Abstract

The relationship between form and function in dryland soils is multi-scale and multi-disciplinary. Organisms as ecosystem engineers can modify and influence soil physical structure, either directly or indirectly, and this in turn can have cascading consequences to the various ecological functions in the impacted soil system. Despite its importance, however, the causes, mechanisms, and consequences of this feedback, which is in the domain of both soil physics and ecology, all remain open questions. This dissertation uses mathematical morphology and topology to provide mechanistic explanations for feedbacks between soil physical structure and ecological function and processes in dryland soils. Two applications are considered, the first of which is flora: in Chapter 2, I develop an open-source software package at Oak Ridge National Laboratory for the automated digital image analysis of plant root morphology in soil and its associated plant-soil characteristics. In Chapter 3, I apply the software developed in Chapter 2 to spatially integrate plant morphological traits and bulk soil water characteristics, and show species-independent soil water properties, dynamics, and uptake across the plant-soil interface, also known as the rhizosphere. In Chapter 4, I switch my ecological focus to that of fauna, where I characterize biophysical effects on soil crack morphology in a faunally active dryland vertisol in Kenya. I find divergent crack morphologies based on macrofauna-based bioturbation or megaherbivore-based biocompaction. I conclude this dissertation by applying the divergent crack morphologies characterized in Chapter 4 to study their consequence on carbon flux dynamics in the same soil system. I show that constraints from particular crack morphologies, combined with limited soil carbon production, create lower mean flux punctuated by outlier fluxes that are orders of magnitude higher. I also show that mechanical enhancements of CO$_2$ efflux caused by thermal convection are induced by soil crack morphology.
Acknowledgements

Thanks to Jan Nordbotten at the University of Bergen, Norway, for help with mathematical modeling of soil fractures; and Hassina Bilheux, Jean-Christophe Bilheux, and Jeffrey Warren for research mentoring at ORNL. Special thanks to my dissertation committee for their invaluable help during my PhD: Ian Bourg, George Scherer, Jeffrey Warren, and Amilcare Porporato. Logistical support was provided by Deanne Brice, Joanne Childs, Steve Childs, Mindy Clark, Cari Ficken, Sara Jawdy, Shafter Powell, Lou Santodonato at ORNL; and Peter Ekomwa, John Gitonga, Boniface Kimathi, Moses Kioko, George Koech, Marcus Spiegel, and the Mpala Research Centre and its staff in Kenya. Thanks are extended to the Caylor Lab, past and present, for their combined logistical, research, and moral support: Stephanie Debats, Cynthia Gerlein-Safdi, Drew Gower, and Hilary Wayland, with special thanks to Cynthia for providing extensive logistical and administrative help while I spent multiple years overseas for my research and work. I am grateful to have mentored several undergraduate students, including Vinicius Amaral, Sylvia Jacobson, and Marcus Spiegel from Princeton University; Anna Lopresti and Valentina Strokopytova from Columbia University; and Natasha Krell from the College of the Atlantic, now a member of the Caylor lab. Finally, I would like to acknowledge my advisor, Kelly Caylor. His unwaivering moral and logistical support have made all the increasingly larger-scale risks and ventures I have undertaken throughout my PhD possible, and my work has been defined by a freedom and leeway that I imagine was not possible otherwise. Thank you. Major funding sources include the Francis R. Upton Graduate Fellowship from Princeton University, the Mary and Randall Hack ’69 Award from the Princeton Environmental Institute, the SCGSR Fellowship from the U.S. Department of Energy, and the David L. Boren Fellowship from the National Security Education Program. This dissertation was made using Jeffrey Dwoskin’s L\LaTeX template.
お母さんへ。

知者不言、言者不知。

《老子、五十六章》

言わずと知れた事からこそ、
感謝の言葉もありません。

Et à Sarah—

Pour tous nos jours de vermeil ensemble,

et pour plus à venir.
# Contents

Abstract .......................................................... iii
Acknowledgements ................................................ iv
List of Tables ...................................................... x
List of Figures ...................................................... xi

1 Introduction ...................................................... 1
  1.1 Motivation and Research Objectives ....................... 1
    1.1.1 Feedbacks Between Soil Form and Function ............ 2
    1.1.2 Mathematical Morphology and Topology ................. 4
    1.1.3 Morphological Applications in Soil .................... 9
    1.1.4 Research objectives and outline ....................... 11
  1.2 Core Chapter Abstracts .................................... 11
  1.3 Contribution to co-authored publications ................ 14

2 RootProcessing: An Open-Source Software Package for Automated Analysis of In-situ Roots in Soil 16
  2.1 Background .................................................. 16
  2.2 Materials and Methods ..................................... 21
    2.2.1 Sample Images and Collection .......................... 21
    2.2.2 Library Features and Environment ...................... 22
    2.2.3 Program Overview ..................................... 23
2.2.4 Segmentation Analysis ............................................. 32
2.3 Results & Discussion ................................................ 35
  2.3.1 Segmentation Comparison – RootReader 2D .............. 35
  2.3.2 Segmentation Comparison – Root Diameter Distribution . 39
2.4 Conclusion ............................................................... 41

3 Integrating Fine Root Morphology and Soil Distance Mapping to Characterize the Plant-Soil Interface 43
  3.1 Background ............................................................. 43
  3.2 Materials and Methods .............................................. 47
    3.2.1 Plant Material and Treatments ............................... 47
    3.2.2 Neutron Radiography Conditions and Experiment ....... 48
    3.2.3 Image Reconstruction and Segmentation .................... 50
    3.2.4 Water Content Determination ................................. 51
    3.2.5 Integrated Plant-Soil Mapping ............................... 51
    3.2.6 Soil Water Characteristics ...................................... 54
  3.3 Results ................................................................. 54
    3.3.1 Integrated Root-Soil Mapping ................................. 54
    3.3.2 Water Content Distribution ................................. 57
    3.3.3 Water Uptake ................................................ 61
  3.4 Discussion ............................................................. 64
    3.4.1 Integrated Plant-Soil Mapping ............................... 64
    3.4.2 Rhizosphere Distribution and Dynamics ..................... 65
    3.4.3 Incorporation of Dynamic Processes ......................... 69
  3.5 Conclusion ............................................................. 70

4 Biophysical Effects on Soil Crack Morphology in a Faunally Active Dryland Vertisol 72
5.4.3 Integrating Soil Mechanics and Soil Carbon Availability . . . 132

5.5 Conclusions . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 136

6 Concluding Remarks 138

6.1 Research Conclusions . . . . . . . . . . . . . . . . . . . . . . . . . . . 138

6.2 Contributions to Knowledge . . . . . . . . . . . . . . . . . . . . . . 140

6.3 Future Work . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 142

Bibliography 146
## List of Tables

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Full list of parameters used for the segmentation comparison. Percentage above threshold was only tested for “half-kernel size = 50”, “minimum threshold = 0”, and “maximum threshold = 100”.</td>
<td>32</td>
</tr>
<tr>
<td>3.1</td>
<td>Gamma distribution parameters of the root distance histograms of each species evaluated in our experiments. Columns 1 and 2 represent mean parameters $k$ and $\theta$ across all samples for a given species, and columns 3 and 4 represent the mean and variance of the gamma distributions.</td>
<td>58</td>
</tr>
<tr>
<td>3.2</td>
<td>Best fit parameters and root mean square error of the sigmoid functions fitted for the five species analyzed in Figure 3.8A, as well as the combined data.</td>
<td>60</td>
</tr>
<tr>
<td>4.1</td>
<td>Statistics of the physicochemical properties of the three soil treatments.</td>
<td>79</td>
</tr>
<tr>
<td>4.2</td>
<td>Frequencies of the four crack layering morphologies in the three soil treatments.</td>
<td>95</td>
</tr>
<tr>
<td>5.1</td>
<td>List of all precipitation events by date and magnitude during the course of experiments.</td>
<td>106</td>
</tr>
<tr>
<td>5.2</td>
<td>Statistics of all visible burrows at each of the BT sites analyzed. Measurements taken in August 2014. N is the number of burrows per BT site. R, d, and k are the mean radius, depth, and fracture permeability of the termite burrows, respectively.</td>
<td>132</td>
</tr>
</tbody>
</table>
List of Figures

1.1 Overview of basic concepts in set theory. (A) Set $A$. (B) Set $B$. (C) Complement of $B$ (written $B^c$). (D) One possible overlapping of sets $A$ and $B$. (E) Union of $A$ and $B$ (written $A \cup B$), based on the positioning of the two sets in (D). (F) Intersection of $A$ and $B$ (written $A \cap B$).

By extension, intersection $C$ are subsets of both $A$ and $B$ (written as $C \subseteq A$ and $C \subseteq B$).

1.2 Basic morphological operations. (A) Set $B$ from Figure 1.1B. (B) Structuring element $S$, with reference pixel $(i, j)$ defined as the black dot in center. (C) Erosion. (D) Dilation. (E) Opening. (F) Closing.

Gray lines indicate the dimensions of the unchanged set $B$.


Gray-colored pixels indicate $S^1$ and the white-colored pixels indicate $S^2$. Note that the structuring elements do not intersect. (I) Set $B$ from Figure 1.1. (J-L). 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ iterations of the thinning operation. Note that the 3$^{rd}$ iteration does not change the object.

1.4 Root morphology analysis. (A) Original root network $X$. (B) Skeletonization $X_n \otimes S_i$. Note that the image shown here is artificially thickened by three pixels for better viewing in figure. (C) Analysis of root diameter distribution.
1.5 Fracture percolation analysis. (A) Original fracture network \( X \). (B) Geodesic propagation \( P_g(X) \). (C-F) 1\(^{st}\), 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) iterations of the percolation operation \( P_g(X) \circ B_n \). (G) After the 5\(^{th}\) iteration, percolation ceases. (H) Analysis of percolation behavior.

2.1 Visual schematic of the analyses available in the RootProcessing package. A sample poplar image is used as an example. Arrows indicate the necessary prerequisite inputs for a given analysis (e.g., the segmentation analysis is necessary prior to running the thickness analysis, distance mapping and water content are both necessary for the integrated root-soil analysis, etc.

2.2 Visual schematic of the alignment process, with the four degrees of freedom outlined, using the reference dataset created by the package.

2.3 Visual schematic of the root segmentation analysis, highlighting three different cases of classification.

2.4 Visual schematic of the root cross-sectional thickness analysis. \( [x, y]_p \) is the evaluated pixel in question, \( [x, y]_c \) is the closest contour pixel, and \( [x, y]_m \) is the medial axis pixel that intersects the line (indicated by the blue and green arrows) between the contour and evaluated pixel. \( L_{MP} \) and \( L_{EP} \) are the distances between the medial axis and evaluated pixel, and the evaluated pixel and contour, respectively. \( H \) is the half-dome thickness, and \( R \) is the root radius at the medial axis of the root. The inset shows the assumed cylindrical distribution of root thickness, centered at the medial axis.
2.5 Visual schematic of the distance mapping procedure, where the black arrow indicates the closest contour pixel to the evaluated pixel, and the blue arrow indicates the closest medial axis pixel from the contour. Regions labeled the same number indicate equivalent root diameter “zones of influence” (i.e., soil regions associated with a given root diameter).

2.6 Comparison of the original corrected neutron image (column 1, row 1) with the segmentation analysis in RootProcessing (column 2, row 1) and in RootReader 2D (all columns, rows 2-4). For the RootReader 2D segmentation images: from column 1 to 5, maximum threshold values are 50, 100, 175, 255, and 255, minimum threshold values are 0, 0, 0, 0, 100. From row 1 to 3 (columns 1-5), half-kernel sizes are 25, 50, and 75. For percentage above threshold (column 6), values correspond to 0.1, 0.5, and 5% (rows 2-4) - min/max threshold are 0 and 100, respectively, and half-kernel size is 50. For fixed threshold (column 7), values are 90, 100, and 120 (rows 2-4). All images use an area filter of 800.

2.7 Cumulative distribution of all topologically distinct objects in the segmented neutron root image (solid line) and segmented flatbed scan root image (dashed line), as sorted by ascending object size fraction. Inset shows segmented neutron root image, with the largest topologically distinct object highlighted in white.
2.8 Root diameter distributions of the root image as segmented by Root-Processing (bar graph), and from the flatbed scanner (dash line). (A, C-E) comprise the Populus deltoides samples, while (B) comprises the *P. trichocarpa* sample. $\mu_{\text{mask}}$ represents mean root diameter of that of the segmentation obtained from the RootProcessing masking algorithm, and $\mu_{\text{scan}}$ represents mean root diameter of that of the segmentation obtained from the flatbed scanned image.  

2.9 Cross-sectional thickness of water and root. Image inset shows the location of where the profile was taken, in red.  

2.10 Comparison of the upper half of the sample root image. (A) Corrected neutron transmission image. (B) Segmented neutron root image. (C) Flatbed root scan. (D) Segmented flatbed scan root image.  

3.1 Experimental set up. (a) sealed seedling at the beamline during experiments. (b) neutron attenuation radiograph of a water content map of a poplar seedling root system.  

3.2 Visual schematic of the plant-soil mapping procedure, and how represented root diameter classes are calculated for each transform. (a) the medial axis transform (MAT), with the root highlighted in green for reference. (b) the root surface (RS) transform. (c) the soil region (SR) transform. Histograms of the represented root diameter values are shown for the (d) MAT, (b) RS, and (c) SR mapping.  

3.3 (a) outline of the three soil subsections in the plant-soil interface. (b) outline of the location of the three soil subsections in the context of a typical water content profile in the plant-soil interface.  

3.4 Distributions of represented root diameter for the MAT, RS, and SR mapping for each sample, with gamma distribution fits shown. Axes are in log-log scale.
3.5 Mean and variance of the represented root diameter data, and their gamma distribution fits for the MAT, RS, and SR mapping. Mean and standard deviation of all parameters for each species is shown. . . . 56

3.6 Mean plant-soil extent of all soil pixels to the closest root surface, sorted by root diameter. (a-f) poplar, (g, h) juniper, (i) maize, (j) maple, and (k-o) grape experiments. . . . . . . . . . . . . . . . . . . 57

3.7 Mean water content over time for the three soil region boundaries in the root-soil interface (labeled as “d = “ in (f)) for the (a-e) poplar and (f) maple species. Dotted vertical lines indicate re-wetting points. . . 59

3.8 (a) difference in water content from root-soil edge to rhizosphere, sorted by root diameter. Green line indicates the sigmoid best fit. (b) difference in water content from rhizosphere to bulk soil, sorted by root diameter. . . . . . . . . . . . . . . . . . . . . . . . . . . . . 60

3.9 Relative water content dynamics, as sorted by root diameter. Top row is difference in water content from root-soil edge to rhizosphere, sorted by bulk soil water content, for root diameters (Droot) (a) < 0.1 cm, (b) 0.1-0.2 cm, and (c) > 0.2 cm. Bottom row is difference from rhizosphere to bulk soil, sorted by bulk soil water content, for root diameters (d) < 0.1 cm, (e) 0.1-0.2 cm, and (f) > 0.2 cm. Dotted lines show reference line where soil subsections at either side of the interface is equal (i.e., difference of zero), and solid lines show best fit linear values for data. 62
3.10 Relative water content dynamics, as sorted by root order. Top row is difference in water content from root-soil edge to rhizosphere, sorted by bulk soil water content, for (a) 1st, (b) 2nd, (c) 3rd, and (d) 4th root order. Bottom row is difference in water content from rhizosphere to bulk soil, sorted by bulk soil water content, for (a) 1st, (b) 2nd, (c) 3rd, and (d) 4th root order. Dotted lines show reference line where soil subsections at either side of the interface is equal (i.e., difference of zero), and solid lines show best fit linear values for data.

3.11 Water uptake characteristics. (a) total water uptake normalized by total root surface area of each root diameter. (b) distribution of total root surface area by root diameter. (c) total water uptake of all dynamics experimental samples plotted by total root surface area.

3.12 Distribution of total root-soil interface data points analyzed in our experimental samples vs of those used in root order analysis.

4.1 Typical experimental setup at one of the BT soil systems, where resin solution is prepared on site in the background. Inset shows a metal-framed soil site.

4.2 Image processing outline. (A) Trinary thresholded image of a cast tomogram, and (B) effect of morphological opening/closing to remove excess resin presence and define integrated soil ped/crack surface. The red line indicates the calculated reference surface level for this cast.
4.3 Crack morphology and topology outline, using a simplified schematic.
(A) morphological parameters of hydraulic radius (RH), shape factor (G), and tortuosity (τ), with A and P respectively corresponding to a crack cross-section area and perimeter, and L and C corresponding to the length of the skeletonized crack edge and the distance between the crack endpoints, respectively, and (B) topological parameters of node degree (ND), branches per path (BPP), and normalized minimum path length (NMPL). Numbers indicate length of each crack edge.

4.4 Schematic of a skeletonized 3D crack structure, with the three colors corresponding to the three individual faces of the crack junction system. At the XY plane (red box at top), the initial starting positions of the three crack faces can be seen, as well as the midpoint at which they join. Along the Z axis, the drifting midpoint is outlined (thin green), as well as a projection (blue) of the terminal midpoint to the XY plane.

4.5 Surface crack characteristics: (A) surface (2D) crack porosity, (B) mean crack aperture, and (C) correlation between 2D crack porosity and 3D crack porosity.

4.6 3D crack porosity in relation to maximum depth of the crack network in the three soil systems. Oversize points represent mean of each system.

4.7 3D profile image of a single major crack observed in one of the BC soil sites – white spaces indicate macropore (i.e., crack) spaces.

4.8 Morphological (A-C) and topological (D-F) characterizations of the 3D crack structure as a network.
4.9 Crack volume-based connectivity characteristics: (A) Euler characteristic after progressive morphological opening/closing of the 3D crack structure, and (B) correlation between 3D crack porosity and the Euler characteristic. ................................................................. 92

4.10 Visualization of the four crack layering morphologies: surface (A, C) and transparent (B, D) visualization of a disjointed crack, and a full crack network with different colors corresponding to different depths; and (E) crack angle distribution and Gaussian fit across all measured crack morphologies. ................................................................. 94

4.11 Schematic of the proposed soil memory/layering process. .................. 97

5.1 Conceptual outline for the link between crack morphology and carbon flux in faunally active vertisols: (a-d) visual schematic flux processes in the four soil classifications outlined in [DeCarlo and Caylor, 2019], and (e) their theoretical inverse cumulative distributions, plotted in log-log space. ................................................................. 104

5.2 (a) Precipitation and (b) volumetric soil moisture of each field soil type during the course of experiments, with the pre-drying, wetting, and drying phases outlined. Error bars indicate standard deviation. .... 107

5.3 Soil temperature profile of a model soil fracture with dimensions of 2.5 \( \times \) 15 \( \times \) 20cm width, length, and depth, respectively, measured at (a) 5cm length and (b) 10cm length position. Temperature at depth was measured in 5cm increments. ................................................................. 113

5.4 Mean frequency and maximal Rayleigh number observed at each crack aperture. Mean crack aperture distributions represent the BC soil crack systems collected in [DeCarlo and Caylor, 2019] at 24.4% mean volumetric soil moisture. Temperature profiles obtained from the model soil fracture are used for Rayleigh number calculation. .... 114
5.5 CO₂ flux distribution of the (a) C, (b) BC, (c) BT, and (d) BT burrow soils over the length of experiments. Dotted lines indicate the boundaries between the pre-wetting, wetting, and drying phases. For each box plot, mean is shown by the square box with black edge, median by the white dash, the 25th and 75th quartile by the box (i.e., the thick central line), and the highest/lowest datum within 1.5 times the inter-quartile range by the whiskers (i.e., the thinner outer lines). All outliers are plotted with a + sign. Note the axis difference between the C and BC soils, and BT and BT burrow soils.

5.6 CO₂ flux distribution of the (a) C and (b) BC soils by time of day in the pre-wetting, wetting, and drying phases.

5.7 CO₂ flux distribution of the (a) BT and (b) BT burrow soils by time of day in the pre-wetting, wetting, and drying phases. Note the axis difference between this and Figure 5.6.

5.8 CO₂ flux distribution of the (a) C, (b) BC, (c) BT, and (d) BT burrow soils by volumetric soil moisture. Note that these do not include fluxes from the pre-wetting, and the first half of the wetting phase due to lack of volumetric soil moisture measurements at this time. Also note axes differences between the C and BC soils, and BT and BT burrow soils.
5.9 Spatial correlation analysis of temperature fluctuations over time within the model soil fracture. (a) example of a strong positive correlation between fluctuations in temperature, observed at daytime between the 5 and 10cm length position and 0cm depth (i.e., horizontally along the surface). (b) example of a strong negative correlation between fluctuations in temperature, observed at nighttime between the 5 and 10cm length position at 5cm depth (i.e., horizontally within the fracture). Spatial maps of strong correlations ($\rho > 0.5$) within the fracture at (c) daytime and (d) nighttime. Figure 5.9A corresponds with the top-most line observed in Figure 5.9C, and Figure 5.9B corresponds with the second top-most line observed in Figure 5.9D.

5.10 Convection cell properties. (a) Distribution of time between convection cell events, with an exponential distribution fit shown. (b) Distribution of the magnitude of cold air intrusion within the fracture.

5.11 Inverse cumulative probability distribution for (a) mechanically-limited/carbon-limited (C), (b) mechanically-enhanced/carbon-limited (BC), (c) mechanically-limited/carbon-enhanced (BT), and (d) mechanically-enhanced/carbon-enhanced (BT burrow) soils.

5.12 Amended conceptual framework for the link between crack morphology and carbon flux in the four soil types classified in our cracked vertisols. Changes made from Figure 5.1 are marked in red.
Chapter 1

Introduction

1.1 Motivation and Research Objectives

Anthropogenic emissions of CO$_2$ have caused a temperature increase of roughly 1°C since 1850 [IPCC, 2014], and a further increase by 2-4 °C is expected under current emission rates. Despite the Paris Climate Accords in 2015, which sought to cap this increase to 2 °C by 2100 via aggressive cuttrailing of emissions, particularly by industrialized countries, none of these signatories have met their pledges 2 years in [Victor et al., 2017]. The advent of significant temperature and climate change throughout the planet thus needs to be considered and evaluated.

Drylands, which are water-limited environments that comprise 40% of the Earth’s land surface and are home to 32% of the world population [Reynolds et al., 2007], will most likely be impacted disproportionately. Climatologically, they are the most vulnerable: global models predict a 44% higher increase in temperature relative to the rest of the planet, even if the entire planet followed the Paris Accords [Huang et al., 2017]. This will result in increased aridification, stagnating precipitation levels, and longer and more persistent droughts [Sheffield and Wood, 2008, IPCC].
These stressors will add to and exacerbate endemic demographic and economic stressors present in many dryland regions of the world. For example, in sub-Saharan Africa, birth rates are the highest in the world, which further stress water and natural resource scarcity [Falkenmark, 1989, Ashton, 2002]. Ultimately, the major controls that affect these above-mentioned feedbacks can ultimately be traced to the soil. Their continued agricultural productivity and water retention ensures a sustainably supplied population, and as climate change continues to expand dryland extent, it will create positive feedback loops that adversely affect soil function, such as water content, plant-associated carbon and emissions [Huang et al., 2016]. Belowground soil/root structure and dynamics likely play a significant role [Breshears and Barnes, 1999, Sankaran et al., 2004], whose coupled biophysical effects can potentially result in increased resilience against climate change in drylands [Bonachela et al., 2015]. Despite this, many of the underlying mechanisms that drive changes in dryland soil function remain an open question.

In this chapter, I will briefly outline the bi-directional feedbacks between soil physical structure and ecological function, gaps in the literature concerning their integration and reasons why this is the case, and the potential role of soil morphology and topology in this integrated analysis. A brief primer on the relevant morphological and topological analyses used in this dissertation is provided, with specific applications to plant and soil phenomena of interest to this dissertation. Research objectives are stated, and a framework for the rest of the dissertation is outlined.

1.1.1 Feedbacks Between Soil Form and Function

Soil structure regulates soil ecological functions and processes via a combination of pore and particulate properties [Rabot et al., 2018]. This includes changes in water infiltration, retention, and percolation, as well as soil gas exchange and aggregation.
behavior. The characteristics of pore networks most relevant to ecological functions include micro/macroporosity, pore distance, and pore connectivity [Rabot et al., 2018]. Changes in macroporosity and connectivity can greatly alter preferential soil water and gas flow [Katuwal et al., 2015, Larsbo et al., 2014, Naveed et al., 2014], as well as soil carbon loss due to changes in organic matter accessibility by microbes [Rabot et al., 2015, Ananyeva et al., 2013, Kravchenko et al., 2015, Strong et al., 2004].

Simultaneously, changes in soil ecological function and processes drive, directly or otherwise, soil structural properties. Organisms can act as “engineers” to modify their physical environment and local habitat, a process broadly referred to as ecosystem engineering [Jones et al., 1994, Jones et al., 1997]. In the soil environment, this is usually focused on soil macrofauna such as earthworms [Blouin et al., 2013a], termites [Holt and Lepage, 2000, Jouquet et al., 2011], and ants [Cammeraat and Risch, 2008], which alter their soil habitat to induce changes in soil microbial communities, organic matter dynamics, and nutrient cycling (e.g., [Bonachela et al., 2015, Bottinelli et al., 2015]). Flora can play a similar role: nearly all roots use exudate and nutrient deposition within the root-adjacent soil region known as the rhizosphere [Hiltner, 1904, Hartmann et al., 2008] to develop microbial and bacterial communities. Finally, this can be extended to environments outside of the soil: large herbivores and mammals can have “rampant indirect effects” on their local environment [Paine, 2000]. These include, but are not limited to: those on trophic cascades [Pringle et al., 2007], rodent-borne disease prevalence [Young et al., 2014], and soil biocompaction and consolidation (e.g., [Drewry et al., 2008, Veldhuis et al., 2014]).

It is thus evident that there exist complex and multi-faceted feedbacks between soil physical structure and ecological function and processes, and many of these
feedbacks can have profound consequences on larger scale water and carbon cycles within the soil. However, despite this, their study is often neglected in the literature, both from soil physicists and ecologists. One reason may be the perceived importance of other soil processes like soil shrink-swelling, freeze-thawing, and/or soil tillage [Bottinelli et al., 2015]. Another is the “balkanization”, i.e., the disciplinary fracturing, of the soil ecological sciences [Blouin et al., 2013b]. It is noted by both soil physicists (e.g., [Rabot et al., 2018]) and soil ecologists (e.g., [Bottinelli et al., 2015]) that the study of the distribution and dynamics of soil pore networks, biotic or otherwise, is required to bridge these gaps.

While several techniques exist to characterize these pore networks, imaging techniques are one of the only ways to enable the direct incorporation of soil geometry visualization. Beyond simple qualitative metrics (e.g., manual counting, identification), quantitative processing of digital imagery often has a large barrier to entry due to the level of expertise required. Nevertheless, it is the effective application of mathematical morphology and topology to images that can yield the most meaningful, direct, and mechanically relevant metrics of integrated soil structure and function [Rabot et al., 2018].

### 1.1.2 Mathematical Morphology and Topology

Mathematical morphology emerged from set theory in the 1960s and was applied to image processing in the 1980s by Jean Serra at the École des Mines in Fontainebleau, France [Serra, 1983, Serra, 1986]. We define a binary image consisting of black (0) and white (1) pixels – for demonstration, we will classify black pixels as the set of interest. Thus, an image can be evaluated as the union of several sets of black pixels, which we label as objects, with white pixels being the complement of the black pixels. Any and all operations on one will affect the other – if a black pixel is removed, a
white pixel is created in its place, and vice versa. A visual overview of the basic concepts in set theory within the context of a binary image are shown in Figure 1.1.

![Figure 1.1](image.png)

**Figure 1.1:** Overview of basic concepts in set theory. (A) Set \(A\). (B) Set \(B\). (C) Complement of \(B\) (written \(B^c\)). (D) One possible overlapping of sets \(A\) and \(B\). (E) Union of \(A\) and \(B\) (written \(A \cup B\)), based on the positioning of the two sets in (D). (F) Intersection of \(A\) and \(B\) (written \(A \cap B\)). By extension, intersection \(C\) are subsets of both \(A\) and \(B\) (written as \(C \subseteq A\) and \(C \subseteq B\)).

We define the three sets \(A\), \(B\), and \(A^c\) as within a single 10×10 pixel image, we can see that these three sets have the same number of black pixels, but different morphological properties. We can characterize this using a sample set, for example a 3×3 set, and conducting a series of intersections with the object to observe whether the sample set is a subset of each object. We define this sample set as a structuring element \(S\). As it is important that \(S\) can be placed for every pixel in the image, we define a reference pixel \((i, j)\) which determines the exact position of \(S\).

Using this notation, we can define key morphological operations whose exten-
sions and applications will be used throughout this dissertation. The first is erosion. If we define $A$ as a set and $S_{(i,j)}$ as the structuring element with reference pixel at $(i,j)$, the erosion of $A$ by $S$ is defined as the set of all pixel locations for which $S$ is placed at that pixel is contained within $A$. Denoted as $A \ominus S$, this is written as follows:

$$A \ominus S = \{(i, j) : S_{i,j} \subseteq A\}$$ (1.1)

Dilation can then be defined as the complement of the erosion of the set complement, i.e.:

$$A \oplus S = (A^c \ominus S)^c$$ (1.2)

Using these two, we can define image opening as the dilation of the erosion of set $A$ by structuring element $S$:

$$A \circ S = (A \ominus S) \oplus S$$ (1.3)

and image closing as the erosion of the dilation of set $A$ by structuring element $S$:

$$A \bullet S = (A \oplus S) \ominus S$$ (1.4)

Applications of erosion, dilation, opening, and closing are shown in Figure 1.2, using set $B$ from Figure 1.1. We use the structuring element $S$ with dimensions shown in Figure 1.2B. Functionally, we can see that erosion and dilation have a uniform size reducing and increasing tendency, respectively. Opening operations remove small objects, while closing operations can fill small holes, and both preserve the overall structure of the object. Finally, we can further generalize the structuring element $S$ as a composite of two structuring elements $S^1$ and $S^2$, such that $S^1 \cap S^2 = \emptyset$ (i.e., the two structuring elements do not intersect). This allows us to satisfy multiple conditions of subset criteria. Using this composite structuring element, we can define
Figure 1.2: Basic morphological operations. (A) Set $B$ from Figure 1.1B. (B) Structuring element $S$, with reference pixel $(i,j)$ defined as the black dot in center. (C) Erosion. (D) Dilation. (E) Opening. (F) Closing. Gray lines indicate the dimensions of the unchanged set $B$. 
We show a specific application for the hit-and-miss transform, referred to as thinning. We define 8 composite structuring elements $S_{1-8}$, shown in Figure 1.3A-H. For set $B_n$ from Figure 1.1 (shown again in Figure 1.3I, the $n + 1$\textsuperscript{th} application of all structuring elements between 1 and 8 is noted as follows:

$$B_{n+1} = B_n \otimes S_i = B_n \setminus (B_n \odot S_i)$$

Where $X \setminus Y$ denotes the relative complement, which indicates all elements in $X$ but not $Y$ (i.e., $X \cap Y^c$), and $B_n \otimes S_i$ denotes the $(n + 1)$\textsuperscript{th} thinning operation, with $i$ indicating one of the 8 composite structuring elements, and $n = 0$ defined as the unchanged set $B$. The 1\textsuperscript{st}, 2\textsuperscript{nd}, and 3\textsuperscript{rd} iterations of the thinning operations are shown in Figure 1.3I-L.
1.1.3 Morphological Applications in Soil

Applying and extending even just these five tools provide a substantial amount of information about the combined morphological and topological properties of objects in a binary image. Examples are numerous, but we show two from soil physics and ecology are shown below, of relevance to this dissertation:

I. Root Morphology

We analyze a washed (i.e., *ex-situ*) root of a young Oak (*Quercus alba*) sapling. We define $X$ to be the root network (Figure 1.4A). We then apply iterative thinning operations $X_n \otimes S_i$, until $X_{n+1} = X_n$ (Figure 1.4B). This is defined as the skeleton $K$ of the root network, which preserves the topology of the original object (i.e., is homotopic). If we measure the Euclidean distance between every pixel and the nearest non-root (i.e., black) pixel in Figure 1.4A, we can obtain a root diameter distribution of $X$ (Figure 1.4C). This analysis on root diameter, and its applications, on *in-situ* roots in soil requires both morphological and topological consideration of the root network, and is a focal point of Chapters 2 and 3.

![Figure 1.4: Root morphology analysis. (A) Original root network $X$. (B) Skeletonization $X_n \otimes S_i$. Note that the image shown here is artificially thickened by three pixels for better viewing in figure. (C) Analysis of root diameter distribution.](image-url)
II. Fracture Percolation

We analyze a vertical cross-section of a biocompacted vertisol fracture network (Chapters 4 and 5). We define $X$ to be the fracture network (Figure 1.5A). We can then define $P_g(X)$ to be the geodesic propagation of $X$, where all objects that are not connected from the upper to lower edge of the image are removed (Figure 1.5B). A morphological opening operation with a structuring element $S$ of pixel size $n$, $P_g(X) \circ B_n$ is applied (Figure 1.5G). This removes all fractures with an opening narrower than $n$ pixels. We then iterate with progressively larger $B$, i.e., $P_g(X \circ B_{n+1})$ (Figure 1.5C-F), until there is no more percolation (Figure 1.5G). We thus combine fracture morphology (image opening) and topology (geodesic propagation) to evaluate the critical narrowing threshold [Moreau et al., 1996] and percolation behavior (Figure 1.5H). A similar analysis is conducted for a 3D fracture network in Chapter 4, where 3D connectivity of a fracture network, represented by the Euler number, is evaluated with progressive opening operations of the fracture (Figure 4.9).

![Fracture percolation analysis](image)

Figure 1.5: Fracture percolation analysis. (A) Original fracture network $X$. (B) Geodesic propagation $P_g(X)$. (C-F) 1st, 2nd, 3rd, and 4th iterations of the percolation operation $P_g(X) \circ B_n$. (G) After the 5th iteration, percolation ceases. (H) Analysis of percolation behavior.
1.1.4 Research objectives and outline

The primary research objective of this dissertation is to provide mechanistic explanations for feedbacks between soil physical structure and ecological function in dryland soils, using mathematical morphology and topology. I use case studies of biological organisms whose ecological functions involve alteration of soil structure, which in turn result in feedbacks between the two. From here, I move on to two broadly defined case studies of this application. The first is that of flora: in Chapter 2 we develop a coding framework to analyze fine root morphology in soil using neutron images collected at Oak Ridge National Laboratory, which we then apply in Chapter 3 to analyze the plant-soil interface in 5 different plant species, where species-independent rhizosphere responses to soil water content dynamics is outlined. The second case study is that of fauna: in Chapter 4 we outline how several classes of fauna can influence the formation of soil fracture in Kenyan drylands. In Chapter 5 we analyze these morphological differences in crack morphology and their linkages with known dynamics of CO₂ production in drylands to create highly dynamic and variable CO₂ flux properties in dryland soil systems.

1.2 Core Chapter Abstracts

The core scientific research of my dissertation is presented in Chapters 2 through 5. The abstracts are presented here.

Chapter 2: RootProcessing: An Open-Source Software Package for Automated Analysis of In-situ Roots in Soil

This chapter provides an overview of RootProcessing, a Python-based software package for the automated image analysis of roots in soil to characterize root morphology and temporal patterns of soil water content dynamics, as well as integrated spa-
tial analyses of the rhizosphere. We collected in situ neutron radiographs of plant roots growing in soil at the HFIR CG-1D neutron imaging beamline at Oak Ridge National Laboratory. Using the raw image output, RootProcessing first conducts a pre-processing procedure of image normalization and stitching of multiple images, then identifies the roots within the soil chamber using a combined fixed/adaptive thresholding segmentation algorithm. Using these data, the software conducts primary measurements of soil water content, root cross-sectional thickness, and root diameter distribution throughout the soil profile. With these products, the software creates a distance map, where all soil pixels within the image are classified under “zones of influence” by nearby roots. This can be used to conduct an integrated spatial analysis of soil water content and its linkages to root distribution. Comparisons of the segmentation product show that RootProcessing has greater accuracy in root classification than RootReader 2D, a similar automated root analysis software. Neutron-based root diameter distribution values are underestimated relative to those obtained from the flatbed scanner images, likely due to extensive root overlap in the latter images. RootProcessing provides a novel, streamlined, and free-to-use software package for the automated image analysis and data extraction of roots in porous media, and can be readily applied to sequential neutron images or other images of roots in soil such as X-ray radiographs or scanned images. The package provides tools for the spatial analysis of the root and soil in tandem as an integrated system.

Chapter 3: Integrating Fine Root Morphology and Soil Distance Mapping to Characterize the Plant-Soil Interface

We integrate plant morphological traits and bulk soil water characteristics using spatial proximity to show species-independent water content distribution and uptake across the plant-soil interface (i.e., rhizosphere). We propagated poplar (*Populus deltoides*), maize (*Zea mays*), juniper (*Juniperus virginiana*), grape (*Vitis rotundii-
folia), and maple (Acer saccharum) seedlings in sand, after which root morphology and soil water dynamics were assessed using neutron radiography. Three local soil regions (root-soil edge, rhizosphere, bulk soil) were classified based on both radial distance from the root surface and diameter of the nearest root, from which changes in water content and distribution were characterized using digital image processing. Water content dynamics across the rhizosphere showed two different species-independent processes: a consistently elevated water content at the root-soil edge interface which increased with root diameter, and plasticity behavior at the bulk soil interface independent of root diameter. Water uptake per unit root surface area declined exponentially with root diameter, independent of species. Results highlight the species-independent hydrologic characteristics of the rhizosphere and the potential for evaluating them in a local spatially-connected soil context. Avenues for improved integration of soil and root characteristics are discussed.

Chapter 4: Biophysical effects on soil crack morphology in a faunally active dryland vertisol

Faunal behavior in dryland ecosystems may physically influence swelling soil hydrologic and pedologic processes due to its contributions to soil crack formation. In order to provide a physical link between this faunal activity and pedological processes, we use a resin visualization technique and X-ray imaging to characterize the biomechanical influences of biocompaction (mega/meso-herbivores) and bioturbation (termites) on 3D crack morphology and topology in a faunally active dryland vertisol system. Results show increased soil cracking intensity due to faunal influence. However, this increased cracking diverged by faunal influence: bioturbation creates “surficial” (shallow, extensive) crack networks while biocompaction creates “systematic” (deep, narrow) crack networks, compared to a reference soil. Biocompacted soils also exhibit a memory of past wetting and drying events via vertically layered crack
morphologies. Despite differences in these crack magnitudes between the faunal influ-
ences, crack structures show morphological and topological similarity as a topological
network. These persistent differences in field crack systems may potentially create
convectively-driven “hotspots” of enhanced water and carbon gas transport in dryland
ecosystems as a result of crack formation, highlighting the importance of considering
fracture behavior in both an ecological, atmospheric, and pedologic context.

Chapter 5: Effects of crack morphology on soil carbon flux
dynamics in a dryland vertisol

CO₂ flux in dryland ecosystems is typically limited by soil carbon availability, result-
ing in pulses of flux with precipitation and transitions between low and high moisture.
Soil crack morphology, which shifts distinctly between these wet and dry periods, can
introduce additional complexity to the magnitude and dynamics of CO₂ flux, though
its full effects remain unknown. In this study, we combine analyses of minute-scale
temperature profile fluctuations in a model soil fracture with CO₂ flux measurements
in Kenyan vertisols with known differences in crack morphology and CO₂ source avail-
ability. We show that flux enhancements due to crack morphology are due to thermal
convection and are driven by depth and aperture. Combined with high soil carbon
availability, fluxes can consistently increase several orders of magnitude. However,
limitations imposed on either axis of soil mechanics or soil carbon availability modu-
late the magnitude of CO₂ flux and increase variability. Our results provide a stronger
integration of the role that soil structure plays on dryland CO₂ flux dynamics.

1.3 Contribution to co-authored publications

The majority of the work presented here is either already published, in review,
or in the process of preparation and submission in peer-reviewed journals. The
manuscripts are co-authored, but the scientific analysis and writing provided here are my own. The references for the works and their publication status is provided below.

Chapter 2 contains research submitted as:


Chapter 3 contains research submitted as:


Chapter 4 contains research published as:


Chapter 5 contains research submitted as:

**K.F. DeCarlo and K.K. Caylor, 2019. Effects of crack morphology on soil carbon flux dynamics in a dryland vertisol. To be submitted to Geoderma.**

The following manuscript has also been co-authored during my time at ORNL:

Chapter 2

RootProcessing: An Open-Source Software Package for Automated Analysis of In-situ Roots in Soil

2.1 Background

The plant root-soil interface is a multidisciplinary component of the plant system which remains poorly understood and understudied. Composed primarily of the root and soil immediately adjacent to it, the interface serves multiple biological, chemical, and physical functions, from facilitating water and nutrient uptake [Darrah, 1993] to fostering dynamic microbial and bacterial communities [Gregory, 2006, Hinsinger et al., 2009]. This critical interface is also referred to as the rhizosphere [Hiltner, 1904], and has unique properties that differ from the bulk soil – properties that often seem contradictory depending on experimental conditions [MacFall et al., 1990, Segal et al., 2008, Nakanishi et al., 2005, Carminati and Vetterlein, 2013].

Given its small size and hidden nature, in-situ quantitative analysis of the rhi-
zosphere is difficult but sorely needed, a demand well filled by neutron imaging (NI; Oswald et al., 2008; Anderson et al., 2009). NI serves as a promising method for furthering our understanding of belowground plant and soil processes. Neutrons are selectively sensitive to hydrogen, which has high concentrations in water and organic matter such as roots, and thus enabling the physical quantification of plant root- and soil-water behavior. Current micrometer-scale resolution of NI provides adequate coverage of the mm-scale rhizosphere. Finally, image acquisition time is on the order of seconds, which enables rapid collection of large amounts of temporal data for capturing high-resolution belowground dynamics. These attributes combined make NI an ideal and increasingly utilized tool for analyzing in tandem the interplay between root structure, soil water distribution, and dynamics at the plant-soil interface (e.g., Carminati et al., 2010; Warren et al., 2013; Zarebanadkouki et al., 2016; Dhiman et al., 2018).

The High Flux Isotope Reactor (HFIR) at Oak Ridge National Laboratory (ORNL) is one of several neutron sources in the Unites States, and provides the strongest continuous source of neutrons in the world. At HFIR, the dedicated NI CG-1D beamline has been operational since January 2011 (Crow et al., 2011; Santodonato et al., 2015). The instrument provides a range of NI capabilities, including a large detector with \( \sim 100\mu m \) spatial resolution and a field of view of 7.4 cm \( \times \) 7.4 cm, producing 2D radiographs on the order of 60 seconds. Larger scale (10s of cm) plant-soil systems can be assessed using automated image capture procedures, including robotic stages and lift tables that progressively display the system within the beamline. Sequential images of the plant-soil system collected in rapid succession, and “stitching” of individual radiographs yields the ability to capture dynamic processes within the plant-soil system. The Versatile Neutron Imaging Beamline (VENUS), located at the Spallation Neutron Source (SNS) at ORNL (Bilheux et al., 2015), will incorporate
simultaneous X-ray and neutron imaging, which will enable analysis of soil pore structure as well. While the majority of experiments conducted at the beamline are used for engineering/energy applications, biological/geoscience/environmental experiments have increased to 5% of applications from 2014-2018, and increased future growth is expected in the plant/soil sciences community.

One of the associated challenges with such a development is the lack of dedicated and/or widely accessible software and tools for extracting the increasingly complex and larger datasets that can be captured and analyzed by a non-expert user. Since beamline inception, user surveys have indicated a need for tools to extract quantitative data from the 2D and 3D images collected at the beamline, which highlight the need for the development of a streamlined image analysis procedure that is relatively simple to use for non-specialized scientists. As root/soil segmentation and skeletonization is a fundamentally persistent problem \cite{Zhang2008, Zeng2008}, no simple solution is present in providing a universal, off-the-shelf image analysis procedure for all datasets \cite{Sezgin2004}, with multiple solutions and degrees of complexity. For image analysis of roots, we can broadly categorize this between manual and automated processes. Manual processes like SmartRoot \cite{IAEA2006, Lobet2011} and DART \cite{LeBot2010} are typically more accurate, though at the cost of an inordinate amount of time for individual images, which introduce subjectivity due to user skill. Automated methods often use threshold methods, typically based on the global histogram of the image \cite{Pierret1999}. While automated methods are relatively straightforward and simple to use, overclassifying is a persistent issue and cleaning is required for connecting and removing spurious or missed root segments \cite{Mooney2012}. Automated procedures typically require the root object to be well-defined by edges, uniform brightness, and contrast \cite{Russ1999}, which can often-times limit their usage in
segmentation to washed roots devoid of soil [Pierret et al., 2013]. However, adaptive thresholding methods based on local values (Gregory et al., 2003; RootReader 2D Clark et al., 2013) serve as potential ways to incorporate porous media analysis alongside root segmentation.

Much of this currently available software is designed with the purpose of analyzing roots ex situ, or washed roots devoid of porous media – few, if any, publicly available and free-to-use software available for in situ analysis of roots. An automated analysis methodology for roots [Menon et al., 2007], which for its segmentation procedure uses an algorithm initially developed for the segmentation of blood vessels in retinal images [Hoover et al., 1998], was later developed for use by the NEUTRA [Lehmann et al., 1999] and ICON [Kuhne et al., 2005] beamlines at the Paul Scherrer Institut in Switzerland where it has been successfully applied to root and soil imaging (e.g., Moradi et al., 2009, Moradi et al., 2012, Carminati et al., 2010, Carminati et al., 2011, Carminati et al., 2016, Ahmed et al., 2016, Ahmed et al., 2018, Zarebanadkouki et al., 2013, Zarebanadkouki et al., 2016). Other studies of roots in soil use manual methods, for example with ROIs [Warren et al., 2013] or by tracing the root in each individual image [Koebernick et al., 2017]. Continued development of automated public domain software will ease the difficulty of image analysis and lower the barrier to entry into this research field, and allow future development of more advanced image-based technical analyses.

The development of such kinds of software at the beamline can also reduce the technical expertise required associated to adapting this laboratory-scale imaging work of belowground root/soil structure work into the larger ecological community. Terrestrial biosphere models show relatively coarse representation of fine-root processes and associated parameters [Warren et al., 2015], and the lack of integrated
analyses limit meaningful linkages between belowground root form and aboveground plant function [Verheijen et al., 2016]. Recently, a global Fine Roots Ecology Database (FRED) has been established at ORNL [Iversen et al., 2017], which provides a global database of fine root (<2 mm) observations and their associated environmental conditions and parameters. Of note is the large fraction of data on root system architecture and morphology, characteristics well-suited for integration with NI data. Of the over 70,000 measurements aggregated in FRED, roughly 10,000 (15%) of measurements include root system architecture and morphology, the most numerous of which is root diameter – this alone consists of roughly 3000 (4%) of all measurements and is furthermore associated with nearly every trait characterized in FRED [Iversen et al., 2017]. NI can add function to form, and thereby reveal dynamics of root water extraction and its association with specific root traits [Dhiman et al., 2018, Ahmed et al., 2018]. Thus providing a more streamlined analysis procedure of root diameter and its associated environmental parameters at the beamline that not just image scientists can process and use would help integrate the data obtained into the larger plant/soil science community.

In response to this need, we have developed RootProcessing, an open source Python package in order to streamline the image processing required for current and future users interested in quantitative analysis of plant/soil systems, both at the beamline and for general use. It provides a series of automated procedures to pre-process raw outputted images at the beamline, whose roots can then be segmented, and used for several analyses of interest to the user, including measurements of root cross-sectional thickness, diameter, and plant/soil water content. Each step is independent, and any person of interest is free to use any component of the package for use on beamline images, or their own root images acquired elsewhere. In this paper, we focus on outlining the software package, and two example analyses of
the segmentation procedure: one is a comparison of software segmentation abilities with RootReader 2D [Clark et al., 2013], a similar automated free-to-use plant root segmentation software; and the second is a comparison of root diameter distribution values with those from flatbed scanned images. Further utilization of the primary measurements and integrated analysis to investigate rhizosphere characteristics is outlined in an Chapter 3.

2.2 Materials and Methods

2.2.1 Sample Images and Collection

The sample roots are month-old poplar (*Populus deltoides*, *Populus trichocarpa*) saplings, numbering five in total, which were grown from cuttings using a hydroponic system with low concentrations of balanced fertilizer – the single P. trichocarpa sample [Dhiman et al., 2018] was grown from seed in sand. After 1 month, they were transplanted into rectangular aluminum containers (20 cm × 18 cm × 1.2 cm). Transplanted roots were carefully rinsed in water, displayed in between moistened layers of pure silica quartz sand (Flint 13, U.S. Silica Company, Berkeley Springs, WV), and then inundated with water amended with balanced N:P:K and micronutrients to fully settle roots and soil. For this exercise, individual roots were displayed to ensure that they did not obstruct each other during neutron beam exposure. For the example plant, there were a high number of roots developed under hydroponics prior to transplanting – in this case, excessive roots were positioned along the top of the chamber to allow more detailed analysis of individual roots for other studies in Chapter 3. Prior to transplanting, sample roots were scanned in a flatbed light scanner to obtain root morphology characteristics un-occluded by porous media.

Neutron radiography images were collected at the CG1D beamline at HFIR, in
ORNL. Neutron attenuation by the plant-soil system was detected with a 25 \( \mu \text{m} \) LiF/ZnS scintillator linked to a charge-coupled detector (CCD) camera system (iKon – L 936 Andor Technology, Belfast, UK). The cold neutron wavelength \( \lambda \) ranged from 0.8 to 6 Å, with a peak neutron intensity of \( 2.2 \times 10^6 \) at 2.6 Å. The field of view (FOV) for this experiment was \( 7.4 \times 7.4 \) cm, with pixel resolution of 32 \( \mu \text{m} \). Aluminum and sand (SiO\(_2\)) have comparatively low attenuation coefficients to cold neutrons compared to water, and as such the plant-soil system enabled accurate imaging and transmission of the neutron flux. As the FOV of the beam was larger than the individual chambers, multiple radiographs were taken of a single chamber by automatically moving the plates up, down, left and right using a motorized lift table. Exposure time for each image was 120-180 s, with consistent beamtime exposures for each experimental sample.

### 2.2.2 Library Features and Environment

RootProcessing is a publicly available Python package written to streamline the image processing pipeline at the CG-1D beamline [Crow et al., 2011, Santodonato et al., 2015]. It is easy to install and run on any machine (Windows, Mac, Linux) designed to introduce, integrate, and expand on the basic image analysis capabilities available and of interest to the plant/soil science community. The package is user-friendly and simple to use, written and documented with a user not highly proficient in image analysis in mind. Each step can be run independently or in any order, so long as necessary input files are available and specified. Furthermore, this allows easy integration with other code and products – non-segmented images not collected at the beamline can use the segmentation code, and segmented images made from other code can use our root/soil analyses, for example. Running each step displays the percentage of analysis completed, and the elapsed time for each step. Users can generate a reference dataset, which generates six images that share features characteristics of
Figure 2.1: Visual schematic of the analyses available in the RootProcessing package. A sample poplar image is used as an example. Arrows indicate the necessary prerequisite inputs for a given analysis (e.g., the segmentation analysis is necessary prior to running the thickness analysis, distance mapping and water content are both necessary for the integrated root-soil analysis, etc.).

those outputted at the beamline (e.g., similar quality/noise, naming format, etc.), which can be used for familiarizing oneself with the library. All descriptions of the library features are done using this reference data. The library also uses a master user-configuration (“user-config”) file, in text format, where all necessary changes to input/output file name and parameters are made. The package inputs all tiff image files (8, 16, and 32-bit), and outputs tiff files (32-bit) for all image-based analyses, with the integrated analysis output in text format.

### 2.2.3 Program Overview

The RootProcessing workflow can be broadly classified into four categories, comprised of eight procedural and executable steps. The package first allows for image pre-processing, consisting of (1) image correction and stitching, (2) and cropping. Following (3) segmentation of the root, the user is able to make primary measure-
Figure 2.2: Visual schematic of the alignment process, with the four degrees of freedom outlined, using the reference dataset created by the package.

Figure 2.1 shows a general schematic of all analyses available in the package using one of the sample root images. Below, using the reference dataset as a simplification for explanation, we explain the eight analyses under these four broad sections:

Part I: Image Pre-Processing (Image Stitching, Cropping)

Image files outputted from the beamline represent neutron counts made at the sensor during neutron white beam exposure. We first normalize these images using an image
of the open beam, which is an image of neutron counts without any object between the beamline and sensor; and of the dark field which accounts for the electronic noise of the detector. We then apply the following formula for normalization:

$$I_{\text{norm}} = \frac{I_{\text{raw}} - DF}{OB - DF}$$  \hspace{1cm} (2.1)$$

where $I_{\text{norm}}$ is the normalized image, $I_{\text{raw}}$ is the beamline-outputted image, $OB$ is the open beam image, and $DF$ is the dark field image. Once the correction is applied, individual images are concatenated to create a single composite image. Given the nature of the automated lift table used at the beamline, four primary degrees of freedom need to be considered:

1. horizontal offset in x-axis (i.e., overlap between two images side by side)
2. vertical offset in x-axis (i.e., table “drift” as it shifts the object side by side)
3. horizontal offset in y-axis (i.e., overlap between two images on top and bottom)
4. vertical offset in y-axis (i.e., table “drift” as it shifts the object up and down)

Numbers 1 and 3 are specified in the automation process available at the beamline, and correspond with the desired overlap between each individual image – 2 and 4 essentially represent the systematic shift of the x- and y-axis separate from the individual image, and are included to increase accuracy of the stitching process. A visual schematic of the offsets is shown in Figure 2.2. Once complete, we include a simple cropping procedure to reduce the image to the desired dimensions.

**Part II: Image Segmentation (Image Masking, Filtering)**

Once the image has been corrected, we apply a simple combined global-local thresholding technique to segment roots from the soil. A visual schematic of this procedure
Figure 2.3: Visual schematic of the root segmentation analysis, highlighting three different cases of classification.
is shown in Figure 2.3. The package takes as input a window size, a local threshold \( \alpha \), and a global threshold value \( \alpha_{\text{global}} \). For every pixel \( T_i \), a local neighborhood of pixels specified by window size is evaluated – if the mean value \( \mu_i \) is higher than the evaluated pixel by the local threshold, it is classified as a root pixel. An optional global threshold is then applied – if the pixel is lower than the global threshold, it will be considered a root pixel, even if the local criteria is not met. This is used to classify certain regions of the root that are larger than the window size, which would otherwise be classified as soil (e.g., soil subsection 3, Figure 2.3). Following the segmentation, a filtering process is applied: the first is an area-based filter, where all mask-labeled objects with a connected pixel count larger than the specified value are removed. The second is a median filter.

Part III: Primary Measurements (Water Content, Root Thickness, Root Diameter)

Water content analysis is based on empirical calibration measurements made at the CG-1D beamline [Kang et al., 2013, Kang et al., 2014]. Using sand and taking into account beam hardening and scattering effects due to hydrogen, the following empirical equation was calculated:

\[
\tau_w = - \frac{\Sigma_w}{2\beta} - \sqrt{\left( \frac{\Sigma_w}{2\beta} \right)^2 - \frac{1}{\beta} \ln \left( \frac{I_{\text{wet}}}{I_{\text{dry}}} \right)}
\]  

(2.2)

where \( \Sigma_w = 5.5542 \text{ cm}^{-1} \) is the attenuation coefficient of water; \( \tau_w \) is the thickness of water in cm; \( \beta = -2.140 \text{ cm}^{-2} \) is the empirical correction factor for beam hardening and scattering effects, and \( I_{\text{wet}} \) and \( I_{\text{dry}} \) are the wet and dry neutron transmission images, respectively [Kang et al., 2013]. By relating the dry image directly from the wet image, this enables analysis of only the change in water content in each individual pixel. As obtaining a completely dry image is practically unfeasible due to
residual water content, and experimental constraints, we do not normalize with the
dry sand image – therefore, we need to correct for the attenuation of aluminum (for
the container) and silica, and thus modify the equation as follows:

$$\frac{\tau_w}{\tau_{Si}} = \frac{\theta_v}{\tau_{Si}} \left( -\frac{\Sigma_w}{2\beta} - \sqrt{\left(\frac{\Sigma_w}{2\beta}\right)^2 - \frac{1}{\beta} \left[ \ln(I_{wet}) + \Sigma_{Al}\tau_{Al} + \Sigma_{Si}\tau_{Si} \right]} \right)$$

(2.3)

where $\Sigma_{Al} = 0.0215 \text{ cm}^{-1}$, and $\Sigma_{Si} = 0.006604 \text{ cm}^{-1}$ are the attenuation coefficients
of aluminum and silica, respectively; $\tau_{Al}$ and $\tau_{Si}$ are the thicknesses of aluminum and
silica, respectively; and $\theta_v$ is the volumetric water content. This equation enables
any given transmission image as an input. The validity of this assumption will de-
pend on the fraction of the porous media that is composed of hydrogen-containing
sources other than water, like organic matter of clay content. We do not need to
include a geometry correction due to the fact that our samples have a constant thick-
ness, wherein our samples are flat plates as opposed to cylindrical samples, where
a geometric correction to account for variability in sand and aluminum thickness is
necessary. The root thickness analysis produces an image of the half-thickness of
the root at a given pixel in the z-axis. Our code uses the same principle outlined in
[Menon et al., 2007] for calculating the volume of a 2D root image by assuming a
series of cylindrical segments – we extend this same principle to every root pixel to
calculate cross-sectional thickness. First, a medial axis skeleton is calculated for the
root. A distance transform of the root is then calculated – the distance value on the
medial axis pixel is labeled the “root radius” value $R$. From here, for every root pixel
$[x, y]_p$, the closest root contour pixel $[x, y]_e$ that doesn’t intersect any other medial
axis pixels is found. We then calculate the edge-pixel distance $L_{EP}$. We then extrap-
olate the line formed between the two points, and extend it towards the medial axis,
identifying the closest medial pixel $[x, y]_m$ whose path consists entirely of the root
(i.e., no medial axes that are located on other root segments). From here, we use the
Figure 2.4: Visual schematic of the root cross-sectional thickness analysis. 

- $[x, y]_p$ is the evaluated pixel in question,
- $[x, y]_e$ is the closest contour pixel, and
- $[x, y]_m$ is the medial axis pixel that intersects the line (indicated by the blue and green arrows) between the contour and evaluated pixel.

$L_{MP}$ and $L_{EP}$ are the distances between the medial axis and evaluated pixel, and the evaluated pixel and contour, respectively.

$H$ is the half-dome thickness, and $R$ is the root radius at the medial axis of the root.

The inset shows the assumed cylindrical distribution of root thickness, centered at the medial axis.
$R$ value assigned to $[x, y]_m$ found earlier. Then, assuming a cylindrical distribution, we can calculate the half-dome height $H$ of the pixel as follows:

$$H^2 = R^2 - (R - L_{EP})^2$$

(2.4)

We show a visual schematic of this analysis in Figure 2.4. The root diameter analysis produces a root diameter of the segmented image. Using the same procedure used to calculate root radius in the root thickness analysis, all medial axis pixels are used to create a distribution of root diameter. The user has the option to determine bin count of their histogram classifications.

**Part IV: Integrated Root/Soil Analysis (Distance Mapping, Water Content/Root Diameter Integration)**

In our integrated analysis of root and soil parameters, we first create a distance map of the plant-soil system – for every soil pixel, an associated root edge pixel is calculated using a Euclidean distance transform, from which a medial axis skeleton is identified. This creates “zones of influence” that are associated with a given root diameter. Figure 2.5 shows a visual schematic of this process – zones of influence with an equivalent root diameter value are labeled with the same number, so Zone 4 on either side of the root for example corresponds to the same root diameter. The integration process combines the water content and root diameter analysis using the distance mapping – the diameter-water content analysis calculates water content values by distance from the root edge for each classified zone of influence, as sorted by root diameter. An application of this spatial analysis to investigate properties of the rhizosphere is elaborated in Chapter 3.
Figure 2.5: Visual schematic of the distance mapping procedure, where the black arrow indicates the closest contour pixel to the evaluated pixel, and the blue arrow indicates the closest medial axis pixel from the contour. Regions labeled the same number indicate equivalent root diameter “zones of influence” (i.e., soil regions associated with a given root diameter).
<table>
<thead>
<tr>
<th>Software Package</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RootReader 2D</td>
<td>Fixed Thresholding</td>
<td>90, 100, 120</td>
</tr>
<tr>
<td></td>
<td>Half-Kernel Size</td>
<td>25, 50, 75</td>
</tr>
<tr>
<td></td>
<td>Min. Threshold</td>
<td>0, 100</td>
</tr>
<tr>
<td></td>
<td>Max. Threshold</td>
<td>50, 100, 175, 255</td>
</tr>
<tr>
<td></td>
<td>Percentage Above Thresh.</td>
<td>0.1, 0.5, 5</td>
</tr>
<tr>
<td></td>
<td>Dust Removal Filter</td>
<td>800</td>
</tr>
<tr>
<td>RootProcessing</td>
<td>Window Size</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>Global Threshold</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Percentage Above Thresh.</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Area Filter</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Median Filter Window</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.1: Full list of parameters used for the segmentation comparison. Percentage above threshold was only tested for “half-kernel size = 50”, “minimum threshold = 0”, and “maximum threshold = 100”.

2.2.4 Segmentation Analysis

Given the role of the segmentation in determining the accuracy and precision of the primary measurements made on the RSA (e.g., root diameter, topology, water content, etc.), we focus on this product in our package. In situ analysis comparisons are limited: roottracker 2D, a popular algorithm developed from [Menon et al., 2007], was not publicly available at the time of this publication, and most other techniques are manual. Thus, we compare one of the *P. deltoides* saplings with RootReader 2D [Clark et al., 2013]. RootReader 2D is a similar automated root analysis software, which uses threshold-based segmentation for classifying roots from images. We test the “Fixed Thresholding” and “Adaptive Min/Max Thresholding” options, under a wide range of available parameters (see Table 2.1 for the full permutation). Note that the “half-kernel size” parameter in RootReader 2D corresponds to half the value of the “window size” parameter used in our masking algorithm. As tiff images cannot be read into RootReader 2D, we converted the images to a JPEG format (8-bit) before analysis. In addition, we compare root diameter distribution extracted from
Figure 2.6: Comparison of the original corrected neutron image (column 1, row 1) with the segmentation analysis in RootProcessing (column 2, row 1) and in RootReader 2D (all columns, rows 2-4). For the RootReader 2D segmentation images: from column 1 to 5, maximum threshold values are 50, 100, 175, 255, and 255, minimum threshold values are 0, 0, 0, 0, 100. From row 1 to 3 (columns 1-5), half-kernel sizes are 25, 50, and 75. For percentage above threshold (column 6), values correspond to 0.1, 0.5, and 5% (rows 2-4) - min/max threshold are 0 and 100, respectively, and half-kernel size is 50. For fixed threshold (column 7), values are 90, 100, and 120 (rows 2-4). All images use an area filter of 800.

our masking data with that extracted from flatbed light scanner images obtained for the five sample poplar images. In order to determine root diameter for the flatbed scanned images, we first use a fixed threshold value to obtain a mask, after which a medial axis is calculated and imposed on a distance transform of the area within the root – the medial axis positions were determined to be root radius and was used to calculate root diameter distribution. For the neutron images, we used the root diameter analysis in our package, which uses the same analysis procedure, as outlined above.
Figure 2.7: Cumulative distribution of all topologically distinct objects in the segmented neutron root image (solid line) and segmented flatbed scan root image (dashed line), as sorted by ascending object size fraction. Inset shows segmented neutron root image, with the largest topologically distinct object highlighted in white.
2.3 Results & Discussion

2.3.1 Segmentation Comparison – RootReader 2D

Segmentation results of RootProcessing and RootReader 2D are shown in Figure 2.6. Note that as RootReader 2D inputs 8-bit JPEG images, the following pixel values range from 0 to 255. Arrows indicate ascending values of the labeled parameter (see Table 2.1 for exact values). Evaluating RootReader 2D, we see that increasing half-kernel size allows for more root segmentation, though the differences between the 25 and 50 are more pronounced than between the 50 and 75 value. Evaluating the segmentation product with no min/max criteria (i.e., min. threshold of 0, max. threshold of 255) in column 4 (panels 10-12), we can see that the software fails to capture the majority of roots – only a small number of roots in the upper, drier half of the image are classified, with virtually no roots in the lower, wetter section, and in the thick stem section as well. Minimum threshold, which corresponds to the value below which all pixels are automatically classified as soil, has a minimum effect, as increasing it would simply remove the few root pixels classified. Maximum threshold, which corresponds to the value above which all pixels are automatically classified as root, has a larger effect. As we progressively decrease maximum threshold, we see that the stem (column 3), and the lower roots (columns 1, 2) are segmented. At the same time, however, larger portions of the sand become classified as roots, due to overlap in the pixel value distribution of the wettest sandy regions and the driest root sections. In effect, given the minimal effect of the local thresholding criteria used, decreasing the maximum threshold serves a similar function as the fixed threshold procedure, which runs into the same issues of distribution overlap as mentioned. Percentage above threshold shows a similar trend as well (column 6). Ultimately, the software provides a trade-off: either a significant loss of root classification, or a significant amount of noise due to wet soil regions. Evaluating RootProcessing, we see that our product
Figure 2.8: Root diameter distributions of the root image as segmented by RootProcessing (bar graph), and from the flatbed scanner (dash line). (A, C-E) comprise the Populus deltoides samples, while (B) comprises the P. trichocarpa sample. $\mu_{\text{mask}}$ represents mean root diameter of that of the segmentation obtained from the RootProcessing masking algorithm, and $\mu_{\text{scan}}$ represents mean root diameter of that of the segmentation obtained from the flatbed scanned image.

shows no issue with the characteristic sandy soil patch artifact present in most of the RootReader segmented products (e.g., Figure 2.6C panels 1-6, 16-19). In addition, our code shows the largest degree of root classification, at rates comparable or better to some of the best results of RootReader 2D, but without any of the accompanying background noise. As the examples in the RootReader 2D documentation show, the software is primarily designed for root system architectures scanned via visual light, such as flatbed scanners – the adaptive threshold algorithm developed here is to account for gradations in the background, which are considerably more even than
the heterogenous signals outputted by partially unsaturated porous media, and the code ultimately primarily relies on fixed thresholding procedures. Roots in soil have a smaller and more complex margin for differentiation, and as such, it is a natural challenge for automated segmentation procedures, though some of these issues can be resolved in part due to dedicated adaptive thresholding techniques. With regards to roots in soil within neutron images, a few characteristic assumptions can be made that can aid in this segmentation. The first is that, while roots may not have a uniform water content throughout the length of its architecture, they tend to be relatively wetter than the immediate soil surrounding it. As such, consistently applying an adaptive threshold using the local neighborhood of every pixel can greatly aid in improving root segmentation. Considering that roots will often be absolutely wetter than the surrounding soil, including a fixed threshold criteria can aid in classification as well — though, this is likely to be more effective in dried plant/soil images, since as the soil gets wetter, its water content value distribution will progressively overlap with that of the root signal value, as we can see in the wetter regions of the image (i.e., the sandy soil patches highlighted earlier). Given the nature of automated segmented processing, discontinuous elements are present in our root segmentations. Visual comparison with the segmentation results seen in [Menon et al., 2007] show similar discontinuous segmentation behavior as well. Figure 2.7 shows a cumulative distribution of topologically individual objects present in the neutron segmented image and the flatbed scan segmented image. A total of 343 objects were identified in the neutron segmentation, while 2105 were identified in the light segmentation. However, 99.3% of pixel area was represented by one object in the light segmentation, as opposed to 24% for the primary object in the neutron segmentation (image inset highlight, Figure 2.7). Lower connectivity per se does not have significant consequences for water content/root diameter analysis — as we do know that the root should be topologically fully connected, this would be problematic to the extent that the user
Figure 2.9: Cross-sectional thickness of water and root. Image inset shows the location of where the profile was taken, in red.
would know that at least some sections of the image classified as soil should be root, which may elevate analyzed soil water content values. In this regard, as considerably more of the root is classified in our software, this error is much less than in other software. However, with regards to topological analyses like branching order, etc., more or less continuous networks are necessary for accurate automated segmentation, which our code does not produce. In this aspect, manual analyses of drawing the root medial axes by hand, like those in DART [Le Bot et al., 2010] are better suited.

2.3.2 Segmentation Comparison – Root Diameter Distribution

Root diameter distributions of the five poplar species, as analyzed by the segmented image and by the flatbed scanned image, are shown in Figure 2.8. With the exception of the P. trichocarpa sample (Figure 2.8B), all root diameter distributions calculated in the segmented image show lower mean values than those from the flatbed scanner, with a higher representation of fine root diameters, primarily in the range 0.7-0.15 cm, where the frequency is almost double in the flatbed scanned images. One potential reason for this discrepancy is due to the different criteria by which root diameter is being calculated for each image. The cross-sectional thickness of water (as calculated by the water content analysis) and of the root (as calculated by the thickness analysis) for stem section of the root is shown in Figure 2.9. Water fraction of root or stem tissue can vary considerably throughout its profile: certain sections, like the root edge show that a majority of the root is composed of water, whereas other sections, particularly in the center of the root, show that water composition is closer to 60-70% of the total root. This is largely a consequence of the relative percentages of tissue that the beam passes through. For the root edge, this tissue is largely water-filled xylem. For the root center, this also included the pith, which can contain air. Combined with the fact that root composition can never truly be 100% water, there will be
Figure 2.10: Comparison of the upper half of the sample root image. (A) Corrected neutron transmission image. (B) Segmented neutron root image. (C) Flatbed root scan. (D) Segmented flatbed scan root image.
some degree of underestimation when analyzing root diameter by its water content. A second potential reason of the underestimation may be representative of artifacts in the flatbed scanning process. In Figure 2.10, we show 4 images of the top half of the sample root image used in our earlier package analysis. In the segmentation (Figure ??B), we can see that while certain sections of the NI root are omitted, a majority of roots are represented. In the flatbed scanner (Figure 2.10C), the root is very clearly defined, and all individual roots are all accurately characterized. However, due to the density of the roots, many of the clustered roots show significant overlap, and once segmented (Figure 2.10D) are classified as singular large roots, which can magnify the root diameter significantly. The P. trichocarpa image sample shows the least degree of overlap, with almost no major root clusters like that seen in Figure 2.10 (note the differences between mask and scan at larger root diameters for Figure 2.8B versus Figure 2.8A, C-E). Root overlap and tangling remains one of the major challenges in root diameter/length analysis in flatbed scanned images [Pierret et al., 2013], and is heavily reliant individual user ability to ensure no root overlap [Zobel, 2003], a fact that is sometimes not possible given certain root morphologies. This may also reflect why we see a relatively large range in mean root diameter in the flatbed scanned images, despite all sample images being of the same age. Visually, we can confirm that minimal overlap is present in the segmented images, which may suggest that the root diameter distributions seen in the masked images are more representative.

2.4 Conclusion

In this chapter, we presented the main features of RootProcessing, a python package that is designed to streamline the image processing framework at the CG-1D beamline, correcting and normalizing the raw output images for primary measurement analyses of water content and root morphology. Users can then use this outputted
data to conduct integrated spatial analyses of root diameter and water content. Our package is publicly available and designed for scientists with relatively little experience in image processing. Each component can be run separately should users be interested in only certain analyses, and as it is open-source based, it is fully modifiable for use by those inclined to do so. Our segmentation procedure shows considerable improvements over similar automated software on classifying roots in situ, namely in the elimination of mis-classification of wet soil patches, which can vary considerably by image and are of a similar value to the roots. Overall, RootProcessing provides an integral framework for those interested in using the output data at the neutron imaging beamline, and also general tools for those interested in conducting primary analyses of scanned images of roots in porous media without the cost of commercial software.
Chapter 3

Integrating Fine Root Morphology and Soil Distance Mapping to Characterize the Plant-Soil Interface

3.1 Background

The fundamental aspects of plant water uptake, namely morphological, physiological and functional responses to soil water availability, remain poorly understood. A key component of this plant-soil dynamic is the region of soil immediately adjacent to the root, known as the rhizosphere [Hiltner, 1904, Hartmann et al., 2008], formed via root penetration through the soil, root water and exudate deposition, and subsequent growth of microbial and bacterial communities [Gregory, 2006, Hinsinger et al., 2009]. Its sphere of influence is defined in context of the specific processes involved and their spatial scale: free-living microbial activity is limited to the sub-mm scale, influence on movement of mobile elements and water can be much greater, cm or above [Darrah, 1993], and linkages to mycorrhizae can lead to meter-scale influences.
Despite the relatively limited scale of root influence on the soil, the rhizosphere has biological, chemical, and physical properties that are often much different from that of the bulk soil, but the difficulty in measurement has limited our understanding of the system. One particular aspect is its spatially- and temporally-dependent behavior in response to excess/deficit soil water conditions, defined as rhizosphere plasticity [Carminati et al., 2011, Carminati and Vetterlein, 2013]: young roots are typically dominated by hydrated mucilage, which increases its water retention capacity [Moradi et al., 2012, Ahmed et al., 2016b]. Eventually decaying and becoming hydrophobic, this mucilage creates shrinkage-induced void spaces as the soil dries [Carminati et al., 2009], and reduces root-soil connectivity and water content, and ability for rapid root uptake following wetting [Carminati et al., 2009, Zarebanadkouki et al., 2018]. The increased aggregation from mucilage activity is also highlighted in the result of increased porosity and pore sizes in the rhizosphere [Whalley et al., 2005, Feeney et al., 2006, Hallett et al., 2003], despite known effects of soil compaction near the root [Dexter, 1987, Aravena et al., 2014, Koebernick et al., 2017]. Chemical quality of exudates or roots, and root turnover size has also been linked to macroaggregated development [Poirier et al., 2018]. Root exudates and mucilage, soil aggregation, and even roots themselves [Dhiman et al., 2018] have impacts on soil hydraulic properties that are likely as dynamic as the root system itself. As a consequence, experimental results of rhizosphere water retention can show seemingly contradictory results, with water content and uptake in the rhizosphere shown to be either higher [MacFall et al., 1990, Segal et al., 2008] or lower [Nakanishi et al., 2005, Tumlinson et al., 2008, Moradi et al., 2011] than the bulk soil with different species and under different experimental conditions.

Furthermore, exudate characteristics can also vary by plant species, manifesting themselves not only in spatial heterogeneity in the radial dimension away from the
root, but also in the lateral dimension along the root. For example, in leguminous species like lentils, exudate deposition is uniform along the root, while maize exudate deposition is concentrated at the root tip [Razavi et al., 2016]. In maize species, water uptake switches from laterals of seminal roots to crown roots as the roots mature and develop in size [Ahmed et al., 2018]. In lupin species, water uptake has been shown to be higher in the upper half of the root architecture, and progressively higher along individual roots from the distal to the proximal sections [Zarebanadkouki et al., 2013, Zarebanadkouki et al., 2014]. Water uptake dynamics vary by root order or size [Rewald et al., 2010, Dhiman et al., 2018], supporting the need to define fine-roots by their functional type, i.e., absorptive versus transportive [McCormack et al., 2015]. Averaging methods commonly seen in studies of the rhizosphere can often lose important details on local water dynamics, distribution, and uptake caused by spatial differences along the lateral dimension.

These properties reflect the intertwined relationships between soil-to-plant inflows like water uptake, and plant-to-soil outflows like mucilage and exudate deposition, and the importance of evaluating them in tandem. Currently, much of the work in the literature has focused on biological and plant characteristics, with less focus on the physical soil properties [Gregory and Hinsinger, 1999, Gregory, 2006], though novel conceptualizations of the rhizosphere from the soil perspective, whereby distance transforms are used to characterize dynamic root system architecture [Koebernick et al., 2014, Schluter et al., 2018]. An integrated analysis of both soil characteristics and root system architecture (RSA) is needed to provide a more comprehensive understanding of rhizosphere characteristics.

Image-based methods can capture such details which can illustrate influences of root order, age, prior plant stress, or morphology, despite similar bulk behavior
Many such methods exist for analyzing in-situ processes at the plant-soil interface, including light transmission [Garrigues et al., 2006], X-ray [Koebernick et al., 2017, Daly et al., 2018], NMR [Pohlmeier et al., 2013], and neutron imaging (e.g., Oswald et al., 2008, Carminati et al., 2010, Warren et al., 2013, Zarebanadkouki et al., 2013, Zarebanadkouki et al., 2016, Dhiman et al., 2018). However, neutron radiography in particular serves as an ideal method for analyzing local spatially- and temporally-dependent plant-soil processes in the rhizosphere due to its high resolution (tens of microns), and image acquisition time on the order of seconds. Combined with high neutron sensitivity to hydrogen, and thus water, neutron radiography allows for the analysis, in tandem, of the interplay between root morphology and soil water distribution and dynamics.

Our primary objective in this study is to integrate disparate components of the plant-soil system by simultaneously quantifying in situ fine root (e.g., root morphology) and soil/water characteristics (e.g., soil-root distance, water content, uptake) in the rhizosphere. Given results in prior studies of links between rhizosphere soil water dynamics and root age/morphology, we hypothesize that general trends may be seen across multiple species when evaluating them in tandem by order of spatial proximity. While recent studies have used distance distributions from soil voxels to the nearest root surface to characterize RSA [Schluter et al., 2018], this is the first study to our knowledge to utilize these spatial relationships between each soil pixel and root surface unit, and their coupled attributes to further characterize the rhizosphere. Given the bi-directional nature of the plant-soil interface, it is intuitive to conceptualize the rhizosphere in this manner. To that end, we extended and further developed the methodologies used in [Dhiman et al., 2018] and used digital image processing to analyze neutron radiographs of transplanted plant seedlings undergoing wetting-drying cycles. Extrapolating surface area from 2D images by
assuming cylindrical-shaped roots, we then use static distance transforms to evaluate spatially-dependent relationships between root diameter/surface area and water content distribution with distance from the root surface, and water extraction (as water volume loss) by root morphology class. Our findings highlight the relationship between the rhizosphere and specific root morphology, and its consequences on plant water uptake processes.

3.2 Materials and Methods

3.2.1 Plant Material and Treatments

Poplar (*Populus deltoides*, *Populus trichocarpa*), maize (*Zea mays*), juniper or Eastern red cedar (*Juniperus virginiana*), grape (*Vitis rotundifolia*), and sugar maple (*Acer saccharum*) species at various stages of morphological development were grown from seed or transplanted into rectangular aluminum containers (20 × 18 × 1.2 cm) and propagated for later neutron imaging experiments.

The *P. trichocarpa* sample [Dhiman et al., 2018] was grown from seed in sand, and then transplanted into the aluminum chambers after 5 weeks, then grown for another 4 weeks. *P. deltoides* samples were propagated from cuttings using a hydroponic system with low concentrations of balanced fertilizer. After one month, they were transplanted into the aluminum containers. For the *Z. mays* samples, seeds were soaked in water for 2 days, germinated in a petri dish on moist paper towels for one week, then transplanted into the containers with the kernel at or just below the soil surface, with new root initials pointed down. These were grown in the aluminum containers for 1-2 weeks before imaging. One-year-old seedlings of *Vitis*. *spp.*, *J. virginiana*, and *A. saccharum* samples were collected at or nearby the Oak Ridge Reservation, then transplanted into the aluminum chambers, and imaged 1-2
weeks later.

Transplanted plant roots were carefully rinsed in water, displayed in between moistened layers of pure silica quartz sand (Flint #13, U.S. Silica Company, Berkeley Springs, WV), and then inundated with water amended with balanced N:P:K and micronutrients to fully settle roots and soil. Individual roots were placed to ensure that they did not obstruct each other during scanning. Once packed, the top and bottom of the containers were sealed with aluminum tape to prevent evaporation. An example of the aluminum chamber set up is shown in Figure 3.1A. During their time in the containers, plants were watered periodically based on mass-based water extraction, every 1-3 days; daily evapotranspiration varied with plant size and density, ranging from 1-10+ ml per day. The plants adapted to the chambers well, with variable axial fine root growth ranging from 0.15 to 0.65 cm per day [Warren et al., 2013]. The age of the rhizosphere around individual roots ranged from <1 day for the newest, finest root tips, and up to 1 month for the larger, older roots. There were 15 unique plant-soil systems analyzed.

### 3.2.2 Neutron Radiography Conditions and Experiment

Neutron radiography experiments were conducted at the CG1D beamline at the High Flux Isotope Reactor (HFIR) in Oak Ridge National Laboratory. Neutron attenuation by the plant-soil system was detected with a 25 μm LiF/ZnS scintillator linked to a charge-coupled detector (CCD) camera system (iKon – L 936 Andor Technology, Belfast, UK). The cold neutron wavelength λ ranged from 0.8 to 6 Å, with a peak neutron intensity of $2.2 \times 10^6$ at 2.6 Å. The field of view (FOV) for this experiment was $7.4 \times 7.4$ cm, with pixel resolution of 32 μm. Aluminum and sand (SiO2) have comparatively low attenuation coefficients to cold neutrons compared to water, and as such the plant-soil system enabled accurate imaging and transmission of the neutron
Figure 3.1: Experimental set up. (a) sealed seedling at the beamline during experiments. (b) neutron attenuation radiograph of a water content map of a poplar seedling root system.
flux. As the FOV of the beam was larger than the individual chambers, multiple radiographs were taken of a single chamber by automatically moving the plates up, down, left and right using a motorized lift table. Exposure time for each image was 120-180 s, with consistent beamtime exposures for each experimental sample. Of the experiments conducted, 6 were dynamic, and 9 were static.

### 3.2.3 Image Reconstruction and Segmentation

Neutron radiographs were normalized with respects to open beam and dark field, effectively converting them into transmission images. Open beam radiographs are images with only the neutron beam, which may not be evenly distributed in a single radiograph. Dark field images are images with the beam turned off, thereby consisting of only background and sensor noise. The 16 radiographs that consist of the chamber were stitched together using open-access code available at the beamline. This consists of identifying identical sections of the individual images and overlaying them into a single composite image. As each image has approximately 5% overlap with all adjacent image, no data is lost during the stitching stage.

We segmented all visible plant roots in the neutron image using mean-based local thresholding and morphological cleaning methods used in [Dhiman et al., 2018](#). Roots were first identified and segmented in the composite image with the driest soil to ensure the largest degree of contrast between the dry soil and wet root. Using digitally scanned flatbed images of the roots as a reference, the masking algorithm used here underestimates the very smallest fine roots (\(\sim 0.01\) cm and below) [Dhiman et al., 2018](#). These results were expected as the finest root sizes were approaching neutron image resolution. Root and soils were difficult to distinguish in some water-saturated sections of the images at the bottom of the chambers and were not used in this study.
3.2.4 Water Content Determination

We followed the methodology outlined and verified by [Kang et al., 2013] to volumetric water content. The water thickness of the plant-soil system is calculated using 2.3. Figure 3.1B shows a neutron radiography converted to a map of water content.

3.2.5 Integrated Plant-Soil Mapping

We created a three-tiered, spatially-integrated map of plant root and soil pixels in order to characterize the bulk soil, various sections of the rhizosphere, and the root, in tandem. We identified three regions in the plant-soil system: (1) the root “skeleton”, from which root morphological traits are derived, (2) the root surface, and (3) the soil region surrounding the root. Figure 3.2 shows a visual schematic of each of the three mapping systems, with the colored regions corresponding to the analyzed root diameter locations for each. We explain each step below. Using the root mask as a base (highlighted in green in Figure 3.2A), we first calculated local root system architecture (RSA) traits, via root morphological characteristics (diameter, surface area, root order). A medial axis transform (MAT) was applied to the root mask, producing an individual pixel-based topological skeleton of the root (Figure 3.2A). We used this MAT to calculate root diameter – the minimum distance of each pixel to the outer edge of the root was multiplied by a factor of 2. Differential root surface area per root diameter class was calculated by multiplying the diameter by \( \pi \) and summing the number of skeleton pixels corresponding to a given root diameter. A key assumption made on the root morphology here is that all roots are cylindrical, and each side of the root is uniformly distributed from the medial axis. Root order analysis was conducted by manually delineating multiple regions of interest (ROIs). 226 ROIs across 5 of the 6 dynamic samples were analyzed.

As an intermediate step, we then created a root surface map, defined as the 8-
Figure 3.2: Visual schematic of the plant-soil mapping procedure, and how represented root diameter classes are calculated for each transform. (a) the medial axis transform (MAT), with the root highlighted in green for reference. (b) the root surface (RS) transform. (c) the soil region (SR) transform. Histograms of the represented root diameter values are shown for the (d) MAT, (b) RS, and (c) SR mapping.

connected perimeter map of the root, where the calculated root diameter value in the MAT is assigned to each pixel in the root surface map based on minimum distance (Figure 3.2B). Finally, we then created a soil region map, where the root diameter value assigned in the root surface map are assigned to each soil pixel, again based on minimum distance (Figure 3.2C). Conceptually, this is similar to the 3D distance transforms first applied by [Koebernick et al., 2014] and modeled by [Schluter et al., 2018], where soil voxels were connected to dynamic RSA characteristics based on histograms of root-soil distance. However, in our analysis, we constraint this to a static RSA, and we calculate both distance to and the root morphological property
Figure 3.3: (a) outline of the three soil subsections in the plant-soil interface. (b) outline of the location of the three soil subsections in the context of a typical water content profile in the plant-soil interface.

of the root surface pixel, for any given soil pixel. This allows us to evaluate each dynamic aspect of the soil in the context of plant root characteristics, and vice versa. Note that we only use the root diameter values determined in the MAT, and furthermore, due to RSA characteristics (e.g., root position, branching degree), the distribution of represented root diameters can change between each mapping system (Figure 3.2D-F). Evaluating the soil physical characteristic values (e.g., water content, water volume change) at each pixel, we can then evaluate root morphology and soil behavior in tandem. All pixels within 1.3 cm (400 pixels) from all root surfaces were evaluated. This procedure thus allows us to assign a local root diameter value to every component of the plant-soil interface. In total, even after averaging across root diameter classes in each image, this outputted 11100 data points.
3.2.6 Soil Water Characteristics

We calculated radial soil water content distribution and dynamics away from the root by dividing each subsection into three classes: 1. the root-soil edge (d = 0-0.01 cm from the root edge), 2. the rhizosphere soil (d = 0-0.15 cm), and 3. the bulk soil (d = 0.5 cm). We note that all soil pixels analyzed represent purely soil values – given the 2D nature of our images, any pixel values that have a root section would be represented by both roots and soils, as soil lies in front and behind of the root. The fine roots not visible by neutron imaging (i.e., < 100 µm) still impact measured pixel saturation across the samples, and thus inject some uncertainty/variability into the analyses. Our subdivision of the rhizosphere zone is a more explicitly outlined formulation of the one often used in the rhizosphere imaging literature (e.g., Carminati et al., 2010, Zarebanadkouki et al., 2018) – the soil region we have defined as the root-soil edge and the rhizosphere is often conflated and quantified interchangeably within the literature. Figure 3.3A illustrates the positions of the three rhizosphere zone soil subdivisions, and Figure 3.3B shows the positions of the three on experimental curves of radial water content distribution. We then calculate volumetric water uptake by calculating water volume change in each specified soil subsection and summing them by root diameter over a unit of time. We additionally normalize this by dividing the water content change by total root surface area of that given root diameter (calculated by assuming a cylindrical root diameter).

3.3 Results

3.3.1 Integrated Root-Soil Mapping

The histograms of represented root diameters at each stage of the integrated plant-soil map, which we label as medial axis transform (MAT), the root surface (RS), and the
surrounding soil region (SR) resembled gamma distributions, with a majority of roots dominated by the fine root classes, and a long tail of thicker roots, though the fits typically underestimate the frequency of the thicker root diameters (Figure 3.4). Good fits to the gamma distribution are often observed in the grape system (Figure 3.4K-M), which often do not have thick roots. While the fine root diameter classes dominate in frequency in all samples, the RS and SR mapping show similar distributions to each other than with the MAT mapping, which shows the most deviation both from the gamma distribution fits and from the other two mapping procedures. This aspect is most notable in a majority of the poplar experimental samples (Figure 3.4A, B, D, E), where the frequency drops almost two orders of magnitude in the thicker root
Figure 3.5: Mean and variance of the represented root diameter data, and their gamma distribution fits for the MAT, RS, and SR mapping. Mean and standard deviation of all parameters for each species is shown.

...diameter classes – this is observed to a lesser extent only in one grape sample (Figure 3.4O), where the drop is smaller and more scattered. Overall, mean represented root diameter in the MAT is higher than both the RS and SR mapping, with a corresponding decrease in variance (Figure 3.5), indicating a disproportionately high representation of the fine root diameter classes in the RS and SR mapping. A visual example of this result can be observed in Figure 3.2 which shows how represented root diameter in the MAT can be higher than the RS/SR mapping. Evaluating the plant-soil system in the radial dimension, root distance histograms (RDHs) of the Euclidean distance transforms used predominantly resembled gamma distributions for all 5 species analyzed [Schluter et al., 2018], suggesting well-developed RSAs – fitted parameters are shown in Table 3.1. This analysis was extended to each soil region for each root diameter class to calculate the mean of each RDH, which we label mean “soil-root extent”, and which we interpret as a measure of accessible soil to the root. We plotted by root diameter for each plant species in Figure 3.6. In a majority of experiments, a relatively high soil-root extent is observed at the smallest root diameters, which then show decreasing trends with increased root diameter. In 6
Figure 3.6: Mean plant-soil extent of all soil pixels to the closest root surface, sorted by root diameter. (a-f) poplar, (g, h) juniper, (i) maize, (j) maple, and (k-o) grape experiments.

of the 15 samples (Figure 3.6B, D, F-H, K, M), an uptick in soil-root extent is observed for the largest root diameters. Again, we use Figure 3.2C as a visual example – the dark zones associated with the finest root diameters extend the longest in the radial direction, which would result in a high accessible soil-root extent. On the other hand, the orange zones, associated with larger root diameters, are “crowded out” by these blue zones, resulting in smaller areas and thus smaller accessible soil-root extent.

3.3.2 Water Content Distribution

Mean water content of the plant-soil system at the three soil regions of the soil for the 6 experimental samples (5 poplar, 1 maple) with dynamic imaging through time are
<table>
<thead>
<tr>
<th></th>
<th>( k ) [cm]</th>
<th>( \theta ) [cm]</th>
<th>mean [cm]</th>
<th>var [cm(^2)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>0.803</td>
<td>0.635</td>
<td>0.509</td>
<td>0.323</td>
</tr>
<tr>
<td>Maple</td>
<td>0.894</td>
<td>2.09</td>
<td>1.87</td>
<td>3.92</td>
</tr>
<tr>
<td>Grape</td>
<td>1.19</td>
<td>0.545</td>
<td>0.650</td>
<td>0.354</td>
</tr>
<tr>
<td>Juniper</td>
<td>1.19</td>
<td>0.717</td>
<td>0.853</td>
<td>0.612</td>
</tr>
<tr>
<td>Maize</td>
<td>0.870</td>
<td>0.377</td>
<td>0.328</td>
<td>0.124</td>
</tr>
</tbody>
</table>

Table 3.1: Gamma distribution parameters of the root distance histograms of each species evaluated in our experiments. Columns 1 and 2 represent mean parameters \( k \) and \( \theta \) across all samples for a given species, and columns 3 and 4 represent the mean and variance of the gamma distributions.

Water content dynamics varied with drying or wetting regimes: under dry conditions, water content at the root-soil edge was greatest amongst the three classes and had the greatest difference between it and the other two soil regions. Following rewetting, this difference decreased, and in the case of one poplar experiment (Figure 3.7e), the bulk soil and rhizosphere water content increased above that of the root-soil edge, though subsequent water content decrease in the latter was minimal compared to the former two regions. While water content distribution was heterogeneous across and within species samples, we observed that inter- and intra-species generalizations can be more readily made when comparing relative differences in water content across the three analyzed soil subsections. We highlight these variable differences in water content distribution in Figure 3.8, where we take the mean water content of the three soil subsections and calculate the difference in water content at the two interfaces between them, for each root diameter class in a single image. Data shown here correspond to the dry image of each experimental sample – this corresponds to only the initial pre-wetting image of the dynamics experiments. We observe that root-soil edge/rhizosphere water content difference is low for the finest root diameters – this difference increases steadily until a root diameter of roughly 0.12cm – beyond this point we observe the value stabilize to roughly 0.1. Given the nature of the distribution, we fit the data with a sigmoid function, with the following fitting parameters:
Figure 3.7: Mean water content over time for the three soil region boundaries in the root-soil interface (labeled as “d = “ in (f)) for the (a-e) poplar and (f) maple species. Dotted vertical lines indicate re-wetting points.

\[ \Delta \theta = \frac{L}{1 + e^{-k(D_{\text{root}} - x_0)}} \]  

(3.1)

where \( \Delta \theta \) is the water content difference between the two soil subsections, \( L \) is the maximum water content difference, \( x_0 \) is the root diameter midpoint of the curve, and \( k \) is the steepness of the curve. Best fit values corresponding to the function are shown in Table 3.2. We observe similar distributions across all five species analyzed, despite the lack of data at the larger root diameters. In contrast, there was no discernable correlation in water content difference between the rhizosphere and bulk soil during the driest period as sorted by root diameter (Figure 3.8B) – unlike the root-soil edge/rhizosphere interface, where water content differences rarely dipped...
Figure 3.8: (a) difference in water content from root-soil edge to rhizosphere, sorted by root diameter. Green line indicates the sigmoid best fit. (b) difference in water content from rhizosphere to bulk soil, sorted by root diameter.

below zero, the rhizosphere/bulk soil interface regularly exhibits both negative and positive water content differences and is centered around a net difference of zero.

<table>
<thead>
<tr>
<th>Species</th>
<th>$L$ [cm$^3$cm$^{-3}$]</th>
<th>$k$ [cm]</th>
<th>$x_0$ [cm]</th>
<th>var rmse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>0.092</td>
<td>21.1</td>
<td>0.11</td>
<td>0.0185</td>
</tr>
<tr>
<td>Poplar</td>
<td>0.0943</td>
<td>23</td>
<td>0.103</td>
<td>0.0199</td>
</tr>
<tr>
<td>Maple</td>
<td>0.113</td>
<td>11.6</td>
<td>0.128</td>
<td>0.0179</td>
</tr>
<tr>
<td>Grape</td>
<td>0.11</td>
<td>15.3</td>
<td>0.175</td>
<td>0.0109</td>
</tr>
<tr>
<td>Juniper</td>
<td>0.147</td>
<td>14.7</td>
<td>0.191</td>
<td>0.011</td>
</tr>
<tr>
<td>Maize</td>
<td>0.0887</td>
<td>20.3</td>
<td>0.0764</td>
<td>0.0196</td>
</tr>
</tbody>
</table>

Table 3.2: Best fit parameters and root mean square error of the sigmoid functions fitted for the five species analyzed in Figure 3.8A, as well as the combined data.

We then extend this analysis of relative change in water content across the entire soil moisture range in Figure 3.9, where we plot mean water content difference in the root-soil edge/rhizosphere interface (top row), and in the rhizosphere/bulk soil interface (bottom row), by root diameter class, using Figure 3.8 as a reference (Figure 3.9A, D: $D_{root} < 0.1$ cm; 3.9B, E: $D_{root} = 0.1$-0.2 cm; 3.9C, F: $D_{root} > 0.2$ cm). Given its organizational structure, a plot can be divided into quadrants: using Figure 3.9D
as an example - the zones above and below the dotted line indicate wetter or drier rhizosphere soil relative to bulk soil, while the left and right zones indicate respectively drier or wetter soil water regimes. The edge-rhizosphere interface relationship highlights what was observed in Figure 3.8A - all points are higher than 0 regardless of root diameter, so this remains the same when plotted against bulk soil water content, and the gradual increase in difference with root diameter is observed as well (Figure 3.9A-C). Very few data points are below the reference zero line, and given the shallow slope of the data, the linear fit never crosses the reference line. In contrast, across the rhizosphere-bulk soil interface, the rhizosphere water content is increasing wetter than the bulk soil as it dries, but drier than the bulk soil under more moist conditions (Figure 3.9D-F) - the negative slope suggests that rhizosphere water content becomes progressively higher and wetter relative to bulk soil water content as the former dries. This relationship appears independent of root diameter. The transition from higher is shown to be 0.11, 0.10, and 0.13 cm$^3$ cm$^{-3}$ for the small, medium, and large root diameter classes, respectively. When classified by root order, we see that these trends are considerably less pronounced (Figure 3.10), though we do observe a similar transition point between rhizosphere and bulk soil water content (3.10E-H) at roughly 0.10 cm$^3$ cm$^{-3}$, and a root-soil edge water content slightly higher than the bulk water content (Figure 3.10A-D), across the full bulk water content range.

### 3.3.3 Water Uptake

We observe a non-linear decrease in water uptake with increased root diameter in the dynamics experimental samples analyzed (Figure 3.11A). The rate of nonlinear decrease of total water uptake is different by species – the difference in the maple experimental sample is less pronounced. We observe that the smallest root diameter classes (D < 0.05 cm) exhibit a similar magnitude of water uptake per unit surface area, as well as a similar decline up until D = 0.1 cm. Beyond this, water uptake is
Figure 3.9: Relative water content dynamics, as sorted by root diameter. Top row is difference in water content from root-soil edge to rhizosphere, sorted by bulk soil water content, for root diameters (D_{root}) (a) < 0.1 cm, (b) 0.1-0.2 cm, and (c) > 0.2 cm. Bottom row is difference from rhizosphere to bulk soil, sorted by bulk soil water content, for root diameters (d) < 0.1 cm, (e) 0.1-0.2 cm, and (f) > 0.2 cm. Dotted lines show reference line where soil subsections at either side of the interface is equal (i.e., difference of zero), and solid lines show best fit linear values for data.
variable – maple species shows an upper bound, with all other poplar samples showing uptake values considerably lower. However, in both species, normalized water uptake decreases exponentially with increased root diameter. Across all samples analyzed, total root surface area of the finest roots are orders of magnitude longer than the larger root diameter classes (Figure 3.11B), though there is a dip at the finest root classes. When evaluating total water uptake by the plant root system for the 6 dynamics experimental samples, we see a strong linear correlation with total calculated surface root area (Figure 3.11C).
Figure 3.11: Water uptake characteristics. (a) total water uptake normalized by total root surface area of each root diameter. (b) distribution of total root surface area by root diameter. (c) total water uptake of all dynamics experimental samples plotted by total root surface area.

3.4 Discussion

3.4.1 Integrated Plant-Soil Mapping

The three-tiered plant-soil mapping procedure outlined in our paper emphasizes the morphological consequence of root system size and distribution on the surrounding soil system. The proportionally higher representation of the fine root classes in the RS and SR mappings are simply by virtue of the root morphological distribution and its spatial relationship to the soil, which favored fine root representation. In one example, a root tip, often the smallest portion of the root, will have access to more soil regions, simply by virtue of being at the end of the root. Another example is that the finest root diameter classes often induce a root crowding effect, where the smaller roots (and their associated zones) surround intermediate-sized roots, which means that less soil is exclusively labeled to the latter. The root tip effect is visible in Figure 3.2C, which subsequently crowds and minimizes the surrounding thicker root soil regions. While a straightforward metric to use for our integrated plant-soil mapping, some constraints apply regarding our implementation of spatial proximity as the sole criteria considered for connecting plant and soil characteristics. While root
water uptake influence can extend to the cm-range and further [Darrah, 1993], some root morphological classes may not have this capability, and thus should be excluded when during the matching process of the root-soil mapping. With regards to the root crowding effect, it is unlikely that only a single root diameter or root surface has exclusive influence on all soil characteristics in that pixel, especially for processes like water uptake. Creating an additive component, where soil pixels can have variable degrees of root morphological assigned value as weighted by spatial proximity, root diameter, etc., may prove fruitful.

3.4.2 Rhizosphere Distribution and Dynamics

In our general analysis of the rhizosphere, we classify two key interfaces: the edge-rhizosphere interface and the rhizosphere-bulk soil interface. Water content differences across these interfaces serve as a simple metric for rhizosphere development, as larger differences in water content and retention between adjacent soil likely indicate more deviations from unaffected soil (e.g., [Carminati et al., 2010]). Using this interpretation, our results suggest that rhizosphere development is consistently pronounced at the edge-rhizosphere interface under the full soil moisture range (Figure 3.9A-C), and shows a sigmoidal relationship with RSA development (i.e., increasing root diameter, Figure 3.8A). In contrast, rhizosphere development shows plasticity behavior that is dependent on local bulk soil moisture content (Figure 3.9D-F), and no discernible influence from RSA properties (Figure 3.8B). Of note, we observe this phenomenon across all five plant species analyzed, despite observed differences in RSA spatial distribution (e.g., Figure 3.6) and biology.

So what may serve to explain these different trends observed at each of the rhizosphere interfaces? For the edge-rhizosphere interface, a potential driving factor is mucilage - a polymeric gel shown to be active in younger, thinner roots [Ahmed...
et al., 2014]. Significant concentrations are typically seen 0.005-0.01 cm from the root surface [Oades, 1978; Foster, 1988; Carminati et al., 2015]. This corresponds to the dimensions of our root-soil edge, which we make explicit in addition to the remaining rhizosphere, typically demarcated between 0-0.15 in prior studies [Carminati et al., 2010]. Along with root hair presence, mucilage is also shown to increase water content at all water potentials [Carminati et al., 2016], which can explain the consistently higher water contents. For the rhizosphere-bulk soil interface, bulk soil characteristics may be more dominant than mucilage behavior, given the consistent transition point independent of root morphology or species. The calculated transition water content corresponds to a matric potential of ∼2kPa using the Brooks and Corey parameters calculated for this soil [Cheng et al., 2012; Kang et al., 2014]. While a relatively low matric potential, this is situated at the lower end of the water retention curve in our coarse sandy soil. Any lower and water availability drops exponentially, quickly approaching residual water content. The fact that edge-rhizosphere interface values are changing with root morphological traits, while rhizosphere-bulk soil interface values are independent of these parameters highlights the multiple processes at play within a single rhizosphere, and the need to clarify appropriate boundary conditions for each end of the rhizosphere.

Although at a much lower magnitude, sorting by root order shows similar trends, and with minimal difference between them (Figure 3.10). This is somewhat contrary to the plant science literature, which has shown non-uniform exudate deposition along the lateral dimension of the root by species (e.g., [Razavi et al., 2016]) and switching of water uptake location by root order as root size increases and matures [Ahmed et al., 2018]. This may be due to species differences (maize vs. maple/poplar). Another aspect may be that our root diameter distribution within the root-order ROIs analyzed fall under a much narrower range of root diameters that are all highly
functional (Figure 3.12), and furthermore approach a diameter range on the order of \(\sim 0.01\text{cm}\), of which our segmentation procedure has been already shown to underestimate \cite{Dhiman2018}. These two aspects limit the extent of our findings on root order and being able to classify the entire RSA via root order may yield results, though segmentation and image processing of this may prove challenging. A promising initial metric is using normal distribution intervals of root diameter as an effective proxy for root order \cite{Liu2018}, which would work well with our plant-soil mapping procedure. Fig. 11 shows that the finest root classes take up significantly more water than the larger root classes. Water uptake is high in the early stages of root development as the root tissue develops, but decreases as they mature and begin to senesce \cite{Wells2003}. Radial conductivity of water into the root varies with distance from root tip, with an estimate 90\% of total water uptake conducted by just 30\% of the root surface \cite{Zwieniecki2003}. In this case, the most distal root tips, which are typically smaller diameter and have less developed xylem vasculature, take up less water than the root sections some distance back from the tip \cite{Zwieniecki2003, Ahmed2016}, but further back the process of suberization of endodermal cells in older roots again limits uptake in this section as roots become more transportive. Younger, smaller roots are more permeable to water and nutrients \cite{Dhiman2018}, and combined with potential suberization of the larger root classes, this may explain the large difference in water uptake we see here. Another potential explanation is differences in mean soil-root extent – in 5 of the 6 poplar experiments, the finest root classes have the largest soil-root extents. While this means that soil water must travel a further distance to reach the root, it also means that more water overall is available to that given root class, given the larger amount of soil (and thus soil water) spatially related and close to the given root class. This was controlled to some degree due to the 1.3 cm (400 pixel) boundary applied, though this does not change the fact that the peripheral
Figure 3.12: Distribution of total root-soil interface data points analyzed in our experimental samples vs of those used in root order analysis.
roots have uninhibited access to soil water on the outer edges of the RSA. This may ultimately result in a higher total water uptake in the fine roots just by virtue of physical proximity to the soil. The soil-root distance for maple is relatively consistent across root diameter, which may explain why its decrease in normalized water uptake with root diameter (Figure 3.11A) is lower in magnitude. An important point to note is that the younger roots in our experiments are underestimates due to resolution and segmentation limitations [Dhiman et al., 2018], which also exclude root hairs. Inclusion of these missed root hairs and superfine fine roots will invariably increase their root surface area, and thus will have the net effect of decreasing water uptake per unit surface area in the smallest root classes, though their total water uptake remains unchanged.

3.4.3 Incorporation of Dynamic Processes

In our study, individual plants were directly transplanted with a developed root system, so all rhizosphere development in the soil commenced simultaneously in an individual sample, allowing us to evaluate coupled root-soil properties more directly. Incorporating a dynamic element to the spatial range of the rhizosphere – commonly assigned a static value of 0.15 cm in the literature (e.g., [Carminati et al., 2010] Zarebanadkouki et al., 2016) may be necessary if evaluating differential shifts in root diameter and morphology.

This is further complicated in the case of naturally growing root systems, whose root tips can produce considerable amounts of mucilage due to abrasion and soil structural resistance [Groleau-Renaud et al., 1998] Read et al., 2003, and which will likely have an effect on rhizosphere development for the rest of the root that grows in the same trajectory. In this regard, incorporating RSA dynamics would provide a more complete picture of rhizosphere development across the full root growth range. In our study, we used static integrated plant-soil maps – our focus was characterizing
plant-soil water dynamics, and root growth was minimal for the duration of our experiments, as the experiment RSAs were already mature by the time of planting (Table 3.1) and maximum duration of experiments was approximately 40 hours (Figure 3.7). Changing this to a dynamic mapping system would provide a more comprehensive analysis of rhizosphere development in the context of both plant and soil characteristics. Given its sensitivity to water, neutron imaging would be invaluable in characterizing these dynamic soil water dimensions of the plant-soil interface (e.g., [Warren et al., 2013, Dhiman et al., 2018]) – while major limitations exist due to 3D neutron image acquisition time ranging in the hour scale, recent advances in ultra-fast neutron tomography for characterizing water flow [Totzke et al., 2017] may provide new avenues in this front. Another promising imaging method for this integrated plant-soil mapping is with X-ray imaging, which has already been utilized to characterize pedological features of the rhizosphere (e.g., [Koebernick et al., 2017]), and whose acquisition time is on the order of seconds for 3D tomography, though the subsequent damage to the plant due to the high flux renders this a destructive/static technique). While soil water characterizations would be challenging, other soil parameters (e.g., porosity, particle size distribution) can be evaluated at a pixel-by-pixel basis in conjunction with root morphological parameters.

3.5 Conclusion

This chapter outlines an integrated analysis of rhizosphere characteristics by incorporating local effects of differential root morphology and bulk soil and water characteristics. By conducting this analysis on five different species across a wide range of soil moisture regimes using neutron radiography, a major objective was to create a generalized, species-independent framework of rhizosphere behavior. Characterizing
the plant-soil region into three domains (root-soil edge, rhizosphere, bulk soil), we calculated relative water content differences between them and evaluated them in the context of differential root diameter. In the edge-rhizosphere interface, we observed a consistently higher water content in the root-soil edge, which increased with root diameter, likely due to mucilage behavior. In the rhizosphere-bulk soil interface, we observed rhizosphere water content plasticity, dependent on local bulk soil water content, and independent of root morphological traits – this was likely due to bulk soil characteristics.
Chapter 4

Biophysical Effects on Soil Crack Morphology in a Faunally Active Dryland Vertisol

4.1 Background

Shrink-swell soils have an extensive presence across the globe, covering approximately 350 million ha [Ahmad and Mermut, 1996]. Also referred to as vertisols, these soils are primarily located in water-limited tropical and sub-tropical zones, with 80% of vertisols located in Australia, India, and East Africa alone [Virmani et al., 1982]. Vertisols’ high fraction of montmorillonite and smectite clay provides high fertility and cation exchange/water retention capacity, and when properly managed, they can serve as highly productive soils in these regions [Ikitoo et al., 2011]. However, these unique properties simultaneously yield a number of complex physical processes: the shrink-swell process induces mechanical failure under desiccating conditions, a phenomenon referred to as desiccation or soil cracking. While these cracks are superficially small (centimeter-scale crack width), they are often systematic and
persistent throughout the soil profile, with meter-scale deep features that persist through multiple soil wetting and sealing events [Stewart et al., 2015]. These features make it a particularly relevant phenomenon in the soil and water sciences: they act as preferential pathways that allow for rapid, non-matrix water flux through the soil profile [Weisbrod et al., 2002, Dragila and Weisbrod, 2004], and enhance vertical redistribution of solutes and contaminants throughout the soil environment [Jamieson et al., 2002], as well as “vents” that cycle carbon and water at rates far greater than from a matrix/pore network-defined soil alone. The magnitude of this venting process, either by forced or free convection, scales non-linearly with crack aperture [Adams et al., 1969, Selim and Kirkham, 1970], and crack depth, which indirectly influences convection by exposing air in the crack space to a progressively larger temperature gradient present in the soil [Nachshon et al., 2008, Weisbrod et al., 2009].

The magnitude and dynamics of these pedological and hydrologic processes are often regulated by variations in crack network topology and morphology. Physico-chemical structural and mechanical changes can play a significant role in influencing these morphological changes in crack networks. When evaluating cracking morphologies in thin swelling clay layers, salinity- [Ren et al., 2016, Panayiotopoulos and Leinas, 2009], or microbially-induced [Preston et al., 2001] aggregation behavior has been shown to create smaller crack networks with lower connectivity. When considering a deeper swelling clay profile, where cracks can propagate at depth, increased aggregation can make crack structures deeper at an equivalent water content [DeCarlo and Shokri, 014b]. The aggregation behavior of the smectite sheets induces larger constraints during the shrinkage phase, creating multi-sheet “stacked” elements that reduce the flexibility of the system [Zabat et al., 1997], and make the consolidated soil systems more prone to mechanical failure (i.e., soil cracking). In the case of salinity, the intercalated cations (and organic compounds) reduce the
electric double layer and chemically induce compaction and consolidation in the soil structure [Luckham and Rossi, 1999, Tessier et al., 1992]. These changes can persist in the soil system, sometimes progressively building on prior alterations. For example, multiple wetting-drying events allow for morphological shifts in crack position, which involve a centralizing tendency of the junction midpoint and an angular shift from 90 to 120 degrees [Tang et al., 2008], with crack angle frequency shifting from a bimodal distribution with peaks at 180 and 90 degrees (corresponding to a “T” shape junction) to a Gaussian distribution with a mean at 120 degrees (corresponding to a “Y” shape junction) [Goehring et al., 2010, Goehring, 2013]. This process may be due to the ability of consolidated systems (“pastes”) retaining a memory of mechanical disturbances and forces, with microscopically anisotropic changes in particle structure and orientation directly reflecting macroscopically anisotropic crack structure [Nakahara and Matsuo, 2005]. This analysis on crack morphology persistence, including changes between multiple wetting-drying cycles, is rarely extended to 3D, owing in part due to the difficulty of effective visualization. Even in the few 3D field crack studies in the literature (e.g., Abou Najm et al., 2010), crack persistence across wetting-drying cycles has been given only qualitative focus, and just on the 2D soil surface.

In natural soil systems, floral and faunal presence can play a role in crack formation that is separate from the purely physicochemical drivers of shrink-swell cycles. Examples of the influence of plant root dynamics on and by soil cracks include preferential root clumping in cracks/macropores [White and Kirkegaard, 2010], cracking behavior between planted crop rows [Dexter, 1988, Oades, 1993], and root shearing/tearing from cracks [Ahmad and Mermut, 1996]. In contrast, studies of faunal influence are often focused on influences on soil quality and structure: grazing behavior by aboveground fauna (“biocompaction”) has been shown to increase soil
bulk density, decrease water infiltration, and increase surface water run-off, resulting in drier and more consolidated soil conditions [Drewry et al., 2008, Veldhuis et al., 2014, van Klink et al., 2015]. On the other hand, mechanical overturning of soil by belowground fauna ("bioturbation") can have contrasting effects by increasing aeration and promoting soil disaggregation [Howison et al., 2016, Howison et al., 2017]. Even in consolidated, “muddy” soil systems where bulk density is high, burrowing animals such as earthworms have been shown to increase macropore density by inducing fractures in the soil medium [Dorgan et al., 2005]. Numerous termite species are capable of physically and chemically altering soil aggregates [Bottinelli et al., 2015], and some species, like Macrotermitinae, can directly alter clay mineralogy at the particle scale [Jouquet et al., 2011, Mujinya et al., 2010]. Bioturbation and biocompaction are both global-scale biotic processes, observed from the Arctic to the savannah [Howison et al., 2017], and furthermore, divergent soil structural properties (e.g., soil bulk density, moisture, structure) are observed as a result of these two processes. Given the known consequences of soil structural changes on soil fracturing behavior, one can expect a link between these biotic processes and crack morphology as well.

To this end, our study investigates how biomechanical elements of biocompaction and bioturbation influences 3D crack structure in a dryland swelling soil, classifying the two elements under the respective categories of aboveground megafauna and belowground termites. We combine crack structural visualization techniques outlined by [Abou Najm et al., 2010] with X-ray imaging to digitally characterize network morphology and topology of affected soils, which show differences in crack intensity and depth-wise distribution despite overall morphological and topological similarity. Finally, we speculate on consequences of altered crack morphology to water and carbon vapor fluxes, specifically the formation of thermally convection processes.
4.2 Materials and Methods

4.2.1 Field Site Identification

Our study sites were located in the Mpala Farm in Laikipia District, central Kenya. They are located in a semi-arid savannah ecosystem, with mean annual precipitation of 600 mm/year and minimum/maximum of 350-1000 mm/year observed in the past two decades. Peak rainfall is usually in April and November, with dry seasons in the summer and early winter. Observations were made in July and August 2014, at the tail end of the dry season. The field site is located within a cattle ranch with large native biodiversity presence, including over 40 large mammal species, as well as an extensive termite presence.

From this, we quantified the biomechanical forces of bioturbation (BT) and biocompaction (BC) in two respective forms at our study site. For BT soils, we used the lenticular soil mounds formed by fungus-cultivating termites (Macrotermiteinae, Odontermes), which range anywhere between <1-10 m, and which are regularly distributed at a spacing of 50-100m [Bonachela et al., 2015]. For the BC soils, we used the long, narrow animal trails regularly utilized by various species including elephants (Loxodonta africana), buffalo (Syncerus caffer), giraffes (Giraffa camelopardalis), plains and Grevy’s zebras (Equus quagga, Equus grevyi), Grant’s gazelles (Gazella granti), elands (Taurotragus oryx), impala (Aepyceros melampus), hartebeests (Alcelaphus buselaphus), and cattle (Bos indicus). Of these, weights range from 40 kg (impala) to 1725 kg (elephants) [Kartzinel et al., 2015]. Due to regular use by all these animals over multiple years, we find it appropriate to aggregate their effect as a non-specific and regular biocompaction event. As a baseline reference soil (C), we used termite-free soils dominated by patchy vegetation inside the Kenya Long-Term Exclusion Experiment plots (KLEE) [Young et al., 1997], which use electric fences to
exclude all large herbivores from a 200 m × 200 m space – other smaller mammals (e.g., mice) can still enter the exclosure through gaps in the fence but have negligible biocompaction influence compared to the mega- and meso-herbivores in this study and are not considered.

Within each classification, several study sites were identified and measured. For BT soils, 4 separate termite mounds were identified, and 2 casts were made at each mound (3 for BT2, due to its larger size), for a total of 11 casts. For the BC soils, a major complication was the highly variable presence of aboveground fauna, and the inability to distinguish (at least, superficially) the prior extent of mega- and meso-herbivoral influence – while animal trails are distinct from the surrounding soil in that there is less vegetation in linear orientations, the degree to which this is the case is not uniform, due to some trails being used more than others. Therefore, individual affected soils were identified immediately after a major rainfall event, where a visible footprint of an animal could be identified, and an active animal trail can be more simply identified. So long as there are no further disturbances, these footprints remain intact within the soil until the next rainfall event, and can be sampled at any time, not necessarily immediately after a rainfall event. Footprints from nearly all herbivore species were used to identify animal trails, and the disparity from the individual animal’s mass will likely play a cofounding factor (e.g., using an elephant’s footprint vs. a gazelle). However, our objective was to quantify the general biocompaction influence, which is the accumulation of years and possibly decades of individual biocompaction events on the animal trail, which are shared by the above herbivore species. Thus, we assume that the cofounding factor from the disparity in the individual footprint to be negligible. Figure 4.1 shows a typical setup for a soil site.
Soil texture and chemical characteristics analysis of a single BC and C soil, and BT samples of each of the four termite mounds were conducted by Crop Nutrition Laboratory Services (ISO 17025 accredited), located in Nairobi, Kenya. Particle size analysis shows clay fractions at a range of 58-70%, indicating clay-rich “black cotton” vertisols, dominated primarily by 2:1 smectites and montmorillonite. The soils in our field sites have an overall higher sand fraction compared to its neighboring Ethiopian highland counterparts [Virmani et al., 1982], which may suggest a lower magnitude of shrink-swell in our soil sites. Full statistics of soil physicochemical properties are shown in Table 4.1.

Soil cracks were characterized using a macropore casting technique [Abou Najm et al.,]
<table>
<thead>
<tr>
<th>Soil Type</th>
<th>BT</th>
<th>Mean</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>BC</th>
<th>Mean</th>
<th>C</th>
<th>Mean</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water Content (g/g)</td>
<td></td>
<td>21.5±3.39</td>
<td>19.9±1.85</td>
<td>22.2±2.21</td>
<td>24.9±2.75</td>
<td>18.3±3.48</td>
<td>24.4±3.92</td>
<td>26.5±4.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sample count</td>
<td></td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N-S/E-W Dim. (m)</td>
<td></td>
<td>N/A</td>
<td>6.44/5.85</td>
<td>4.39/2.26</td>
<td>6.95/4.9</td>
<td>1.45/2.1</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td></td>
<td>24.8</td>
<td>21.4</td>
<td>23.3</td>
<td>27.4</td>
<td>27.2</td>
<td>22.9</td>
<td>29.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silt</td>
<td></td>
<td>10.6</td>
<td>8.56</td>
<td>12.7</td>
<td>8.42</td>
<td>12.8</td>
<td>15.1</td>
<td>12.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td></td>
<td>64.5</td>
<td>70</td>
<td>64</td>
<td>64.1</td>
<td>60</td>
<td>62</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td>7.84</td>
<td>7.82</td>
<td>8.40</td>
<td>7.68</td>
<td>7.46</td>
<td>6.89</td>
<td>6.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC (µS/cm)</td>
<td></td>
<td>167.3</td>
<td>229</td>
<td>157</td>
<td>135</td>
<td>148</td>
<td>68</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P (ppm)</td>
<td></td>
<td>7.63</td>
<td>3.73</td>
<td>8.18</td>
<td>7.51</td>
<td>11.1</td>
<td>7.13</td>
<td>4.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K (ppm)</td>
<td></td>
<td>798</td>
<td>750</td>
<td>713</td>
<td>905</td>
<td>825</td>
<td>1040</td>
<td>1050</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ca (ppm)</td>
<td></td>
<td>7070</td>
<td>5930</td>
<td>8380</td>
<td>6810</td>
<td>7150</td>
<td>4440</td>
<td>4230</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mg (ppm)</td>
<td></td>
<td>751</td>
<td>886</td>
<td>714</td>
<td>685</td>
<td>720</td>
<td>1020</td>
<td>945</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S (ppm)</td>
<td></td>
<td>1.02</td>
<td>1.49</td>
<td>0.83</td>
<td>0.74</td>
<td>&lt; 0.5</td>
<td>4.35</td>
<td>&lt; 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na (ppm)</td>
<td></td>
<td>334.8</td>
<td>881</td>
<td>321</td>
<td>49.7</td>
<td>87.6</td>
<td>207</td>
<td>174</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fe (ppm)</td>
<td></td>
<td>67.8</td>
<td>82.4</td>
<td>56.6</td>
<td>65.5</td>
<td>66.8</td>
<td>132</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mn (ppm)</td>
<td></td>
<td>323.5</td>
<td>280</td>
<td>338</td>
<td>330</td>
<td>346</td>
<td>359</td>
<td>238</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B (ppm)</td>
<td></td>
<td>1.21</td>
<td>1.34</td>
<td>1.46</td>
<td>1.02</td>
<td>1.03</td>
<td>0.45</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu (ppm)</td>
<td></td>
<td>2.71</td>
<td>2.3</td>
<td>3.18</td>
<td>2.66</td>
<td>2.71</td>
<td>2.11</td>
<td>1.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn (ppm)</td>
<td></td>
<td>5.09</td>
<td>&lt; 0.20</td>
<td>5.04</td>
<td>4.66</td>
<td>2.71</td>
<td>5.72</td>
<td>6.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CEC (meq/100g)</td>
<td></td>
<td>46.8</td>
<td>44.4</td>
<td>52.7</td>
<td>43.9</td>
<td>46.1</td>
<td>36.5</td>
<td>34.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N (%)</td>
<td></td>
<td>0.11</td>
<td>0.11</td>
<td>0.1</td>
<td>0.12</td>
<td>0.11</td>
<td>0.1</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OM (%)</td>
<td></td>
<td>4.11</td>
<td>3.82</td>
<td>3.92</td>
<td>4.08</td>
<td>4.44</td>
<td>3.72</td>
<td>2.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C:N</td>
<td></td>
<td>21.6</td>
<td>20.2</td>
<td>22.8</td>
<td>19.8</td>
<td>23.5</td>
<td>21.6</td>
<td>14.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Statistics of the physicochemical properties of the three soil treatments.

2010 [Stewart et al., 2015], whereby low viscosity epoxy resin with hardener was poured into a 0.25m × 0.25 m demarcated square frame, until the entire surface of interest was covered. Additional epoxy was poured as needed. Most casts were top-filled after approximately 30 minutes from the onset of pouring. Casts were left in the ground for at least 2 days to allow for sufficient hardening, after which they were excavated from the ground and lightly cleaned. The soil casts were then packaged and brought to a private hospital in Nairobi, where they were scanned using the Aquilon ONE CT Scanner, which has a z-axis resolution of 0.5 mm/pixel. Scanning energy level and XY resolution were adjusted between different casts due to the large range in size of each individual cast – resolution ranged between 0.601-1.05 mm/pixel, but the final resolution of each cast was adjusted to form uniform resolution in all dimensions.
for each cast. Gravimetric soil moisture was determined for each soil using a soil corer. Samples were dried at 105 °C for a minimum of 48 hours to ensure complete drying and extended if there were still mass losses at the end of drying.

### 4.2.3 Image Processing

Images from X-ray scans were processed using standard segmentation methods seen in prior analyses of soil cracking [DeCarlo and Shokri, 014a, DeCarlo and Shokri, 014b] – in our study, images were first segmented into trinary images due to the three distinct classes of soil, resin, and void space pixels (Figure 4.2A). In our images, the resin class corresponds to the cracks present in the soil system. Using simple morphological opening/closing operations, we can eliminate “false hits”, which are the excess resin layers of variable thickness that pooled on the soil surface, and “misses”, which are the trapped air bubbles and pockets and the void spaces not captured by the resin (Figure 4.2B), arguably some of the major experimental limitations associated with crack characterization with the casting technique. One important characteristic of this method is that any and all crack void spaces that are topologically isolated from the overlying atmosphere are not characterized, as any resin poured from the surface cannot reach that void space. Once the surface resin was removed, the surface level of the crack structure was defined differently by soil treatment: the surface of BC and C soils was defined as the mean elevation of the non-cracked surface due to their flat topography, while the surface of BT soils was defined as the low position of the undulating topography due to the “sub-mounds” present within the larger mound.

### 4.2.4 Crack Morphology/Topology Characterization

We characterized metrics of 2D and 3D crack porosity using standard segmentation methods [Taina et al., 2008, DeCarlo and Shokri, 014a, DeCarlo and Shokri, 014b, Noiriel, 2015]. Surface crack aperture was determined by calculating the
Figure 4.2: Image processing outline. (A) Trinary thresholded image of a cast tomo-
gram, and (B) effect of morphological opening/closing to remove excess resin presence
and define integrated soil ped/crack surface. The red line indicates the calculated ref-
ence surface level for this cast.
minimum distance from the medial axis of each crack to the crack edge adjacent to
the soil and doubling it to account for total width. Crack volume-based connectivity
was characterized by calculating the Euler characteristic for each crack structure,
and connectivity functions were determined by calculating the Euler characteristic
($\chi = N - C + H$, where N, C and H are the isolated pores, redundant connections,
and isolated solids, respectively) after morphological operations of progressively
increasing diameter structural element [Vogel, 2002].

Porous media network characteristics of the crack structure was calculated by
using a medial axis transformation (MAT) “skeleton” to produce a mathematical
graph of edges and nodes [Lindquist et al., 2000, Jiang et al., 2007, Dong and M.J.,
2009]. All crack void space pixels were assigned to their respective edge or node
based on Euclidean distance, thus linking the geometry of the crack network with its
topology – every crack “voxel” (3D pixel) was assigned a unique network node or edge
label. Despite being a crack network, several nodes and edges are directly adjacent
to the overlying atmosphere, which serve as the primary point of entry to and from
the underlying crack network in the soil. Thus, these “surface” crack edges and nodes
were defined as those whose corresponding crack voxels were directly adjacent to the
overlying void space class (i.e., overlying atmosphere). Minimum path lengths were
calculated using these surface nodes as the terminal nodes.

A suite of network parameters was calculated to quantify the crack network,
the nature of each of which is highlighted in simplified diagrams in Figure 4.3.
Morphological parameters investigated include: a.) hydraulic radius ($RH = A/P$,
where A and P are the area and perimeter of the crack cross-section), b.) shape
factor ($G = A/P^2$), and c.) tortuosity ($\tau = L/C$, where L is the length of the crack
edge and C is the distance between the two endpoints of the crack). Topological
Figure 4.3: Crack morphology and topology outline, using a simplified schematic. (A) morphological parameters of hydraulic radius (RH), shape factor (G), and tortuosity (τ), with A and P respectively corresponding to a crack cross-section area and perimeter, and L and C corresponding to the length of the skeletonized crack edge and the distance between the crack endpoints, respectively, and (B) topological parameters of node degree (ND), branches per path (BPP), and normalized minimum path length (NMPL). Numbers indicate length of each crack edge.

parameters investigated include: a.) node degree, defined as the number of crack edges connected to a single crack node, b.) branches/path, which indicate the number of diverging crack edges on a minimum path length between a given node and a surface node, and c.) normalized minimum path length, which is the minimum path length between a given node and a surface node normalized by the maximum minimum path length in the crack network.

4.2.5 Crack Layering Morphology Characterization

We extend prior analyses on 2D mud-crack vertex/angle shift under multiple wetting-drying cycles [Tang et al., 2008, Goehring et al., 2010, Goehring, 2013] into a 3D
crack structure to observe morphological shifts, and ultimately extent of wetting-drying hysteresis with depth. In prior studies, the statistical distribution of the crack angles present in the 2D layer is used as a metric for the number of wetting-drying cycles that the soil layer has experienced since the last mechanical disturbance. In the experimental studies, this was when the pastes were formed and laid out. So, for example, rectilinear T-shape crack junctions dominate the final crack pattern present in the dried 2D layer in the first few wetting-drying cycles. In later cycles, this shifts to equilateral Y-shape crack junctions. Numerous additional complexities arise when extending this to 3D and to the field. Cracks in the field, far from the idealized parallel straight-down cracks of theory, show a large degree of vertical spatial variability, at times gradual, and at times sudden and discontinuous. These are due to structural, mechanical, and wetting/drying heterogeneities in a natural soil system.

In our studies, while cracks propagate in 3D, they are primarily top-down oriented due to the drying front. Additionally, given the nature of tomography, we have hundreds of 2D tomograms perpendicular to depth. Thus, we extend the 2D crack angle analysis seen in the literature to every 2D layer present in the tomogram. In both the 2D case seen in the literature and our 3D application, the mechanics of two cracks joining is the same. Crack displacement was determined by calculating the 2D distance in the XY plane from the surface midpoint (i.e., the position where the 3 cracks meet at the surface to the midpoint at the terminal depth of the junction (i.e., the position where the 3 cracks meet at the bottom of the crack). All visible crack junctions at the surface were calculated for each digital 3D image. Figure 4.4 shows a schematic of the analysis conducted on a sample crack morphology.
Figure 4.4: Schematic of a skeletonized 3D crack structure, with the three colors corresponding to the three individual faces of the crack junction system. At the XY plane (red box at top), the initial starting positions of the three crack faces can be seen, as well as the midpoint at which they join. Along the Z axis, the drifting midpoint is outlined (thin green), as well as a projection (blue) of the terminal midpoint to the XY plane (thick green).
4.3 Results & Discussion

4.3.1 Cracking Morphology and Topology

Surface observations show enhanced soil crack fraction with higher faunal activity, with both BT and BC soil crack fractions roughly double that of C (Figure 4.5A); mean crack aperture is also slightly higher in the faunally influenced soils (Figure 4.5B). Surface crack fraction also shows moderate correlation with total crack porosity across all soil systems (Figure 4.5C), suggesting that total crack porosity can be partially predicted by surface observations. However, 3D crack porosity results diverge based on the particular biomechanical force (Figure 4.6), splitting between below- and aboveground fauna. BT soils exhibit extensive but surficial crack networks despite their systematic disturbance of soil structure, with high crack porosity confined in the top 5cm of the soil. BC soils exhibit more systematic crack networks despite their surficial trampling disturbance, with deeper cracks of moderate/high crack porosity throughout the depth of the soil. Overall crack magnitude shows large variance despite a narrow gravimetric water content range, between 0.22-0.27.

One important point that must be considered at this stage is the multi-faceted
Figure 4.6: 3D crack porosity in relation to maximum depth of the crack network in the three soil systems. Oversize points represent mean of each system.
influence of soil moisture on the pedological processes involved. Water content has a significant influence on soil compaction – it is well known that trampling-induced soil compaction and consolidation is greater under waterlogged as opposed to dry conditions \[\text{Hamza and Anderson, 2005}, \text{van Klink et al., 2015}\], and the soil compaction induced by individual biocompaction events is indeed dependent on soil moisture. However, the biocompaction events evaluated here occur year-round due to regular animal movement, and the animal trails which we used for our casting sites reflect years (and perhaps decades) of accumulated biocompaction events, both in the wet and dry season. This correlation between water content and compaction is separate from that with crack formation – soil crack magnitude is inevitably correlated with decreased water content, primarily to the extent that constrained shrinkage drives fracture formation in brittle/rigid structures \[\text{Scherer, 1990}\]. Divergences in crack magnitude, despite similar soil moisture values, absent chemical or biological changes, is thus driven by changes in physical soil structure. These changes in soil structure are in turn induced by biocompaction, which happens to have a greater impact on soil compaction/compression under wetter soil conditions. Thus, what is creating these divergent crack structures is likely not differences in soil moisture, but differences in soil microstructure and mechanics, which can produce crack networks with widely varying depth, magnitude, and dynamics at a similar soil moisture range \[\text{DeCarlo and Shokri, 014b}\]. In the case of fauna: the dispersed but unconsolidated soil systems created by bioturbation behavior typical of Macrotermitinae would create mechanical conditions conducive to surficial cracking networks. Conversely, the soil consolidation induced by megafaunal biocompaction would create a mechanically “stiff” soil structure that is more prone to failure \[\text{DeCarlo and Shokri, 014b}\], and which ultimately increases crack volume.

One possible complicating factor of this analysis is the differing cation concen-
trations between the soil systems, which have a known effect on soil structural and cracking morphology. Previous studies have used cation-induced montmorillonite permeability changes as a proxy for understanding shifts in cracking morphology [DeCarlo and Shokri, 2014b]: following this, studies have shown that Na influence is observed at 5700-11500 ppm, with appreciable effects observed at 6000 ppm [Quirk and Schofield, 1955], which is over an order of magnitude higher than that seen in our soils (174-335 ppm). With regards to Ca, no major changes are seen beyond concentrations of 60ppm [Quirk, 1952, Quirk and Schofield, 1955], which is two orders of magnitude lower than those observed in our soils (4230-7070 ppm), and also typical of the vertisols in Africa, with Ethiopian vertisols showing 3400-8800 ppm [Ahmad and Mermut, 1996]. We can thus assume that cation-induced effects on soil structure and crack morphology are uniform across all systems.

Another physical consideration with regards to the structural analysis of these cracking structures in field soil conditions is that these crack void spaces are rarely, if ever, entirely “unfilled”, parallel crack structures. While sites were chosen where the soil crack surface is intact, soil surface particles, rocks, etc. frequently fall deep into the soil profile through cracks in the dry period in a phenomenon referred to as “self-swallowing” [Fagan and Nanson, 2004]. This not only obviously alters volumetric properties like crack porosity, but also likely network morphological and topological properties. It only takes a single rock or ped to bisect, branch, divide, and otherwise morphologically and topologically alter a single fracture. This in turn converts singular fractures, both large and small, into a highly divided and sinuous network of smaller sub-fractures and pores (Figure 4.7), also making individual cracks difficult to quantify.

Despite the divergent faunal influences with regards to crack volume and depth,
Figure 4.7: 3D profile image of a single major crack observed in one of the BC soil sites – white spaces indicate macropore (i.e., crack) spaces.
Figure 4.8: Morphological (A-C) and topological (D-F) characterizations of the 3D crack structure as a network.

The crack structure showed a large degree of morphological and topological uniformity when analyzed as a topological network. Mean hydraulic radius (Figure 4.8A) is on the order of mm for all three networks, with a similar shape factor (Figure 4.8B) and tortuosity (Figure 4.8C). Topology-wise, node degree shows a network dominated by a low node degree (Figure 4.8D), and long tails for both branch length (Figure 4.8E) and normalized minimum path length (Figure 4.8F). The extensive self-swallowing mechanism observed in all of our study sites may suggest that despite large differences in overall cracking intensity and dynamics, from a network viewpoint, soil crack structures in faunally and biologically active regions may act less as typical “fractures” per se and more as the individual pore-like subsections that are present within the individual fractures. This morphological and topological similarity was observed despite the extensive presence of debris within the analyzed cracks, though debris presence and the new void space subsections created may have had a
Figure 4.9: Crack volume-based connectivity characteristics: (A) Euler characteristic after progressive morphological opening/closing of the 3D crack structure, and (B) correlation between 3D crack porosity and the Euler characteristic.

The topologically homogenizing effect on the original crack network. While we have used the most commonly used metrics of pore network morphology and topology from the literature (e.g., Lindquist et al., 2000, Jiang et al., 2007, Dong and M.J., 2009), comparatively less work has been done on crack networks than pore networks, and there may be additional morphological and topological metrics that are less-known and more appropriate for the former.

Connectivity functions for the three soil systems show increased connectivity due to faunal activity, with BT soils showing the highest connectivity, though BC soils show higher connectivity after a minimum crack aperture range of 0.3cm (Figure 4.9A). With regards to physical interpretation of the Euler characteristic $\chi$, specified in Section 4.2.4, $H = 0$ since this would correspond to solids floating in air, which is unlikely under environmental conditions, $C$ corresponds to the robustness of the connected network (more redundant connections equals a more well-connected network) and $N$ corresponds to the isolated pockets of air in the soil system (e.g.,
naturally present void spaces, cracks that formed at depth and never connected to the surface or the overlying network, etc.). As a soil wets and swells, the smallest crack apertures will close and seal, creating more and more isolated pockets of cracks and air in the soil (increasing N) reducing the overall connectivity of the network (decreasing C). As it is a simple arithmetic calculation of the three parameters, $\chi < 0$ would indicate more connections than isolated pockets, and vice versa for $\chi > 0$. As such, the progressively increasing structural element methodology used for this connectivity analysis corresponds exactly with the “hydraulic diameter” of a macro-pore \cite{Vogel2002}. Furthermore, increases in the minimum crack aperture threshold can be physically interpreted as a dynamic of a swelling, and thus narrowing, crack aperture – as the soil wets and swells, cracks begin to seal up, decreasing connectivity (i.e., increasing Euler characteristic) of the crack network. Therefore, the connectivity function highlights the percolation persistence of the crack networks with progressive infiltration and soil swelling/sealing. Surface crack sealing rates have been shown to potentially be significantly different from those in the subsurface, and nonlinearly responsive to water content \cite{Stewart2015}, so crack connectivity during a sealing event is likely higher than suggested here. Additionally, crack volume-based connectivity shows a strong linear correlation with crack porosity (Figure 4.9B), which is expected, as larger crack volumes and magnitudes would result in more robust individual connections.

### 4.3.2 Soil Memory Effects: Crack Layering Morphology

In our 3D crack structures, far from the idealized parallel straight-down cracks of theory and the laboratory, cracks in the field show a large degree of vertical spatial variability, at times gradual, and at times sudden and discontinuous. We therefore extend these prior analyses conducted over time in the vertical dimension, thereby creating a continuous profile of crack vertex/angle shift.
Figure 4.10: Visualization of the four crack layering morphologies: surface (A, C) and transparent (B, D) visualization of a disjointed crack, and a full crack network with different colors corresponding to different depths; and (E) crack angle distribution and Gaussian fit across all measured crack morphologies.

When extending crack vertex/angle analysis to 3D, three possible crack morphologies were identified: straight cracks, whose total midpoint displacement is negligible (defined as <1cm with depth); drifting cracks, whose displacement is non-negligible (defined as >1 cm with depth); and splintering cracks, which exhibited more than one crack junction total with depth. A number of these morphologies are visible in Figure 4.10C and D – Figure 4.4 shows a 3D visualization of a “drifting” crack morphology. A fourth crack morphology, which we labeled as the disjointed crack morphology, exhibited a discontinuously large degree of displacement within a short vertical distance, an example of which can be seen in Figure 4.10A and B. Crack angle distribution of all crack structures from surface to depth show a smooth Gaussian distribution centered at 120 degrees (Figure 4.10E), which suggest that a
The majority of the fractures observed are the result of multiple wetting-drying cycles. Table 2 shows the occurrence of each of the crack morphologies. Of the four crack junction morphologies identified, the formation of straight, drifting, and splintering cracks can be explained as the natural result of cracks propagating in a heterogeneous soil, with cracks propagating through regions most prone to failure. The disjointed crack morphology may indicate a novel type of “soil memory”, a record of prior cracks formed during past wetting-drying cycles. The deeper a given crack, the less likely its entirety will be sealed during any given wetting event (given its distance from the surface, tortuosity, etc.), and as such is more likely to persist through multiple wetting-drying events. Furthermore, as crack propagation is invariably correlated with drying extent, the deep crack will be indicative of the intensity of a given drying event that has occurred in the soil. While crack initiation and propagation are stochastic processes, in an undisturbed soil, crack formation will often occur in the same location, as the sealed crack regions tend to be less cohesive and more likely prone to initial failure in subsequent wetting-drying cycles [Tang et al., 2008], meaning that subsequent crack formation is often deterministic to an extent and related to the initial cracks in the first wetting-drying cycle. However, in our field sites, regular surficial disturbance, like that from animal trampling, will homogenize and reconsolidate the upper soil layer, allowing for spatially unrelated cracks to form alongside, above, and below the older cracks in the soil.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Soil type</th>
<th>Total count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT</td>
<td>BC</td>
</tr>
<tr>
<td>Straight</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Drifting</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>Splintering</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Disjoint</td>
<td>1</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 4.2: Frequencies of the four crack layering morphologies in the three soil treatments.
allowing for the formation of the disjointed crack morphology. The predominance of 120-degree “Y” crack junctions throughout the soil profile suggests that cracks at depth propagate through soils that have experienced multiple wetting-drying cycles since the last disturbance, and that the mechanical homogenization and reconsolidation process is confined to the surface layer and has not influenced the bulk of the soil.

Consolidation in the BC soils can be introduced in a number of ways: direct mechanical consequences from grazing and trampling are well known, with soil structural collapse and consolidation into a low porosity muddy “paste” [Warren et al., 1986], and compaction effects extending up to 25 cm despite the surficial occurrence of trampling [Pietola et al., 2005]. Furthermore, elephant movement in wetter soil conditions (as was the case in our experiments) can allow for the transfer and deposition of up to a m$^3$ of mud, which they carry on their bodies after wallowing in mud [Haynes, 2012]. This will not only introduce a new consolidated layer, but also allow for a completely different set of mechanical initial conditions from which unrelated cracks can form and extend through depth.

From these observations, we make two assumptions: (a) debris presence in cracks minimize preferential water seepage via cracks, thereby keeping the wetting extent relatively uniform; (b) consolidation events from trampling occur regularly, minimizing memory effects near the soil surface, and allowing novel crack morphologies to form in each drying event. From this we reiterate and outline the crack layering process as a schematic in Figure 4.11 In year 1, when the extent of a wetting event is deeper than that of a drying event, all cracks that formed will seal. In year 2, when the wetting event is shallower, any crack extremities that were not exposed to water will be left behind in the soil profile at depth. This process, as it continues to occur in subsequent years (year 3, 4), can persist across multiple wetting-drying cycles.
Figure 4.11: Schematic of the proposed soil memory/layering process.

After several years (year 10, arbitrarily set), this process allows for the possibility of multiple cracks to “layer” on the other, thereby forming a single crack with multiple disjoints (highlighted in purple), with subsections formed in multiple drying-wetting cycles. Furthermore, this also allows cracks formed from two different drying events to form side by side. Note that not all cracks may be linked to the surface (highlighted in green), and also will not be captured by the methodology used in this study. One observation is that while most of the characterized morphologies were observed in the BC soils, none were observed in the C soils while a few were seen in the BT soils. This is surprising considering that the C soils will still experience moderate degrees of compaction without faunal trampling effects due to kinetic impacts from rainfall events [Norton, 1987, Assouline, 2004]. Surface crusts and seals in silty soils formed
as a result of episodic rainfall events have been shown to be up to 25% higher than
unsealed soils [Fohrer et al., 1999] — this is likely to be considerably higher in clay-rich
soils, whose aggregates can break down further, and under torrential rainfall, which
is typical of our study sites. However, the lack of crack layering morphologies may
be due to the extremely shallow, and more importantly, isolated, crack networks
observed in the C soils, and the cracks need to form junctions in order for us to
conduct a crack junction analysis. However, this does not mean that the vertical
movement of a single crack cannot show wetting-drying tendencies and conducting
these experiments under further drier conditionss may enable the formation of deeper
and better-connected cracks that may yield more information on the wetting-drying
mechanics of these soils.

4.3.3 Potential Consequences to Water and Carbon Fluxes

The influence of crack morphology on soil water and carbon flux is often non-linear.
Small changes in crack aperture can increase hydraulic conductivity by orders of
magnitude [McKay et al., 1993, Øygarden et al., 1997]. Vapor outflows can be
enhanced by processes like pressure pumping [Luo and Zhou, 2006, Rey, 2015],
which can elevate flux by 3-7 times at the field plot scale [Takle et al., 2004]. These
enhancements, driven mainly by changes in crack morphology at the soil surface, are
likely to be observed in all three soil treatments, with greater magnitudes observed
in the biocompacted and bioturbated soils, owing to their larger mean crack aperture.

Thermally-induced convection has been shown in prior laboratory and fieldwork
[Nachshon et al., 2008, Weisbrod et al., 2009] to have a large potential for soil vapor
mixing and flux in the crack space, related to thresholds of the Rayleigh number
(Ra). Onset of convection in cracks is defined when the Ra exceeds a critical value
of $\text{Ra} = 4\pi^2$ [Nield, 1982]. Nachshon et al. [Nachshon et al., 2008] uses a modified Ra
dependent on soil temperature gradient and crack aperture to calculate the extent of
free convection in a fracture, as well as the maximum velocity of the convective air
plume. Results showed that larger gradients in temperature induced progressively
larger convective (and even turbulent) cells, with faster air replacement rate. There-
fore, because deeper cracks will have larger temperature gradients, biocompacted
soils may have larger fluxes due to convective processes within the fracture space.
These fluxes are most likely to occur in the nighttime hours due to the inverted
temperature profile in the soil layer [Weisbrod et al., 2009].

A major complication is the variable supply of soil carbon under different soil
conditions, either due to differences in vegetation, microbial activity, or faunal
presence. Termite mounds are often ecological “hotspots”, showing enriched nitrogen
and phosphorus and elevated grass production [Pringle et al., 2010]. In contrast,
biocompressed soils will likely exhibit decreased vegetation due to regular animal
trampling. Combined with the persistent respired carbon from termite presence,
bioturbated soils will likely exhibit enhanced soil carbon and water flux compared
to biocompacted soils. Prior studies on thermally-induced convection in cracks are
focused primarily on geological fractures, with a regular and stable carbon vapor
source (e.g., fractures along volcanic fault lines [Rey, 2015]). As such, application of
this phenomenon in biologically active (and variable) field soils will require not only
consideration of physical properties (e.g., temperature gradient, crack morphology),
which will serve to enhance any present carbon flux, but also biological properties
(e.g., soil microbial activity, root presence), which will ultimately serve as the carbon
source.
4.4 Conclusions

Faunal biomechanical factors of biocompaction and bioturbation – well studied phenomena in ecology – were used as variables to investigate their influence on 3D crack structure in a faunally active dryland soil using combined resin visualization techniques and X-ray imaging. Our field soil sites were located in a faunally active wildlife preserve/ranch in Kenya, using animal trails left behind by megafauna for biocompactive soil sites, and using soil mounds created by termite colonies for bioturbative soils. The Kenya Long-Term Exclosure Experiment (KLEE) plots, which have electric fences to prevent faunal presence, were used as a fauna-free control soil. With regards to crack magnitude (volume, depth, aperture), we find enhanced cracking intensity with faunal presence. However, the structural nature of the formed cracks diverged according to the faunal influence: bioturbated soils exhibited a “surficial” crack network, with extensive cracks constrained in the topmost layers of the soil profile; while biocompacted soils exhibited a “systematic” crack network, with moderate cracks present through the length of the soil profile. However, despite these differences, the crack structures showed remarkable morphological and topological similarity when analyzed as mathematical graphs/networks. In the consolidated biocompacted soils, a mechanism for “soil memory” of prior drying-wetting events in the soil environment was theorized through the crack layering morphology. Four crack morphologies were outlined, the first three (straight, drifting, splintering) of which form through known mechanisms of crack fracture mechanics, and the fourth (disjointed) of which forms as a result of continuous mechanical disturbance and reconsolidation of the soil surface, which enables the “imprinting” of multiple wetting-drying cycle-persistent cracks at varying depth, whose morphologies are structurally unrelated to those currently present at the surface.
Chapter 5

Effects of Crack Morphology on Soil Carbon Flux Dynamics in a Dryland Vertisol

5.1 Background

Soil respiration is the largest and most variable terrestrial source of CO$_2$ flux to the atmosphere, with a net annual flux of 75-77 Pg C/yr [Raich and Potter, 1995, Schlesinger and Andrews, 2000] and an estimated increase of 3.3 Pg / yr [Follett, 2001]. Terrestrial carbon pools are estimated at 1550, 750-950, and 600 Pg C for soil organic/inorganic carbon [Batjes, 1996, Eswaran et al., 1993, Schlesinger, 1995] and terrestrial vegetation [Houghton, 1995, Schimel, 1995], respectively. Fluxes to and from them are highly sensitive to changes in climate [Cox et al., 2000, Raich et al., 2002] and understanding their governing mechanisms is crucial for delineating future changes to soil-atmosphere CO$_2$ flux.

However, the contribution to global atmospheric CO$_2$ by dryland soils, which
cover 40% of the Earth’s land surface [Reynolds et al., 2007], remains an open question due to its under-representation in the literature (e.g., [Bond-Lamberty and Thomson, 2010]). In many mesic ecosystems, soil respiration is typically modeled using exponential relationships between temperature, water content, and substrate supply [Davidson et al., 2006], showing co-varying relationships between precipitation and temperature in response to phenological changes [Davidson et al., 1998]. However, in dryland soils, these individual parameters may have separate temporal relationships [Reichstein et al., 2002, Tang et al., 2005]. In particular, dryland ecological processes exhibit a pulsed nature to precipitation and soil moisture, being tightly coupled to seasonal distribution of rainfall but also magnitude of individual rainfall events [Loik et al., 2004]. For example, re-wetting induced fluxes can be up to 5 times greater than from soils that were continually wetted [Fierer and Schimel, 2003, Huxman et al., 2004], often characterized as the Birch effect [Birch, 1958]. These pulses contribute to a significant portion of the total CO$_2$ efflux from dryland soils [Liu et al., 2002, Thomas et al., 2008, Xu et al., 2004]. This pulse is high but often short-lived, returning back to pre-hydrated levels within days [Thomas and Hoon, 2010]. Several biotic factors have been theorized, including soil organic matter exposure following break up of aggregates [Appel, 1998, Denef et al., 2001], the use of soil microorganisms that died during desiccation as substrate for the newly forming soil microfauna [Luo and Zhou, 2006].

Abiotic factors can also make a large contribution to soil-atmosphere CO$_2$ flux, particularly in the form of changes to gas transfer mechanisms. Diffusion occurs across all permeable interfaces [Allaire et al., 2008], and is often considered the dominant or only process for gas transport in low-permeability porous media [Moldrup et al., 2004]. However, under higher permeability ranges, advective processes can play a larger role [You and Zhan, 2013], and can even dominate fluxes [Massman et al., 2004].
et al., 1997, Weisbrod and Dragila, 2006. Specific examples of advective gas transfer include barometric pumping [Weeks, 1993, Weisbrod and Dragila, 2006], surface winds [Lewicki et al., 2003, Lewicki et al., 2007], and thermal convection [Nachshon et al., 2008, Weisbrod et al., 2009]. The consequences of these processes to CO$_2$ flux can be significant, as they often do not follow simple temperature/soil moisture relationships, display erratic and more variable flux distributions, and have fluxes several orders of magnitude higher than those typically observed with diffusion-dominated flux (e.g., Etiope, 1999, Sanchez-Canete et al., 2011, Breecker et al., 2013). Contributions to net flux can be variable: at times, these advective fluxes can directly vent into the atmosphere [Weisbrod et al., 2009], and at others they can occur entirely within the subsurface, only increasing concentration gradients and elevating diffusive fluxes [Levintal et al., 2017]. These complex dynamics are due to the close relationship between flux and macropore morphology, often in the form of faults or fracture [Kuang et al., 2013]. However, field studies have often focused on particular geological cases like those in rock faults [Weisbrod et al., 2009] or karst systems/subterranean caves [Sanchez-Canete et al., 2011, Kuang et al., 2013]. Often reliant on steep temperature gradients and regular shifts in moisture content or porosity, these gas transfer processes likely play a significant role in the alteration of flux in dryland soils [Kowalski et al., 2008, Serrano-Ortiz et al., 2010], but few studies have quantified their relative importance or temporal and spatial variation [Rey, 2015].

These processes may be particularly present and dominant in shrink-swell soils. Also known as vertisols, they have an extensive presence across the globe [Ahmad and Mermut, 1996], but are also primarily located in water-limited tropical and subtropical zones, with 80% of vertisols in Australia, India, and East Africa [Virmani et al., 1982]. Their high montmorillonite and smectite clay fraction results in high soil fertility and productivity (e.g., [Ikitoo et al., 2011]), but can also develop deep
soil cracks under drying conditions which persist throughout the soil profile through multiple wetting/drying events [Miller et al., 2010, Stewart et al., 2015]. These soil cracks display the permeability properties necessary for significant advective gas transfer (e.g., Kishne et al., 2009, Miller et al., 2010), and have exhibited anomalous and variable subsoil CO$_2$ concentrations, associated with subsidence behavior and thus crack development [Breecker et al., 2013]. Crack morphology and connectivity in the upper soil layer (0-0.25m), where most biogenic sources of carbon are located, have been shown to exhibit large variability despite similar soil moisture ranges due to biophysical effects [DeCarlo and Caylor, 2019], but to our knowledge, studies on cracked dryland soils that combine in-situ morphological properties and CO$_2$ flux dynamics have not been published.

Figure 5.1: Conceptual outline for the link between crack morphology and carbon flux in faunally active vertisols: (a-d) visual schematic flux processes in the four soil classifications outlined in [DeCarlo and Caylor, 2019], and (e) their theoretical inverse cumulative distributions, plotted in log-log space.

We outline a theoretical conceptualization of how these two elements may be linked, using [DeCarlo and Caylor, 2019] as a reference (Figure 5.1). Two major controls
associated with these biologically active vertisols are first defined: 1.) soil crack mechanics, and 2.) soil carbon availability. In the control (C) soils, where carbon availability is limited and cracking is minimal, fluxes are dominated by standard diffusion processes (Figure 5.1A). In the biocompacted (BC) soils, where the soil is biologically similar but deep cracks are present due to faunal activity on the surface, carbon availability is also relatively low, but fluxes are mechanically enhanced due to thermal gradients present during the night-time hours (Figure 5.1B). In the bioturbated (BT) soils, where termites are active, carbon availability is relatively enhanced, but fluxes are mechanically limited due to the insufficient depth of the otherwise wide cracks formed (Figure 5.1C). Finally, in the deep termite burrows of the BT soils, carbon availability is enhanced and are dominated by convective fluxes (Figure 5.1D). Figure 5.1F shows how these four permutations may result in different carbon flux signatures, given otherwise uniform conditions in terms of water content, microbial content, etc. Distributions here are shown in the form of inverse cumulative probability distributions. In mechanically-limited/carbon-limited cracked soil systems (C), we expect fluxes to result in a fairly uniform distribution due to relatively low-variability diffusive fluxes; assuming consistent termite activity, enhanced carbon availability (BT) would result in a higher-magnitude distribution that otherwise retains the diffusion flux signature. Shifting to low-carbon but mechanically-enhanced (BC) soil systems, we would expect periodically large fluxes as a result of the venting process interspersed between lower diffusive fluxes, resulting in a wide distribution of flux. A similar signature, albeit with a higher magnitude and range due to high carbon availability, would be expected in the carbon-enhanced variant (BT burrows). The objective of this present study is to experimentally elucidate these links between diurnal-scale CO₂ flux and different crack morphologies and connectivity, using the same soil systems and conditions.
<table>
<thead>
<tr>
<th>Date (2019)</th>
<th>Precipitation [mm/day]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wetting Phase</td>
<td></td>
</tr>
<tr>
<td>17 Jun</td>
<td>1.81</td>
</tr>
<tr>
<td>18 Jun</td>
<td>7.03</td>
</tr>
<tr>
<td>19 Jun</td>
<td>2.27</td>
</tr>
<tr>
<td>20 Jun</td>
<td>4.54</td>
</tr>
<tr>
<td>22 Jun</td>
<td>7.25</td>
</tr>
<tr>
<td>23 Jun</td>
<td>6.12</td>
</tr>
<tr>
<td>24 Jun</td>
<td>0.452</td>
</tr>
<tr>
<td>25 Jun</td>
<td>14.2</td>
</tr>
<tr>
<td>29 Jun</td>
<td>3.17</td>
</tr>
<tr>
<td>01 Jul</td>
<td>3.63</td>
</tr>
<tr>
<td>03 Jul</td>
<td>5.78</td>
</tr>
<tr>
<td>04 Jul</td>
<td>14.7</td>
</tr>
<tr>
<td>05 Jul</td>
<td>5.10</td>
</tr>
<tr>
<td>Drying Phase</td>
<td></td>
</tr>
<tr>
<td>12 Jul</td>
<td>0.452</td>
</tr>
<tr>
<td>28 Jul</td>
<td>3.52</td>
</tr>
<tr>
<td>29 Jul</td>
<td>2.38</td>
</tr>
<tr>
<td>30 Jul</td>
<td>1.70</td>
</tr>
<tr>
<td>31 Jul</td>
<td>4.53</td>
</tr>
</tbody>
</table>

Table 5.1: List of all precipitation events by date and magnitude during the course of experiments.

5.2 Materials and Methods

5.2.1 Crack Field Site Selection

Our soils were located on vertisols located in the Mpala Farm in Laikipia District, central Kenya. They are part of a semi-arid savannah ecosystem, with mean annual precipitation of 600 mm/year, though annual variability is high, ranging between 350-1000 mm/year observed in the past two decades. Peak rainfall is usually in April and November, with dry seasons in the summer and early winter. On 13 of the 19 days between June 17th to July 5th, an uncharacteristic series of small to moderate rainfall events took place, totaling 76 mm. Prior to this, the field site had not seen a major or minor rainfall event since May 13\textsuperscript{th} (25.7 mm) and May 24\textsuperscript{th} (1 mm), respectively. Afterwards, rainfall effectively ceased until the tail end of experiments. As such, we used this well-timed event to characterize the effect of a naturally-induced
precipitation and soil moisture pulse on crack-induced CO$_2$ flux. We classify the data collected prior to June 17th, between June 17$^{th}$ and July 5$^{th}$, and July 5$^{th}$ onwards as pre-wetting, wetting, and drying data, respectively (Figure 5.2A). We show the temporal distribution and magnitude of all precipitation events during the course of experiments in Table 5.1.

The vertisols in our field sites have a large native biodiversity presence, including over 40 large mammal species, and an extensive termite habitation. This variable faunal activity is capable of inducing divergent crack morphologies in the vertisol.
despite otherwise comparable soil physicochemical properties, including soil moisture and cation content [DeCarlo and Caylor, 2019]. The faunal biophysical activity (or lack thereof) and the resultant crack morphology is as follows:

1. Reference soil (C): these are termite-free soils dominated by patchy vegetation inside the Kenya Long-Term Exclusion Experiment (KLEE) plots [Young et al., 1997], which use electric fences to exclude all large herbivores from a 200 m × 200 m space. Relative to the other soil types investigated, crack porosity and maximal depth are minimal.

2. Biocompacted soil (BC): these are the long, narrow animal trails regularly used by various megaherbivores in the region over multiple years. Cracking in these soils are deep, with a moderate crack porosity and large maximal depth.

3. Bioturbated (BT) soil: these are the lenticular soil mounds formed by fungus-cultivating termites (Macrotermiteinae, Odontermes), which range anywhere between <1-10 m, and which are regularly distributed at a spacing of 50-100 m [Bonachela et al., 2015]. Maximal crack depth is low, but crack porosity is highest of the three soil types. Within the BT soils, termites also manually dig several deep, stable burrows used for entry and exit of their nests.

Within this classification, we chose 4 sites in the C soil and 6 sites in the BT soils, located in 3 of the 4 termite mounds used in [DeCarlo and Caylor, 2019], including 3 sites of BT burrows, located in the largest termite mound. Given the regular megafaunal presence and the mechanical consequences on intact crack morphology, our BC soil sites were more variable, and consequently more numerous. We measured a total of 17 sites, of which 5 commenced from the beginning of the season, 2 commenced 2 weeks after start, and the last 10 commenced after the end of the rain events, 1 month after start. Of these, 2 sites were trampled by megafauna during the season and were rendered unusable. Volumetric soil moisture content is measured
using a Hydrosense probe (CD/CS620, Campbell Scientific), immediately adjacent to the flux measurement. Due to equipment problems, we could not start making soil moisture measurements until the middle of the wetting phase of our experiments (Figure 5.2B).

### 5.2.2 Fracture Temperature Profile

We dug a square hole measuring $15 \times 15 \times 20$ cm in sandy red soil, covering all boundaries with sealed plastic tarp, and packed the hole with BC soil gathered from our field sites to create a stable model fracture measuring $2.5 \times 15 \times 20$ cm. The soil was collected dry, and then wetted to help create the appropriate shape, and left to dry and set for 5 days. During this time, cracks measuring $\sim 0.3$ cm in aperture formed along the face of the fracture, though the structure was largely intact. Note that as this soil was excavated, reconsolidated, and manually packed into the hole, it retains virtually none of the fauna-influenced structural properties characterized prior in the BC soils. We then coiled 5 thermocouples (Type T SLE – 0.81 mm diameter, Omega) each on 4 metal rods, with 5 cm spacing between each, and placed them 5 cm apart within the fracture, thereby creating a grid of temperature measurements at 5 cm increments in all directions. We record temperature using a datalogger (CR1000X, Campbell Scientific) and thermocouple multiplexer (AM25T, Campbell Scientific) every minute for 24 hours starting at 8h30 on August 11th, 2015. We assume temperature profiles between our model fracture and our field sites to be comparable, and thus valid for comparison.

We analyze the temperature profile of the model fracture to find potential evidence of mechanical enhancements to fracture air flux. Specifically, we use the Rayleigh number, which is a dimensionless number that compares buoyancy and viscosity, to evaluate the potential for fractures to induce free convection:
\[ Ra = \frac{\Delta T \alpha g k L}{\nu \kappa} \]  

(5.1)

where \( \Delta T \) (°C) is the temperature difference between fracture air across length scale \( L \) (m), \( \alpha = 0.00367 \) (1/°C) is the thermal expansion coefficient, \( g \) is the gravitational constant (m/s²), \( k \) is the fracture permeability (m²), \( \nu = 1.5 \times 10^{-5} \) (m²/s) is the kinematic viscosity, and \( \kappa = 2 \times 10^{-5} \) (m²/s) is the thermal diffusivity. Free convection in fracture will theoretically occur when \( Ra \) exceeds a critical value of \( 4\pi^2 \) [Lapwood, 1948, Nield, 1982], roughly equal to 40; the \( Ra \) value is linearly correlated with the temperature gradient and quadratically related to fracture permeability.

5.2.3 CO₂ Flux Measurements

Our CO₂ flux measurements were made between June 13th and July 29th, 2016 – thus measurements were made in the midst of the dry season. A steady-state, dynamic closed chamber system (14.7cm height, 15cm internal diameter) attached to an infrared gas analyzer (IRGA; LI-840, Li-Cor, Lincoln, NE, USA) and a miniature diaphragm pump (1.0L/min flow rate; 3014 series, Denver-Gardner, Milwaukee, WI, USA) was used for CO₂ efflux measurement. We made manual measurements 5 times a day, at 6h00, 12h00, 18h00, 22h00, and 2h00 the following day – a minority of time points are missing due to the logistical difficulty of making the number of measurements made every 4-6 hours. Measurements were taken by setting the chamber on the soil site for 2-4 min and recording measurements at 5 second intervals. Soil efflux rate was calculated using a least-squares fit on the rate of CO₂ concentration increase in the chamber for at least the last minute of data, corrected for mean experimental temperature and pressure, and the volume of the chamber. We only used measurements that showed a consistent linear trend in CO₂ accumulation within the chamber – a small minority of data where concentration rate was erratic was
We did not place soil collars into our soil sites. We justify this action given the unique experimental considerations of our study and vertisol properties, and how the known consequences will likely outweigh the potential benefits of collar insertion. Firstly, given how hard the soil becomes, it is logistically impossible to place soil collars into dry vertisols without destroying the crack structure we seek to study. Carefully excavating the soil to place collars is not an option either given the large soil peds formed by the dried vertisol – attempts to manually make smooth cuts into the soil requires breaking the aggregates, which will not only greatly disturb the soil structure, but also create large artificial “fractures” along both the inside and outside of the collar, due to the uneven fit of the collar. Inserting the collars when wet, while facilitating placement of the collars, is also not an option, as this would destroy the very crack structures we seek to study, and furthermore, would still create the aforementioned artificial “fractures” along the collar. As such, we opted to firmly but carefully push our chambers on top of our field sites and cover the surrounding 1m of the soil in all directions with a tarp sealed around the chamber, to minimize air entry and exit between the chamber and the atmosphere. Furthermore, we filled the crack on both sides outside of the chamber for approximately 10-20cm to further minimize air leakage and entry.

5.3 Results

5.3.1 Fracture Air Thermal Dynamics

Temperature values of fracture air measured in the model crack are shown by depth for the two central horizontal length positions, at x = 5 (Figure 5.3A) and 10cm (Figure 5.3B) – equipment failure for 0 and 15cm length precluded their data usage.
Like other typical soil temperature profiles, we observe high amplitude at the surface (x = 0cm), with larger daytime variability and smaller nighttime variability. Likewise, we observe low amplitudes and variability at 20cm depth for both horizontal lengths. Two sharp drops in surface temperature are observed at 12h and 15h, possibly due to cloud cover (no rain was observed on this day). A large upward spike of 12°C occurs at 12h46 at 5cm length, 10cm depth for 3 minutes, bringing it up to surface air temperature, after which it returns to its pre-spike value. As we do not observe it at any other depth and is for a few minutes, we assume it to be due to falling surface debris. However, several distinct phenomena are observed in our profile, mostly in the nighttime hours. The temperature profile at 5cm depth and 5cm length shows regular downward dips in temperature by 2-5°C, roughly between the hours of 18h and 8h. These events lasted for a single time point (1 min) and bring them to a temperature 1-2°C of that of the surface. We also observe regular upward spikes in temperature at 5cm and 10cm length during the same hours, though the magnitude is lower, at around 1.5-2°C. Finally, the temperature profile at 10 and 15cm are virtually identical at the nighttime hours for both 5 and 10cm length, only decoupling in the daytime.

We calculate maximum Ra observed at each soil depth as a function of crack aperture and compare them with crack aperture distribution of the BC soil sites analyzed in DeCarlo and Caylor, 2019 in Figure 5.4 - we assume that a similar temperature profile is present in both the model fracture and the BC soil sites. Mean crack aperture is 0.27cm, with 14% of crack apertures at 0.3cm and above, distributed as a long tail. Meanwhile, maximum Ra first exceeds that of the critical value at a crack aperture of 0.28cm, between 5-10cm depth, which corresponds to a fracture permeability of $6.53 \times 10^{-7}$ m$^2$ – the minimum crack aperture above which Ra first exceeds the critical value at all depths is approximately 0.6cm.
Figure 5.3: Soil temperature profile of a model soil fracture with dimensions of 2.5 × 15 × 20cm width, length, and depth, respectively, measured at (a) 5cm length and (b) 10cm length position. Temperature at depth was measured in 5cm increments.

### 5.3.2 Wetting Effects on CO$_2$ Flux

CO$_2$ mean efflux rates in the C and BC soils were comparable during the pre-wetting phase (Figure 5.5A-B). Following the initial series of precipitation events (June 17$^{\text{th}}$-20$^{\text{th}}$), CO$_2$ flux declined (June 21$^{\text{st}}$). After a second series of precipitation events (June 22$^{\text{nd}}$-25$^{\text{th}}$), mean efflux in the C soils increased and remained elevated through the rest of the wetting phase, with no decreases in mean flux observed. However, the BC soil flux initially spiked by a factor of 5, after which flux decreased, though at remaining at a flux rate higher than that of the C soil. In the drying phase, the C soils showed a steady decline in mean flux, with a slight uptick for the last two values, coinciding with the onset of rainfall on July 28$^{\text{th}}$. The BC soils, however, show more erratic behavior – the first week in the drying phase remains elevated and higher than those in the C soils, after which flux values decrease markedly below that of the C soils on July 19$^{\text{th}}$; the fluxes increase significantly once more, doubling those of the C soils between July 21$^{\text{st}}$-23$^{\text{rd}}$. No uptick is observed in the BC soils following the onset of rainfall at the end of the drying phase. While a rainfall event
Figure 5.4: Mean frequency and maximal Rayleigh number observed at each crack aperture. Mean crack aperture distributions represent the BC soil crack systems collected in [DeCarlo and Caylor, 2019] at 24.4% mean volumetric soil moisture. Temperature profiles obtained from the model soil fracture are used for Rayleigh number calculation.
occurs in the midst of the drying phase (July 12\textsuperscript{th}), the effects seem negligible, likely in part due to its small magnitude.

The BT soils show mean CO\textsubscript{2} flux that is considerably more elevated than those in the C or BC soils (Figure 5.5C-D). Pre-wetting phase fluxes vary by a factor of 4, while two large flux spikes are observed in the BT soils on June 30\textsuperscript{th} and July 7\textsuperscript{th}, 5 and 3 days after the two largest rainfall events observed in the wetting phase, respectively. Mean flux rates decrease during the drying phase, though values do not show a steady decrease like those in the C and BC soils and remain consistent. The burrows, which still are part of active termite mounds, show the largest fluxes overall – during the wetting phase, mean flux values are almost two orders of magnitude higher than even the BT soils themselves, with a large spike that doubles mean flux observed on July 5\textsuperscript{th}, a day after the largest rainfall event in the wetting phase. Mean flux values decrease just as dramatically in the drying phase, dropping an order of magnitude at the start of the drying phase, and decreasing to a value comparable to that of the regular outlier fluxes observed in BT soils near the end of the drying phase.

Mean flux rate changes do not highlight the change in CO\textsubscript{2} flux distribution observed in the soil types, which explain some of the variability observed in our results. BC and C soils show comparable distributions in the pre-wetting phase, and after the first series of precipitation events in the wetting phase. We observe that mean and median CO\textsubscript{2} flux values are relatively similar for these time points. However, following the second series of precipitation events, we begin to observe a shift in this trend: in the C soils, we observe an increase in mean CO\textsubscript{2} flux relative to that of the median, with outlier CO\textsubscript{2} fluxes higher than the mean flux by a factor of 2 observed immediately after the two major precipitation events in the wetting phase. This effect is considerably more pronounced in the BC soils – following the
Figure 5.5: CO$_2$ flux distribution of the (a) C, (b) BC, (c) BT, and (d) BT burrow soils over the length of experiments. Dotted lines indicate the boundaries between the pre-wetting, wetting, and drying phases. For each box plot, mean is shown by the square box with black edge, median by the white dash, the 25$^{th}$ and 75$^{th}$ quartile by the box (i.e., the thick central line), and the highest/lowest datum within 1.5 times the inter-quartile range by the whiskers (i.e., the thinner outer lines). All outliers are plotted with a + sign. Note the axis difference between the C and BC soils, and BT and BT burrow soils.
June 25th rainfall event, we observe outlier flux values that are an order of magnitude higher than the mean CO$_2$ flux, which was already double that of the C soil. A series of outlier fluxes that are 2-8 times larger than the mean flux are also observed after the July 4th rainfall event as well. Flux distributions diverge during the drying phase as well: in the C soils, while flux variability is higher than in the pre-wetting phase, overall magnitudes decline. In the BC soils, outlier fluxes that are higher than mean flux by a factor ranging between 4-20 persist throughout the drying phase. A slight uptick in outlier count and values are observed between July 21st-23rd, which account for the increase in mean flux during this time. No discernible differences in outlier count or value are observed at the tail end of experiments, where rainfall resumes.

The BT soils show a different distribution pattern from those observed in the C and BC soils. Outlier BT fluxes that exceed even the highest values observed in BC soil are present even during the pre-wetting phase – outlier flux magnitudes do not increase during the wetting phase, though a higher number of elevated fluxes are observed, as evidenced by the higher quartile values. Aside for an outlier flux observed after the July 4th rainfall event, the range of CO$_2$ fluxes observed during the drying phase are comparable to those observed in both the pre-wetting and wetting phase, though occurring at a higher frequency. The burrow flux distributions, on the other hand, show an almost step-like behavior – during the wetting phase, flux range is several orders of magnitude higher than those observed in all soils, after which flux values diminish to a level comparable to the rest of the BT soils. Like that of the C soils, the burrows flux distribution is unique in that it is not dominated by outlier flux presence, at least to the extent that the BC and BT soils are.
5.3.3 Diurnal Effects on CO₂ Flux

Diurnal CO₂ variability in the C and BC soils, and the BT soils and their burrows within the three moisture regimes is plotted in Figure 5.6 and 5.7. Measurements from the pre-wetting phase in the BT burrow soils, and from 2h in the wetting phase in the C and BT soils are missing due to either logistical or equipment issues.

In the pre-wetting phase in the C soils, we observe little variability between hour of day measured, though measurements taken at 6h are slightly lower. In the wetting phase, mean flux values are highest at 12h, with its range almost double that of those at the other time points. This trend diminishes slightly in the drying phase, though the highest outlier fluxes are observed at 12h. In the BC soils, we observe that mean flux and distribution are comparable to those in the C soils at all hours of the day save for those at 2h, whose flux values are higher than the others by a factor of 3. In the wetting phase, this trend is reversed – fluxes are roughly 3 times higher at 12h compared to the other time points, with the highest outlier flux value observed at this time point. In the drying phase, this trend diminishes, with outlier flux values observed at all time points, though the highest values are still observed at 12h.

BT soils do not show significant diurnal effects: during the pre-wetting and drying phase, outlier flux values are spread across time points; during the wetting phase, outlier fluxes are present at 6h and 12h. In the burrow soils, during the wetting phase, flux values seem to show significant divergence at 22h, where mean flux is approximately 3 times larger than at other time points, and with virtually no variability (n=2). Distribution magnitude decreases in the drying phase to a level comparable to that of the drying phase in the BT soils, and outlier fluxes are similar as well.
Figure 5.6: CO$_2$ flux distribution of the (a) C and (b) BC soils by time of day in the pre-wetting, wetting, and drying phases.
Figure 5.7: CO$_2$ flux distribution of the (a) BT and (b) BT burrow soils by time of day in the pre-wetting, wetting, and drying phases. Note the axis difference between this and Figure 5.6.
5.3.4 Soil Moisture Effects on CO₂ Flux

CO₂ flux variability in the soil types by soil moisture range is plotted in Figure 5.8. Note that this only compares fluxes from the second half of the wetting phase and the drying phase – no pre-wetting phase values are included here. Mean Flux values and distribution remain relatively constant between the soil moisture range of 0.2 and higher – flux values progressively decrease at soil moisture ranges of 0.1-0.2, and <0.1. In the BC soils, flux variability seems to increase with progressively lower soil moisture below 0.2-0.3, with the lower quartiles of CO₂ flux steadily decreasing. A large number of outlier fluxes are observed at soil moisture ranges of 0.1-0.2, and 0.5-0.6. BT soils show flux variability ranging between several orders of magnitude across all soil moisture ranges, and mean flux shows an exponentially increasing trend with higher soil moisture. A large number of outlier fluxes are observed at a soil moisture range of 0.1-0.2. BT burrow soils show a similar trend overall as well, albeit with higher values.

5.4 Discussion

Despite originating from the same vertisol in a single dryland environment within a single drying season, with individual sites all within 2-3 kilometers of one another, mean CO₂ efflux ranged from a minimum of 1.13 µmol m⁻² s⁻¹, observed in the BC soils 2 weeks into the drying phase, to a maximum of 1465.3 µmol m⁻² s⁻¹, observed in the BT burrows at the end of the wetting phase. Minimum mean CO₂ efflux rate is on the lower end of mean soil efflux rates in water-limited environments compiled in [Thomas et al., 2011], while maximum mean efflux rate is 4 times greater than its largest spike, which was measured after a wetting event of velvet mesquite [Sponseller, 2007]. It is evident that a multitude of soil physical and biological processes govern the complex CO₂ dynamics observed in our field site. Here, we use
Figure 5.8: CO$_2$ flux distribution of the (a) C, (b) BC, (c) BT, and (d) BT burrow soils by volumetric soil moisture. Note that these do not include fluxes from the pre-wetting, and the first half of the wetting phase due to lack of volumetric soil moisture measurements at this time. Also note axes differences between the C and BC soils, and BT and BT burrow soils.
the conceptual framework outlined in the beginning of this study (Figure 5.1), out-
lining the two major relevant controls: crack mechanics, which is abiotic in nature; 
as well as soil carbon availability, which is biotic in nature. Both operate on gradients 
that can either limit or enhance CO₂ flux. We outline the relevant environmental 
conditions that describe our CO₂ efflux results, and finally evaluate each permu-
tation (mechanically-limited/carbon-limited, mechanically-enhanced/carbon-limited, 
mechanically-limited/carbon-enhanced, mechanically-enhanced/carbon-enhanced).

5.4.1 Abiotic Factors: Soil Structural Mechanics

Thermal Convection

We find evidence that the temperature profiles and dynamics exhibited in our model 
soil fracture, which shares similar morphological properties to our BC soils, displays 
properties that reflect thermal convection. Acute dips observed in the temperature 
profile at 5cm depth, observed only at night, are likely to be individual cold air 
intrusions from surface air at localized points, as we only see them at the 10cm 
length. To further quantify this, we correlate minute-scale temperature fluctuations 
between spatially adjacent soil subsections using the Pearson correlation coefficient 
– spatially adjacent locations that both show simultaneous increases or decreases in 
temperature will have a positive value, while those that show opposing responses 
will have a negative value. The closer to -1 or 1, the stronger the correlation. 
Two strong correlations between fluctuations are shown in Figure 5.9A (positive 
correlation, between 5 and 10cm length at depth of 0cm at daytime) and 5.9B 
(negative correlation, between 5 and 10cm length at depth of 5cm at nighttime).

We plot the correlation between each spatial location with a line in Figure 5.9C and 
D for the day and night, where color indicates a positive or negative correlation – 
only strong correlations, which we arbitrarily set at an absolute value above 0.5, 
are shown. Daytime correlations show that increases or decreases in temperature
are positively correlated throughout the soil profile, likely due to natural gradual changes observed throughout the course of the day. At night, we observe a shift in the upper half of the soil: at 10cm length, between 0 and 5cm depth (hereby labeled as location 1 and 2), a strong positive correlation is observed, which means that the individual dips in temperature observed in the soil profile at location 2 correspond with dips observed only at the surface immediately above it. Note that no correlation is observed between location 2 and the other surface temperature site, which means that this effect is localized. Simultaneously, at the same depth, but at 5cm length (hereby labeled as location 3), we observe a corresponding increase in temperature, quantified by the strong negative correlation between the two locations. Note that as location 2 is positively correlated with location 1, location 3 is also negatively correlated with location 1 as well. This result suggests that cold air intrusion, initiating at location 1, penetrates the fracture to a depth between 5 and 10cm, which then displaces the warmer air below location 2 laterally and upwards, resulting in warmer air at location 3 and thus higher temperature. Given the strong correlation and our timescale, this process occurs within a minute-timescale. We plot convection cell magnitude and temporal distribution in Figure 5.10. We define the onset and cessation of a convection cell as when the flux fluctuation dips and rises above 0.5°C/min, respectively. A total of 121 individual convection cell events were observed, of which all lasted 3 minutes or less (N_{1min} = 113, N_{2min} = 6, N_{3min} = 2). The distribution of temporal spacing between convection cell events and the frequency of intrusion magnitude are exponential in nature, with a mean event spacing of 5.09 min and a mean intrusion value of 1.37°C/min.

Finger-flow patterns, whereby air is laterally displaced along the crack profile, is typical of fracture convection cells [Nachshon et al., 2008, Weisbrod et al., 2009], though our size and temporal dynamics vary. This is despite considerably steeper
Figure 5.9: Spatial correlation analysis of temperature fluctuations over time within the model soil fracture. (a) example of a strong positive correlation between fluctuations in temperature, observed at daytime between the 5 and 10cm length position and 0cm depth (i.e., horizontally along the surface). (b) example of a strong negative correlation between fluctuations in temperature, observed at nighttime between the 5 and 10cm length position at 5cm depth (i.e., horizontally within the fracture). Spatial maps of strong correlations ($\rho > 0.5$) within the fracture at (c) daytime and (d) nighttime. Figure 5.9A corresponds with the top-most line observed in Figure 5.9C, and Figure 5.9B corresponds with the second top-most line observed in Figure 5.9D.
Figure 5.10: Convection cell properties. (a) Distribution of time between convection cell events, with an exponential distribution fit shown. (b) Distribution of the magnitude of cold air intrusion within the fracture.

temperature gradients observed in our studies – surface measurements at night in our field studies dropped to around 15°C at night, creating a fracture gradient of 10°C over 20cm, approximately 2-4 times larger than those characterized above. Thus, differences may be in part due to differences in crack morphology – [Weisbrod et al., 2009] evaluated rock fractures that had a variable aperture of 1-5cm, length of 2m, and depth of >1m, while [Nachshon et al., 2008] used Hele-Shaw cells with aperture of 1-2cm, and 50cm length and depth. Similarly, minimum fracture permeability for thermal convection onset is 4 times smaller in [Levintal et al., 2017], which used artificial fractures with a depth of 1m but lower temperature gradients than our model fracture, further enforcing this fact. The regular onset and arrest of convection cells in our model fracture may be due to transient disruptions in the soil air temperature profile by the convection cells themselves, which serve as a natural negative feedback to the convection process – however, given the large surface area of the fracture, any cold air introduced in the soil is likely quickly warmed by the adjacent soil face, re-establishing the vertical temperature profile.
Larger fracture apertures show a non-linear increase in maximum Ra, which suggests that the magnitude of convection observed in the fracture, and therefore that of air displacement and flux, at all soil profiles, will also increase non-linearly. This will likely result in exponentially higher fluxes from larger surface crack apertures. However, the consequences to overall air displacement and flux are offset by the exponential decrease of progressively larger crack apertures observed in a typical cracked soil surface. Therefore, assuming a uniform distribution and immediate availability of a given gas in fracture, we can expect to see a highly non-linear distribution of that gas fluxes for soil sites affected by thermal convection, in the form of a multitude of small, non-convection influenced fluxes, and a small number of large, convection-influenced fluxes. Combined with the fact that convection cells may not necessarily reach the surface and be confined within the soil subsurface \cite{Levintal et al., 2017}, which still nevertheless enhance gas flux by reducing the diffusion length, we would expect to see a large spread in fracture-induced gas flux.

In addition to the deep soil fractures that characterize BC soils, BT burrows are likely to generate thermally-induced convection cells, which have the function of venting their natural habitats. Though the physical process is identical, wherein cold dense air displaces underlying the less dense hot air, the environmental conditions under which they form are slightly different – we expand further on this in Section 5.3.2.

Vertisol Morphology

Despite similar mean CO₂ flux observed during the pre-wetting and drying phase, a number of outlier fluxes ranging between 20-100 μmol m⁻² s⁻¹ are observed in the BC soils, only during the drying phase. This may be due to particularities in vertisol
crack morphology. [Breecker et al., 2013] observed a steep, step-like decline in subsoil CO$_2$ concentration, from 10% to 0.1%, with steady declines in soil moisture. This was hypothesized to be due to the formation of soil cracks, which increase vertisol porosity and decrease tortuosity. This increases the coefficient of variation in subsoil CO$_2$ concentrations to almost double that of non-vertic soils [Flechard et al., 2007, Breecker et al., 2013], and likely contributes to the characteristic flux distribution spanning several orders of magnitude observed in other faulted/fractured soil systems (e.g., [Etiope, 1999]). In addition, a soil crack layering effect has been observed in our BC soils, where surface disturbance and swelling events seal only the upper sections of the crack and leave a crack void space at relatively shallow depth of 0-0.5m [DeCarlo and Caylor, 2019]. As new cracks form on a drying surface, a series of sustained outlier flux events would occur as the advancing cracks progressively open and expose deeper and deeper void spaces. This may also explain why pre-wetting phase flux shows lower variability and virtually no outlier fluxes, as maximum shrinkage of the soil has been reached, and all easily accessible air pockets were exposed.

5.4.2 Biotic Factors: Soil Carbon Availability

The primary controls of soil respiration at the diel timescale are typically considered to be moisture pulse, temperature, and photosynthesis rate [Craine et al., 1999, Hogberg et al., 2001, Huxman et al., 2004, Xu et al., 2004, Tang et al., 2005, Baldocchi et al., 2006]. We assume photosynthesis rate and autotrophic activity to be negligible given the timing of the dry season and that virtually all annual grasses had died by this stage, and we also note that moisture pulse and temperature are physical processes that also have abiotic consequences, as highlighted elsewhere in this paper. However, we focus here on its biotic influences and consequences of soil carbon supply, availability, and utilization by heterotrophic communities.
Moisture Pulse

Soil CO$_2$ efflux in the C and BC soils show a positive response to precipitation events, though the magnitude between the two vary. Precipitation events, even those of light rainfall, have been shown to have a sustained increase in CO$_2$ efflux the persist over 24 hours, which contribute significantly to total CO$_2$ efflux from soils [Huxman et al., 2004, Liu et al., 2002, Xu et al., 2004]. Increases by a factor of 5-10 are usually observed only after a re-wetting pulse, and not when the soil was continually moist [Fierer and Schimel, 2003, Wang et al., 2007]. [Thomas and Hoon, 2010] artificially applied water to dry crusted soils – simulation of light rainfall (1.4 mm) resulted in a saturation depth of 0.004 m, which increased daytime fluxes from 2.6 $\mu$mol m$^{-2}$ s$^{-1}$ to a peak of 11.5 $\mu$mol m$^{-2}$ s$^{-1}$, a 4 factor increase, while heavy rainfall (120 mm) resulted in a saturation depth of 0.31 m and an increase in daytime fluxes to 59.3 $\mu$mol m$^{-2}$ s$^{-1}$, a 23 factor increase, and rainfall events of over 20 mm was characterized to be of sufficient size necessary to wet subsoil and result in sustained increases in CO$_2$ concentration beyond that of just superficial increases in flux. The largest rainfall events were 14.1 and 14.7 mm/hr, which are about an order of magnitude lower than the heavy rainfall simulation. However, soil cracks are likely to lower this minimum threshold – C and BC soils have shown a depth range of 0.01-0.08 m and 0.04-0.25 m, respectively, at a volumetric soil moisture range of 21-25% [DeCarlo and Caylor, 2019], with deeper depths likely observed under drier conditions, like the pre-wetting phase observed. Cracks act as preferential pathways for rapid water flow into the subsurface [Hoogmoed and Bouma, 1980, Romkens and Prasad, 2006], thereby bypassing absorption by the surface layer of the soil, and create saturation depths comparable, if not far deeper, than the ones observed in [Thomas and Hoon, 2010]. Soil moisture measurements recorded in our experiments reflect those at the surface, and may not reflect differences in soil moisture at depth.
Temperature

At the diel scale, the highest fluxes were observed in the day time (12h00) during the wetting phase, and to a lesser extent in the drying phase, for the C and BC soils. In the pre-wetting phase, this trend is no longer present – C soils show comparable mean flux throughout the day (though minimum flux is variable and fluxes in the early morning show slightly lower values), while BC soil fluxes show an increase in the nighttime (2h00), consistently showing a flux increases by a factor of about 2.

Temperature is positively correlated with soil respiration under wet conditions, but the correlation becomes weaker with lower soil moisture due to substrate limitation on autotrophic and heterotrophic sources [Skopp et al., 1990]. This soil moisture dependency for positive correlations with temperature is also present in dryland soils [Conant et al., 2004, Thomas et al., 2011], which has also been attributed to changes in soil microbial community activity. Low dryland organic C content, rapidly exhausted by microbial communities, in dryland soils is likely to play a role in the transient nature of surface and subsoil pulse of CO₂ after even heavy wetting. Dryland soils investigated in [Thomas, 2012] have total C of 0.39-0.56%, as opposed to our soils which have a total C of 1.6-2.4%, which may also contribute to some of the higher fluxes observed in our studies.

Termite Activity

The BT soils and their burrows exhibit different CO₂ characteristics, both in terms of magnitude and dynamics due to subsoil termite presence. The termite species present in our soils (M. Odontermes) create active subterranean colonies as deep as 2m below the surface and characterized by the cultivation of fungus gardens. In virtually all soils, they create hollow chimneys several meters high, which is capable of inducing the Venturi effect due to differentials in wind velocity at the top and
base of the chimney [Turner, 1994] – however, in our field sites, the chimneys are absent, with mounds less than half a meter tall [Pringle et al., 2010]. This is most likely due to the shrink-swell process unique to vertisols. Additionally, external winds can establish positive hydrostatic pressure gradients [Turner, 2001], but in African termite mounds, this has been shown to have no effect on CO$_2$ flux [Ocko et al., 2017], instead suggesting that free convection induced by thermal gradients in the soil profile is likely the primary mechanism for the observed elevated fluxes. These are likely further enhanced by increased subsoil temperature due to metabolic activity [Turner, 1994, Ganot et al., 2012].

We see evidence of convection in the BT burrows, particularly during the wetting phase, when CO$_2$ concentrations are elevated – CO$_2$ fluxes are lowest in the daytime (12h00), increasing in the evening (18h00) and peaking at night (22h00), after which fluxes gradually decrease (6h00). We also observe that daytime mean fluxes are lower than all mean night time fluxes. We note that the origin of the burrows created by the termite species is physically different from that of the fractures generated in the BC soils, and furthermore the particularities of vertisol morphology observed in the BC soils (e.g., soil layering effects) is unlikely to be present in these soils. However, functionally, these burrows play the same role in terms of soil-gas exchange, as they both mechanically enhance flux via thermal convection. Thus, we evaluate burrows in the context of fracture morphology, and furthermore evaluate both the BC and BT burrows soils as those with mechanical enhancements present. Theoretically, BT burrow morphology meets the minimum criteria for thermally-induced convection (Table 5.2), showing a fracture permeability of $7.5 \times 10^{-5} - 3.6 \times 10^{-4}$ m$^2$ if we approximate it as a fracture (using the burrow diameter as a proxy for crack aperture), 2-3 orders of magnitude higher than those observed in the BC soils. Large morphological variability is observed, however, with depth.
and diameter ranging between 0.1-1.03 m and 1-28.6 cm, respectively. Furthermore, due to a combination of termite activity and shrink-swell, burrows were regularly created and/or sealed, and at times we were able to observe burrows within some of the BT soil sites, primarily during the drying phase. These two factors are likely to contribute to the wide range in flux observed.

<table>
<thead>
<tr>
<th>BT site</th>
<th>N</th>
<th>R [cm]</th>
<th>d [cm]</th>
<th>k [m$^2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>1.9</td>
<td>44.2</td>
<td>1.2 $\times 10^{-4}$</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>1.5</td>
<td>42.1</td>
<td>7.5 $\times 10^{-5}$</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>1.8</td>
<td>39.2</td>
<td>1.1 $\times 10^{-4}$</td>
</tr>
</tbody>
</table>

Table 5.2: Statistics of all visible burrows at each of the BT sites analyzed. Measurements taken in August 2014. N is the number of burrows per BT site. R, d, and k are the mean radius, depth, and fracture permeability of the termite burrows, respectively.

CO$_2$ flux shows a nonlinearly positive relationship with soil moisture in the BT burrows, with fluxes during the wetting phase about an order of magnitude higher than those during the drying phase. Similar positive correlations were observed between CO$_2$ flux and soil moisture [Jamali et al., 2013], though not all species show this correlation. The relationship is less clear in the BT soils, which may be due to a combination of the hidden influence of BT burrows below the BT soil sites, which changes with time, and the negative correlation between decreasing soil moisture and increasing crack porosity and width, which increase the soil surface area available for diffusive fluxes. Several outlier BT fluxes that are comparable in magnitude to values observed in the BT burrows are present in the drying phase, which may further suggest that BT burrows contribute to BT flux, albeit at a lesser degree.

5.4.3 Integrating Soil Mechanics and Soil Carbon Availability

In the beginning of this study, we defined two primary controls in the context of CO$_2$ flux dynamics and distribution: soil crack mechanics, and soil carbon availability.
Limitations on either control can inhibit CO₂ flux magnitude and distribution in particular ways. Crack-induced enhancement occurs primarily by the extent of thermal convection, which is a function of crack depth due to the exposure of vertical thermal gradients in the soil. Larger crack apertures also simultaneously non-linearly increase CO₂ fluxes and are exponentially less frequent than smaller crack apertures, creating a long tail of outlier fluxes that can be orders of magnitude larger. Decreasing soil moisture increases both crack depth and aperture for a given soil and enhances flux, though outlier fluxes may still occur in undisturbed vertisols due to the sudden degassing of CO₂ from trapped crack air spaces in the soil layer. Soil carbon availability enhancement shows positive tendencies to precipitation pulse and shifting from wetting/drying regimes, a tendency seen in most water-limited environments. CO₂ flux rates in carbon-limited soils experience large pulses following rainfall events and the re-wetting of the soil, the magnitude and fluctuation of which is positively correlated with the intensity of rainfall. Diurnal temperature fluctuations can affect soil carbon production and flux as well, with warmer environments generating higher microbial activity, though a sufficiently high soil moisture is required. Meanwhile, termite activity and their cultivation of fungi can provide consistently elevated levels of CO₂ in an otherwise organically poor soil environment. Fluxes in these carbon-enhanced soils are much higher in magnitude. Note that the effect that these controls have on CO₂ flux are not entirely independent from one another (e.g., precipitation pulses, particularly heavy ones, can enhance carbon flux pulse, but may also seal sections of the soil crack system due to shrink-swell).

The consequences of how these affect overall distribution of flux are shown in Figure 5.11 where we plot the inverse cumulative probability distributions of the four soil classifications for each individual soil site. The results are mostly in agreement with our original conceptual framework. First evaluating the two extremes: in the
Figure 5.11: Inverse cumulative probability distribution for (a) mechanically-limited/carbon-limited (C), (b) mechanically-enhanced/carbon-limited (BC), (c) mechanically-limited/carbon-enhanced (BT), and (d) mechanically-enhanced/carbon-enhanced (BT-burrow) soils.
mechanically-limited/carbon-limited soils, flux range and magnitude were low at each individual site, while in the mechanically-enhanced/carbon-enhanced soils, site flux range and magnitude were both high; both soil types had low site-to-site variability. When both of the limitations on soil crack mechanics and carbon availability were removed, as was the case in the burrows of the BT soils, the carbon-rich soil air is consistently vented out of the soil system via convective processes. This extended the CO$_2$ flux magnitude to its maximal range, and reduced site-to-site variability; a wide range of flux is observed due to the periodic nature of the convection process. In the mechanically-enhanced/carbon-limited soils, we observe an intermediate flux range, magnitude, and site-to-site variability; although all sites exhibit a wide range of flux, which suggests a convection process, flux magnitudes are variable, with some (but not all) sites over an order of magnitude higher than the mechanically-limited soils. In these cases, limitations on carbon availability mean that crack enhancement cannot consistently provide high fluxes of CO$_2$ rich air. However, in the mechanically-limited/carbon-enhanced soils, half of the measured sites exhibit a diffusive flux signature and magnitude identical to that of the mechanically-limited/carbon-limited soils, whereas the other half exhibit a convective flux signature with a magnitude between that of the low- and high-carbon soils. We hypothesize that this may be due to the regular termite-induced structural changes occurring at depth. We qualitatively observed the regular cycling of old and new burrows within active termite mounds, with activity particularly increased following rainfall events; it is likely that similar activity is taking place at depth, where burrows may become topologically linked with the superficial crack network, inducing a muted convection response. Even if the burrows are not topologically connected with the crack network, the reduced distance between these two morphologies can reduce the effective diffusion length necessary for outflux into the atmosphere [Levintal et al., 2017], which may also result in a muted version of the high-carbon convection signature. That there was no effect of high
carbon availability on flux magnitude in these soil sites suggests that a majority of the carbon produced by the termites may be vented through their burrows, and the flux measured is what the soil would have contributed regardless of termite presence. An amended conceptualization of the effect of these two controls on cracked vertisol flux is shown in Figure 5.12 – as can be seen in Figure 5.12C, if newly formed termite burrows are in close proximity to, or even connected with, the superficial crack network, the associated convective processes may dominate the otherwise diffusive flux signature of the BT crack network.

5.5 Conclusions

We show that crack morphology in faunally active vertisols enhances CO$_2$ flux by several orders of magnitude. This enhancement is primarily governed by crack depth and aperture, which enhances the onset of thermally-induced convection within the fracture. However, this enhancement is also modulated by the biotic dynamics present in dryland ecosystems, which is characterized by low soil carbon availability. These fluxes show pulse behavior to precipitation and soil moisture increase, most likely due to the activation of soil heterotrophic communities. In certain sections of the soil, termite colonies can provide a persistently high source of soil CO$_2$ otherwise
not present. The interaction of these two axes can produce distinct CO$_2$ flux distribution profiles: we see a large increase in flux range and magnitude when simultaneously shifting from a mechanically-limited/carbon-limited soil to a mechanically-enhanced/carbon-enhanced soil, with both exhibiting minimal site-to-site variability; while mechanically-enhanced/carbon-limited and mechanically-limited/carbon-enhanced soils exhibited intermediate flux signatures. Our results highlight how soil crack and macropore morphology can play a large role in the selective modulation and enhancement of CO$_2$ flux dynamics in dryland ecosystems.
Chapter 6

Concluding Remarks

In this chapter, I outline the primary research questions in this dissertation, and how the findings and conclusions in Chapters 2-5 answer them in a larger context. I then outline major contributions to the greater scientific community as a result of my dissertation. Finally, I outline potential avenues for future work.

6.1 Research Conclusions

The primary research objective of this dissertation was to provide mechanistic explanations for feedbacks between soil physical structure and ecological functions in dryland soils, using mathematical morphology and topology. To address this, I investigated the role of certain ecosystem engineers and their impact on these soil physical-ecological feedbacks.

The first two chapters focus on the fine roots of plants as ecosystem engineers and their active interaction with the soil physical environment via the rhizosphere. In Chapter 2, I provided the plant and soil sciences community with a user-friendly image processing software package for the morphological segmentation and analysis of roots in soil, as well as the measurements of associated biophysical properties. In
Chapter 3, I applied this software package to investigate how differential changes in root diameter can impact soil water properties and dynamics within the rhizosphere. Here, I observe a dominance of water uptake by the finest roots (<0.1 cm diameter), and water content plasticity in response to variable soil water conditions, both independent of species. These two chapters highlight the impact of flora and the soil structural and functional linkages induced by them: they show how changes in root exudation behavior, which varies by root morphology, influences rhizosphere and bulk soil water content distribution, which in turn influences rhizosphere water retention and soil water uptake by roots.

The next two chapters focus on the biophysical consequences of soil macrofauna and megaherbivores as ecosystem engineers in a Kenyan dryland vertisol. In Chapter 4, I show how macrofauna-induced bioturbation and megaherbivore-induced biocompaction can create divergent soil crack morphologies relative to a faunally-unaffected vertisol. Compared to no faunal influence, which shows minimal cracking in both volume and maximal depth, biocompacted soils have deep crack morphologies that have moderate aperture. Meanwhile, bioturbated soils have shallow crack morphologies with wider apertures. In Chapter 5, we show that these differences in soil crack morphology can create highly complex CO$_2$ dynamics. Fluxes in non-faunally impacted soils, which are limited by both soil mechanics and soil carbon production, are characterized by relatively low flux variability and magnitude. Meanwhile, fluxes in biocompacted soils are mechanically enhanced by thermal convection but limited by low soil carbon production during drying periods. Bioturbated soils show similar moderate limitation, with fluxes enhanced by high soil carbon production due to termite activity, but mechanically limited by their shallower crack morphologies. Fluxes in both are characterized by a relatively low mean flux that is punctuated by outlier fluxes that are orders of magnitude higher. However, when both abiotic (e.g.,
soil mechanics) and biotic (e.g., soil carbon production) limitations are removed, as is the case in the termite burrows of the bioturbated soils, fluxes become persistently high at the outlier flux magnitude, with low variability. These two chapters highlight how even indirect changes by faunal ecosystem engineers to soil physical structure can have cascading impacts on coupled physical-ecological processes in soil, exemplified by soil $\text{CO}_2$ flux.

In both case studies, analysis of soil morphology and topology is shown to provide mechanistic relationships between soil physical properties and ecological organisms, processes, and functions, and reveals how one shapes and is shaped by the other.

### 6.2 Contributions to Knowledge

The application of morphological and topological metrics to specific case studies within the physical-ecology interface in dryland soils has enabled several contributions to the literature.

The software package outlined in Chapter 2, publicly available at [https://pypi.org/project/rootprocessing/](https://pypi.org/project/rootprocessing/), effectively lowers the barrier to entry for the plant and sciences community that wishes to do more with their image data, enabling them to conduct morphological analysis of in-situ root and soil water characteristics, as well as integrate these plant and soil properties into study of the rhizosphere. Digital image processing, particularly that of morphological and topological features, can extract a multitude of metrics but also requires specialized skill and expertise, and this package attempts to remove some of these requirements. Furthermore, our package provides a simple but effective segmentation procedure of in-situ roots in soil, which is a fundamental challenge in digital image processing (e.g., Zhang et al., 2014).
in the literature, and our package outperforms similar publicly available software by a wide margin (c.f., Figure 2.6). The segmentation and software analysis features have already been used in a co-authored publication, which analyzed changes in water uptake by a drought-affected root system [Dhiman et al., 2018].

Broadly speaking, an improved segmentation enables us to characterize in-situ root morphological properties like root diameter directly within the context of associated soil water properties. This in turn enables a generalized morphological analysis of the rhizosphere, which forms the basis of Chapter 3. Biophysical properties of the rhizosphere (e.g., water retention, aggregation, etc.) directly control regulation of nutrient and root water uptake, but show complex plasticity response to soil water conditions [Carminati and Vetterlein, 2013]. Exudate characteristics of the rhizosphere also seem to vary by species (e.g., [Razavi et al., 2016, Ahmed et al., 2018]). In this chapter, I show that these differences in rhizosphere water characteristics and dynamics between five species disappear when sorted by differential changes in root diameter, and that the finest roots dominate water uptake, again independent of species. Chapter 3 contributes to the literature by suggesting that many of the species-variable rhizosphere processes regarding exudation behavior and its soil physical consequences are explained by differences in plant root morphology distribution.

Chapter 4 reveals the hidden abiotic consequences of fauna (soil macrofauna, megaherbivores) on soil crack morphology in a Kenyan vertisol. Ecosystem engineers are known to have wide-ranging effects on their local habitats, though their abiotic effects on soil structure are less studied, and to our knowledge, never studied on soil fracture. I show for the first time a combined direct visualization and morpho-
logical/topological characterization of field-scale (centimeter-meter scale) fracture networks due to the indirect influence of biotic faunal processes. In addition to metrics of crack depth and porosity (e.g., Figure 4.6), I fully characterize the morphological and topological properties of the fracture network in both faunally impacted soil systems. This work has been published in the literature as DeCarlo and Caylor, 2019.

These morphological and topological differences in turn have been shown to have consequences on soil water and carbon cycle balances, which I show in Chapter 5. CO\(_2\) flux dynamics in drylands are an open question due to their under-representation in the literature (e.g., Bond-Lamberty and Thomson, 2010). Studies on the contributions of soil structural differences, much less those of cracks, are fewer still, though the significant impact of crack enhancement on CO\(_2\) flux has been observed in the literature (e.g., Etiope, 1999, Breecker et al., 2013). I quantify for the first time the presence and statistics of thermally-induced free convection within biologically active field-scale soil cracks (Figure 5.8, 5.9). I also relate for the first time direct changes in soil crack morphology and its interactions with variable soil carbon production in field-scale dryland soils. These interactions can either diminish or increase CO\(_2\) flux dynamics to levels comparable to the extremes typically observed in dryland soils (e.g., Thomas et al., 2011). I also outline a general framework of interplay between soil mechanics and soil carbon production in fractured soils and its effects on soil CO\(_2\) efflux dynamics.

### 6.3 Future Work

As this dissertation focuses on the application of mathematical morphology and topology, which are universal characterizations of form, within the soil sciences,
future work is multifaceted, and numerous other case studies focusing on different ecosystem engineers or processes can be proposed. Here, I focus on extensions of the case studies investigated in this dissertation.

First focusing on flora: the application of our software packages to other soil classes is a logical next step. Sample images used in Chapters 2 and 3 were transplanted root systems in sandy porous media. Heavily clayey soils or those with high organic matter content, both of which will have a higher attenuation response due to higher hydrogen content, can potentially diminish segmentation efficacy [Menon et al., 2007]. As such, more sophisticated segmentation procedures may be necessary (e.g., [Hoover et al., 1998]). Transition points with regards to rhizosphere water dynamics were observed at a water content directly associated with the residual water content of the porous medium used (Section 3.4.2), and as such may also vary with different soil water retention curves and porosity. Expanding on root morphological properties is another logical step. Root order characterizations are a widely sought-after topological metric of roots, but the small sample size limited the conclusions we were able to make regarding them. While notoriously difficult to conduct automatically, root order classification can potentially be conducted using root diameter intervals [Liu et al., 2018], and provides a natural extension to our present work, which also used root diameter as a morphological parameter.

Perhaps the most promising method for upscaling our work lies with the Fine Roots Ecology Database (FRED), which provides a global database of fine root (<2 mm diameter) observations and their associated environmental conditions and parameters, and which was established at ORNL in 2017 [Iversen et al., 2017]. 15% of measurements include root system architecture and morphology, and 4% of measurements, which still consists of 3000 measurements total, include root diame-
ter. Furthermore, the fine root diameter range outlined above is within the bounds investigated in Chapter 3. As such, the coupled physical-ecological morphological relationships elucidated here, both in the forms of the trends observed in Chapter 3 and in the new types of analyses that can be performed with the software in Chapter 2, can be quantified as parameters to be compared with other fine roots in a wide range of environmental conditions. This provides a fast-lane for integrating these mechanisms into the larger plant and soil sciences community, as well as a way to provide further mechanistic explanations for the environmental conditions observed in other fine roots of the world.

Secondly, focusing on fauna: it is evident that the abiotic changes of dryland soil crack morphology can now be added to the ever-increasing list of “rampant indirect effects” [Paine, 2000] of fauna, particularly those of large herbivores, in their role of ecosystem engineers. Arguably the most interesting aspect is the potential ramifications that fauna previously unassociated with the carbon cycle may have on carbon flux dynamics. Climate change within the next century is virtually certain, and with it will come increases in temperature, precipitation variability, and higher microbial activity, resulting in higher soil CO$_2$ production [Huang et al., 2016, Huang et al., 2017]. The major framework outlined in Chapter 5 was that soil mechanics and carbon production influence CO$_2$ flux dynamics in fractured dryland soils.

Several questions emerge from this outlined framework. The first is that of up-scaling and generalization. Vertisols comprise approximately 2% of global land cover, but 80% are located within Australia, India, and East Africa, all of which can be characterized as drylands, and at least two of these regions are experiencing significant changes in land-use and development (e.g., Ikitoo et al., 2011 [Ahmad and Mermut, 1996]). Furthermore, the soil crack morphologies seen in our field sites
are on the smaller end of extent and magnitude, and other vertisols can be several cm wide and meters deep [Ahmad and Mermut, 1996]. Is the above mechanism present or relevant in these types of vertisols as well, and if so, are they even larger than the ones seen in our field sites? And with its global distribution, are these regions sufficient to have global impacts on carbon dynamics with climate change? This latter point leads us to our second line of questioning, which is the consequences of changes in soil carbon production. If carbon production increases disproportionately in drylands, due to its relatively higher temperature increases, increased precipitation variability/intensity, etc., will this be enough to remove the carbon production limitation imposed in the organically poor dryland soils exemplified in our vertisols? If both limitations are removed, as will be the case if carbon production sufficiently increases, these soils persistently exhibit fluxes at that outlier magnitude, effectively resulting in an increase of CO₂ flux at a logarithmic scale. The removal of even one of these limitations can increase maximum CO₂ flux values by orders of magnitude. Further research is needed to answer these open questions.
Bibliography


