3.1 presents an illustration of the aggregate image (Figure 3.1(a)) and its incorrectly delineated boundary that classifies the pore connected to the aggregate's surface as an outside background (Figure 3.1(b)).

**Figure 3.1:** Example of (a) 2D unprocessed soil aggregate grayscale image slice; (b) the same image with the background in black and the aggregate in white where aggregate boundary has been identified using an automated flood/seed fill algorithm; note that the intra-aggregate pore connected to the outside is incorrectly classified as belonging to the background (red square); (c) the same image after boundary sealing algorithm has been applied with r = 12; (d) the final processed gray-scale image with the background in white; (e) the final processed grey-scale image with the background in white with r = 15; note the artificial pore pixels added to the sides of the image (red squares).


In order to ensure proper inclusion of such pores in the aggregate body we utilized an image closing technique (Gonzalez et al., 2004), that allows sealing pore "necks" located at the aggregate boundaries (Figure 3.1(c)). Image closing is a two-step procedure which involves image dilation followed by image erosion (Gonzalez et al., 2004). An illustration of the image closing procedure applied to a simple 2D image using a circular structuring element is shown in Figure 3.2. Details of the image closing technique can be found at Image Processing Learning Resources website (HIPR2, 2003). All image processing and data analyses were conducted using 3D aggregate images.

Outcomes of image closing are sensitive to the parameter called neighborhood size, r, which defines the size of the structuring element used for image closing operation (MATLAB, Version 7.8.0). The structuring element is a cluster consisting of a certain combination of pixels/voxels that is used to probe the surface of the unevenly-shaped image (Gonzalez et al., 2004). Detailed illustration of image processing using structuring elements with different sizes is provided by Vogel et al. (2010). In this study we used 3D spherically shaped structuring elements, from which the neighborhood size parameter r is defined by its radius. An example shows a spherical structuring element with r equal to 2 (Figure 3.3).

**Figure 3.2:** An example of 2D image closing using a disk-shaped structuring element: a) the original image; b) dilating the image a) (the portion added in a course of dilation is shown in light blue); c) eroding the image b) (the pixels that were not present in the original image are shown in red); d) the final image.



**Figure 3.3:** An example of a spherical structuring element with r = 2. a) Voxels of the structuring element are showing in red; b) central portion of the spherical structuring element (a) is showing in red.





If the selected parameter r is too small, it will lead to an insufficient closure of the pore necks. However, large values of r will result in over-smoothing of the aggregate boundary and inflation of the aggregate volume. In order to identify the optimal r value we examined values ranging from 0 to 20. The r of 0 corresponds to the original image where no sealing has been done.

The main issue is what should be used as a criterion for identifying the optimal r value. In this study we explored the usefulness and performance of two different potential criteria for rvalue selection. The criteria are the change in the overall aggregate volume as a function of r, and the change in the fractal dimension of the aggregate boundary as a function of r values. For analysis of the performance of these criteria in assessing the optimal value of r parameter, we used 3D images from four aggregates, one from each treatment in each experiment.

To determine the aggregate volume we used the total number of voxels classified as those belonging to the aggregate. When r = 0, the volume is equal to that of the original image. As r increases, so does the size of the structuring element and, hence, the aggregate's volume.

To determine the fractal dimension of the aggregate surface, the boundary was first identified using Canny edge detector (Canny, 1986) implemented in MATLAB. The fractal dimension of the boundary was then calculated using 3D box-counting algorithm (Mandelbrot, 1982). The aggregate boundary is the most irregular and has the highest fractal dimension when r = 0. As r increases, the boundary becomes smoother and fractal dimension decreases.

Once we have identified an optimal r value, it was applied to all aggregate images used in this study. Image closing with pre-selected r value has been conducted using *Imclose* in MATLAB Image Processing Toolbox (Gonzalez et al., 2004). The "closed" pores (Figure 3.1(c))

were then converted into parts of aggregate bodies (Figure 3.1(d)) using *Imfill* in MATLAB Image Processing Toolbox (Gonzalez et al., 2004).

#### **Image segmentaiton**

After boundary delineation, the stacks of grayscale images were segmented into pores and solids using indicator kriging segmentation module in 3DMA-Rock software (Lindquist et al., 2000). Multiple sets of the two thresholds in indicator kriging segmentation were chosen by the parameters of the fitted mixed Gaussian distribution of GV within the aggregate domain using Expectation-Maximization algorithm (Wang et al., 2010). The segmented images were assessed using region non-uniformity measure proposed by Levine and Nazif (1985) and evaluated by Wang et al. (2011). Detailed description of region non-uniformity calculation and applicability in soil image analyses is provided in Wang et al. (2011). All the calculations were conducted using 3D data sets.

# **Pore characteristics**

The segmented soil aggregate images were used to extract pore characteristics, including macro-porosity, medial axes, and burn numbers (BN) (Lindquist et al., 2000; Peth et al., 2008). Macro-porosity is defined as the percent of the pore voxels within the aggregate image, and in this study it is the porosity visible at scanning resolution of 14.6  $\mu$ m, i.e. pores >14.6  $\mu$ m. Medial axis is a digitized curve which represents the skeleton of the pore space. Burn number is the distance from the medial axis pore voxel to the closest solid voxel. A schematic representation of medial axes and burn numbers is demonstrated in Figure 3.4. The units for BN are the numbers

of voxels. Note that BN values are directly related to pore sizes, e.g., in this study, BN equal to 1 is equivalent to pore size of 15-30  $\mu$ m, BN equal to 2 is equivalent to pore size of 45-60  $\mu$ m, BN equal to 3 is equivalent to pore size of 75-90  $\mu$ m, etc. In this study the BN distribution was standardized by dividing the number of BN voxels of each size by the total number of pore voxels and we will refer to it as standardized fraction for BN (std. fraction). Calculations of the pore characteristics were conducted in 3DMA-Rock software.

**Figure 3.4:** A schematic representation of medial axes and burn numbers. Black pixels represent solid materials and white pixels represent pore space. Rainbow color scale pixels are medial axis paths with red for burn number equal to 2, yellow for burn number equal to 3, green for burn number equal to 4, etc.



The aggregate total porosity was determined from aggregate weights and volumes (Rossi et al., 2008). The aggregate volume was obtained using 3D aggregate images. Particle density for total porosity determination was measured using pycnometer method following procedure described in Bielders et al. (1990) using ~10 g samples consisting of several 4-6.3 mm aggregates. The particle density was not different among the treatments and was equal to 2.65 g/cm<sup>3</sup>.

# **Identification of aggregate layers**

Since one of the hypotheses of this study is that aggregate pore networks may differ in their characteristics depending on the distance from the aggregate boundaries, we conducted pore characterizations in three aggregate layers. This layer classification was consistent with and thus allowed direct comparisons with the study by Park and Smucker (2005b). The layers were identified based on the shortest distance between each voxel inside the aggregate and the aggregate's surface (Figure 3.5). The determination of the layers was conducted in 3DMA-Rock software using the same technique as the one applied for the BN determination. For that, first the maximum BN of each aggregate was determined. This number represented the distance between aggregates' surfaces and their centers. Three layers, namely, exterior, transitional and interior, were determined as each layer constituted about 1/3 of the aggregate's volume. The width for each layer was calculated based on three concentric spheres:

$$W_{1} = \sqrt[3]{\frac{1}{3}}R_{1}W_{2} = \left(\sqrt[3]{\frac{2}{3}} - \sqrt[3]{\frac{1}{3}}\right)R_{1}W_{3} = \left(1 - \sqrt[3]{\frac{2}{3}}\right)R$$
(3.1)

Where  $W_1$ ,  $W_2$ , and  $W_3$  are the radius of the interior, transitional and exterior layer of the aggregates, R is the distance between aggregate's surface and its center.

**Figure 3.5:** An example of the three layers of aggregates in a 2D image slice. Light, medium, and dark gray intensities represent, respectively, exterior, transitional, and interior layers. Notice that the layers were identified in 3D, therefore they might not look like they are of equal width when shown in a 2D representation.



# Statistical analyses

Statistical analyses for comparing of pore characteristics of the whole aggregates from different experiments and treatments were conducted as a completely randomized design with treatments in LTER or CERN experiments, respectively. For analyses of pore characteristics in exterior, transitional and interior layers of the aggregates, layer was used as an additional factor in a split-plot arrangement. The data analyses were conducted using PROC MIXED in SAS 9.2 (SAS Institute, 2008).

#### **RESULTS AND DISCUSSION**

#### Aggregate boundary identification

The relationship between the aggregate volume and the values of the parameter r is presented on Figure 3.6(a). The aggregate volumes and r values were positively related. However, while the magnitude of the relationship appeared to be inconsistent at 0-10 range of rvalues, the aggregate volumes were slowly approaching plateaus at r values greater than 10. This indicated that structuring element with small r values ( $r \le 10$ ) was not sufficient to 'close' all the necessary pores connected to aggregate boundaries, e.g., cracks and channels induced by pneumatic pressures and/or plant roots and fungi hyphae. On the other hand, when the size of the structuring element became too large ( $r \ge 15$ ), we observed artificial layers of pores added to the aggregate boundary (Figure 3.1e). This result indicates that substantial caution must be exercised when selecting optimal r value since large r values significantly increases the porosities which are falsely taken from the background. Figure 3.6(c) showed that the aggregates' volume increase rates were generally declining until r value of 12, after which the increase rates went up a bit. We need to point out that there exists a large aggregate-to-aggregate variability in the relationship between aggregate volume and r value, so the best strategy is to determine the optimal r value based on each individual aggregate in the study. However, considering the large number of samples in this study (49 aggregates) and significant computer processing time for large r values (r > = 12), we decide to choose a universal r value for all the aggregates in this study.

The complexity of the aggregate boundary as described by fractal dimension decreased with increasing r values (Figure 3.6(b)). As expected, the aggregate boundaries became much smoother when large structuring elements were probing the image. Notice that at  $r \ge 15$  the

aggregate boundary had become sufficiently smooth not to be strongly affected by further increase in r values. The reduction in the fractal dimension for different r values mostly agreed with the corresponding increase in aggregate volumes (Figure 3.6(b)(d)).

**Figure 3.6:** (a) Total volume of the aggregates after sealing the boundaries with different r values in four studied aggregates. Solid lines were fitted to the linear parts of the volume/r relationships; (b) fractal dimension of the aggregate boundaries of the four studied aggregates at different r values; (c) aggregate volume increase rate<sup>+</sup> at different r values; (d) reduction rate<sup>+</sup> of fractal dimension at different r values.



Figure 3.6 (cont'd).



Figure 3.6 (cont'd).



<sup>†</sup> Increase rate and reduction rate are calculated as the difference between the value at current r and the value at (r-1), and then divided by the value at (r-1).

Although there was a large variability among the volume and fractal dimension changes as a function of r values in different aggregates, r value of 12 appeared to be suitable for boundary sealing in all four aggregates. The r value of 12 ensured the inclusion of boundary pores having opening less than about 350 µm (14.6x12x2 µm, where 14.6µm is the image resolution and is multiplied by optimal image closing diameter value (2x12)) but at the same time maintained sufficiently irregular aggregate boundaries. Once optimal r value (r = 12) was determined based on the four aggregates, it was applied to all aggregate images of the study. However, we need to point out that still further caution should be exercised during boundary sealing. We observed that in some of the studied aggregates, sealing with r = 12 still resulted in several voxels of an artificial layer of pores added to the aggregate boundaries. Removing 5 layers of pore voxels from the entire aggregate boundary remediated the problem. Please note that there is a possible implication that doing this could result in fewer pores in the aggregates' exterior layer, so we carefully checked several aggregates' images to ensure that removal of 5 layers of pores from the aggregate boundary is only sufficient for removing the artificial layer.

Overall, we recommend examination of aggregate volumes as a main tool and fractal dimension as an auxiliary tool for identifying appropriate r values. We expect that the optimal r value will depend on the size of the studied aggregates, the scanned resolution and the average size of pore connected to aggregate surface.

#### Pore characteristics in whole aggregates

Total porosity values for LTER-CT and LTER-NS aggregates were equal to 34.4% and 36.4%, respectively (Table 3.1). Although total porosity for LTER-NS aggregates was numerically higher than that of LTER-CT aggregates, the difference was not statistically significant. The tendency for lower total porosity in macro-aggregates from conventional tillage as compared to those from no-till and forest soils was reported by Park and Smucker (2005a). They attributed lower total porosities in the conventional tillage aggregates to more frequent aggregate turnover rates, and accumulation of clay particles inside the aggregates. Munkholm and Kay (2002) found that aggregates of 4-8 mm in size had lower porosity in soils under continuous corn management than in soils from a dairy farm with 5-year crop grass rotation management. Lower total porosities of the aggregates from conventionally tilled as compared to native vegetation or pasture soils appear to be associated with lower organic matter content and lower aggregate stability (Six et al., 1999; Lal, 2002). In this study LTER-CT has significantly lower C concentration than LTER-NS (Table 1). Total aggregate porosity values for CERN-NS and CERN-BS aggregates were 45.5% and 46.7%, respectively (Table 3.1), and also not significantly different from each other.

**Table 3.1:** List of soil aggregates physical and chemical properties from top soil (0-20cm), including texture, total C, N, total porosities, macro-porosities (pores  $> 14.6 \mu$ m), and porosities of pores  $< 14.6 \mu$ m.

Location	Treatment	Sand, %	Silt, %	Clay, %	Total C, %	Total N, %	Total porosity, %	Macro- porosity, %	Porosity < 14.6 μm, %
LTER	CT	21.3	44.1	34.6	0.78	0.07	34.4 (2.3) <b>‡</b>	10.4 (3.2) a†	24.0 b
	NS	26.9	33.8	39.4	1.02	0.09	36.4 (3.7)	5.9 (1.7) b	31.5 a
CERN	NS	32.7	35.6	31.7	1.31	0.12	45.5 (4.3)	14.2 (5.4) a	31.3 a
	BS	24.4	46.3	29.4	1.19	0.10	46.7 (2.9)	15.3 (5.8) a	31.4 a

\* Means within the same columns of the same location followed by the same lowercase letter are not significantly different (p<0.05). Standard deviations are shown in parentheses.

Even though there were no differences in total porosity between LTER-CT and LTER-NS aggregates, pore size distributions of the two treatments were noticeably different. First, the macro-porosity, that is  $\mu$ CT measured porosity based on visible pores >14.6 µm, was found to be significantly higher in the aggregates from LTER-CT than in the aggregates from LTER-NS soils (Table 3.1). No information on intra-aggregate porosity directly comparable to the results of this study is available from literature. One example is the study by Peth et al. (2008) who investigated the microtomographic images of two aggregates with resolution of 5.4 µm and 3.2 µm, representing a conventionally tilled soil and a long-term (>40 years) grassland Alfisol on a loess parent material. They observed that the µCT measured porosity (pores~>5µm) was lower in the aggregate from the conventionally tilled soil. Papadopoulos et al. (2009) compared soil aggregate porosities using µCT with resolution of 8 µm for a silty clay loam soil from organic and conventional management. They reported higher porosity values of aggregates in organic management in one of the two studied fields. However, both studies only selected limited regions of interest within the aggregates and only two aggregates (one from each land use) were used. Note that in this study, the macro-porosity is based on the proportion of pores >14.6µm thus direct comparisons with the results of Peth et al. (2008) and Papadopoulos et al. (2009) are difficult.

Together with numerically greater total porosity values of LTER-NS aggregates, their lower macro-porosity indicates that the small pores (<14.6 $\mu$ m) were more abundant in LTER-NS than in LTER-CT aggregates (Table 3.1). The proportion of pores 15-30  $\mu$ m in size (pores with BN=1) were found to be similar in LTER-NS and LTER-CT soil (Figure 3.7(a)). However, pores of intermediate size, 45-60  $\mu$ m (pores with BN=2) and 75-90  $\mu$ m (pores with BN=3), were more abundant in LTER-NS soil (Figure 3.7(a)). The largest pores (>90  $\mu$ m,

BN>=4) were much more abundant in LTER-NS than in LTER-CT aggregates and proportion of pores 195-210  $\mu$ m (BN=7) and >225  $\mu$ m (BN>=8) were 3-4 times greater in LTER-NS than in LTER-CT (Figure 3.7(a)). These results indicated that aggregates in long-established (>20 years) native succession vegetation had pore size distributions different from those of the aggregates in conventionally tilled agricultural management. Pore size distributions of the aggregates from long term native vegetation were characterized by greater proportions of very small and very large pores, but by lower proportions of intermediate pores.

Visual inspection of the aggregate images indicated that most of the large pores present in LTER-NS aggregates are round pores of likely biological origin, while elongated pores and cracks of non-biological origin were prevalent in LTER-CT aggregates. Six representative 2D aggregate images from LTER-CT and LTER-NS aggregates are shown on Figure 3.8(a-f).

**Figure 3.7:** Percent of pore space visible at 14.6 $\mu$ m resolution occupied by pores of different burn numbers in aggregates from (a) LTER-CT and LTER-NS treatments; and (b) CERN-BS and CERN-NS treatments. Burn number equal to 1 is equivalent to pore size of 15-30 $\mu$ m, BN equal to 2 is equivalent to pore size of 45-60 $\mu$ m, BN equal to 3 is equivalent to pore size of 75-90 $\mu$ m, etc. For each burn number, the letters mark the significant differences between land uses while 'ns' indicates no statistically significant difference (p<0.05). Std. fraction on the y-axis is the standardized burn number distribution. Note that for clarity purposes different scales of y-axis values were used for fractions of pores with different burn numbers.



Figure 3.7 (cont'd).



Figure 3.7 (cont'd).



**Figure 3.8:** Examples of 2D image slices from central portions of the aggregates from LTER-CT (a-c), LTER-NS (d-f), CERN-NS (g-h) and CERN-BS (i-j) soils.









Figure 3.8 (cont'd).



Marked differences in the aggregate size distributions in the LTER-CT and LTER-NS treatments were reported by Grandy and Robertson (2006), who observed that aggregates from LTER-CT had lower mean weight diameter, and LTER-CT soil had lower proportion of macro-aggregates (2-8 mm) and higher proportion of micro-aggregates ( $<250 \mu$ m) as compared to LTER-NS. De Gryze et al. (2004) reported lower stability of LTER-CT than LTER-NS aggregates with aggregate mean weight diameter being significantly lower in soil of LTER-CT as compared to LTER-NS. These lower stabilities of the LTER-CT aggregates could be caused

by the prevalence of the intermediate sized (45-90  $\mu$ m) pores that we observed in this study. Such pores might possibly serve as planes of aggregate breakage. Arguably, if a compressive force is applied to LTER-CT and LTER-NS aggregates shown on Figure 3.8(a-f), the LTER-CT aggregates will easily break into smaller fractions along the numerous micro-cracks, while greater force would be necessary to break the LTER-NS aggregates. Additionally, CERES modeling predicts surface soil aggregates from LTER-CT are exposed to 53 to 57 severe, eg, >10% volumetric soil water contents and drying and wetting (DW) cycles annually in contract to 35 to 37 DW cycles for LTER-NS aggregates (Smucker and Park, 2006). These significantly greater numbers and greater ranges of soil water content explain the larger number of planes of weakness in the LTER-CT aggregates.

Greater abundance of large pores of biological origin in LTER-NS aggregates (Figs. 3.7(a) and 3.8(d-f)) can be explained in part by greater numbers of plant roots and greater activity of soil fauna in LTER-NS soil as compared to LTER-CT (Smith et al, 2008). Once the roots are decomposed they leave behind pores with smoother curved walls enriched by presence of stable 1-10 µm organo-mineral microaggregate formations and root residues (Oades, 1993; Rasse and Smucker, 1998; Six et al., 2004; Watteau et al., 2006). In this study, roots and pieces of particulate organic matter were observed within pores of many LTER-NS aggregates. It also should be pointed out that significant differences in soil textural percentages (Table 3.1) could lead to significant difference in pore size distributions between LTER-CT and LTER-NS aggregates as reported by Park and Smucker (2005ab). It could be speculated that higher clay content in LTER-NS aggregates could be responsible for greater number of larger pores and less number of smaller pores, since clay particles could possibly fill in the small space and create smoother yet stable walls favoring for larger pore formation in LTER-NS.

No significant differences in pore size distributions were observed between CERN-NS and CERN-BS aggregates (Table 3.1 and Figure 3.7(b)). Likewise, visual examination of the aggregate images did not reveal any notable differences between the aggregates of the two treatments (Figure 3.8(g-j)). This lack of differences probably reflected a relatively young age of CERN experiment, insufficient for the differences to develop.

# Pore characteristics in aggregate layers

At exterior, transitional and interior layers, macro-porosity of LTER-CT aggregates was significantly higher than that of LTER-NS aggregates, consistently with the discussed above macro-porosity results for the whole aggregates (Table 3.2(a)). However, in either LTER-CT or LTER-NS aggregates there were no significant differences in macro-porosity among exterior, transitional and interior layers. The possible explanation for lack of macro-porosity gradient in LTER-CT is that aggregates were pieces of former compacted fragments, resulting from repeated mechanical stress exerted by tillage, transport and harvest processes. In contrast, there was a tendency for macro-porosity to increase from exterior to interior in both CERN-NS and CERN-BS aggregates (Table 3.2(a)).

While no previous studies have examined the differences in macro-porosities of the aggregate layers, the total porosity has been measured. E.g., Park and Smucker (2005b) reported greater total porosity in exterior layer for 4-6.3 mm aggregates from silty clay loam soils of Wooster (fine-loamy, mesic Typic Fragiudalf) and 6.3-9.5 mm aggregates from silt loam soils of Hoytville (fine, illitic, mesic Mollic Epiaqualf) series under conventional tillage, and no tillage management. Park and Smucker (2005b) attributed the more profound spatial gradient in total

porosity found in CT aggregates to compression of repeated tillage, which led to condensed center of the aggregates, compared to less obvious porosity gradient in NT aggregates.

	(a) Macro-porosity						
	Location	Treatment	Exterior	Transitional	Interior		
	I TED	СТ	9.95 (0.73) a†A‡	10.76 (0.73)aA	10.45 (0.73)aA		
	LIEK	NS	5.65 (0.76)bA	5.78 (0.76)bA	6.27 (0.76)bA		
(	CEDN	NS	12.45 (1.64)aA	13.65 (1.64)aA	15.38 (1.64)aB		
	CEKN	BS	12.98 (1.71)aA	15.52 (1.71)aB	16.56 (1.71)aB		

**Table 3.2:** Pore characteristics within exterior, transitional and interior layer of the aggregates. (a) Macro-porosity; (b) Mean burn number on MA.

(b) Mean burn number on MA						
Location	Treatment	Exterior	Transitional	Interior		
I TED	СТ	1.64 (0.05)aA	1.58 (0.05)aA	1.59 (0.05)aA		
LIEK	NS	1.61 (0.05)aA	1.63 (0.05)aA	1.73 (0.05)aB		
CEDN	NS	1.60 (0.05)aA	1.54 (0.05)aA	1.62 (0.05)aA		
CERN	BS	1.63 (0.05)aA	1.60 (0.05)aA	1.65 (0.05)aA		

<sup>†</sup> Means within the same columns of the same location followed by the same lower case letter are not significantly different (p<0.05).

 $\ddagger$  Means within the same row followed by the same upper case letter are not significantly different (p<0.05). Standard errors for means are shown in parentheses.

A strong spatial trend in mean burn number values was present in LTER-NS aggregates, where the highest burn numbers were observed in the interior, followed by the transitional and then by the exterior layer (Table 3.2(b)). This result provides further quantitative support to the visual observations of large pores being often observed in the central parts of the LTER-NS aggregates (Figure 3.8), reflecting the most complete absence of soil disturbance and high biological activity for the longest period of time in this treatment.

Pore size distributions in the aggregate layers of the two LTER treatments had different spatial gradients (Figure 3.9(a)). In LTER-NS the exterior layers had more pores of intermediate sizes of 45-60  $\mu$ m (BN = 2) and 75-90  $\mu$ m (BN = 3) while noticeably fewer large pores

(pores >120  $\mu$ m (BN>=5)) than in the interior layers. In contrast, in LTER-CT the exterior layers were somewhat more abundant in 75-90  $\mu$ m (BN = 3) and 105-120  $\mu$ m (BN = 4) pores. However no differences in terms of either pores with BN<=2 or BN>=5 were observed among the LTER-CT layers. These differences point to greater spatial uniformity in pore distributions in aggregates from this heavily disturbed conventionally tilled management as compared to the undisturbed native vegetation.

The trends for higher proportions of 45-90  $\mu$ m pores (BN=2 and 3) and lower proportions of pores 135-150  $\mu$ m (BN = 5) in LTER-CT than in LTER-NS that were previously mentioned in the whole aggregate data also consistently appeared in the data from all three individual layers. However, the greater abundance of the large pores, that is pores >165  $\mu$ m (BN>=6), in LTER-NS than in LTER-CT was only present in the transitional and interior layers. The differences between the two treatments in terms of large pore presences were not significant in the aggregate exteriors.

The tendency for more 75-90  $\mu$ m (BN = 3) pores in the aggregate exteriors than in transitional and interior layers noted for LTER-CT and LTER-NS aggregates was also present in both CERN-NS and CERN-BS aggregates. Also similar to the native vegetation LTER-NS treatment, large pores of 165-180  $\mu$ m (BN = 6) and 195-210  $\mu$ m (BN = 7) in size were more abundant in the interiors of the native vegetation CERN-NS aggregates. These similarities point to possible ubiquity in aggregate formation processes acting in the studied soils despite substantial differences in their parent materials and the duration of the native vegetation presence. No differences in pore size distribution between CERN-BS and CERN-NS aggregates were observed in the individual layers (Figure 3.9(b)).

**Figure 3.9:** Percent of pore space visible at >14.6 µm resolution occupied by pores of different burn numbers in aggregates exterior (Ext), transitional (Tran), and interior (Int) layers of (a) LTER-CT and LTER-NS treatments; and (b) CERN-BS and CERN-NS treatments. Burn number equal to 1 is equivalent to pore size of 15-30 µm, BN equal to 2 is equivalent to pore size of 45-60 µm, BN equal to 3 is equivalent to pore size of 75-90 µm, etc. For each burn number, the lowercase letters mark significant differences among the three layers within each treatment. The differences among the layers are not statistically significant (p<0.05) if lowercase letters are not displayed. For each burn number, the uppercase letters mark significant (p<0.05). The cases when no statistically significant difference existed both among layers and between treatments (p<0.05) are marked with 'ns'. Std. fraction on the y-axis is the standardized burn number distribution. Note that for clarity purposes different scales of y-axis values were used for fractions of pores with different burn numbers.





Figure3.9 (cont'd).



Figure3.9 (cont'd).



# CONCLUSION

Automatic flood fill algorithm for identifying aggregate boundaries on 3D  $\mu$ CT images often fails to include pores connected to the aggregate outsides. Image closing is an effective technique to delineate soil aggregate boundaries from  $\mu$ CT images. However, successful use of

image closing technique requires determining an optimal value of the neighborhood size parameter, r. We found that examining the plots of the total aggregate volume, aggregate boundary fractal dimension, and the rates of change in total volume and fractal dimension versus r could serve as useful criteria for selecting the optimal r value. However, close attention should be paid to the artificial layers of pores that might be added to aggregates' boundary. The optimal r value might depend on a number of factors including the size of the studied aggregates, the scanned resolution and the average sizes of pores connected to aggregate surface. In this study the r value of 12 appeared to be adequate for boundary delineation.

Twenty years of contrasting management in the LTER experiment led to pronounced differences in the intra-aggregate pore size distributions. We observed significantly greater percent of pores > 15  $\mu$ m in LTER-CT aggregates as compared to those of LTER-NS. LTER-NS had more large pores (pore size of > 90 $\mu$ m) than LTER-CT aggregates, while more medium size pores (45-90  $\mu$ m) were found in LTER-CT aggregates. Visual examination of aggregate images indicated that large pores of biological origin were often present in the interiors of the LTER-NS aggregates while medium-sized cracks were commonly found in the LTER-CT aggregates. Further studies describing and quantifying the role of intra-aggregate pore size distributions in a number of soil processes, including, but not limited to C sequestration are necessary.

The differences in pore size distributions between LTER-CT and LTER-NS were more pronounced in the aggregate interiors, as compared to the exterior layers. For aggregates from LTER and CERN, pores were found to be larger in the aggregate interiors than in the exterior layers; while intermediate pores (45-90  $\mu$ m) were more abundant in the aggregate exterior layers. This trend was present in all land use and management treatments of the study and was most pronounced in LTER-NS. Future research is needed to confirm spatial pattern in pore size distributions within soil aggregates of other sizes.

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# Chapter 4: *ESCHERICHIA COLI* REDISTRIBUTTION AND TRANSPORT IN INTACT SOIL MACRO-AGGREGATES

#### ABSTRACT

Past research had demonstrated the ability of E. coli to survive and regrow in the soil environment, and further to infiltrate with water flow under saturated conditions. Knowledge of the exact pore locations within soil aggregates opens new opportunities for understanding soil microorganism movement and functioning. The first objective of this study was to assess E.coli spatial distribution potential release within soil aggregates from three studied soil treatments, namely, conventionally tilled (T1) and no-till (T2) corn/soybean/wheat rotation and native succession vegetation (T7) on Typic Hapludalfs of the Kalamazoo (fine-loamy) series at NSF Long-Term Ecological Research site, southwest Michigan. The second objective was to study the redistribution pattern of *E.coli* within soil aggregates and relate *E. coli* movement within aggregates to the aggregates' internal pore structures. The results confirmed that E.coli movement in soil aggregates were mainly driven by water flow via capillary forces. Most of the seeding E.coli cells were remained within the aggregates (97%-99%) under the saturated condition even when water was evacuated, however, they were found to be differentially redistributed within the soil aggregates from three studied treatments. Redistribution was most pronounced in T1 aggregates, followed by T2, and was almost negligible in T7 aggregates. Principal component analysis showed that T1 aggregates are quite different from T2/T7 aggregates in pore characteristics, which was possibly responsible for *E.coil* redistribution pattern. E.coli transport from top to other parts of soil aggregates were related to pore

characteristics of aggregates middle part, however, due to high variability in pore characteristics among soil aggregates, the relationship was weak. *E.coli* redistribution was somehow related to pore tortuoisities and connectivities of the aggregates' center portion.

## **INTRODUCTION**

*Escherichia coli* (*E.coli*), especially *E.coli* O157:H7, has been regarded as an important indicator of environmental quality and public health risk (CDC; Vogt and Dippold, 2005; Hellweger and Masopust, 2008). Effective strategies need to be developed to control the passage of *E.coli* to waterways; yet thorough understanding of its survival and transport mechanisms is indispensible. In agricultural fields, transport of manure-borne *E.coli* into soils, surface and ground water can cause severe in-field contamination thus threatening food safety (Jamieson et al., 2002; Guber et al., 2005; Jay et al., 2007). The soil structure and nutrient distribution at macro and micro scale is believed to be responsible for *E.coli* survival and transport within soil matrix (Pachepsky et al., 2006), and precipitation facilitates infiltration of bacteria suspensions through flow pathways into deeper ground (Jenkins et al., 2008).

Soil matrix is among the most complex media in that it controls many physical, chemical and biological processes at various scales. Past research had shown soil processes should be studied at the scale where they function with applicable modeling techniques (Vogel and Roth, 2002; Vogel et al., 2003; Lin, 2003). Among various scales, it was believed that pore arrangement within soil aggregates and their connectivity to the inter-aggregate pore space are recognized to be among the main factors controlling soil processes (Revil and Cathles, 1999). When entering soil matrix, *E.coli* will transport with water flow and/or attach to soil particles.

There were ample research published on *E.coli* movement and attachment in soils (Hagedorn et al., 1978; Lindqvist and Bengtsson, 1991; Johnson and Logan, 1996; Ling et al., 2002; Guber et al., 2005a&b). Soil organic matter can hinder *E.coli* attaching to soil particles due to its negative charges (Johnson and Logan (1996)), whereas high clay content in soils may promote bacteria attachment (Huysman and Verstrate, 1993).

Though findings above were mostly at field and soil column scale, it is highly possible that *E.coli* travel within aggregates and further release from the aggregates are strongly associated with pore structures they passed en route. However, lacking of appropriate tools and modeling techniques, few past studies addressed *E.coli* distribution and transport at micro scale in soils.

Recent advance of X-ray computed microtomography ( $\mu$ CT) imaging enabled us to quantify the intra-aggregate structures in 3 dimensions (3D) with a resolution of several microns (Gibson et al., 2006; Nunan et al., 2006; Dathe et al., 2005, 2006; Peth et al., 2008). Nunan et al. (2006) investigated semivariograms and pore shape parameters of microbial habitat for soil aggregates 1-3 mm in size at a scanning resolution of 4.4 $\mu$ m. Our previous work (Kravchenko et al. 2011) showed that soil aggregates under different land use managements displayed contrasting pore distribution patterns, thus impacting on *E. coli* fate and transport differentially.

The objectives of this study were 1) to compare *E.coli* spatial distribution within intact soil aggregates from the same soil type but under long-term (>18 years) differential land use and management settings, namely, conventionally plowed row crop agriculture, conservationally managed (no-till) row crop agriculture, and native vegetation on land with previous agricultural use under air dry and saturated flow conditions; 2) to explore the potential of *E.coli* release from

the aggregates and relate *E.coli* spatial redistribution under saturated flow to internal soil aggregate pore structures quantified from  $\mu$ CT analysis.

## **MATERIALS AND METHODS**

## Soil sample collection

Soil samples were collected from the Long Term Ecological Research site (LTER), Kellogg Biological Station, established in 1988 in southwest Michigan (85° 24` W, 42° 24` N). Soils are well-drained Typic Hapludalfs of the Kalamazoo (fine-loamy, mixed, mesic) and Oshtemo (coarse-loamy, mixed, mesic) series, developed on glacial outwash. For details on site description, experimental design and research protocols of the LTER experiment see http://lter.kbs.msu.edu (Kellogg Biological Station, 2009). The three LTER treatments that were sampled are corn-soybean-wheat rotation either conventionally plowed (T1) or no-till (T2) and native succession vegetation (T7) treatment, abandoned from agricultural management after spring plowing in 1989. Prior to setting up the experiment in 1988 the entire area belonged to a uniformly conventionally tilled and managed agricultural field that has been in agricultural production for at least past 100 years. Soil samples from this site were collected in spring of 2009 from three adjacent 1-ha plots. The distance between the sampling locations of these plots was approximately 25 m. Maintaining a short distance between the samples of the two studied treatments allowed us to reduce effects of the local soil spatial variability on soil aggregate characteristics. Three sites were sampled at each of the treatments.

At each sampling site, a soil block approximately  $15x15 \text{ cm}^2$  in size was extracted from 0-20 cm depth using a sharp flat spade. Samples were air-dried and manually sieved for 30 s and

the aggregate fraction, 4-6.3 mm, was retained for this study. 15 scanned aggregates (5 per treatment) were used in the flow experiment. 9 scanned aggregates (3 per treatment) were used in the slicing experiment.

#### Soil aggregate image acquisition

Aggregate image data were collected on the bending magnet beam line, station 13-BM-D of the GeoSoilEnvironCARS (GSECARS) at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), IL. Data were collected with the Si (111) channel cut monochromator tuned to 28 keV incident energy. 2D image slices were taken at 1° rotation angle steps and combined into a 3D image consisting of 520 slices with 696x696 pixels per slice. The voxel size was 15.51 µm. Based on our preliminary results, this resolution was found to be optimal for the 4-6.3 mm aggregate size fraction. A snapshot of the image on the mirror was taken with the CCD camera representing a depth-integrated grayscale map of the linear attenuation of the X-ray beam as it passed through the sample. After an image was collected, the column was rotated 1°, and the process was repeated. Detailed information on the procedure is given in Rivers et al. (1999).

The data were preprocessed by removing ring artifacts (Ketcham and Carlson, 2001) and adjusting for backfield projections and reconstructed using Fast Fourier Transformation. After reconstruction, the grayscale values in the image ranged from 0 (black) to 255 (white) corresponding to low and high X-ray attenuations, respectively.

### Aggregate image analysis

Soil aggregate boundaries were identified using a combination of image closing and filling techniques and specific parameterization was applied to each individual aggregate to ensure the best volume inclusion and boundary roughness (Wang et al., in preparation). After boundary delineation, the stacks of grayscale images were segmented into pores and solids using indicator kriging segmentation module in 3DMA-Rock software (Lindquist et al., 2000). Multiple sets of the two thresholds in indicator kriging segmentation were chosen based on the parameters of the fitted mixed Gaussian distribution of grayscale values within the aggregate domain using Expectation-Maximization algorithm (Wang et al., 2011). The segmented images were assessed using region non-uniformity measure proposed by Levine and Nazif (1985) and evaluated by Wang et al. (2011). Detailed description of region non-uniformity calculation and applicability in soil image analyses is provided in Wang et al. (2011). All the calculations were conducted using 3D data sets.

The segmented soil aggregate images were used to extract pore characteristics, including macro-porosity, medial axes, and burn numbers (BN) (Lindquist et al., 2000; Peth et al., 2008). Macro-porosity is defined as the percent of the pore voxels within the aggregate image, and in this study it is the porosity visible at scanning resolution of 15  $\mu$ m, i.e. pores >15  $\mu$ m. Medial axis is a digitized curve which represents the skeleton of the pore space. Burn number is the distance from the medial axis pore voxel to the closest solid voxel. The units for BN are the numbers of voxels. Please note that BN values are directly related to pore sizes, e.g., in this study, BN equal to 1 is equivalent to pore size of 15-30  $\mu$ m, BN equal to 2 is equivalent to pore size of 45-60  $\mu$ m, BN equal to 3 is equivalent to pore size of 75-90  $\mu$ m, etc. The BN distribution is standardized as dividing the number of BN voxels for each size by the total number of pore

voxels. And we will refer it to standardized fraction for BN (std. fraction). Calculations of the pore characteristics were conducted in 3DMA-Rock software (Lindquist et al., 2000).

Based on medial axes information, the pore network connectivity was calculated as the maximum flow in a network N = (V, E) using Edmonds-Karp algorithm (Edmonds and Karp, 1972). Medial axis voxels were used as the vertex sets V and burn numbers on medial axes were used as capacities on edge sets E. The super-source node and super-sink node were artificially superimposed to the pore network to avoid difficulty in multi-source and multi-sink maximum flow calculation, where infinity capacity was assigned on each edge (Ford and Fulkerson, 1962). The super-source node was set so that it connected to all the medial axis voxels on the first slice of the 3D volume. The super-sink node was set so that it connected to all the medial axis voxels on the last slice of the 3D volume. The MATLAB code for generating capacity matrix is in Appendix 4.1. The C++ code for maximum flow calculation implementing Edmonds-Karp algorithm is in Appendix 4.2.

Due to the very large size of the aggregate images, all the image data and codes are run at High Performance Computing Center (HPCC) at Michigan State University.

#### Alignment of the physical and virtual cuttings on aggregates

Before cutting, the orientation of an aggregate was matched to that of the scanned data. During actually cutting notes were taken on the order of cuts and sizes of resulting pieces. Then based on the notes, the cutting planes on aggregate images were identified so as to ensure maximal correspondence with the physical cuts within each individual aggregate. Final assessment of quality of the cuts was made by visually examining the aggregate images along with cutting planes and by calculating density of the aggregate slices. Density of each slice was calculated based on the slice's weight and volume as determined based on the cut location. The density estimates could serve only as an approximate assessment tool since aggregate slices were moist when weighted. A schematic representation of aggregates cutting was shown in Figure 4.1.

**Figure 4.1:** Schematic representation of the aggregate cutting. Numbers 1, 2, 3, 4, 5, 6 and 7 correspond to top, bottom, left, right, front, back and center slices, respectively. The sequence of the numbers represents the cuttings in order.



## E.coli application experiments

Two *E.coil* application experiments, namely, *E.coli* flow experiment and *E.coli* slicing experiment, were conducted.

In the *E.coli* flow experiment, 15 scanned aggregates, 5 from each treatment, were used. This experiment was conducted in the glass beads matrix chamber system designed for the study (Chun et al., 2011 in preparation). Pictures of the system were shown in Figure 4.2. **Figure 4.2:** A picture shows the glass beads matrix chamber system – glass beads matrix chamber for adding bacteria then flushing and vacuum extracting *E.coli* from soil aggregates.



Approximately 6 mm<sup>3</sup> of glass beads (1mm in diameter, with density of 2.5 g/cc) was measured using centrifuge tubes, and the weight ( $W_{beads}$ ) was recorded. A single layer of glass beads were added into the chamber, and then aggregates were carefully transferred into it. Prior to placing an aggregate in the chamber its image was examined and then, consistent with the image, the orientation of the aggregate (e.g., top, right, left, etc.) was marked with colored permanent markers. This ensured that after the flow experiment relative positions of the aggregate slices corresponding to the image could be identified for further analyses of the relationships between intra-aggregate pores and *E.coli* distributions. Once in the chamber, *E. coli* was added to the top of the aggregate at a concentration of approximately 10<sup>7</sup> CFU/ml. The volume of *E. coli* culture dilution to be added to each aggregate was calculated as 30% of the

total porosity of the aggregate determined from the aggregate's image. Then remaining glass beads were added to cover the aggregate.

The whole system was allowed to equilibrate in the fridge (4  $^{\circ}$ C) for 0.5 hour. Then Phosphate Buffered Water (PBW) (pH 7-7.2) was added into the system. The volume of PBW was determined as the in-between glass beads space volume:

$$V_{PBW} = (V_{GB_{in}\_tube} - \frac{W_{GB}}{\rho_{GB}}) + 70\% \times V_{pore}$$
(4.1)

where  $V_{GB\_in\_tube}$  is the volume of glass beads placed in the centrifuge tube (6 mm<sup>3</sup>),  $W_{GB}$  is the weight of the glass beads,  $\rho_{GB}$  is the density of glass beads (2.5 g/cc) and  $V_{pore}$  is the pore volume based on total porosity calculation.

After PBW application, the system was further equilibrated in the fridge (4  $^{\circ}$ C) for 2 hours. Then the effluent was extracted from the system using a vacuum and collected into centrifuge tubes. During the extraction process, the same suction pressure (120 mbar) was applied to all the aggregates for approximately 8 mins. This pressure ensured the maximum extraction of the liquid from the system. About 69% of the added PBW was extracted. The flow system was kept in the ice box during the effluent extraction process. Effluent was then stored in the freezer at -20  $^{\circ}$ C for qPCR analysis.

The flow system was dried using compressed air for approximately 50 mins, while chambers were surrounded by ice to minimize *E. coli* growth. Aggregates were cut into 7 pieces

using medical scalpels, flame-ethanol sterilizing in between each cut. Positions of the cutting planes were recorded as described above. Aggregate slices were transferred into soil DNA kits for qPCR analysis. Glass beads were transferred into centrifuge tubes with 5 ml PBW in. All the samples were stored in the freezer (-20  $^{\circ}$ C) for qPCR analysis.

In the *E.coli* slicing experiment, a total of 9 not-scanned aggregates, 3 from each treatment, were used. We added 20  $\mu$ L of *E. coli* culture dilution at concentration of approximately 10<sup>7</sup> CFU/ml on top of each aggregate. Based on the assessment of scanned aggregates, 20  $\mu$ L is the average volume of the 30% aggregate pore space. Aggregates were allowed to equilibrate for 2 hours after *E.coli* application and kept in the fridge (4 <sup>o</sup>C) inside closed containers within beakers containing water to avoid evaporation. Aggregates were cut into 7 pieces and transferred into soil DNA kits for qPCR analysis.

## **qPCR** analysis

DNA was extracted from the seeded soil aggregates using UltraClean<sup>TM</sup> Soil DNA kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer's protocol. The extracted DNA was stored at  $-20^{\circ}$ C until further analysis. These samples were analyzed by qPCR method at Research Technology Support Facility (RTSF), Michigan State University (MSU) using the assay targeting uidA gene of *E. coli* (Srinivasan et al. 2011). The primers and probe sequences used for the analysis is listed in Table 4.1. The final 15 µL reaction mixture for amplification of E. coli uidA gene consisted of 10µL of Roche Light Cycler Taqman Mastermix,

 $0.5 \mu$ M of forward and reverse primers each,  $0.2 \mu$ M probe, MgCl2 (3.2 mM) and nuclease free water. Cycle threshold (Ct) was measured during each amplification and target gene concentration was analyzed automatically by absolute quantification method by the LightCycler® Software 4.0.

Table 4.1: Primer and probe sequence used in this study.

		Primer/probe
Bacteria/gene	Primer/probe sequence	concentrations
E. coli/uidA	5'-CAATGGTGATGTCAGCGTT-3'	0.5 µM
	5'-ACACTCTGTCCGGCTTTTG-3'	0.5 µM
	6FAM-TTGCAACTGGACAAGGCACCAGC-BBQ	0.2 µM

To assess *E.coli* distribution within aggregate slices the number of *E.coli* cells observed in each piece, *i*, were expressed per unit of mass and then divided by the total amount of *E.coli* cells in the aggregate expressed per unit of mass:

$$\operatorname{Re} \operatorname{lative} \operatorname{E.coli} \operatorname{ratio} = \frac{\operatorname{obs}_{i}}{\operatorname{Weight}_{i}} / \frac{\sum_{i=1}^{n} \operatorname{obs}_{i}}{\sum_{i=1}^{n} \operatorname{Weight}_{i}} \quad (4.2)$$

The resulting relative *E.coli* ratio was used in all further analyses.

#### **Statistical analysis**

Descriptive statistics of pore characteristics were summarized using PROC MEANS (SAS Inc., V9.2). The pore characteristic data were analyzed using principal component analysis with soil management as qualitative variables using PROC PRINQUAL (SAS Inc., V9.2). Categorical principal component analysis maximizes relationship among variables having numerical, ordinal or nominal measurements using alternating lease squares (Young et al., 1978). The univariate relationship between *E.coli* spatial distribution and pore characteristics in aggregates pieces was analyzed using linear regression model in PROC REG (SAS Inc., V9.2). The multivariate relationship between *E.coli* spatial distribution and pore characteristics in aggregates pieces was analyzed using partial least regression (PLSR) method in PROC PLS (SAS Inc, V9.2). Transformation of the variables was applied when normality assumption was violated.

#### **RESULTS AND DISCUSSION**

### E.coli distribution and transport in the flow and slicing experiment

The breakthrough of *E.coli* found in effluents and glass beads were ranging from 1.5% to 3.2% for aggregates with large variability observed from T1, T2 and T7 treatments (Figure 4.3). Most of the *E.coli* cells were retained in soil aggregate matrix even though negatively charged *E.coli* cell surface will probably repel clay particles in soils. Therefore, there are possibly other mechanisms that control *E.coli* transport in soil aggregates. Moreover, the retention of *E.coli* within soil aggregates is not a function of soil management in this study.





E.coli in effluents and glass beads

It shows that *E.coli* cells distributed spatially heterogeneous within soil aggregates under different water conditions and soil managements (Figure 4.4). Here we use differences in relative *E.coil* ratio between soil aggregates pieces to compare the *E.coil* distribution in the flow and slicing experiments. *E.coli* distribution in slicing experiment showed high degree of spatial heterogeneity (Figure 4.4(a)). This is consistent with another set of similar experiments, where we have shown that at air-dry condition *E.coli* transport in soil aggregates was mainly driven by water flow via capillary suctions and by specific configuration of the intra-aggregate pores (Kravchenko et al., submitted). This mechanism had been confirmed in previous studies on the same and larger scales, such as Artz et al. (2005) and Brennan et al. (2010), who reported that bacteria travels fast through macro-pore in soil columns with preferential flow; and Guber et al. (2009) who found that *E.coli* entering air-day soil aggregates along with water flow was mainly driven by capillary forces.

After saturation, equilibration, and water extraction the E.coli redistributed within the aggregate primarily in vertical direction and became more homogeneously spread (Figure 4.4(a)). Redistribution was most pronounced in T1 aggregates, followed by T2, and was almost negligible in T7 aggregates. Top slices of soil aggregates retained more *E.coli* cells than other parts of the aggregates for all soil aggregate managements in both the flow and slicing experiments (Figure 4.4(a)). There were significant greater differences between top and other portion of aggregates in relative *E.coli* ratio found in the slicing experiment, while no difference among aggregates' center, sides and bottom was observed (Figure 4.4(a)). However, the notable differences in *E. coli* between top and other portion of the aggregates were found differentially in three soil managements. There were significant greater differences between top and bottom/center of aggregates in relative *E.coli* ratio found in the slicing experiment in T1 and T2, while no such difference was found in T7 (Figure 4.4(b,d)). There were significant greater differences between top and sides of aggregates in relative E.coli ratio found in the slicing experiment in T1, while no such difference was found in T2 and T7 (Figure 4.4(c)). This suggested that saturated water flow during rainfall and runoff events, could lead to significant *E.coli* redistribution within soils. Moreover, the magnitude of redistribution highly depends on soil managements; specifically conventional tilled soils will facilitate E.coli transport while soils under native succession are more likely to retard its transport. The reason could be presence of organic matter in T7 aggregates which will reduce their hydrophilicity. These differences observed in the two experiments among soil managements could be attributed to pore structure differences, such as total porosity, macro porosity and pore size distributions.

Figure 4.4: Comparison of relative E.coli ratio in flow and slicing experiments.



(b) Difference in relative E. coli ratio between aggregates' top and bottom



Figure 4.4 (cont'd).



(d) Difference in relative *E.coli* ratio between aggregates' top and center



#### Relationship between E.coli distribution and pore characteristics

Table 4.2 summarized pore characteristics for soil aggregates from T1, T2 and T7. Total porosity values for T1, T2 and T7 aggregates were similar, equal to 39.3%, 35.2% and 37.9% (Table 4.2). The macro-porosity for T1, T2 and T7 aggregates were 10.2%, 6.3% and 5.4% (Table 4.2). Statistical analysis showed that T1 aggregates has significant greater macro-porosity than those of T2 and T7 aggregates, where no difference found between T2 and T7 aggregates in macro-porosity values. Pore size distributions for T1, T2 and T7 showed a declining trend with increasing pore sizes, yet no differences were found among treatments for each pore size.

**Table 4.2:** Descriptive statistics for pore characteristics for soil aggregates with size 4-6.3mm from conventional tillage (T1), no-till (T2) and native succession (T7). Standard deviations are shown in parentheses. Burn number equal to 1 (BN1) is equivalent to pore size of 15-30  $\mu$ m, BN equal to 2 (BN2) is equivalent to pore size of 45-60  $\mu$ m, BN equal to 3 (BN3) is equivalent to pore size of 75-90  $\mu$ m, and BN equal to or greater than 4 (BN4g) is equivalent to pore size > 105  $\mu$ m.

Treatment	Total	Macro-	BN1	BN2	BN3	BN4g
СТ	39.3 (3.4)	10.2 (2.5)	0.025	0.011	0.003	0.001
			(0.023)	(0.002)	(0.0003)	(0.001)
NT	35.2 (4.7)	6.3 (2.8)	0.028	0.009	0.002	0.002
			(0.009)	(0.001)	(0.0006)	(0.0007)
NS	37.9 (4.1)	5.4 (1.6)	0.032	0.008	0.002	0.002
			(0.012)	(0.002)	(0.0002)	(0.0006)

Univariate analysis using each pore characteristics individually was suffering small sample size issues. Thus we explored multivariate relationship between soil aggregates and their pore characteristics. The two-dimensional solution with eigenvalues greater than 1 accounted for 73% of the variance from categorical PCA using soil pore characteristics variables were plotted in Figure 4.5(a). The scores for each soil aggregates (soil aggregate id) were also plotted in the

same figure. It is clear that T1 aggregates were well separated from T2 and T7 aggregates with the first PC, which accounts for 41.27% of the total variability (Figure 4.5(a)). However, there is no distinction between T2 and T7 aggregates.

On the first dimension, total porosity, macro-porosity, BN2, BN3 and CT had positive component loadings (Figure 4.5(a)). Therefore, these features are more favorable to T1 aggregates. Large projection of macro-porosity on the first principal component indicated that it contributed a significant portion in explaining the variability in T1 aggregates. BN3 and BN2 are the second and third largest positively contributing variable for T1 aggregates, indicating prevalence of medium size pores (45-90 µm) in conventionally tilled soil aggregates. Similar findings were reported in Wang et al. (2011) who studied another set of 4-6.3 mm T1 and T7 aggregates sampled from the same KBS-LTER experiment, but at different time. These results perhaps point to a universal nature of observed tendency for CT aggregates which contain a large amount of cracks and elongated pores, leading to low aggregate stability reported in other studies (DeGryze et al., 2004; Grandy and Robertson, 2006). However, BN1 and BN4g were found not correlated to neither T1 nor T7 aggregates, which demonstrate small pore size (15-30 µm) and large pore size (> 105  $\mu$ m) are probably uniformly distributed in all soil aggregates in this study. This is inconsistent with Wang et al. (2011) for pore size distributions found in T7 soil aggregates where more large pores were present. However, with only 5 aggregates from T7 from this study, it is not clear how this results could be comparable to 12 T7 aggregates used in that study. The differences in *E.coli* redistribution observed between T1/T2 and T7 (on Figure 4.4) possibly are related to the differences in pore structures. It is also possible that higher organic matter content and higher clay percentage in T7 aggregates (data not shown) could be the reason for relatively high E.coli retention.

**Figure 4.5:** Bi-plot of soil aggregate samples and pore characteristics from PCA for a) the whole soil aggregates and b) middle portion of the soil aggregates including center and sides. The three-digit numbers are labels for soil aggregates with '1xx' representing T1 aggregates, '2xx' representing T2 aggregates and '7xx' representing T7 aggregates. Soil management was converted to indicator dummy variables CT (T1) and NS (T7). Burn number equal to 1 (BN1) is equivalent to pore size of 15-30  $\mu$ m, BN equal to 2 (BN2) is equivalent to pore size of 45-60  $\mu$ m, BN equal to 3 (BN3) is equivalent to pore size of 75-90  $\mu$ m, and BN equal to or greater than 4 (BN4g) is equivalent to pore size > 105  $\mu$ m.



## Figure 4.5(cont'd).



To relate *E.coil* transport from soil aggregates' top to bottom part, we examine the pore characteristics that connect these two parts, i.e. pore characteristics in the center and side portion. Decomposing all variables in two-dimensional solutions using PCA with eigenvalues greater than 1 accounted for 71% of the variance from soil pore characteristics in the center and sides (Figure 4.5(b)). Similar as those of the whole soil aggregates' pore characteristics, porosity, BN2 and BN3 are the most positive contributing variables to T1 aggregates' center and sides. Moreover, flow and connectivity related features, i.e., the number of paths, max flow and tortuosity had positive loadings on T1 aggregates (Figure 4.5(b)). In general, this indicates pore

networks in center of T1 aggregates are more convoluted yet connected. This is in line with aggregate pore characteristics (5  $\mu$ m) from conventional tillage from another soil type reported by Peth et al. (2008).

The relationship between *E.coli* spatial distribution in soil aggregates from the flow experiment and soil pore characteristics were investigated using partial least square regression (Figure 4.6(a)). Because of high variability of the intra-aggregate pores, only 49% of total variation in pore characteristics and 31% of total variation in *E.coil* spatial distribution were account using the first two PLSR factors. The  $R^2$  against number of factors in PLSR was plotted in Figure 4.6(b). While three independent factors could explain 89% of total variation in pore characteristics, only about 40% of total variation in *E.coil* data could be explained (Figure 4.6(b)). This indicates very high variability in *E.coli* data which could not be revealed by pore configuration investigated in this study.

Among *E.coli* distribution variables, top was mostly correlated to pore characteristics, followed by center, then sides and bottom (Figure 4.6(a)). At top of the aggregates, where *E.coli* was applied, *E.coli* concentration was negatively related to max flow in the center portion of the soil aggregates. *E.coli* retained in the center of the soil aggregates was somehow positively related to porosity, medium to large pore sizes (BN3 and BN4g), and tortuosity of the pore space. At aggregates sides, small pores (BN1) were more responsible for *E.coli* distribution.

**Figure 4.6:** (a) Correlation loading plot from PLSR between pore characteristics (porosity, tortuosity, maxflow, paths, BN1-BN4g) and *E.coli* spatial distribution (TOP, CENTER, SIDES, BOTTOM). The numbers are representing aggregate ids: 1-5 represent T1 aggregates, 6-10 represent T2 aggregates and 11-15 represent T7 aggregates. (b) R<sup>2</sup> plots for percent of variation explained vs. number of factors in PLSR.



Figure 4.6(cont'd).



It seems that max flow and tortuosity of the pore networks in aggregate center are mostly related to *E.coli* distribution. Figure 4.7 shows that the difference in *E.coli* concentration between top and bottom slices are somehow negatively related to max flow/tortuosity ratio in the center portion of the pore space. However, the  $R^2$  is quite low mainly because of several high influential points (marked as 125, 722 and 230). Visual assessment of aggregate 230 indicates that there exist large pores connected from top and center portion to the bottom, which may facilitate *E.coli* transport (Aggregate 230, Figure 4.8(a)). However, pores in the center is not well connected which leads to low connectivity in aggregate 230 (Figure 4.7). Aggregate 722 shows opposite trend in difference between top and bottom in *E.coil* concentration, where *E.coli* are

still mostly trapped at top with only a minor translocation to other parts of the aggregate (Figure 4.8(b)). There were disconnected medium to large pores found in the center of aggregate 722. Abundance of randomly scattered pores of various sizes penetrating the entire aggregate led to substantial *E.coli* redistribution to the aggregate's sides as well as center and bottom (Aggregate 125, Figure4.8(c)).

**Figure 4.7:** The relationship between difference in E.coli concentration between top and bottom slices and max flow/tortuosity ratio. Gray circles mark high influential points. Numbers along with the circles are aggregates IDs.



**Figure 4.8:** 3D visualization of aggregates pore space for pieces. Pores with bright color indicate high E.coli concentration. (a) Aggregate 230; (b) Aggregate 722; (c) Aggregate 125.





## CONCLUSION

Despite high variability in enumeration of *E.coli* cells using qPCR analysis, *E.coli* redistribution and transport within soil aggregates display a significant different spatial distribution pattern under air-dry and saturated flow condition. When *E.coli* was first applied to an air-dry aggregate, its resulting spatial distribution was highly heterogeneous in aggregates of all management practices. After saturation, equilibration, and water extraction the *E.coli* redistributed within the aggregate primarily in vertical direction and became more homogeneously spread. However, only relatively small percent (T1: 2.5%, T2: 3.2%, T7: 1.5%) of *E.coli* has completely left the aggregates. Redistribution was most pronounced in T1 aggregates, followed by T2, and was almost negligible in T7 aggregates.

T1, T2 and T7 aggregates are well separated by aggregate pore characteristics. There was greater macro-porosity and medium sized pores (75-120 microns, BN2 and BN3) found in T1 aggregates compared to those of T2 and T7 aggregates. The differences in *E.coli* redistribution observed between T1/T2 and T7 possibly are related to the differences in pore structures.

*E.coli* redistribution was related to intra-aggregate pore characteristics, however, because of high variability of the intra-aggregate pores the relationships were weak. Only 49% of total variation in pore characteristics and 31% of total variation in *E.coil* spatial distribution were account using the first two partial least square regression factors. In aggregates' top, *E.coli* concentration was negatively related to max flow in the center portion of the soil aggregates. *E.coli* retained in the aggregate's centers was positively related to porosity, medium to large pore sizes (BN3 and BN4g), and tortuosity of the pore space. Max flow to tortuosity ratio seems to relate to difference in *E.coli* concentration between top and bottom slices, however, this relationship is week ( $R^2=0.13$ ). It also should be pointed out that other than pore characteristics, higher organic matter content in NS aggregates could be the reason for relatively high *E.coli* retention. Future analyses are needed to address *E.coli* release and redistribution from soil aggregates with repeated saturation and extraction processes, which simulates multiple rain events.

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## **Chapter 5: CONCLUSION**

Soil aggregates play an important role in several soil processes, e.g., C sequestration, water flow and solute transport, and microbial activities. Advancement in X-ray microtomography ( $\mu$ CT) imaging provides us opportunity to understand the mechanisms behind these processes. Overall, the dissertation aims to effectively utilize soil aggregate  $\mu$ CT imaging, to understand soil aggregate structure difference under contrasting land use and managements, and to investigate bacteria redistribution and transport pattern and its relation to aggregate pore characteristics.

Lack of ground-truth images poses great challenge to soil image segmentation. Proposed simulation method successfully generated soil aggregate grey scale images from pore/solid binary images, thus provided ground-truth information. Performance of several commonly used image segmentation methods, including entropy, iterative, Otsu's, and indicator kriging (IK) were assessed using ground-truth based (ME) and non-ground-truth based criteria (NU). IK was found preferable to other methods when the image histogram is clearly bimodal. NU performs sufficiently well as a segmentation criterion, which could be used in assessing soil  $\mu$ CT image segmentation results in future. Identification of irregular soil aggregate boundaries from  $\mu$ CT images can be done using image closing technique with a properly determined neighborhood size parameter, *r*. Examining the plots of the total aggregate volume, aggregate boundary fractal dimension against *r* could serve as useful criteria for selecting the optimal *r* value. The optimal *r* value might depend on a number of factors including the size of the studied aggregates, the scanned resolution and the average sizes of pores connected to aggregate surface.

Using processed soil aggregate µCT images, soil aggregates pore structures from contrasting land use and management as well as spatial gradient of pores in individual aggregate was addressed. 20 years of contrasting management in the LTER experiment led to pronounced differences in the intra-aggregate pore size distributions. Significantly greater percent of pores > 15 µm in LTER-CT aggregates was observed as compared to those of LTER-NS. LTER-NS aggregates had more large pores (> 90µm) than LTER-CT aggregates, while more medium sized pores (45-90 µm) were found in LTER-CT aggregates. With respect to spatial distributions, pores were larger in the aggregate interiors than in the exterior layers; while intermediate pores (45-90 µm) were more abundant in the aggregate exterior layers. This trend was present in all land use and management treatments of the study and was most pronounced in LTER-NS. These results implied that a general mechanism maybe responsible for aggregates formation, but long term land use could alter the magnitude and intensity of involved soil processes. One implication of aggregates' pore size distribution on aggregate stability may serve as a warning indicator of soil degradation on large scale. The difference in pore size distribution at aggregate scale between contrasting land uses could possibly upscale to soil cores/fields to understand macropore flow, C sequestration mechanisms and water retention properties.

*E.coli* redistribution and transport within soil aggregates display a significant different spatial distribution pattern under air-dry and saturated flow condition. When *E.coli* was first applied to an air-dry aggregate, its resulting spatial distribution was highly heterogeneous in aggregates of all management practices (CT, NT and NS). After saturation, equilibration, and water extraction the *E.coli* redistributed within the aggregate primarily in vertical direction and became more homogeneously spread. Only relatively small percent of *E.coli* has completely left the aggregates. Redistribution was most pronounced in CT aggregates, followed by NT, and was
almost negligible in NS aggregates. *E.coli* spatial distribution was related to intra-aggregate pore characteristics; however, because of high variability of the intra-aggregate pores the relationships were weak. In aggregates' top, *E.coli* concentration was negatively related to pore connectivity in the center portion of the soil aggregates. *E.coli* retained in the aggregate's centers was positively related to porosity, medium to large pore sizes and tortuosity of the pore space. It could be speculated that other than pore characteristics, complex processes are also involved for *E.coli* transport in soil aggregates. Implication of these results may help us understand potential in-field contamination caused by *E.coli* movement to groundwater under precipitation events.

Further research is necessary in several aspects: developing new approaches for multiphase (e.g., pore, water, and solid; pore, organic matter, and solid) segmentation in soil images; modeling pore scale flow using fluid dynamic theories and related theory to lab measurements in order to understand water and solute transport mechanisms; describing and quantifying the role of intra-aggregate pore size distributions in a number of soil processes, including, but not limited to C sequestration; confirming spatial pattern in pore size distributions within soil aggregates of other sizes; evaluating *E.coli* release and redistribution from soil aggregates with repeated saturation and extraction processes, which simulates multiple rain events.

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