

**IMPACTS OF SOIL STRUCTURAL HETEROGENEITY ON  
BIOGEOCHEMICAL PROCESSES ACROSS SCALES**

by

Shane M. Franklin

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Water Science and Policy

Fall 2023

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## ACKNOWLEDGMENTS

What an experience! I have been afforded many privileges during my Ph.D. and throughout my life. I would have never made it to this point without my family's support from childhood to present. Thanks to my father for always telling me, "The world is your oyster." Thanks to my mother for her endless love. And thanks to my sister for giving me someone to look up to growing up and even now. Our weekly Sunday family video calls lifted my spirits and helped me through the following week.

It is with the sincerest gratitude that I thank my advisor Dr. Yan Jin and my co-advisor Dr. Bruce Vasilas. Thank you for giving me this opportunity, allowing me to follow my interests, and training me how to be a soil scientist. You have both helped me grow professionally and as a person. There are very few people that have invested the amount of time and energy in helping me achieve my goals as you have, and that, I will never forget. In addition, I would like to thank my committee members, Dr. Jeffrey J. Fuhrmann, Dr. Alexandra Kravchenko, and Dr. Rodrigo Vargas for their time and efforts in this process.

Others I would like to thank are Dr. Andrey Guber for serving as a collaborator and providing training on time-lapse zymography. Thanks to Dr. Chin Chen Kou for running the IC. Thanks to Dr. Taozhu Sun, Dr. Mohammad Afsar, Fatema Kaniz and Dr. Jing Yan for providing friendship, support, and scientific input. And of course, thanks to my dog Mav for always happily greeting me when coming home from the lab.

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## **ABSTRACT**

Understanding soil carbon dynamics is necessary to ensure long term soil health and improved understanding of soil-atmosphere interaction. Soil scientists are tasked with understanding how shifts in climate, a soil forming factor, will affect soil carbon stocks so that management decisions and predictions can be better informed. This is of significant interest and importance as shifts in weather patterns are already occurring. These shifts in climate alter soil structure in timescales as short as years, adding a new layer of complexity to understanding soil structure. To achieve the goal of better management and prediction, first, soil structure must be understood as a stationary arrangement. Second, the evolution of soil structure must be understood under varying conditions, however the latter remains a frontier. Finally, once sufficient evidence is gathered, the new information must be incorporated into mechanistic models, which implement a holistic framework.

This dissertation explores the aforementioned experimentally and with in-depth literature reviews. Model experimental systems were created to mimic macropore and preferential flow effects on oxygen dynamics and the spatial distribution phosphatase and  $\beta$ -glucosidase activity after glucose addition. Results show that oxygen

consumption and enzyme activity are greatest along macropore linings. This information along with preexisting literature was used to create a conceptual model relating pore size, flow, and carbon processing. In addition, this dissertation outlines how hydrologic connectivity of soil pore networks controls biogeochemical processes across scales. Furthermore, while connectivity and pore size are perhaps the most important factors in controlling biogeochemical processes, it has been found that either are rarely incorporated into current models. Therefore, a conclusion that connectivity and pore size distribution should be further evaluated and considered as model parameters for representing carbon turnover and storage can be made

## **Chapter 1**

### **INTRODUCTION**

The years of my Ph.D. (2017-2023) have been an exciting time to enter the field of soil science. Technological advancement of various imaging techniques and recent paradigm shifts have drastically changed the way that soil carbon dynamics are understood. For decades, the prevailing belief has been that soil carbon persists due to biochemical recalcitrance (Dungait et al., 2012; Lehmann and Kleber, 2015).

However, it has now become well accepted that connectivity and accessibility are the primary controllers of the fate of soil carbon. This has made the study of soil structure, the spatial arrangement of solids and pores (Juma, 1993; Letey, 1991; Rabot et al., 2018), increasingly important. Within this three-dimensional framework, water moves, the microbial habitat is shaped, and biochemical processes occur. Therefore, soil structure should be studied holistically, using methods from soil physics, biology, and chemistry to generate novel insights into soil functions such as carbon storage and decomposition (Baveye et al., 2018; Vogel et al., 2022).

Substantial progress on the understanding of the fate of carbon in soil pores has occurred, with pore size emerging as a significant controlling factor (Kravchenko and Guber, 2017). This provides a valuable step in generalizing pore scale biogeochemical

heterogeneity. However, heterogeneity occurs at every scale, creating one soil science's greatest challenges, from experimental design to upscaling (Baveye and Laba, 2015). Therefore, a significant focus of this dissertation has been on heterogeneity across scales. Of particular interest has been preferential and macropore flow, where a substantial amount of water passes through small volumes of the soil matrix (Beven and Germann, 2013, 1982; Flury et al., 1994). These flow paths, in some cases, have been documented to persist for periods as long as decades (Hagedorn and Bundt, 2002), which may explain why preferential flow paths have often been found to be enriched with carbon, microorganisms, and others (Bak et al., 2019; Bogner et al., 2012; Bundt et al., 2001; Chabbi et al., 2009). The unique characteristics of flow paths have unknown implications for macroscopic soil functionality, therefore the work presented here has been done in effort to shed light on the ubiquitous nature of flow induced resource gradients and explore what this could mean for soil functionality across scales.

Chapter 1 examines oxygen dynamics along artificially created preferential flow paths by using different textures of sand. A soil slurry with an addition of glucose was flowed through the sands and oxygen consumption by means of heterotrophic respiration was monitored using planar optodes. Results showed that oxygen consumption occurs at the textural interface between fine and coarse sand or the "macropore linings." In addition, oxygen profiles in soil aggregates have previously been found to have an anaerobic center with oxygen content gradually increasing as

moving outward to the exterior of the aggregate. In this chapter nearly identical oxygen profiles were documented in one of the treatments. Therefore, the work may provide mechanistic explanation for why oxygen gradients can be found in field sampled aggregates.

Chapter 2 provides a review of literature that ultimately leads to the creation of a conceptual model relating flow, pore size, and carbon turnover. The focus of the chapter, which also serves as the chapter title is, “The unexplored role of preferential flow in soil carbon dynamics.” Preferential flow has long been documented and has primarily been studied with the aim to understand how this phenomenon may control the transport of contaminants. However, here preferential flow is shown to play an important, yet poorly understood, role in shaping soil biogeochemical functionality. It is concluded that there is potential to upscale soil carbon dynamics if hydrologic connectivity across scales can be accurately determined.

Chapter 3 builds upon the conclusion found in Chapter 2. The role of hydrologic connectivity of soil pore networks across scales is further examined. Water scalars such as water filled pore space and matric potential are discussed with a primary finding that matric potential appears to be the most suitable water scalar to compare the biogeochemical function of different soils. This is because matric potential at a given value is equal in all soils and is related to pore size. This chapter expresses that pore size and connectivity are documented as key controllers of soil carbon dynamics, yet only a few models attempt to make use of these parameters. Ultimately, this

chapter continues to argue that further understanding of hydrologic connectivity across scales is needed to better understand and model soil carbon dynamics.

The dissertation ends with Chapter 4, which outlines overall conclusions and future directions. Appendix A shows incomplete work that builds upon Chapter 1. Another model system was created to induce varied degrees of preferential flow, this time using sieved soil rather than sand. Breakthrough curves were measured using  $\text{KBr}^-$ , a conservative tracer, for homogenous, 1 path, and 2 path treatments. In addition, the activity of  $\beta$ -glucosidase and phosphate was determined using time-lapse zymography. Like the oxygen profiles found in Chapter 1, enzyme activity was found to be greatest at the macropore linings following glucose addition. The results further express how dispersion and preferential flow can significantly shape microenvironments, which ultimately plays a role in carbon transport and storage across scales.

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## Chapter 2

### **MORE THAN MEETS THE DYE: EVALUATING PREFERENTIAL FLOW PATHS AS MICROBIAL HOTSPOTS**

#### **2.1 Abstract**

Preferential flow paths have been suggested to function as microbial hotspots. This idea is speculative, based on biomass counts rather than assessments of the activity of the microbial population. In this study we used O<sub>2</sub>-sensitive optodes, or optical sensor devices, to observe O<sub>2</sub> depletion by soil microbes as a reference for microbial activity along three artificially constructed preferential flow path geometries. The flow paths were constructed using contrasting fine (210–297 μm) and coarse (595–841 μm) sand textures in the geometries of (i) coarse sand in the center surrounded by fine sand, (ii) half coarse sand on the left and half fine sand on the right, and (iii) fine sand in the center surrounded by coarse sand. A soil slurry, containing soil microbes and glucose as an added source of C, was flowed through the sands to create nutrient gradients due to nonuniform flow. Results suggest that O<sub>2</sub> depletion is greatest along the boundary between preferential flow paths (coarse sand) and the bulk matrix (fine sand). While the results offer insight into the locations of microbial activity, the exact positioning remains unclear. Our work suggests that preferential flow plays a key role in the spatial distribution of microbial hotspots in soil and demonstrates the nexus between

soil physical and microbial processes. We show that O<sub>2</sub>-sensitive optodes were effective in monitoring O<sub>2</sub> depletion due to microbial activity along the paths. To address issues related to C cycling under the changing environment, biophysical processes in flow paths must be better understood.

## **2.2 Introduction**

Preferential flow is generally defined as the movement of water through preferred pathways, bypassing much of the soil matrix (e.g., Flury et al., 1994). This phenomenon has been studied extensively, however, most studies focus on the physical and hydrological processes associated with these pathways (e.g., characterization, quantification/modeling, and mechanisms of on-set) and their impacts on water management and contaminant transport (Jury 1999; Šimůnek et al., 2003; Jarvis et al., 2012; Guo and Lin, 2018; Nimmo, 2012; Beven and Germann, 2013; Beven, 2018; Gerke et al., 2010). Given the importance of preferential flow in soil and its contribution to water distribution, water retention, and transport of contaminants and nutrients, it is not difficult to imagine that preferential flow could play an important role in soil biogeochemical processes as well. This notion has been increasingly recognized (Liu and Lin, 2015; Morales et al., 2010; Hagedorn et al., 2015; Leue et al., 2017) and lends a new frontier in preferential flow research as the need for accurate biogeochemical models has become paramount in recent years.

Pioneering work by Bundt et al. (2001) sampled soil from preferential flow paths, marked with blue dye, and the matrix soil independently of each other. Results showed that the dyed flow paths were enriched in organic carbon, organic nitrogen, and microbial biomass, likely due to surface applied ash. Thus, preferential flow paths were termed continuous biological hotspots in soil due to favorable microbial living conditions associated with the paths, such as higher oxygen concentrations and a continual supply of water and fresh organics. Microbial hotspots are small volumes of soil with much faster process rates and intensive reactions than the average rates of the bulk soil (Kuzyakov, 2010). At these locations the active fraction of microorganisms is 2-20 times greater than the bulk soil and microbial process rates of soil organic matter (SOM) are 10-100 times greater (Kuzyakov and Blagodatskaya, 2015). Less quantitatively, hotspots have been described as locations where hydrologic flow paths converge with substrates or where flow paths containing complementary or missing reactants meet (McClain et al., 2003). The Bundt et al. (2001) conclusion, however, has been questioned and termed anecdotal and credited to biological history from decaying roots and biopores rather than hydrologic function (Tecon and Or, 2017). While roots and biopores have a noted role in carbon dynamics and hotspot development (Hoang et al., 2016; Dallinger and Horn, 2014; Kuzyakov and Blagodatskaya, 2015; Leue et al., 2018; Jarvis, 2007), other literature suggests hydrology and soil physical structure alone can create nutrient gradients and enhanced rates of microbial activity.

The results from Bundt et al. (2001) have been corroborated as higher concentrations of carbon have been found in tongues (Chabbi et al., 2009) and in the cracks of clay rice paddies (Fuhrmann et al., 2019). This was again the case in a coniferous forest, though here attributed to roots (Bogner et al., 2012). Further evidence shows that preferential flow paths could serve as locations that propagate antibiotic resistance in microbes due to the accumulation of antibiotics in the flow paths (Lüneberg et al., 2018). Additionally, macropores, commonly associated with preferential flow display high levels of biogeochemical potential. Pivetz et al. (1996) showed that a constructed macropore channel quickly degraded p-nitrophenol (PNP) despite rapid flow velocity. Macropore linings, primarily formed by shrink-swell processes, exhibited elevated sorption capacity, an increased resistance to desorption, and a large potential to mineralize pesticides (Mallawatantri et al., 1996). Similar results were found by Vinther et al. (2001). Decomposition of organic carbon varied in a systematic manner with more mineralization occurring in larger pores where fructose was added at the matric potential of -1 kPa, than pores inoculated at -3.15 kPa and -100 kPa (Ruamps et al., 2011). Furthermore, atmospherically connected pores, determined by X-ray computed micro-tomography, exhibited decomposition rates of particulate organic carbon (POC) 3-15 times higher than those that were not atmospherically connected (Kravchenko et al., 2015). Pore sizes of 40-90  $\mu\text{m}$  have been termed “easy come easy go” locations for carbon (Quigley et al., 2018), and a review paper indicates that pore sizes of 30-90  $\mu\text{m}$  may be the most important for the cycling of carbon (Kravchenko and Guber, 2017). Interestingly, macropores fall into the 30-90  $\mu\text{m}$  range at 75  $\mu\text{m}$  and

larger according to Soil Science Society of America Glossary (2008), though other sizes have been proposed making the term somewhat ambiguous (Luxmoore, 1981). Other studies conclude that the pore size is arbitrary, and air-water interfaces are the cause of increased rates of carbon mineralization (Strong et al., 2004; Bouckaert et al., 2013), but this too suggests preferential flow networks could play a key role in the carbon cycle as air-water interfaces will frequently be present in these networks.

Because preferential flow paths have been shown to be stable, persisting for decades in some cases (Ritsema and Dekker, 2000; Hagedorn and Bundt, 2002; Leinemann et al., 2016), assessing their biogeochemical function is necessary. In fact, Liu and Lin (2015) suggest that in order to accurately represent and predict hotspots and hot moments (high rates in time) of greenhouse gas emissions we must model and identify large-scale preferential flow networks, which will aid in the elucidation of chemical fluxes. Additionally, Smith et al. (2017) propose that carbon models should treat soil moisture as a network, emphasizing pathways for resource distribution and diffusion. Despite conclusions from the Bundt et al. (2001) paper and the significance of hotspots, few have attempted to examine the topic further (Rubol et al., 2014; Hagedorn et al., 2015). While the number of investigations on preferential flow has been on an upward trajectory since the 1980s, there continues to be a large number of unknowns associated with preferential flow resulting from theoretical and technological bottlenecks (Guo and Lin, 2018). These unknowns range from

quantification to predictive modelling, and while not mentioned here, the biophysical properties of preferential flow paths.

To examine hotspot development along artificially constructed preferential flow paths we employed an oxygen sensitive optode imaging system (Presens, GmbH, Regensburg, Germany). This allowed us to visually observe the development of anoxic regions arising from oxygen utilization by microbes along and adjacent to preferential flow paths. Our objectives were to 1) determine if preferential flow paths function as microbial hotspots and determine hydrology's role in hotspot development and 2) demonstrate the feasibility of using oxygen sensitive optodes to monitor oxygen depletion by microbes along artificially constructed flow paths. We hypothesized that anoxic regions would develop along the flow paths due to a carbon gradient created by preferential transport of input solution through the coarse sand.

## **2.3 Methods**

### **2.3.1 Chamber Experiment**

A custom flow cell composed of three separate chambers and a removable front panel was fabricated from polycarbonate (Fig. 2.1). In this experiment only the middle chamber was used. To ensure the chamber functioned as separate entity a rubber gasket was glued to the dividing walls so that when the front panel was screwed on an air-tight seal was achieved. The chamber dimensions were 6 cm x 1 cm x 17 cm and

contained a screened drain at the bottom. Sensor foils (SF-RPSU4, Presens GmbH, Regensburg, Germany) were glued to the inside of the removable front panel at three depths so that the sensor foils would be in contact with the sand. They were arranged 1 cm apart from each other and 1 cm from the edges of the chamber. To simulate preferential flow, we used contrasting fine (210-297  $\mu\text{m}$ ) and coarse (595-841  $\mu\text{m}$ ) sand textures so that flow would primarily take place in the coarse sand. The chamber was packed in three textural configurations while lying flat: 1) coarse sand in the center surrounded by fine sand, 2) half coarse sand on the left, half fine sand on the right and 3) fine sand in the center surrounded by coarse sand. Sand was packed around barriers made from cardstock paper to form the textural contrasts for each configuration. The barriers were lifted out upon completion and the sand was gently tamped to achieve consolidation. An additional barrier was placed at the top of the chamber to keep the sand in place. Once the chamber was packed with sand the front panel was screwed on while still lying flat. After the panel was screwed on the cell was turned upright and the barriers at the top were removed. The use of sand allowed us to control texture as well as functioning as a nutrient free medium so that carbon gradients would be created by preferential flow through the coarse sand as has been noted in previous literature. Additionally, while we have termed the 210-297  $\mu\text{m}$  sand “fine” it is still quite coarse and drained easily due to gravitational force at the conclusion of the flow event. Therefore, oxygen diffused readily into the chambers from the openings at the top and bottom (drain). Thus, regions of anoxia could be attributed to high microbial activity.

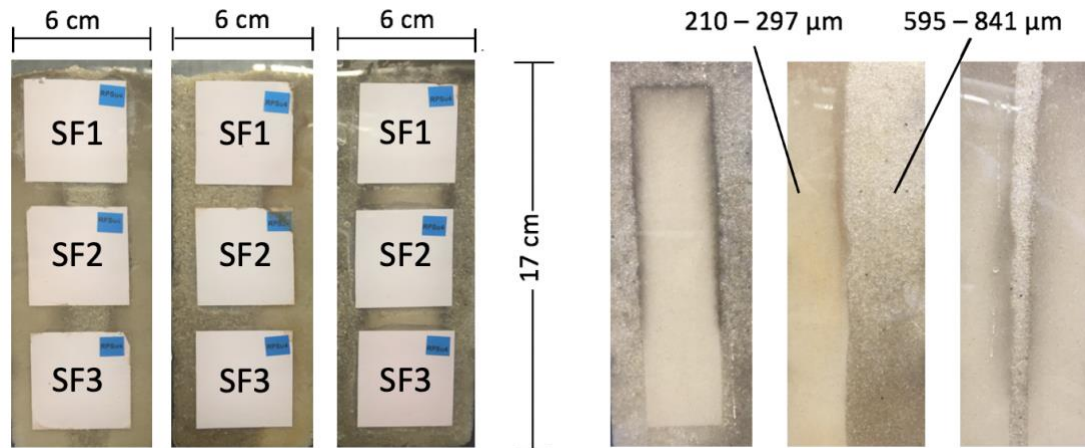


Figure 2.1. Schematic of the experimental chambers. Sensor foils (SFs) were located at 0 to 4, 5 to 9, and 10 to 14 cm. Foils had 1 cm of space on each side. The right-hand image shows the view of the backside where accumulation of input solution can clearly be seen at the textural interfaces.

### 2.3.2 Input Solution

To introduce a microbial community to the chambers 100 g wet soil was stirred in 1000 ml DI water overnight. The next day the soil suspension was removed from the stir and allowed to settle for 30 minutes. One-hundred ml (~3-4 pore volumes) of remaining solution was then collected. Average pore volumes were  $32.10 \text{ cm}^3$ ,  $29.30 \text{ cm}^3$ , and  $26.04 \text{ cm}^3$  corresponding to packing geometries 1-3 respectively. The volumes of fine and coarse sand used in geometries 1-3 were  $77.5 \text{ cm}^3$  fine and  $15.5 \text{ cm}^3$  coarse,  $46.5 \text{ cm}^3$  fine and  $46.5 \text{ cm}^3$  coarse, and  $40.5 \text{ cm}^3$  fine and  $52.5 \text{ cm}^3$  coarse. Glucose concentrations of 0.04 – 0.06 g were added to the solution to serve as a

carbon source for the microbes. This was done right before the initiation of each flow event so that microbial use of glucose when in suspension was negligible. A single flow event of 100 ml was selected to have more control of the flow regime, thus nutrient gradient, with total flow lasting ~45 seconds. This limited the total flow in the fine textured sand, providing a more representative simulation of preferential flow. Before initiating flow, the chambers were fully saturated. Once saturated, a Mariotte bottle applied the input solution at a constant head of 1 cm. Immediately after flowing the solution through the chamber, time series imaging at 15-minute intervals was initiated to monitor oxygen depletion due to microbial consumption over time (Fig. 2.2). The experiment was conducted at room temperature (21-23 °C) and the chamber was kept in the dark for the duration of the experiment.

### **2.3.3 Image Capture and Processing**

A USB-Microscope, “Visisens A1” (PreSens GmbH, Regensburg, Germany), was used to capture spatial heterogeneities in oxygen saturation from oxygen sensitive sensor foils (SF-RPSU4, Presens GmbH, Regensburg, Germany). The USB-microscope was externally positioned so that it was in alignment with the sensor foil. The LED lights contained in the microscope excite the luminescent oxygen sensitive and reference dye, contained in the sensor foil, which is translated into color images. For more detailed descriptions see Rubol et al. (2016); Holst and Grunwald (2001); and Tschiersch et al. (2012). To calibrate the camera a droplet of oxygen-free water,

made with 100 ml DI, 1 g sodium sulfite, and 50  $\mu$ L cobalt nitrate standard solution, was pipetted onto the foil. Then regions of interest were selected for 0% oxygen saturation (the droplet) and 100% oxygen saturation (free air). The wide view AT-4 adaptor was used to capture the trends of oxygen saturation. Time series images displayed in Fig. 2.2 were taken from the sensor foil location SF2 at 15-minute intervals. Due to the frequency of image capture, we were only able to monitor one sensor foil at a time, however, our set up offers the possibility to monitor activity at all sensor foil locations if images are taken manually, but that was outside the scope of this study.

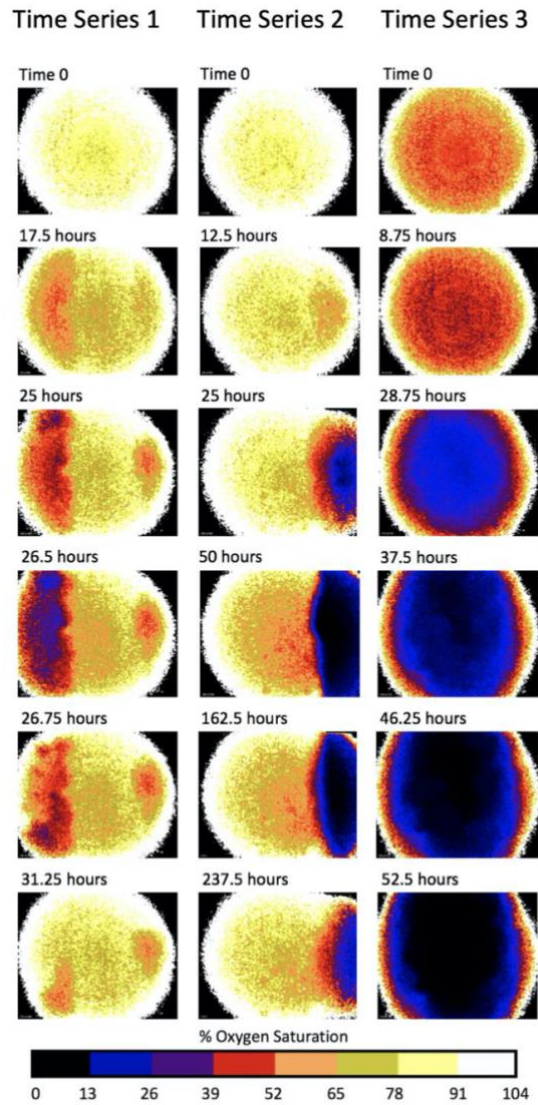


Figure 2.2. Oxygen maps with time for three textural configurations: Time Series 1, coarse sand in the center surrounded by fine sand; Time Series 2, coarse sand on the left half and fine sand on the right half; and Time Series 3, fine sand in the center surrounded by coarse sand. Darker colors represent lower oxygen concentrations and lighter colors represent higher oxygen concentrations. The frame of view is 3 by 2.5 cm at a resolution of 23.4 mm per pixel.

All images from the VisiSens AnalytiCal 1 software were converted to RAW image files using an application provided by the system manufacturer. This conversion assigned gray values for each pixel, resulting in files that could be uploaded to ImageJ open access software for analysis. To reveal the locations at which percent oxygen saturation was the lowest, time series image sequences were averaged and analyzed at 8-bit gray scale. For time-series 1, images 20-129 corresponding to the start and end of microbial activity (27.5 h) were averaged together. By excluding images prior to 20 the averages were not affected by periods where little to no oxygen consumption was occurring. Time-series 2 and 3 were analyzed following the same methodology, over images 91-200 and 40-139, respectively, although we did not record periods long enough to document the end in activity. Custom look-up tables (LUT's) or color scales were created to accentuate the variability in the averaged images. A selected region from the resultant averaged images was then used to plot the profile of oxygen saturation over distance (Fig. 2.3). This data was superimposed on idealized images of the textural configurations to clearly show the trends of oxygen saturation in space. To demonstrate spatial heterogeneities in oxygen saturation over time, the VisiSens AnalytiCal 1 software was used to select three regions of interest (ROIs) on individual images from each time series (Fig. 2.4 top). The data was then exported and plotted (Fig. 2.4 bottom).

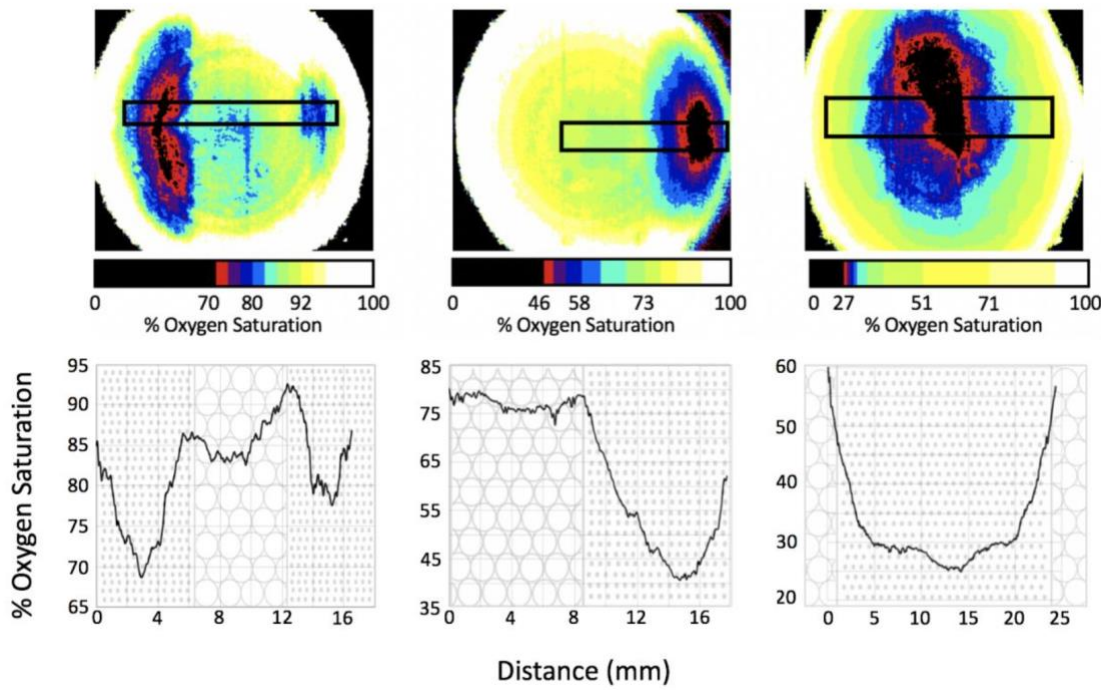


Figure 2.3. The average of a select 110 images from each time series. Color scales were made to accentuate the most oxygen-depleted regions. The color images correspond to the graphs below them. Each black rectangle is the selected area from which the graphs were created, showing oxygen saturation percentage with distance.

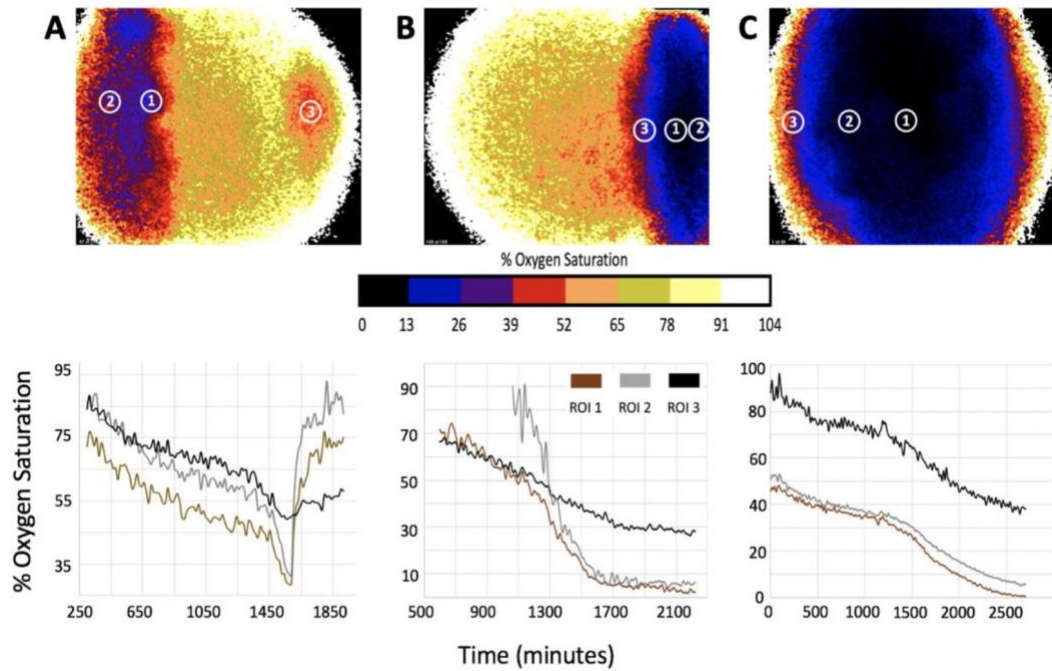


Figure 2.4. Trends in oxygen saturation with time for a select three regions of interest (ROIs). The ROIs are shown by the numbers on each image. Images A, B, and C correspond to the packing geometries 1 to 3, respectively. The most representative image from each time series is shown. Image A was taken at 1590 min, B was taken at 2250 min, and C was taken at 2925 min for each individual time series.

## 2.4 Results and Discussion

Time series image sequences in Fig. 2.2 show formation of anoxic zones due to oxygen consumption by microbes. The coarse sand flow paths conducted a higher volume of input solution than the fine sand as expected. This resulted in non-uniform spatial distribution of microbial activity and oxygen saturation likely as due to uneven

distribution of glucose and microbes. Rapid flow through the coarse sand coupled with limited surface area offered poor conditions for the accumulation of glucose and microbes in the flow paths themselves, and in our study the flow paths remained fully oxygenated throughout the experiment. Higher surface area and frictional forces at the textural interface caused slower velocities allowing accumulation in the fine sand. In both time series 1 and 2 show similar oxygen depletion patterns occur near and along the textural interface. In time series 1, oxygen depletion begins directly at the textural interface of fine and coarse sand. This is again seen in time series 2. Higher flow velocities in time series 2 due to half the chamber being coarse sand likely caused an increased occurrence of lateral transmission of the input solution into the fine sand, therefore widening the area of oxygen depletion. However, in the averaged images, we can still note that oxygen depletion is highest near the textural interface and becomes more oxygenated moving toward the edge of the frame of view for time series 1 and 2 (Fig. 2.3) confirming our hypothesis. In other words, activity is concentrated near and along the interface and activity does not continue outside of the frame of view. Time series 3 follows a different formation pattern as oxygen is depleted from the center first. While counterintuitive based on the oxygen depletion pattern, it is likely that microbial activity is in fact localized near the textural interface as was the case with time series 1 and 2, but due to the presence microbes on both sides of the fine sand, oxygen in the center is depleted first. While we did not directly measure microbial parameters, previous literature is in support of our conclusion. The observed oxygen depletion pattern we observed is nearly identical to oxygen gradients measured in soil

aggregates by Sexstone et al. (1985). This effect is demonstrated again by Borer et al. (2018) when examining carbon and oxygen gradients in an artificial pore network where it was found that aerobic microbes consume oxygen at the edge resulting in an anoxic center. Other work states aggregate surfaces are microbial hotspots (Kuzyakov and Blagodatskaya, 2015), and furthermore, a study examining the transport and fate of *E. coli* in the presence of preferential flow simulated using contrasting sand textures found significant cell retention at the textural interface (Wang et al., 2013).

The averaged images enabled us to determine what locations were most anoxic and measure the distances into the fine sand at which anoxia was occurring. For time series 1, oxygen depletion is greatest at 3 mm from the textural interface and activity clearly lessens moving away farther from the textural interface. In time series 2, oxygen depletion is greatest at 6 mm off the textural interface and then begins to become more oxygenated. In time series 3, oxygen depletion is greatest at the center of the fine sand at first and then becomes relatively uniform when reaching a quasi-stable state. It was noted that there is an additional centimeter of sand outside of the imaged frame view in time series 1 and 2, which should be highly oxygenated based on the trend of the averaged data. Additionally, with more significant textural contrasts than what was tested in this study, we would expect to see hotspots taking place more directly at the textural interfaces due to reduced transport into the fine material.

Our averaged time series results are supported by the ROI data (Fig. 2.4 bottom).

Images selected from time series 1 and 2 show that oxygen saturation over time is

lowest near the textural interface. The depletion of oxygen is not as intense when moving further away from the textural interface. Again, consistent with our averaged images, the ROIs selected for an image from time series 3 show that oxygen depletion is nearly uniform across the fine sand. Although duration of oxygen depletion varied between time series, between 1300-1500 minutes, oxygen consumption began to rapidly decrease in each case in our study.

Our results indicate that the effects of preferential flow extend beyond the physical processes of water flow and contaminant/nutrient transport, and are likely to play important roles in biogeochemical processes in soil. Work from Hagedorn et al. (1999) show that preferential flow paths exhibit enhanced rates of denitrification when wet, and early onset of nitrification and at higher intensity than that of the matrix soil. At microbial hotspots, the resultant oxygen gradients create micro-niche environments (Rubol et al., 2016). Carbon and oxygen gradients such as these have been shown to create intimate spatial segregation of aerobes and facultative anaerobes (Borer et al., 2018), which may offer explanation for what was seen by Hagedorn et al. (1999). Given that carbon and nitrogen in flow paths is often much fresher than that of the matrix (Bundt et al., 2001b; Chabbi et al., 2009) we can infer that it is more labile, and thus, more susceptible to microbial utilization. Nonetheless, we are aware of only one study that has attempted to link CO<sub>2</sub> emissions and preferential flow (Hagedorn et al., 2015), though results from greenhouse gas studies seem to offer analogous conclusions (Jarecke et al., 2016; Frouz and Bujalský, 2018).

Hydrology plays an important role in the formation and distribution of microbial hotspots. This is in agreeance with other work as water flow has been noted to play an important role on distribution of microbes and hotspots of reactivity at varying scales. A small scale lab experiment examining oxygen dynamics at a fluctuating capillary fringe found that *E. coli* were predominantly deposited in the transition region of the fringe (Jost et al., 2015). A review article terms ecohydrological interfaces hotspots (Krause et al., 2017), drawing conclusions similar to McClain et al. (2003). Modelling results for a heterogenous aquifer system also suggest that zones of higher conductivity result in hotspots of chemical reactivity due to enhanced mixing (Pool and Dentz, 2018). Microscale modelling work again suggests that biofilm distribution and biogeochemical activity is increased along preferential flow paths (Yan et al., 2017).

## **2.5 Conclusions**

We provide visual evidence that microbial hotspots are localized along and adjacent to preferential flow pathways. The use of the AT-4 wide view adaptor enabled observations of microbial hotspot development over a relatively large frame of view (3 cm x 2.5 cm) giving general trends of oxygen saturation in the system. It should be noted that we were only able to offer an estimation of the spatial distribution of microbial activity using this methodology. This is apparent from time series 3, where active microbes are likely coating the outside of the fine sand on opposing sides, thus

causing oxygen depletion to be highest in the center of the fine sand like what is seen in soil aggregates. Hydrology played a significant role on the spatial distribution of microbial hotspots. We do not discount the importance of root exudates and biopores in hotspot formation, but our results suggest that flow patterns arising from structural heterogeneities can also play a significant role in the spatial distribution of hotspots. Additional studies following our experimental design are necessary to assess how the microbial community arranges in the presence of preferential flow and more direct measurements of other microbial parameters should be addressed. Preferential flow paths function as dynamic biogeochemical entities, but the biophysical aspects of preferential flow paths remain largely unknown and deserve further investigation.

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## Chapter 3

### THE UNEXPLORED ROLE OF PREFERENTIAL FLOW IN SOIL CARBON DYNAMICS

#### 3.1 Abstract

Water is a crucial factor controlling the fate and processing of soil organics. Water commonly flows through the vadose zone via preferential flow pathways, resulting in nonuniform and rapid infiltration. Hence, a large portion of the soil matrix is bypassed. Preferential flow paths, often associated with well-connected macropore networks ( $>300 \mu\text{m } \emptyset$ ), offer a unique balance between water availability, nutrient delivery, and re-oxygenation upon drainage. The heightened concentrations of moisture, nutrients, and oxygen make these locations optimal for high rates of microbial activity. Flow paths often display temporal stability. This stability results in repeated wetting and biogeochemical reactivation through time creating a lasting impact on micro-environmental conditions relevant to microbial functioning and carbon cycling in soil. Despite decades of research on preferential flow, there is still a need to link flow paths and the resultant heterogeneous moisture distributions to soil function. In this review, we discuss how preferential flow can serve as a framework of reference for the spatially and temporally heterogeneous biogeochemical cycling of soil carbon. We highlight the importance of combining current knowledge of pore-

scale carbon dynamics with an appreciation of connected networks of hydraulically active pores/paths within the soil profile. Such combination opens new possibilities for upscaling pore-scale processes with the inclusion of resource heterogeneity at the macroscale. Working within this hydraulically connected framework can provide insight for the mechanistic representation of hot moments, which are temporally isolated large pulses of CO<sub>2</sub> after rewetting or thawing events. We conclude with suggestions on knowledge gaps and stress the critical need of linking soil physics with biology to mechanistically understand soil functions.

## **3.2 Introduction**

### **3.2.1 Emerging biophysical relationships**

In their landmark review, Beven and Germann (1982) posed the question, “How do macropores operate hydrologically?” highlighting the need to incorporate spatial heterogeneity into theoretical approaches to model water flow and contaminant transport in soil. Macropores are pores  $>300 \mu\text{m } \varnothing$  (Jarvis, 2007) that arise biotically from roots and worm burrows, or abiotically from shrink-swell processes and erosive subsurface flow. Even when constituting less than 1% of the soil matrix, macropores are capable of transmitting more than 90% of infiltrating water (Sanders et al., 2012; Watson and Luxmoore, 1986). The outcome of this non-equilibrium phenomenon, termed preferential flow, is that portions of the soil matrix are bypassed (Flury et al., 1994). In the years following Beven and Germann (1982), research on macropores

rapidly grew, generating important insights into their physical properties and associated hydrology (Luxmoore et al., 1990; McDonnell, 1990; Nielsen et al., 1986; Richard and Steenhuis, 1988). Interest in predicting and modeling preferential flow as a soil physical process has continued (Jury, 1999; Šimůnek et al., 2003; Guo and Lin, 2018; Nimmo, 2012; Beven, 2018; Gerke et al., 2010; Jarvis, 2016), but the role of preferential flow phenomena in soil biogeochemical cycling has so far received little attention (Baveye and Laba, 2015). We argue that despite decades of research on preferential flow, there is a need to link flow paths and the resultant heterogeneous soil moisture distributions to soil functions, especially, to biogeochemical processes.

One may ask, why does preferential flow need to be accounted for in studies of soil biogeochemical processes? After all, soil biology and biochemistry have already been making meaningful strides in understanding soil functioning without considering preferential flow. The short answer is – upscaling. Accurate upscaling of non-linear microscale biogeochemical processes that drive soil heterogeneity and their conversion into relevant predictions of macroscale functions has remained a "holy grail" of soil science (Baveye et al., 2018; Clark et al., 2015; Falconer et al., 2015). Translating the knowledge of key drivers of biochemical processes into whole soil profile predictions requires information on the spatial distribution patterns of such drivers. We highlight that preferential flow fundamentally affects the whole profile distribution of the most important of such drivers, soil moisture, and substantially influences carbon distribution and microbial activity as well.

While the role of soil moisture for individual biogeochemical phenomena such as soil oxygenation (Jost et al., 2015; Schlüter et al., 2018), microbial activity and motility (Skopp et al., 1990; Manzoni et al., 2012; Kleyer et al., 2020; Wang and Or, 2013), or soil respiration (Leon et al., 2014; Sponseller, 2007; Moore and Dalva, 1993) has been researched at length, a general understanding of the interplay between soil physical and biological processes remains fragmented and incomplete. The culprit is the prevalence of narrow intra-discipline foci. For example, soil physicists study soil moisture in terms of amount, flow speed, and the related transport processes (Nielsen et al., 2010; Wang et al., 2013; Villholth et al., 2000). Soil microbiologists are interested in how moisture affects microbial ecology (Wilpiseski et al., 2019; Zhao et al., 2019), and soil biogeochemists examine how soil moisture controls greenhouse gas efflux (Kim et al., 2012; Leitner et al., 2017; Warner et al., 2018). As we will discuss, the influence of preferential flow on soil functioning is a complex outcome of interacting physics, hydrology, geochemistry, and biology. Thus, further progress in studying them will require interdisciplinary efforts.

Water is crucial for the processing and mineralization of soil carbon. Thus, following the water as it preferentially travels through the soil profile may offer explanations for carbon dynamics that have otherwise been challenging to describe. Such an approach requires soil to be viewed as an interconnected 3-dimensional space (Smith et al., 2017) where individual pores combine to form larger connected hydraulic networks (Sidle et al., 2001) that control the overall fluxes of nutrients and elemental budgets in

catchments (Liu and Lin, 2015). Taking a 3D structural approach with a focus on hydraulic connectivity is of great importance as efforts to link biodiversity and microbial activity to soil function are “doomed to failure” without taking into consideration the physical habitat that controls resource delivery (Young and Bengough, 2018). Recognition of the importance of soil structure and connectivity of the liquid phase for carbon cycling is ubiquitous. However, efforts to integrate knowledge from various disciplines and across scales are not (Baveye et al., 2018).

### **3.2.2 Biogeochemical heterogeneity in the soil matrix**

The majority of ecologically relevant biogeochemical processes are thought to occur within microbial hotspots, i.e., small volumes of soil where carbon turnover rates can be 10-100 times greater than the bulk soil (Kuzyakov and Blagodatskaya, 2015). There is ambiguity in the terminology of hotspots (Bernhardt et al., 2017), which includes hotspots as locations of high activity within the soil (Kuzyakov and Blagodatskaya, 2015) or across the landscape (Leon, et al 2014). That said, there is increasing evidence that processes occurring in very active (i.e., “hot”) spatially defined locations within the soil matrix should be of primary interest when investigating biogeochemical processes. Heterogeneity in resource supply, e.g., fresh organic inputs from plant and animal sources, is the main cause of hotspot formation, thus, the main driver of micro-scale spatial heterogeneity in biogeochemical processes. The other cause is heterogeneity of the physical micro-environments where the

processes take place, which are largely dictated by the size and connectivity of soil pores. Recent attempts in identifying structure-function relationships at the pore-scale have attributed functional soil properties, e.g., carbon mineralization, to specific features of soil structure and pore architecture (Young and Crawford, 2004; Crawford et al., 2012; Nunan et al., 2017; Rabot et al., 2018). Bridging the analyses of hotspot activities with characteristics of the pores that surround them (e.g., Kravchenko et al., 2017), and elucidating microbial interactions with soil structure and the aqueous phase (Tecon and Or, 2017) are important steps in deciphering the drivers of soil biogeochemical heterogeneity. Unfortunately, it remains unclear how these relationships can be translated into meaningful predictions of macroscale soil function. While technical difficulties, e.g., coarse model resolution of upscaling plays a role, we suggest that the lack of accounting for perhaps the most dominant factor controlling moisture and resource distributions in soil – preferential flow, is a conceptual drawback halting progress for mechanistic models.

### **3.2.3 Preferential flow paths and carbon cycling: a baseline for new hypotheses**

We postulate that the following features make preferential flow paths crucial components of soil functioning: 1) substantial inputs of water and nutrients that likely stimulate microbial activity (Bundt et al., 2001a; Franklin et al., 2019); 2) temporal stability enabling reactivation for long periods, providing a defined network of pores where carbon cycling will most readily occur (Ritsema and Dekker, 2000; Hagedorn

and Bundt, 2002; Leinemann et al., 2016; Kapetas et al., 2014); 3) sizeable spatial extent, often spanning the entire depth of the soil profile (Chabbi et al., 2009; Bak et al., 2019); and 4) pore architecture beneficial for enhanced microbial activity as well as for soil carbon protection by means of increased aggregation and chemical bonding of microbially processed organic matter (Anthony et al., 2020; Cotrufo et al., 2013). Preferential flow paths are, in essence, spatially well-connected and highly active hotspots continuously operating within the soil profile (Bundt et al., 2001a). Furthermore, they offer links to subsoil regions resulting in horizontal stratification of the age and concentration of carbon distributions at depths (Chabbi et al., 2009; Fontaine et al., 2007; Rumpel and Kögel-Knabner, 2011).

Examination of preferential flow's impact on soil carbon cycling is becoming increasingly important. In the coming years, prolonged periods of drought caused by global environmental change are expected in many regions worldwide (IPCC, 2021). Drought conditions can induce increases in soil macroporosity and will likely contribute to an intensified hydrologic cycle (Hirmas et al., 2018). Increased macroporosity, in turn, can promote the occurrence of preferential flow. But how preferential flow will affect the hydrologic and carbon cycle remains difficult to predict. Therefore, addressing biogeochemical heterogeneity and potential climatic feedback loops linked with increased macroporosity and preferential flow will be an important step forward. In this review, we specifically focus on the role of preferential flow in soil carbon cycling as a biogeochemical cycle of major importance for the

Earth System (Falkowski et al., 2000) with pertinent policy implications (Clothier et al., 2008).

### **3.3 Preferential Flow**

#### **3.3.1 Types of preferential flow and associated moisture conditions**

Preferential flow refers to the uneven and rapid infiltration of water, which bypasses portions of the soil matrix. This phenomenon is omnipresent; a soil that does not exhibit preferential flow would be considered an outlier (Flury et al., 1994). Up to fifteen different types of preferential flow have been identified (Lin, 2010), but generally, by its origin and properties, preferential flow can be classified into three main categories: macropore flow, unstable flow, and funnel flow (Fig 3.1). *Macropore flow* primarily occurs along root channels, worm burrows, cracks, and fissures.

Macropore flow can also occur through voids created by termites (Chen et al., 2019; Jouquet et al., 2016), ants (Li et al., 2019), crabs (Xiao et al., 2019), and soil-dwelling mammals (Wilson and Smith, 2015), but for simplicity, we have chosen not to represent them in Fig. 3.1. *Unstable flow*, often referred to as fingered flow or fingering, occurs through paths that have formed when a uniform horizontal (stable) wetting front breaks into individual fingers. Unlike macropore flow, fingered flow is not directly dependent on soil structural properties and has even been shown to occur in homogeneously packed media (Wang et al., 2003a). Fingers arise due to flow-inhibiting interfaces such as pressure gradients, air entrapment, or regions of water

repellency (Nimmo, 2020). *Funnel flow* is driven by the redirection of water into distinct pathways due to sporadic textural heterogeneities and inclusions such as lenses (Kung, 1990). The occurrence, spatial patterns, and volumes of preferential flow depend on soil properties such as soil type, soil structure, antecedent moisture, soil water repellency, and others including rainfall intensity. Thus, the effects of preferential flow on soil biogeochemistry will vary depending on the flow regime and characteristics of the soil profile.

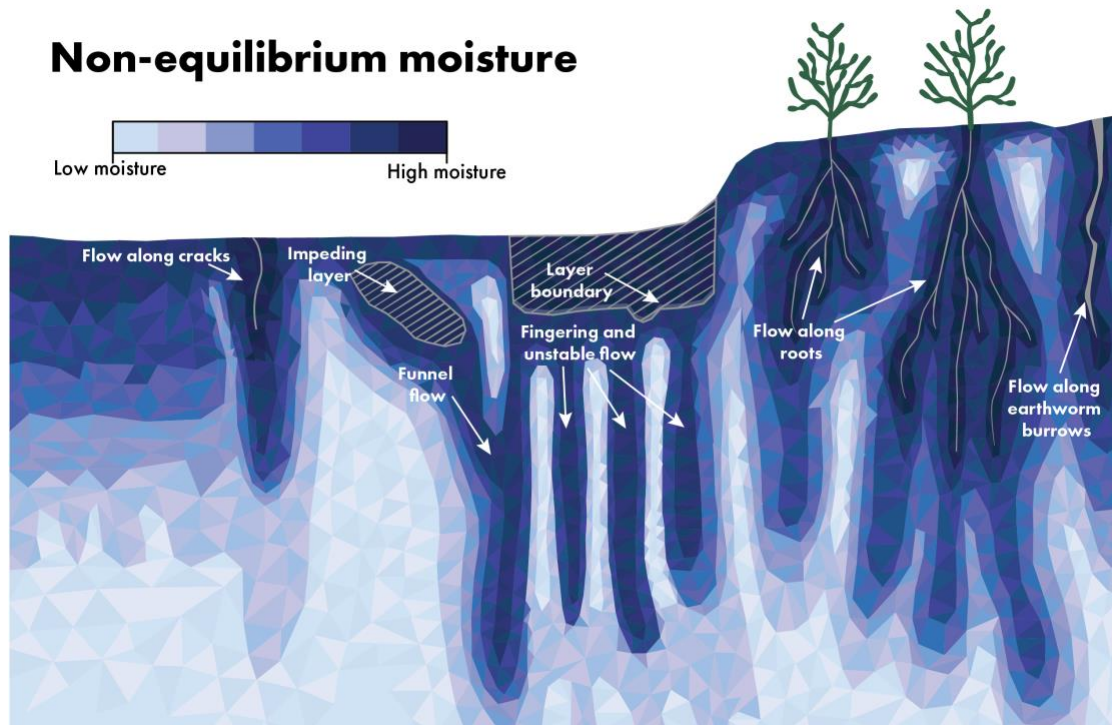


Figure 3.1. Heterogeneous moisture distributions caused by different types of preferential flow: macropore flow along cracks, roots and worm burrows, unstable flow, and funnel flow. Regions that have high interaction with water are dark blue, while regions with little interaction are light blue. Adapted from Hendrickx and Flury (2001).

Preferential flow paths can represent small percentages of the soil matrix but account for the transmission of considerable portions of infiltrating water (Watson and Luxmoore, 1986). Thus, preferential flow is a key mechanism controlling moisture and nutrient distributions in soil and should be expected to play a substantial role in shaping microbial ecology both locally and across the landscape (Bak et al., 2019; Tecon and Or, 2016; Wang et al., 2013; Young and Bengough, 2018). The moisture conditions a microbe may experience are quite diverse depending on its location in the soil profile and on the size (Nunan et al., 2003) and connectivity (Lee and Kang, 2020) of the pore space it inhabits. For example, hydrologic studies showed that ~80% of rainfall traveled through < 25% of the soil matrix (Quisenberry et al., 1993). Another study employing X-ray computed tomography on intact soil cores revealed that the hydrologically active macropore network accounted for less than 10% of the total porosity (Sammartino et al., 2015), and others showed that only ~30% of macropores significantly contributed to the infiltration process (Mori et al., 1999). This is noteworthy as the primary mode of nutrient acquisition for bacteria is often determined by the arrival of resources rather than motility (Nunan et al., 2020; Lehmann et al., 2020). The question remains, however, how do these small volumes of soil that transmit the majority of water and solutes shape the soil microbiome and overall soil function? We refer readers who have further interest in the physical and hydrological characteristics of preferential flow to other published reviews (Gerke, 2006; Beven and Germann, 2013; Jarvis, 2007; Nimmo, 2020). Additionally, a review

of the methods commonly used to study preferential flow can be found in Allaire et al. (2009).

### **3.3.2 Flow Path Stability**

In undisturbed soils, preferential flow paths often exhibit temporal stability, meaning that flow repeatedly takes place through the same pore networks (Quisenberry et al., 1994). This observation has been reported in field studies (Leinemann et al., 2016) and across scales (Lin, 2006). Despite temporal changes in pore size distributions, the overall structure of soil has a much longer “memory” (Targulian and Bronnikova, 2019). Layers of cutans (finely textured deposits) and mottling (redoximorphic features) coating macropore walls in well-structured soil are further evidence of the longevity and active functioning of flow paths (Beven and Germann, 1982; Quisenberry et al., 1993). The persistence of flow paths certainly depends on soil characteristics, structural development, and the presence of management disturbances, but as demonstrated via isotopic analysis, preferential flow paths in undisturbed soils can exist for decades (Hagedorn and Bundt, 2002).

Another cause for flow path stability is hysteresis in the soil moisture characteristic (SMC) curve where at the same matric potential the water content in the drying limb of SMC is higher than that in the wetting limb. The effect of hysteresis is evident in the physical evolution of fingered flow paths. A finger can be thought of as having two components, the finger core where the majority of flow takes place, and the finger

fringe, which is a small wetting zone that surrounds the finger core (Glass et al., 1989). Initially, the finger core and finger fringe have the same moisture content, but as the fingertip infiltrates downward, the pores in the finger core empty. At this moment, the finger core and the finger fringe have equivalent matric potentials. However, the finger core coincides with the drying portion of the SMC, whereas the finger fringe remains on the wetting curve (DiCarlo et al., 1999; Glass et al., 1989). This induces sustained lateral movement of water from the finger fringe back into the finger core, and thus the fingers remain narrow (Jury et al., 2003; Wang et al., 2003b). In addition to hysteresis, fingers have been shown to initiate where water repellency was the lowest (Ritsema and Dekker, 1994). Hydrophobic compounds may create near impermeable coatings around soil aggregates (Capriel, 1997) that reinforce the locations of flow paths. Furthermore, if the matrix soil outside of the fingers remains dry, reactivation of the fingers is expected to occur during subsequent rewetting events due to higher hydraulic conductivities offered by the previously wetted finger regions.

A third mechanism that may create flow path stability is microbial engineering through the excretion of extracellular polymeric substances (EPS) (Morales et al., 2010). For example, Rubol et al. (2014) observed the development of a preferential flow path due to bioclogging of the surrounding matrix. The reduction in porosity caused by cell proliferation resulted in the rapid preferential movement of water at 1.4 times the velocity of the matrix. The high flow velocity impeded microbial growth in the path itself, creating a positive feedback cycle where oxygen and nutrients were

channeled along the path, thereby further increasing microbial growth and activity at the periphery of the flow path. The presence of EPS helps maintain optimal conditions for microbial activity (Guo et al., 2018) and can create micro-hydrological niches by bridging multiple pores and creating continuity of the liquid phase upon soil drying (Benard et al., 2019). EPS increases soil water retention (Zheng et al., 2018; Benard et al., 2019; Guo et al., 2018) resulting in local micro-sites with higher water content. The increased water content combined with redirections of water from bioclogging and wetting instabilities (hydrophobicity) created by EPS, may generate a similar wetting effect (Morales et al., 2010) as previously discussed - the wetter, and more conductive, regions offer a path of least resistance for the next flow event.

The stability of macropores of biological origin can have seasonal (Schneider et al., 2018) and species dependencies (Capowiez et al., 2015; Felten and Emmerling, 2009). For example, anecic earthworms eat their way through the soil and eject surface casts creating compacted burrows (Lavelle, 1988), while endogeic earthworms are more likely to refill their burrows with their casts (Capowiez et al., 2014). The differences in life strategy impact the hydrology of the burrows (Whalen et al., 2015), but burrows of the compacted variety are expected to invoke an influence on hydrology and carbon dynamics as long as decades (Brown et al., 2000). Even plant roots can create stable macropores that last for years (Hoang et al., 2016). Despite documentation of flow path stability and multiple ascribed mechanisms, the persistence of flow paths and moisture distributions is poorly documented. Perhaps, this is because the traditional

belief has largely been that preferential flow is simply unpredictable (Jarvis et al., 2012). However, emerging evidence and theory suggest that due to the widespread occurrence of preferential flow across all soils, the existence of a persistent network of fast flow pathways is likely (Jarvis et al., 2016). Even in tilled fields, flow paths can form and remain stable for the length of a growing season or until physically altered (Ritsema and Dekker, 2000). The persistence of flow patterns and their interconnectivity remains challenging to describe (Wessolek et al., 2009), but there are important modeling implications that should be taken into consideration as quasi-stable states of oxygen saturation, nutrient gradients, bacterial organization, and others can result (Franklin et al., 2019; Kuzyakov and Razavi, 2019). Such stability supports the idea that soils behave in a self-organizing fashion, where order arises out of a seemingly random system (Borer et al., 2018; Crawford et al., 2012; Young and Crawford, 2004; Sidle et al., 2001; Beven and Germann, 2013).

### **3.3.3 Biogeochemical properties of preferential flow paths**

The stability of flow paths and their recurring activation can lead to local accumulation of nutrients within the flow path domains, ultimately influencing soil biogeochemistry and carbon cycling. Preferential flow paths often contain significantly greater concentrations of carbon and nitrogen as compared to non-flow path regions. This occurs in both managed and natural soils, with carbon concentrations in flow paths as much as 70% greater than the matrix soil in some cases

(Bundt et al., 2001a; Chabbi et al., 2009; Fuhrmann et al., 2019; Bogner et al., 2012). Moreover, the carbon and nitrogen associated with flow paths tends to be of recent age while those located within the surrounding soil matrix are several millennia older (Bundt et al., 2001b; Chabbi et al., 2009; Marin-Spiotta et al., 2011). Continuous inputs of new labile carbon into flow path domains (Leinemann et al., 2016) combined with the lack of physical protection of the added inputs due to high pore connectivity, offer conditions suitable for enhanced microbial activity, development of biofilms, and associated accretion of EPS (Yan et al., 2017).

Biofilms are hydrated dense clusters of cells composed of organisms having various geno- and phenotypes (Stewart and Franklin, 2008). While biofilms embedded in an EPS matrix play a role in soil hydrology and perhaps even flow path stability as discussed earlier, they also operate as key drivers of biogeochemical cycling (Stewart and Franklin, 2008). High levels of microbial activity and high rates of carbon mineralization within preferential flow paths (Hagedorn et al., 2015; Fuhrmann et al., 2019) ensure that they often function as microbial hotspots (Bundt et al., 2001a). As demonstrated experimentally, localized high rates of microbial activity can develop very quickly after preferential flow path establishment (Franklin et al., 2019).

Modeling work has further demonstrated that biofilm growth and activity are maximal along preferential flow paths (Yan et al., 2017). Preferential flow paths can also turn into localities with high rates of pesticide mineralization (Mallawatantri et al., 1996; Pivetz et al., 1996; Vinther et al., 2001; Dechesne et al., 2014; Monard et al., 2008;

Liu et al., 2011). The latter is likely due to high organic carbon concentrations allowing for increased sorption of pesticides in combination with exceptionally high microbial activity (Bundt et al., 2001b). Moreover, microbial communities have been shown to adapt to the prevailing conditions (Lüneberg et al., 2018), thus flow paths can become enriched in pesticides degraders (Monard et al., 2008).

Variations in microbial habitat characteristics within a flow path itself can be as large as those between the flow path and the adjacent soil matrix. Franklin et al. (2019) demonstrated using oxygen-sensitive planar optodes, that micro-environmental conditions within preferential flow paths can exhibit high degrees of heterogeneity not only in nutrient and carbon inputs but also in oxygenation. To simulate preferential flow, coarse (595–841  $\mu\text{m}$ ) and fine (210–297  $\mu\text{m}$ ) sands were used as proxies for macropore channels and the pores that surround them, respectively. After a single flow event containing glucose and a soil slurry (to introduce microorganisms), oxygen depletion resulting from microbial utilization was found at the textural interfaces, or “linings,” of the macropore channels (Fig. 3.2A and C). When flow occurred through coarse sand on both sides of the fine sand, oxygen consumption outpaced oxygen diffusion resulting in an anaerobic center in the fine sand (Fig. 3.2B), like oxygen depletion patterns found in soil aggregates (Sexstone et al., 1985). As soil drains a “big gulp” occurs, which describes the downward suction of oxygen (Jarecke et al., 2016). Water flow through macropores often occurs in films, meaning the pore space is not entirely full of water (Nimmo, 2020), allowing for quick gaseous exchange. In

addition, macropores cannot hold water against gravitational forces (Soil Science Society of America, 2008) so they and the pores nearby are the first to become oxygenated upon drainage. Thus, macropore networks that have just experienced a nutrient-laden water flux will have the first opportunity to mineralize carbon upon drainage. Further exploration of the spatial patterns in microbial activity and oxygen gradients caused by soil structure and resource delivery may help in understanding community organization and the formation of anaerobic microsites (Keiluweit et al., 2018; Owens et al., 2017).

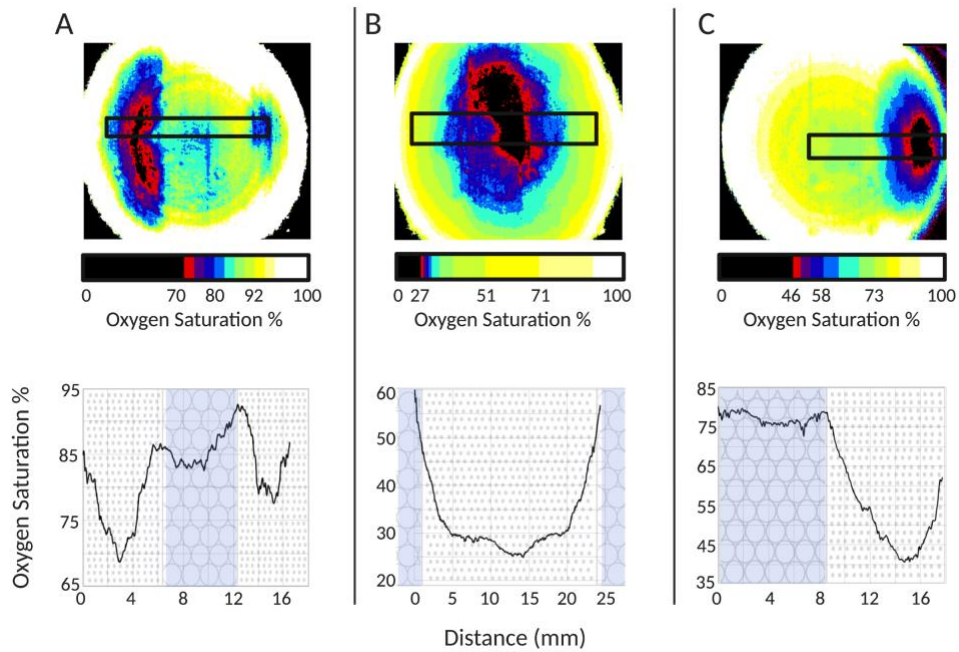


Figure 3.2. The top row shows averaged oxygen depletion over time and highlights the creation of unique and dynamic microbial niches. Color scales were made separately to accentuate the averaged differences in oxygen saturation. The black rectangles are the regions of interest that are plotted on the second row of graphs that express spatial oxygen saturation in the two sand types. Panel A shows coarse sand (circles) surrounded by fine sand (dots) on both sides. Panel B shows fine sand surrounded by coarse sand on both sides. Panel C shows half coarse and fine sand. Flow (shaded blue) primarily took place in coarse sand. Adapted from Franklin et al. (2019).

The formation of microbial activity gradients in flow paths of non- biological origin is highly dependent on flow velocity (Rubol et al., 2014) and the delivery of nutrients. As a result, not the entire body of the flow path functions as a microbial hotspot.

Rather, the smaller pores adjacent to the main flow path that allow for the accumulation of nutrients and protection from high flow velocities (Nunan et al., 2003) become the locations where microbial activities are the highest (Franklin et al., 2019). In macropores of biological origin, such as earthworm burrows, the intra-path heterogeneity begins with deposition of organic residues on the pore walls, and then likely continues to develop with the formation of the above-mentioned heterogeneity of flow and nutrient delivery. Different sources of organic materials involved in pore formation can affect subsequent flow path functioning and contribution to biochemical processes within the soil profile (Leue et al., 2017, 2018). Organic coatings along worm burrows are more hydrophobic than coatings along cracks of non-biological origin (Eller- brock et al., 2016), and the type of organics present along the walls can have significant effects on wettability and flow regime (Gerke, 2006; Haas et al., 2018; Leue et al., 2019; Larsbo et al., 2016). Recent work has begun to explore the composition and processing of organic matter in earthworm and root biopores further (Banfield et al., 2018; Hoang et al, 2016, 2017), but it must be noted that studies on biopores and their associated carbon compositions and biological processes are being viewed largely under discipline-specific lenses, rather than holistically.

Macropores originated by earthworms or root-originated macropores that have been subsequently populated by earthworms can have very different characteristics from macropores solely populated by plant roots (Hoang et al., 2016). Earthworm-populated macropores have a considerable amount of coating of earthworm origin on their walls.

The coating layer closes pore openings into the macropore reducing lateral connectivity between the macropore and the remaining soil matrix (Pagenkemper et al., 2015). These coatings can be further compacted after subsequent earthworm passage (Binet and Curmi, 1992) leading to substantial increases in the bulk density of the soil surrounding macropores of earthworm origin (Schrader et al., 2007). Thus, preferential flow paths of earthworm origin might have the least influence on the surrounding soil as there is less water exchange with the surrounding matrix (Bastardie et al., 2005). But they can have a great influence on the carbon processes of the entire soil profile by serving as a direct route for dissolved organic carbon movement to deeper soil horizons (Kodesova et al., 2012). Different carbon contents and microbial activities observed in earthworm burrows as opposed to pores created by roots further increase variability in the contribution of biological macropores to soil carbon processing - the walls of earthworm burrows have higher carbon levels and greater microbial activity than the walls of biopores generated solely by plant roots (Hoang et al., 2016).

Sustained contrasts in micro-environmental conditions within preferential flow paths might lead to the formation of contrasting microbial communities characterized by different ecological functions and carbon use efficiencies. Recently, Bak et al. (2019) sampled flow paths (biopores, fractures, and sand lenses) and the associated soil matrix to depths of 6 m to compare bacterial community structure. They found that flow paths contained greater bacterial abundance and diversity in comparison to the

matrix soil. Moreover, they reported that aerobic processes could take place in flow paths at depths of over 5 m, highlighting the need to include this information when modeling nutrient turnover in well-structured clayey tills. In related work, the active dispersing potential of bacterial communities coming from flow path regions and the matrix soil was evaluated using porous surface models (Krüger et al., 2019). The models allow the assessment of distances traveled by bacteria by controlling water film thickness on a soil-like surface. Results showed that bacteria from flow path regions expressed greater dispersive potential when compared to bacteria from the matrix soil. The greater dispersive potential expressed by bacteria in flow path regions may be used to colonize new habitats (Krüger et al., 2019) perhaps reflecting a necessary survival strategy needed to endure repeated and rapid changes in moisture conditions.

#### **3.4 Pores: the building blocks of flow paths and their contribution to carbon dynamics**

Macropore flow has varied levels of interaction with surrounding pores, involving volumes much greater than just a few pores (Weiler and Flühler, 2004). The same is true for unstable and funnel flow where many pores are involved in the infiltration process. Thus, complex pore networks of varying size and connectivity are working in conjunction to maximize infiltration through the soil profile, often converging to form larger preferential flow systems across the landscape (Sidle et al., 2001). The best way to conceptualize this comes from recent work by van der Linden et al. (2019) that

describes a self-organized group of primary pathways that have inter- and intra-connections with secondary and tertiary pathways. Here we denote primary pathways as large macro- pores  $>300 \mu\text{m } \emptyset$  (Jarvis, 2007), secondary pathways as  $30\text{--}300 \mu\text{m } \emptyset$  pores (Kravchenko et al., 2019), and tertiary pathways as  $<30 \mu\text{m } \emptyset$  pores (Yao et al., 2011). This classification accounts both for a pore's ability to transmit water and the living space it provides for microorganisms (Fig. 3). It is likely representative of pore systems in many soil types, although in some cases where permeability is very low, pores as small as  $30 \mu\text{m } \emptyset$  can function as the main flow channels (Tokunaga and Wan, 2001). Poiseuille's Law stipulates that laminar fluid transmission through a narrow tube is dependent on the radius of the tube raised to the fourth power. Thus, a  $300 \mu\text{m } \emptyset$  pore could transmit 16 times more water than a  $150 \mu\text{m } \emptyset$  pore, resulting in preferential flow at the pore scale (Bottero et al., 2013; Sanders et al., 2012). Although applying Poiseuille's Law to soil water flow is overly simplistic, it nevertheless clearly demonstrates the significant influence that even a small change in pore size can have on flow velocity and associated nutrient delivery, thus their effects on microbial habitat and function.

From a microbial perspective, primary pathways such as larger macropores ( $>300 \mu\text{m } \emptyset$ ) offer less than favorable conditions for communities to thrive due to extreme wetting and drying regimes and little protection from predators (Nunan et al., 2003; Kravchenko et al., 2019). Additionally, nutrients are easily lost from these pores through leaching. Work from soil aggregates and intact cores suggests that  $\sim 30\text{--}300$

$\mu\text{m } \emptyset$  pores generate the most substantial influence on the decomposition of organics and soil respiration (Kravchenko and Guber, 2017; Quigley et al., 2018; Kravchenko et al., 2019) indicating that secondary pathways (30–300  $\mu\text{m } \emptyset$ ) are of main importance for microbial carbon processing (Fig. 3). Secondary pathways experience reduced flow velocities in comparison to primary pathways, offer moderate water retention, allow for ready oxygen influx, provide sufficient space for biofilm building (Nunan et al., 2003; Bouckaert et al., 2013), and thus, for active functioning of the resident microorganisms. That contrasts with tertiary pathways (i.e., small  $<30 \mu\text{m } \emptyset$  pores), which are not of sufficient size to accommodate a large microbial community, and further, may remain anaerobic due to water retention and limited oxygen diffusion. Tertiary paths would be more suitable for entombment and protective storage of the organic inputs arriving via the primary and secondary pathways, especially, the decomposition products from the organisms inhabiting secondary pathways. Observations of more labile carbon coming from larger pores than from smaller pores (Smith et al., 2017; Bailey et al., 2017) support this suggestion. Thus, preferential flow paths present a unique spatial arrangement of tightly knit micro-environments where: 1) the primary paths ( $>300 \mu\text{m } \emptyset$ ) supply new inputs, including organic substrates; 2) secondary paths (30–300  $\mu\text{m } \emptyset$ ) offer inter- and intra-connections that host microbial communities benefiting from the inputs; and 3) tertiary paths ( $<30 \mu\text{m } \emptyset$ ) serve as localities for carbon storage and protection (Fig. 3.3).

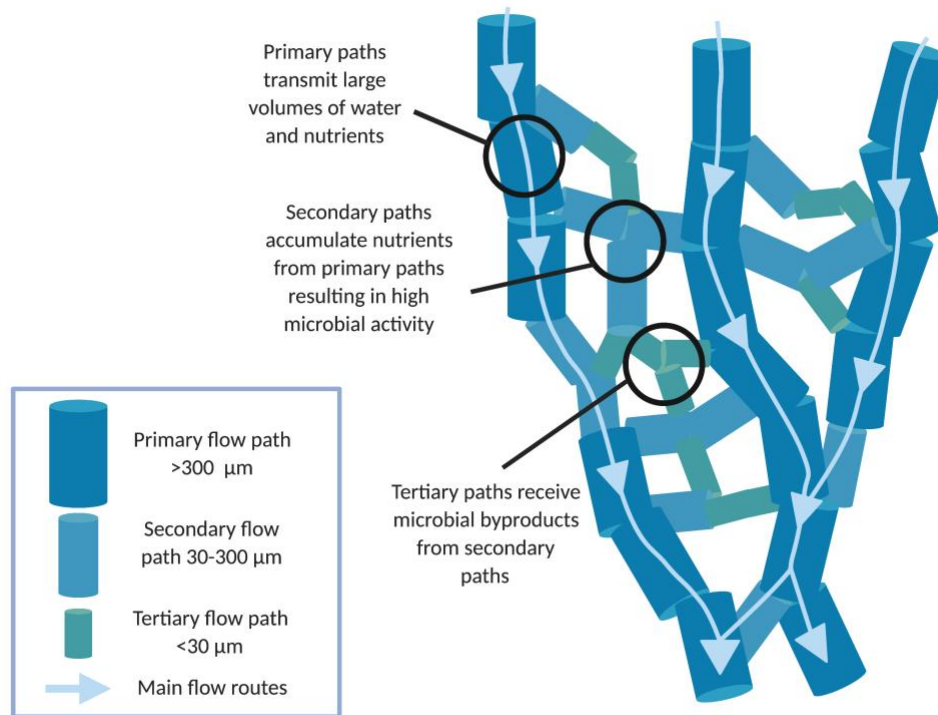


Figure 3.3. Conceptual model showing the relationship between pore size, water flow, and carbon dynamics within preferential flow paths. Primary paths transmit large volumes of water at high flow velocities. The nutrients carried in primary pathways are deposited in secondary pathways and processed microbially. Microbial byproducts from secondary pathways are introduced to tertiary pathways where they may be protected and stored.

The microbial communities populating preferential flow path regions likely differ in their composition depending on the distance from the primary path and on the prevailing properties of the pore spaces that they inhabit. However, what effect such variations in microbial community composition might have on carbon processing is

unclear. There has been considerable interest in whether pore characteristics or microbial community composition has a greater effect on carbon mineralization in soil pores. To address this, Nunan et al. (2017) performed a reciprocal transplant experiment showing that connectivity and diffusion pathways were greater determinants of carbon mineralization than was microbial community structure. X-ray tomography has revealed that atmospherically connected pores exhibit decomposition rates up to 15 times higher than those that are not atmospherically connected (Rabbi et al., 2016; Kravchenko et al., 2015). In addition, while there is evidence that microbial communities in differently sized pores are unique, the structural properties of the pore architecture and connectivity of water (Treves et al., 2003) tend to have a greater influence on carbon mineralization (Negassa et al., 2015; Kravchenko et al., 2014; Ruamps et al., 2011). The findings from pore-scale studies have greatly improved our understanding of soil functionality. Arguably, they can provide insight as we consider heterogeneous carbon and moisture distributions resulting from preferential flow to assess carbon (and others) dynamics more accurately at the catchment and continental scales (Crowther et al., 2019).

### **3.5 Upscaling Biogeochemical Heterogeneity**

Processes of ecological relevance occur in hotspots heterogeneously located in the soil profile, which are often formed and activated by the presence of water (Kuzyakov and Blagodatskaya, 2015; Krause et al., 2017; McClain et al., 2003; Lee and Kang, 2020).

Preferential flow serves as an important local scale phenomenon that affects field-scale processes (Vervoort et al., 1999) making further study of the topic an appealing course of action to combine efforts for the accurate upscaling and representation of pore-scale processes in Earth System Models (ESMs) (Band et al., 2014; Cotrufo et al., 2013). ESMs are guided by pedotransfer functions (PTFs), which are estimations of soil processes based on easy to measure soil properties such as texture (Bouma, 1981, 1989; Van Looy et al., 2017). However, current PTFs lack the inclusion of soil structure (Lin, 2003) leaving ESMs unable to extrapolate the climatic impacts of small-scale soil hydrology to the global scale (Fatichi et al., 2020; Vereecken et al., 2019). Thus, to achieve more representative PTFs, they must reflect our knowledge of the soil structural influence on soil hydrology and preferential flow (Castellano et al., 2012; Lin, 2012; Lin et al., 2005; Pachepsky et al., 2006).

Generation of PTFs and ESMs with consideration given to soil structure has been executed with promising results (Quisenberry et al., 1993; Shaw et al., 2000; Fatichi et al., 2020; Rahman and Rosolem, 2017), but an overall lack of studies of this kind highlight the need for additional development. To generate and assess the performance of new PTFs, innovation is necessary to get past the bottlenecks associated with the study of preferential flow (Guo and Lin, 2018). Some improvement could be achieved by long-term monitoring using grid lysimeters *in situ*, allowing for the high spatial and temporal sampling of effluent during rewetting events (Andreini and Steenhuis, 1990; Bowman et al., 1994; De Rooij and Stagnitti, 2002; Mohanty et al., 2016; Quisenberry

et al., 1994). Determining the isotopic signature of water at high temporal and spatial frequency with grid lysimeters offers insight into residence times and will provide benchmark information for testing and developing models (Dwivedi et al., 2020; Eriksson, 1971). This will allow a mechanistic understanding that avoids highly parameterized equations that can only fit, as opposed to validate, data (Kirchner, 2006). Modeling efforts using Maximum Flow, Minimum Cost algorithms to complement imaging techniques such as X-ray tomography (van der Linden et al., 2019) may be a promising way forward in determining spatial arrangement and connectivity of active flow paths. Similarly, ESMs that incorporate mechanistic microscale processes in the development of macroscopic heterotrophic respiration models (Yan et al., 2018; Yan et al., 2016) could provide important insight into heterogeneous biogeochemical processes, though continued experimental validation for model development and benchmarking is needed.

Rabot et al. (2018) state there is a need for a soil structure library and suggest compiling standardized X-ray tomography data from undisturbed soil cores from which structure-function relationships could be assigned. We believe there is value to this. However, we must note potential limitations related to representative elementary volumes (REVs) for different soils (Koestel et al., 2020) and the tradeoff between scale and resolution, i.e., larger samples result in lower resolution images. Recent work has performed scale fusion, where large soil cores (~7.5 cm Ø) are imaged and then subsampled and scanned at finer resolutions to uncover the full pore size

distribution (Kohne et al., 2011; Lucas et al., 2020; Schlüter et al., 2011; Vogel et al., 2010); however, this seems daunting to include in such a soil structure library. At larger scales, the use of electrical resistivity tomography (ERT) to monitor soil moisture and guide spatial analysis (Calamita et al., 2017) is promising, along with ongoing advancements in remote sensing (Mohanty, 2013).

It is unlikely that all the intricacies of soil biology could be fully encompassed by PTFs, so it is likely necessary to represent biophysical interactions explicitly in ESMs (Wieder et al., 2013, 2015). As shown in Fig. 3.3, the microbiological and physical properties of soil are intimately tied. Therefore, our knowledge of pore-scale carbon dynamics can be used by placing it in macroscopically defined moisture networks with emphasis on connectivity and preferential flow. This may provide a new theoretical modeling framework to work from or aid in further development of pre-existing models such as CIPS (Kuka et al., 2007), PROMISE (Waring et al., 2020) or the dynamic interaction model proposed by Meurer et al. (2020). We hope this will allow for better quantification of the spatial distribution and volumes of active microhabitats and provide greater insight into their overall influence on soil function (Smercina et al., 2021). In addition, the conceptualization presented in Fig. 3, can be used to guide experimental designs and sampling schemes to verify theoretical predictions. There are exciting opportunities to examine soil ecology *in situ* using microfluidic chips with controlled pore sizes and geometries (Harvey et al., 2020; Mafla-Endara et al., 2021). Furthermore, evaluation of the active fraction of

microorganisms will aid in relating microbial diversity to soil function (Blagodatskaya and Kuzyakov, 2013; Couradeau et al., 2019; Heitkotter and Marschner, 2018). There is increasing awareness that long-term soil carbon persistence is due to connectivity constraints (Smith et al., 2017) and physical protection (Lehmann and Kleber, 2015; Lehmann et al., 2020) as opposed to chemical recalcitrance. Comparably, connectivity constraints have been expressed in dual-porosity models that assume the existence of mobile and immobile domains of water (Gerke and van Genuchten, 1993; Simunek et al., 2003). Ultimately, to progress, there must be coordinated efforts in terms of techniques and data availability (i.e., technical aspects), conceptual developments (i.e., how to do it), and organizational aspects (i.e., who will do it) to increase interoperability (Vargas et al., 2017) and move towards an integration of preferential flow in soil carbon science.

### **3.6 Preferential Flow and CO<sub>2</sub> Emissions**

#### **3.6.1 Hot Moments**

Of particular importance for an accurate representation of preferential flow and the associated biogeochemical response in ESMs is the prediction of hot moments. After dry soils are rewet, they often experience a sudden burst, or hot moment, of CO<sub>2</sub> emissions known as the “The Birch Effect” (Birch, 1958). The underlying mechanisms driving these large pulses of CO<sub>2</sub> have been recently reviewed by Barnard et al. (2020). The five predominant mechanisms are: 1) microbial cells accumulate solutes

during drying to remain hydrated, which are then released as easily degradable compounds upon rewetting to avoid rupturing (Fierer and Schimel, 2003), 2) dead microbial cells are mobilized (Blazewicz et al., 2014), 3) mobilization of desorbed organic compounds (Blankinship and Schimel, 2018; Deneff et al., 2001), 4) increased connectivity of the liquid phase allows microbes to access substrate (Smith et al., 2017) and 5) infiltrating water causes degasification whereby CO<sub>2</sub> is pushed out of pore spaces where it has been previously accumulated (Maranon-Jimenez et al., 2011). Hot moments driven by water pulses contribute substantially to total annual CO<sub>2</sub> budgets. In drylands, hot moments can result in increases of CO<sub>2</sub> efflux upwards of 15,000% (Groffman et al., 2009; Kim et al., 2012), and >200% even in temperate wetlands (Petraakis et al., 2017). The magnitude of CO<sub>2</sub> efflux after rewetting is less pronounced in wetter ecosystems, but even in a mesic temperate deciduous forest, pulses of CO<sub>2</sub> after rainfall have been shown to account for 16–21% of the annual efflux (Lee et al., 2002). Hot moments are often missed due to a lack of continuous measurements *in situ* or misrepresented by models due to the use of semi-empirical functions based on overly simplistic relationships between averaged values of soil temperature and soil moisture (Vargas et al., 2018). We suggest that given the challenges in predicting ecosystem response to rewetting, taking into consideration preferential flow might contribute to a better mechanistic understanding of hot moments of CO<sub>2</sub> emissions.

### **3.6.2 Effects of Heterogeneous Moisture and Rainfall Variability on CO<sub>2</sub> Efflux**

In ecosystems undergoing prolonged periods of drought soils will often display hydrophobic characteristics (Doerr and Ritsema, 2005). Hydrophobicity can result in the development of unstable preferential flow patterns (Dekker and Ritsema, 1994; Ritsema and Dekker, 1994). It has been suggested that a reduction of CO<sub>2</sub> efflux could occur as a result of incomplete rewetting due to preferential flow caused by hydrophobicity (Muhr and Borken, 2009). This appears to be true during the rewetting period as infiltrating water degasses fewer soil pores when channeled through smaller soil volumes (i.e., preferential flow paths) (Sanchez-García et al., 2020a). However, the effect of preferential flow on the 24–48 h post wetting efflux (Rey et al., 2017; Wang et al., 2016) when hydraulic redistribution is occurring remains unclear. High rainfall intensities often lead to the increased occurrence of preferential flow (Buckingham et al., 2008; Jarvis, 2007), and larger rainfall events commonly result in larger CO<sub>2</sub> effluxes (Barnard et al., 2020; Chen et al., 2009; Sponseller, 2007). These observations leave us with paradoxical conclusions on the effects of preferential flow on CO<sub>2</sub> efflux. While preferential flow may cause an initial reduction in total CO<sub>2</sub> efflux, the preferentially wetted regions will likely constitute the most biologically active locations (Franklin et al., 2019).

As research on this topic remains in its infancy, considerable uncertainty remains as to how soil water repellency and preferential flow affects carbon dynamics in various soil types and under different precipitation patterns (Sanchez-García et al., 2020b). As

an example, we present a scenario where CO<sub>2</sub> efflux is not proportional to rainfall intensity (Fig. 3.4; Vargas et al., 2018). In Case I and II, the ecosystem response to rewetting after the long dry season is shown. While the day of the year is the same and both represent the first rainfall after a long drought, there are large differences in the responses of CO<sub>2</sub> efflux. In Case I, the precipitation event was 25 mm at a rate of 5 mm/h, whereas in Case II the precipitation event was 5 mm at a rate of 1 mm/h but resulted in greater CO<sub>2</sub> efflux. These results support the hypothesis that preferential flow will cause a reduction in CO<sub>2</sub> efflux (Muhr and Borcken, 2009). In Case II, lower rainfall intensity resulted in more uniform wetting and greater CO<sub>2</sub> efflux in comparison to Case I. Also, note that in Case II, bare soil resulted in greater CO<sub>2</sub> efflux when compared to vegetated soil. This was likely due to the increased occurrence of preferential flow along plant roots causing incomplete wetting, thus less degassing.

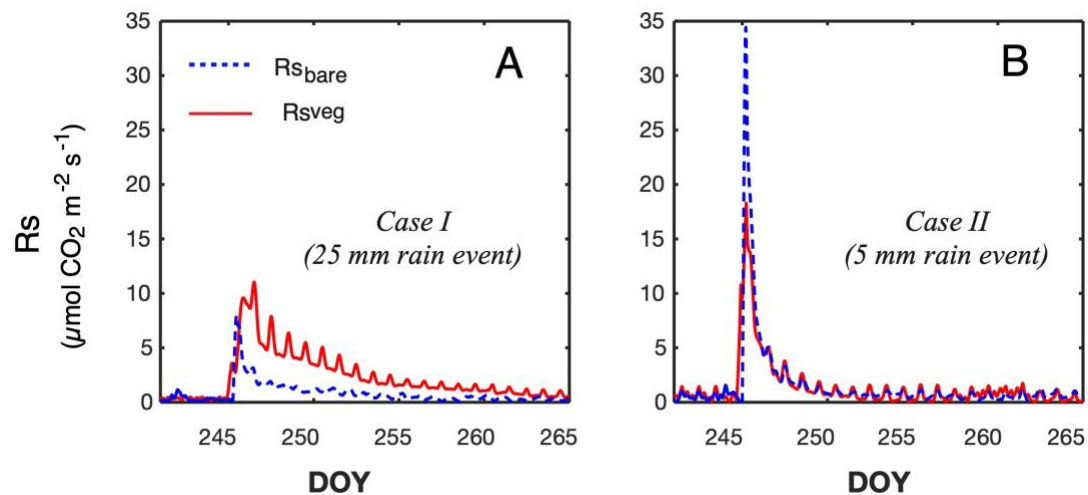


Figure 3.4. Pulse responses of soil CO<sub>2</sub> efflux (Rs) under vegetation (Rs<sub>veg</sub>) or in bare soil (Rs<sub>bare</sub>) following the first rainfall after a long drought in a water-limited grassland. Solid lines represent Rs measured under vegetation (Rs<sub>veg</sub>) and dashed lines represent Rs measured in bare soil (Rs<sub>bare</sub>). The numbers in the x-axis represent the day of the year (DOY) for years 2011 (a) and 2012 (b) for each panel. Adapted from Vargas et al. (2018).

To overcome the apparent paradox and inconsistent relationships between CO<sub>2</sub> efflux and rainfall intensity, rather than simply reporting event size as total rainfall, complementary analyses are needed. Information on antecedent soil conditions, storm composition, and the type of flow events that will be brought on by a specific storm should aid in this effort. This type of information can help predict the extent and timing of preferential flow onset, which has been shown to affect the concentrations of mobilized solutes (McGrath et al., 2008). Taking measures such as these into account will offer a more comprehensive and accurate determination of how precipitation events influence soil CO<sub>2</sub> production and efflux. General moisture metrics such as the water status index (Yan et al., 2014), volumetric water content (Leon et al., 2014), water-filled pore space (Franzluebbers, 1999), matric potential (Castellano et al., 2011), relative extractable soil water (Vargas et al., 2013), soil moisture characteristic curve (Ghezzehei et al., 2019), and mean precipitation within a season (Warner et al., 2019) have been used with moderate success in predicting CO<sub>2</sub> efflux at different spatial and temporal scales. Nevertheless, uncertainty remains as none of these metrics can fully account for the heterogeneity of water movement and its effects on carbon cycling in soils.

Even though the influence of preferential flow on CO<sub>2</sub> efflux remains unknown, it certainly plays a role. In an infiltration experiment, <sup>14</sup>C- labeled dissolved organic matter (DOM) was used to track how much DOM was leached, how much was retained, and how much was mineralized to CO<sub>2</sub>. The results showed that 80% of the labeled DOM was bound to mineral surfaces, and on average, only 2–9% was mineralized to CO<sub>2</sub> (Hagedorn et al., 2015). Due to the increased presence of labeled DOM found along flow path regions, Hagedorn et al. (2015) determined that DOM accumulation over time would result in flow paths functioning as hotspots of organic matter degradation, though the majority of the carbon being processed may be of older age. Their results are in agreement with the “DOM cycling downward” conceptual model proposed by Kaiser and Kalbitz (2012), in which they assume DOM is temporarily immobilized on mineral surfaces, processed microbially, and then released by desorption and dissolution, and finally leached deeper into the soil profile (Afsar, 2020).

### **3.7 Conclusions**

The works outlined in this review highlight the role of preferential flow in the carbon cycle. Preferential flow paths exhibit temporal stability, which allows for their repeated activation over periods as long as decades. Recurrence of flow results in heightened concentrations of carbon and microbial biomass. Flow paths often self-organize, forming large hydraulic networks across the landscape. These macroscopic

networks can be viewed as assemblages of well-connected pores rather than large singular units. These assemblages of pores harbor highly active and diverse microbial communities. A conceptual model (Fig. 3.3) was developed describing a hierarchical classification of pores based on their hydrology and contribution to soil carbon processing. The conceptual model categorizes 1) primary paths as large macropores  $>300 \mu\text{m } \emptyset$ ; 2) secondary paths as pores  $30\text{--}300 \mu\text{m } \emptyset$ ; and 3) tertiary paths as pores  $<30 \mu\text{m } \emptyset$ . Primary paths function as the main flow channels, supplying water and nutrients. Secondary paths provide protection from high flow velocities, ample levels of oxygen, and accumulate inputs from the primary paths. These factors allow secondary paths to host active microbial communities that play a significant role in carbon processing. Tertiary paths are too small for significant growth of biomass and may serve as anaerobic microsites due to oxygen limitations. This makes tertiary paths viable localities for carbon storage and protection.

Micro-habitat is significantly shaped by pore size, pore arrangement, pore connectivity, and the associated moisture conditions. This information can be used to develop new experimental and modeling frameworks that will offer improved quantification of the volumes of active micro-sites. Elucidating such biophysical interactions will be crucial to increase ESM accuracy. The importance of preferential flow has been implicitly recognized in soil and biogeochemical research for years, but almost no work explicitly accounts for its role in microbial ecology and overall soil function. This has likely stemmed from narrow disciplinary foci and the difficulty of

studying both preferential flow and microbiology *in situ*. However, with theoretical and technological advancements there are now ways to achieve this. There is undeniable synergy between soil physics and biology that has been largely unexplored. We conclude that the use of preferential flow networks as a framework of interest for the study of microbial ecology and biogeochemical processes will reveal new insights into soil functionality.

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## Chapter 4

### HYDROLOGIC CONNECTIVITY OF SOIL PORE NETWORKS CONTROLS BIOGEOCHEMICAL PROCESSES ACROSS SCALES

#### 4.1 Abstract

All pores imbedded in the soil matrix are not equal. Pore size and pore connectivity have a significant influence on water flow and retention, microbial habitat, and the chemistries and amounts of carbon processed and stored. Pores from ~30-300  $\mu\text{m}$   $\emptyset$  are hotspots for the microbial processing of soil carbon while pores above and below this range appear to function more so for carbon delivery and storage respectively. Due to the unique microbial niches created by the size and arrangement of the pore system, strong log-linear relationships between matric potential and heterotrophic respiration of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  are well established. However, the studies that demonstrate this relationship have been performed in the lab setting under controlled conditions. In the field using this relationship, along with other moisture functions, is limited due to challenges in accounting for heterogeneous dynamics of soil moisture in both time and space. Antecedent soil moisture and precipitation input largely controls which pore networks become activated yet there have been few attempts to define the hydrologic activation of assorted pore networks and their subsequent biogeochemical response. To build upon our ever-increasing knowledge of pore scale carbon

dynamics, viewing pores as interconnected assemblages that bridge scales and defining when and how these pore networks are activated under different antecedent conditions will help fill the research gap at the interface of hydrology and biogeochemistry. Further, while pore size and pore connectivity are key parameters in controlling carbon and other biogeochemical processes, modeling efforts attempting to utilize these parameters are rare. Therefore, the aim of this opinion paper is to explore how hydrologic connection of soil pore networks affects biogeochemical processes across scales.

## **4.2 Finding Order in Heterogeneity**

Soil is one of the most heterogeneous mediums on Earth. This heterogeneity can be observed at virtually every scale (Lin et al., 2005; Schulz et al., 2006) and is believed to support the tremendous amount of microbial diversity found in soil (Crawford et al., 2005; Or et al., 2007). Finding meaningful generalities within this complex system for various purposes, including modeling, has proven to be one of soil science's greatest challenges (Baveye and Laba, 2015). This problem remains even with the vast amount of literature that has been produced since the field's inception and has led to a point where there is a clear need for a shift towards holistic understanding of hydrology and biogeochemistry (Lexartza-Artza and Wainwright, 2009; Lin, 2003; Sivapalan, 2005; Vogel et al., 2022). This holistic perspective comes alongside a now well accepted paradigm shift that carbon decomposition is governed by microbial accessibility to

carbon within pore space more so than by chemical recalcitrance (Dungait et al., 2012; Lehmann et al., 2020; Lehmann and Kleber, 2015). Discussion of accessibility typically comes from a microbial perspective and in reference to the pore scale (Smercina et al., 2021), however accessibility and connectivity manifests an influence on biogeochemistry across scales when considering pore networks, flow paths, and macroscopic soil architecture.

When searching for order within soil, perhaps reflecting on the rise of ancient human civilizations can provide a fitting analogy for where microorganisms choose to reside and the functions they carry out. All ancient civilizations had one thing in common: they were located along major rivers. These rivers supplied a consistent supply of water, created fertile lands, and enabled the coexistence of a diverse group of species. In soil water functions as the biogeochemical “on and off” switch (Birch, 1958; Linn and Doran, 1984; Orchard and Cook, 1983; Skopp et al., 1990) by regulating microbial community structure, oxidation-reduction, and microbial accessibility to OM by means of transport and (dis)connected water films. All of these processes take place at the microscale within the physical soil environment created by the spatial arrangement of solids and pores - soil structure or architecture (Baveye et al., 2018; Juma, 1993; Letey, 1991; Lin, 2010; Marshall, 1962; Nunan et al., 2002; Rabot et al., 2018; Vogel et al., 2006). The physical microenvironments created by soil structure are heterogeneously distributed yet the apparent impact on microbial functionality is beginning to appear somewhat generalizable as a certain degree of order and

organization arises at the pore scale (Borer et al., 2018; Crawford et al., 2012; Kravchenko et al., 2019; Young and Crawford, 2004). A significant driver of this organization comes from the ways in which soil structure creates unique niches by shaping diffusion pathways and the extent to which they are connected under different water contents. These diffusion pathways serve as the primary mechanism of nutrient transport to soil microbes and control their motility and activity (Manzoni et al., 2012; Or et al., 2007; Purcell, 1977; Tecon and Or, 2016; Young and Bengough, 2018). Thus, for microbial communities, the  $\mu\text{m}$ -cm scale connectivity of different pore sizes matters (Carson et al., 2010; Kravchenko et al., 2015; Treves et al., 2003). However, there is also larger scale influence on microbial communities expressed in hydrologically connected sections of the soil pedon (Bak et al., 2019; Lüneberg et al., 2018; Dechesne et al., 2014; Krüger et al., 2019). Consequently, some have raised the need to “go with the flow” in new aged models of carbon processing and storage when moving from the pore scale to larger scales (Waring et al., 2020).

Increased recognition of the biophysical controls on soil functionality has led to an interdisciplinary convergence (Baveye et al., 2018), which can be extended further as there is a convergence occurring between scales of study as well. This convergence centers around hydrologic connectivity and the emergent unique properties of pores and flow paths (Lin, 2003; Schlüter et al., 2022; Smith et al., 2017; van der Ploeg et al., 2018; Young et al., 2008; Young and Bengough, 2018). Hydrologic connectivity is broadly defined and increasingly found in the literature (Ali and Roy, 2010, 2009;

Bracken et al., 2013; James and Roulet, 2007; Lexartza-Artza and Wainwright, 2009; McGuire and McDonnell, 2010; Pringle, 2003; Stieglitz et al., 2003), however there has been little discussion addressing how hydrologic connectivity regulates biogeochemical processes across scales (Bauke et al., 2022; Covino, 2017; Franklin et al., 2021; Li et al., 2021; Lin, 2012a; Raymond et al., 2016; Védère et al., 2022; Wen et al., 2022). For the purpose of this article we primarily focus on the definitions coming from Knudby and Carrera (2005) and Western et al. (2001) who define hydrologic connectivity as “spatially connected features which concentrate flow and reduce travel times” and “the extent to which connected features, such as arbitrarily shaped bands or pathways having similar (e.g., high) values, are present in a hydrologically relevant spatial pattern.” These definitions are essentially describing preferential flow (Beven and Germann, 1982; Flury et al., 1994), a process that occurs from the pore to field scale and remains one of hydrology’s biggest stumbling blocks (Blöschl et al., 2019). Making progress in this area necessitates new hydrological theories (Beven, 2018) with emphasis on pattern, process, and function (Beven, 2019; Sivapalan, 2005). Hydrologic connectivity is central to these issues (Ali and Roy, 2009) and with further study may improve both hydrologic and biogeochemical theory as they are intimately tied, yet often decoupled in experimental investigations and models (Jia et al., 2021; Li et al., 2021).

The need to elucidate the organizing principles of flow networks and determine simplistic hydrologic laws has been expressed before (McDonnell et al., 2007; Jury,

1982; Dooge, 1986; Morel-Seytoux, 1988). However, it seems there remains continued stagnation in this arena (Beven, 2018; Beven and Germann, 2013; Weiler, 2017) in part due to the challenging nature of studying hydrologic connectivity and perhaps also in part due to an increasing shift towards modeling studies as opposed to experimental field studies at the field scale (Burt and McDonnell, 2015). Here, it is important to raise this point once again as the organizing principles of biogeochemical processes in soil are becoming increasingly known and depend on hydrologic connectivity through the physical soil structure. Therefore, overcoming the apparent bottlenecks associated with our understanding of the manifestation and hydrologic activation of pore networks at various scales would provide a substantial advancement in Earth Sciences (Guo and Lin, 2018; Lin, 2012b). In the remaining sections of the paper, the importance of hydrologic connectivity in biogeochemical processes across scales will be discussed.

### **4.3 Pores Make Pathways: Connectivity Drives Functions Across Scales**

Soil structure is defined as the spatial arrangement of solids and pores (Marshall, 1962; Letey, 1991; Rabot et al., 2018). Alternatively, structure has been defined as the hierarchical grouping of soil particles forming porous aggregates (Dexter, 1988). These definitions are reasonably synonymous yet they have created a divergence in the ways in which biogeochemical processes, as a function of soil architecture, are conceived (Kravchenko et al., 2019; Letey, 1991; Rabot et al., 2018; Wang et al.,

2019; Yudina and Kuzyakov, 2019). Soil architecture does play a significant role in shaping biogeochemical cycles, but ultimately for these cycles to operate the presence of water is necessary (Birch, 1958). This is a well-known fact, but water, and more specifically the connectedness of water across scales has been neglected in discussions of soil architecture's influence on biogeochemical cycling in soil. At the pore scale this dictates the microbial niche and at larger scales drives mass fluxes of elements through soil. In recent years, research has developed novel understanding of carbon dynamics in soil pores (Kravchenko and Guber, 2017) resulting in four emerging patterns linked to pore size classes and flow networks: 1) carbon in smaller pores is chemically more recalcitrant and more difficult for microbes to access than carbon in larger pores (Adu and Oades, 1978; Bailey et al., 2017; Chenu and Stotzky, 2001; Smith et al., 2017), 2) bacterial and enzyme activity is highest in pores  $\sim 30\text{-}300\ \mu\text{m}\ \varnothing$ , which may be extended down as far as  $10\ \mu\text{m}$  (Franklin et al., 2021; Kravchenko et al., 2019; Schlüter et al., 2022b, 2019), 3) microbial hotspots form near macropores, which serve as localities for high carbon and oxygen inputs (Franklin et al., 2019; Kuzyakov and Blagodatskaya, 2015; Schlüter et al., 2019; Stewart et al., 1999), and 4) macroscopic regions of soil shown to be hydrologically connected express increased concentrations of microbial biomass, carbon, and other unique properties in comparison to the bulk soil matrix (Table 4.1).

Pores  $>300\ \mu\text{m}\ \varnothing$  create a biological limitation likely due to adverse conditions caused by predation (Erktan et al., 2020; Nunan et al., 2003) and shear stress as  $300\ \mu\text{m}\ \varnothing$  is a

pore size commonly attributed to rapid preferential flow (Jarvis, 2007). The minimum 30  $\mu\text{m}$   $\emptyset$  considers the size of bacterial communities (Kuzyakov and Mason-Jones, 2018) and is one of the smallest values that has been used to define macropores (Marshall 1959). Further, 30  $\mu\text{m}$  is the distance required for bacteria to “outrun diffusion” in search of new and more favorable environmental conditions (Purcell, 1977). The 30-300  $\mu\text{m}$  range is quite close to those defined over 40 years ago by Greenland (1977) who assigned transmission pores 50-500  $\mu\text{m}$ , storage pores 0.5-50  $\mu\text{m}$ , and residual pores  $<0.5$   $\mu\text{m}$  and also pairs well with findings that the isotopic age of water in larger pore sizes is younger than that of smaller pores (Geris et al., 2015; Sprenger et al., 2019). What makes the 30-300  $\mu\text{m}$  range unique is that it takes into consideration both the hydraulic function as well as biogeochemical function of the pore space. Beyond functionality, pores  $> 30$   $\mu\text{m}$   $\emptyset$  have been related to the degree of structural development of soil aggregates (Yudina et al., 2022). This suggests a close, if not definite, link between structural development, hydraulic functionality, transport and storage of carbon, and the “Goldilocks” type of environment pores in the 30-300  $\mu\text{m}$  range provides for microorganisms.

The importance of pore size is further expressed in relationships of matric potential with  $\text{CO}_2$  and  $\text{N}_2\text{O}$  efflux. Bhaumik and Clark (1948) found maximum  $\text{CO}_2$  efflux at 50 cm of water tension for each soil studied, which corresponds to 4.9 kPa. They also showed that the water tension influences the microbial community. A log-linear relationship between matric potential and  $\text{CO}_2$  efflux has been established for some

time (Sommers et al., 1981; Orchard and Cook, 1983). Van der Weerden et al. (2012) concluded that pores of 60-300  $\mu\text{m}$   $\emptyset$  were particularly important for the production of  $\text{N}_2\text{O}$ . Castellano et al. (2011, 2010) found that maximum production of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  occurred at very similar matric potential ranges from around -1 to -5 kPa, which corresponds to maximum pore sizes of 300  $\mu\text{m}$   $\emptyset$  and 100  $\mu\text{m}$   $\emptyset$ . Modeling work also shows that -5kPa water tension creates a biological limitation as capillary pinning foci and insufficient film thickness limit bacterial colony expansion (Wang and Or, 2010). An interesting point regarding the contribution of individual pore size classes to  $\text{CO}_2$  and  $\text{N}_2\text{O}$  is that the same results have been reported using various methodologies. For example, some studies that investigate the role of matric potential and GHG efflux have been performed by adding a carbon source directly to the pore space at a controlled matric potential (Kravchenko et al., 2021). Alternatively, some of the above-mentioned studies have started with saturated samples and then allowed to desaturate while monitoring matric potentials changes and efflux (Castellano et al., 2011, 2010).

Aside from the relationship with matric potential, numerous relationships between water content and greenhouse gas efflux have been used to represent SOC dynamics. These include water-filled pore space, volumetric and gravimetric water content, relative soil water content, and diffusion based functions (Abramoff et al., 2018; Moyano et al., 2012; Zheng et al., 2022). These relationships have proved to be inconsistent however, which can in part be attributed to structural composition as two

soils with nearly identical bulk densities can have dissimilar outputs of CO<sub>2</sub> and N<sub>2</sub>O (Van Der Weerden et al., 2012). In other words parameters like volumetric water content are less relatable to efflux because two soils at the same volumetric water content can experience saturation of different pore sizes depending on the overall pore size distribution (Ball et al., 1988; Liang et al., 2022). Matric potential differs in this regard as it is a measure of thermodynamically available water, thus matric potential can be viewed as a universal water scaler (Ghezzehei et al., 2019; Miller and Johnson, 1964; Moyano et al., 2013; Sommers et al., 1981). Further, because matric potential is a universal property there is the potential to draw relationships to the microbial niche making it feasible to generalize the functioning of microbial communities as a function of water availability (Lennon et al., 2012; Ruamps et al., 2011). Other recent attempts using x-ray CT linked the distance of particulate organic matter from pores to N<sub>2</sub>O efflux with good correlation advancing overall process understanding (Ortega-Ramírez et al., 2022; Rohe et al., 2021), but traditional measures of water content have explained efflux just as well (Linn and Doran, 1984; Orchard and Cook, 1983; Skopp et al., 1990).

Understanding individual pores contribution to soil functioning is becoming clearer, but pores do not exist as solitary entities. Rather, they are close knit assemblages with varying degrees of connectivity, which in turn create large connected networks or flow paths (van der Linden et al., 2019; Nieber and Sidle, 2010; Noguchi et al., 1999; Sidle et al., 2001). These networks and flow paths appear to play significant roles in shaping

larger scale biogeochemistry (Table 4.1). Therefore, there is need to identify emergent patterns and feedbacks in hydrology and biology that are driven by soil structure (Jury, 1982; Morales et al., 2010; Phillips, 2022). A recent review on soil structure's impact on infiltration finds that there is an absence of theoretical frameworks attempting to link soil structure with infiltration (Basset et al., 2023). The lack of work in this area is disappointing as Lin (2003) advocated for incorporating structural information into infiltration theory 20 years ago. Nevertheless technological advancement has facilitated recent efforts to collect and compile structural information from imaging techniques such as x-ray tomography (Weller et al., 2022), but this is limited to relatively small samples due to resolution constraints. Undoubtedly, structural libraries created from soil images will provide new insight and can provide connectivity metrics such as Euler-Poincaré characteristic (Vogel et al., 2010; Vogel and Kretzschmar, 1996), but there is scale dependence of connectivity (Lucas et al., 2020) which ultimately leads to questions regarding the existence of REV (Koestel et al., 2020; Schlüter et al., 2020).

Our knowledge of structural connectivity in soil largely comes from small scale imaging techniques, but as outlined connectivity is a multi-scale property. This has led some to believe that vertical connectivity through the soil pedon is perhaps the most meaningful for the understanding of soil functions because within these scales local controls dominate, while at larger scales regional controls take over (Lin, 2012b; Vogel, 2019). Pedon level connectivity expresses important controls on

biogeochemical functionality (Radolinski et al., 2022), but vertical connectivity is not commonly measured (Li et al., 2021; Xiao et al., 2021). Therefore, not only is our understanding of hydrologic connectivity as a function of soil structure lacking, but it is lacking in a time where rapid structural changes due to shifts in climate appears certain (Hirmas et al., 2018; Sullivan et al., 2022). These structural changes along with changes in other properties, such as hydrophobicity, have the potential to shift soil to alternative steady states of hydrologic functionality (Robinson et al., 2019, 2016) which has unknown ramifications on microbial response to wetting (Evans et al., 2022; Manzoni et al., 2012). Thus, a complex issue presents itself where there is need to incorporate soil structure (Fatichi et al., 2020; Schulz et al., 2006) and connectivity into hydrological theory (Sidle, 2021; Sidle et al., 2017), understand how structure changes over time (Vereecken et al., 2022), and combine this information with biogeochemical theory (Jia et al., 2021; Li et al., 2021; Moyano et al., 2013).

Table 4.1. Flow path and soil structure influence on biogeochemistry across scales

<b>Properties of Flow Path vs. Matrix</b>	<b>Microbial Analysis</b>	<b>Depth</b>	<b>Flow Path Identifier</b>	<b>Soil Type</b>	<b>System</b>	<b>Reference</b>
- Highest degradation of 2,4 dichloropbenoxyacetic acid occurred in macropores	N/A	0-10 cm	Open macropore channel and textural contrast of silt loam matrix and sand flow path	Fluvaque-ntic Eutrochre-pt	Undisturbed and repacked soil columns	(Pivetz and Steenhuis, 1995)
- Increased pesticide mineralization in Bt macropore linings - Increased OC and microbial biomass in linings of Bt macropores and 45% greater clay content	- Total cells by plate counts	1.4-3.5 m	Dark color of macropore linings	Xeric Argialbol-Is	Agriculture	Mallawatantri et al., 1996
- Pesticide was degraded rapidly in preferential flow path	N/A	0-5 cm	Open macropore channel and textural contrast of silt loam matrix and sand flow path	Fluvaque-ntic Eutrochre-pt	Repacked	Pivetz et al., 1996
- Macropore soil contained larger concentrations of NO <sub>3</sub> -N, water soluble carbon, and bacterial biomass	- Total cells by direct microscopy and plate counts. - Nitrate reducers and denitrifying bacteria identified using most	50-120 cm	Visible redoximorphic features on macropore channels	Sandy loams	Agriculture and forest	Vinther et al., 1999

	probable number analysis on soil dilutions					
- Higher NO <sub>3</sub> concentrations in flow paths - Enhanced denitrification in flow paths - Nitrification started earlier and was more pronounced in flow paths	N/A	0-5 cm	Suction cups	Humaquept	Forest	Hagedorn et al., 1999
- C concentrations were 10–70% higher - Enriched in N - 9-92% greater microbial biomass	-Fumigation extraction - Genetic fingerprinting using agarose gels	0-1 m	Dye staining	Typic Haplumbrept	Forest	Bundt et al., 2001a
- OC and total N were 15 to 75% higher - OC was younger and N cycling was more rapid	N/A	0-1 m	Dye staining	Typic Haplumbrept	Forest	Bundt et al., 2001b
- Activities of C <sub>s</sub> <sup>137</sup> , Pb <sup>210, 239</sup> , Pu <sup>240</sup> , and concentrations of OC were enriched up to 3.5 times indicating flow paths were stable for decades	N/A	0-1 m	Dye staining	Dystric Cambisol	Forest	Hagedorn and Bundt, 2002
- Two times higher carbon content and depleted in N - Carbon age thousands of years younger than matrix soil	N/A	60-140 cm	Eluvial tongue	Dystric Cambisol	Agriculture	Chabbi et al., 2009

- Channel infillings and crack surfaces enriched in OM - Lower C:N ratios - Higher ammonium oxalate-extractable Al - Lower ammonium oxalate-extractable Fe - OM infillings at >50 cm depth were significantly younger than matrix soil	-Fumigation extraction - CO <sub>2</sub> incubation	0-1m	Cracks and dark color of macropore linings	Hydric Placudands	Rainforest	Marin-Spiotta et al., 2011
- 5.0 g kg <sup>-1</sup> more C and 0.24 g kg <sup>-1</sup> more N - C:N ratio larger by 2 - 32% more Ca, 57% more Mg, and 67% more Fe	N/A	0-1 m	Dye staining	Haplic podzol	Forest	Bogner et al., 2012
- Microorganisms primarily grew on the surfaces of grains and aggregates near preferential flow paths	Biofilm growth simulation	0-10 cm	Artificial pore structures derived from x-ray computed tomography	N/A	Model simulation	Yan et al., 2017
- Clay coatings in Bt horizons are enriched in heterocyclic organic matter compounds	N/A	40-80 cm	Cracks, pinhole fillings, root channels, earthworm burrows	Hapludalf	Agriculture	Leue et al., 2017
- Greater bacterial abundance and diversity at all depths - Bacterial lifestyles likely differ from those of bacteria in matrix communities	16S rRNA community analysis	0-6 m	Biopores, fractures, sand lenses	Anthric Luvisol	Agriculture	Bak et al., 2019

- Increase in shared dispersers among the preferential flow paths	- 16S rRNA community analysis - Motility on porous surface models	0-3.5 m	Biopores and fractures	Anthric Luvisol	Agriculture	Krüger et al., 2019
- Oxygen depletion by microbes was greatest near the flow path linings - Oxygen patterns resembled those shown in aggregates by Sexstone et al., 1985	- Oxygen mapping as proxy for heterotrophic respiration	0-4 cm	Textural contrast of coarse sand flow path and fine sand matrix	Artificial packed sands	Lab study	Franklin et al., 2019
- Paths enriched in OC (+12%) and plant nutrients (N: +21%, Ca <sup>2+</sup> : +59%, K <sup>+</sup> : +39%, Mg <sup>2+</sup> : +39%) - Larger microbial activities along cracks	- 16S rRNA community analysis - Gel electrophoresis	0-60 cm	Dye staining	Anthraquic Gleysol	Rice paddy	Fuhrmann et al., 2019
- Highest CEC values were found for clay rich coatings and pinhole fillings - CEC values of uncoated cracks and earthworm burrows were similar to those of bulk soil	N/A	34-60 cm	Cracks, pinhole fillings, earthworm burrows	Haplic Luvisol	Agriculture	Leue et al., 2019
- Higher xylanase activities - Macropores show type-specific and site-specific differences in xylanase and phenol oxidase activities	- Zymography - Enzyme assays - Phospholipid fatty acid analysis	25-85 cm	Cracks, biopores, pinhole fillings	Haplic Luvisol	Agriculture	Leue et al., 2021

- Distance from flow path was a significant predictor of $\Delta^{14}\text{C}$ and improved model fit ( $R^2 = 0.97$ ) - Decreased microbial abundance with distance from flow paths in deep mineral horizons	16S rRNA community analysis	0-1 m	Cracks and dark color of macropore linings	Hydric Placidands	Rainforest	Lacroix et al., 2022
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#### 4.4 Connectivity in Hydrological and Carbon Models

All carbon models are pool models (Azizi-Rad et al., 2021). Ultimately the labile and recalcitrant pools of carbon can be theorized as being in pores of different sizes. To our knowledge, there are few carbon models that make explicit use of pore size distributions in their model structure (Kuka et al., 2007; Meurer et al., 2020; Waring et al., 2020), but none of them incorporate a measure of connectivity (Baveye et al., 2021). The model by Kuka et al. (2007) distributes carbon into three groups of pore classes, macro-, meso-, and micropores. Micropores are represented by the volume of pore space at permanent wilting point, mesopores as the volume of pores at field capacity, and macropores as the remaining pore volume. The model by Meurer et al. (2020) splits the pore size distribution into 80% textural pores and 20% aggregation pores. In this model macropores are ignored because they are assumed to have little impact of SOM storage, which is in concurrence with the conceptual model put

forward in Franklin et al. (2021). The model by Waring et al. (2020) separates the pore domain into three pore size classes, those being flow permitting pores, interparticle porosity of clay domains, and intraparticle porosity. There are other approaches to model carbon processes such as 3D Lattice-Boltzman and pore network models that do represent connectivity, but validating them remains challenging (Ebrahimi and Or, 2015; Mbé et al., 2022; Pot et al., 2022, 2021).

Most SOM models simply use water scalars, such as volumetric water content, rather than combining with transient models of water flow and those that do often use the Richard's equation (Herbst et al., 2008; Meurer et al., 2020; Zhang et al., 2021).

Richard's equation was developed to quantify unsaturated water flow under an imposed equilibrium condition and homogenous porous media. Therefore, Richard's equation and the models that are based on it do not apply to more general cases of water flow in heterogeneous soils, which is unsteady and preferential (Bauser et al., 2022; Beven, 2019, 2018; Davies et al., 2011; Davies and Beven, 2012). In addition to assumptions made in Richard's equation, it also lumps the contribution of all individual pathways into a single conductivity value. The lumping of pore networks and flow paths contribution to flow into a single conductivity value motivated the work by Kung et al. (2005, 2006) where they examine the contribution of individual pore networks to the overall flow process. To derive their model tracers were sequentially applied at increasing wetness. The experiment shows that preferential flow domains are formed by clustered pore networks and was able to provide

information on the hydrologic connectivity of the pore networks. They showed that water in small pores contributes very little to the overall flow process as their matric potential quickly goes to zero. In addition, they derived pore size and how many pores of that size contributed to flow. When the model was tested in a field site hundreds of miles away with similar soil type and management the model fit quite well. The drawback of the model by Kung et al. (2005, 2006), and a significant one at that, is that soil is conceptualized as a group of capillary bundles which allows the use of Poiseuille's law. This causes large underestimation of the pore radii contributing to flow (Tuller and Or, 2001; Nimmo, 2016). Nonetheless, these works are a good example describing how field scale soil moisture is dictated by the activation and connection of different pore networks. The implications of such pore network activation and connection on biogeochemical functioning remain poorly understood, although literature repeatedly expresses that locations where flow paths concentrate and converge result in zones of high reactivity (Krause et al., 2017; McClain et al., 2003; Yan et al., 2017)

Given what is known about pore size and biogeochemical function, this should underscore the importance of further understanding hydrologic connectivity and experimental and modeling approaches that can capture the occurrence. Hydrologic connectivity has unknown implications in regards to priming (Fontaine et al., 2007; Kuzyakov et al., 2000), hot moments of greenhouse gas efflux following soil rewetting (Birch, 1958; Groffman et al., 2009; Sánchez-García et al., 2020, 2020b), and subsoil

carbon dynamics (Chabbi et al., 2009; Kaiser and Kalbitz, 2012; Rumpel and Kögel-Knabner, 2011). Therefore, there is need to synergistically evaluate connectivity from both hydrologic and biogeochemical points of view. Additional carbon models using pore size distributions to constrain carbon transformations are expected to appear in the coming years, but deliberation should be given to how and if connectivity should be represented. As mentioned, measures of connectivity from x-ray tomography are relatively simple to acquire, but much less is known about how vertical connectivity of through the soil pedon affects soil functioning. Furthermore, there has been little incorporation of microbial population dynamics into biogeochemical models, although doing so may be a necessary advancement for accurate projections soil carbon (Sokol et al., 2022; Wieder et al., 2015, 2013). Using structural parameters such as pore size has shown potential in describing the structure and function of microbial communities (Ruamps et al., 2013; Schimel and Schaeffer, 2012) therefore, modeling approaches that emphasize soil structure appear promising. As Baveye (2023) suggest, advancement of biogeochemical models should begin at the pore scale and move upward. One way to examine this upward movement could be done by performing multiscale studies going from pores to pore networks, to the connections and interactions of distinct pore networks.

#### **4.5 Summary**

The opinion presented here has been shaped by research from various fields of study covering scales ranging from individual soil pores to the entire Earth System. Despite the breadth of work covered a consistent message on the way to make progress in Earth Science transcends disciplinary bounds and centers on understanding hydrologic connectivity across scales. Hydrologic connectivity is a function of soil architecture and controls biogeochemical processes. This begins at the pore scale as (dis)connection of liquid films plays an integral role in structuring microbial communities and their functioning. At larger scales the activation and connection of pore networks drive mass fluxes of elements and macroscopic water distributions. Pore scale functioning is beginning to be well understood, but there is still work to be done, particularly on the microbial front as studying microbiology in undisturbed environments is challenging. At larger scales many questions remain as to the ways in which hydrologic flow paths connect and interact. Further, work needs to be done to integrate hydrologic and biogeochemical theory into a cohesive framework. Doing such integration seems feasible using structural model parameters such as pore connectivity or perhaps a measure of connectivity at a larger scale. If a bottom-up approach is the way forward, then multi-scale experimental studies focusing on connectivity should be strongly considered.

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## **Chapter 5**

### **CONCLUSIONS**

#### **5.1 Summary of Research**

This dissertation aimed to explore the impact of soil structural heterogeneity on biogeochemical functions from pore to pedon scales, particularly those related to carbon cycling. An emphasis was placed on macropores and preferential flow paths because they are arguably the most significant generators of heterogeneity at large scales. Heterogeneity seems almost impossible to fully understand and resolve, yet patterns are emerging.

Chapter 2 examines the generation of oxygen profiles due to heterotrophic respiration. Planar optodes were used to monitor oxygen levels in artificial flow systems composed of contrasting sand textures. The contrast in sand texture encouraged preferential flow. The results from this study show that oxygen consumption is greatest along the textural interface of the two sands. The consumption was localized and distinct. In addition, the results have recreated oxygen profiles found in naturally occurring soil aggregates, which may offer mechanistic explanation for why this is seen in the field.

Chapter 3 reviews literature on preferential flow and carbon cycling finding that very little is known about how preferential flow controls biogeochemical functions in soil. Multiple scales are discussed including the pore scale. A conceptual model relating flow, microbial activity, and carbon turnover and storage was created. Pores  $> 300 \mu\text{m}$  and larger were considered macropores and noted as major conduits nutrient laden water fluxes when activated. These macropores deliver nutrients to nearby pores of  $30\text{-}300 \mu\text{m}$ , which function as microbial hotspots, while pores  $< 30 \mu\text{m}$  were defined as locations where carbon can be stored. Another major conclusion from this study was that pore scale biogeochemistry can be upscaled if consideration is given to the connectivity of water at larger scales, aka accounting for large scale heterogeneity.

Chapter 4 digs deeper into the scaling issue concluded in Chapter 3. Matric potential is identified as the most powerful water predictor of  $\text{CO}_2$  efflux. This further confirms that understanding where the water is located and connected pathways of water would be powerful to create large scale carbon models. Unfortunately, very little effort has been made to explore pore size or connectivity as model parameters. In addition to the continued discussion on hydrologic connectivity, a table documenting available literature on how flow dictates biogeochemical heterogeneity was composed. The table should make it abundantly clear that preferential flow is a ubiquitous process and that its control on biogeochemical heterogeneity is equally ubiquitous.

Appendix A experimentally examines how enzyme activity of phosphatase and  $\beta$ -glucosidase are controlled by dispersion and preferential flow in repacked soil cores.

Soil was packed around metal tubes to create voids upon tube removal. The voids were filled with sand to create flow pathways. Breakthrough curves show the degree of dispersion in each of the three treatments, those being homogenous, one path, and two path. Enzyme activity was visually examined along the sand flow paths using time-lapse zymography. Increased dispersion and decreased flow velocity was shown to control enzyme activity. Phosphatase activity was greatest near the macropore linings while less of a trend was apparent for  $\beta$ -glucosidase. Activity of both enzymes was more evenly distributed in the homogenous treatment, and phosphatase activity was greater than  $\beta$ -glucosidase. Management strategies aimed at carbon gains requires knowledge of the pore system. The key to gains appears to be well structured soil with an abundance of well-connected transmission pores that allow for flow, nutrient delivery, and processing of new carbon which can then diffuse into smaller pores in close association with the transmission pores.

## **5.2 Future Research**

This dissertation has identified knowledge gaps, which are arguably significant. Ultimately, many of the knowledge gaps center around hydrologic connectivity across scales. At the pore scale as Chapter 4 points out, biogeochemical organizational patterns are becoming apparent at the pore scale. There does, however, remain significant room to continue pore scale research. Information on microbial interactions in pore space are perhaps the most needed. And while some generalizable patterns

have been recognized, such as which pores contribute the most to CO<sub>2</sub> efflux, considerable efforts remain as to comparing such impacts across different soil types and management practices.

Very little information on connectivity exists at any scale. However, if the community will continue chasing the “holy grail” of upscaling pore scale processes, connectivity is a topic that can no longer be avoided. The logical way to go about this would be to systematically perform multiscale experiments to see which factors were most meaningful at each scale. Shockingly, very few studies attempt this kind of work. To continually discuss upscaling without experimentally performing multiscale studies is ludicrous. Clearly, there is continued work to be done on hydrologic connectivity across scales and as Dr. Keith Beven concluded his 2018 paper “A Century of Denial: Preferential and Nonequilibrium Water Flow in Soils, 1864-1984,” “there is much for the next generation to think deeply about.”



## Appendix A

### IMPACTS OF DISPERSION ON ENZYME ACTIVITY

#### A.1 Abstract

Dissolved organic matter (DOM) is a mobile and labile component of the soil carbon pool. Relationships between water content and microbial activity are well-known, however few studies attempt to link flow and *in situ* microbial response. The goal of this study was to determine how dispersion controls the intensity and spatial distribution of  $\beta$ -glucosidase and phosphatase activity. Three soil columns were repacked from sieved soil into three geometries homogenous, one path, and two paths. The first contained only sieved soil representing a homogenous case. The other two contained the same soil, but also vertical sand flow paths. For each of the three columns, breakthrough curves of  $\text{Br}^-$  were determined and glucose solution was added under saturated flow conditions to induce microbial activity. Once flow concluded the soil columns were given a 24-hour incubation period before performing time-lapse zymography to examine spatial activity of phosphatase and  $\beta$ -glucosidase. In all cases phosphatase activity was highest near surface and decreased with depth. The opposite trend was observed for  $\beta$ -glucosidase, which increased with depth. Zymograms showed that in the flow path systems phosphatase activity was greatest near the macropore linings while in the homogenous case the activity was more evenly

distributed. Results show that residence time of carbon in pore space is a significant control on enzymatic activity.

## **A.2 Introduction**

Dissolved organic matter (DOM) is a mobile and labile component of the soil carbon pool (Buettner et al., 2014; Yan et al., 2018; Zsolnay, 1996) making hydrology a key contributor to the fate and distribution of DOM in soil (Jardine et al., 2006, 1990; Marin-Spiotta et al., 2011; Schlüter et al., 2022). In fact, hydrologic regime has been concluded to be more important for DOM fluxes than biotic factors, especially with increasing time scales (Kalbitz et al., 2000). This however does not mean that biotic factors are unimportant. Microbes, and more specifically, the extracellular enzymes they secrete, drive biogeochemical processes in soil, which mediate plant growth and soil-atmosphere exchange of carbon (Burns et al., 2013). For these reasons extracellular enzyme activities have often been used to index soil quality, as changes in their activity and abundance can trigger substantial shifts in biogeochemical cycles (Dick, 1994). Therefore, while enzymes are the drivers of biogeochemical functioning, water is the facilitator of it (Birch, 1958; Linn and Doran, 1984; Orchard and Cook, 1983). Consequently, enzyme kinetics are increasingly found in soil carbon models projecting future global carbon stocks (Davidson et al., 2012; Wang et al., 2022; Wieder et al., 2015, 2013).

Current soil carbon model assumptions are often made from bulk soil characteristics, ignoring the ubiquitous micro- and macroscale heterogeneities that dictate microbial habitat and activity (Baveye et al., 2018; Bogner et al., 2012; Chabbi et al., 2009; Ruamps et al., 2011; Young et al., 2008). This creates a great deal of uncertainty on the microbial representation in these models (Sokol et al., 2022). Thus, understanding the factors that control the heterogeneous distribution of enzyme activity in soil is required to fully understand organic matter fate and ecological function (Baldrian, 2014). Solute transport, determined by convection, hydrodynamic dispersion flux, and diffusion (Jury and Horton, 2004), is one cause of enzymatic heterogeneity as it induces hotspot formation via the delivery of substrate during precipitation events (Kuzyakov and Blagodatskaya, 2015; McClain et al., 2003). As DOM traverses the soil matrix its fate is a function of input rate and soil structure (Franklin et al., 2021; Jardine et al., 2006; Letey, 1991; Rabot et al., 2018; Zsolnay, 1996), which plays a key role in defining hydrologic properties (Fatichi et al., 2020; Lin, 2003; Schlüter et al., 2012) and microbial accessibility of mobilized carbon (Dungait et al., 2012; Lehmann et al., 2020; Lehmann and Kleber, 2015; Tipping, 1998).

One of the most influential components of soil structure in terms of both hydrology and biology is soil macropores. Beven and Germann (2013) concluded that preferential flow through macropores, where small volumes of soil transmit large volumes of water (Beven and Germann, 1982; Flury et al., 1994; Watson and Luxmoore, 1986), may be the most significant cause of physical, chemical, and

biological heterogeneity within the soil matrix. Preferential flow paths are often found to be enriched with fresh organic matter (Bogner et al., 2012; Bundt et al., 2001a; 2001b; Chabbi et al., 2009) likely in part due to the increased DOM load during heavy rainfall events. Yet, to this day macropore's physical (Beven, 2018; Weiler, 2017) and biogeochemical influence often remains conveniently ignored (Franklin et al., 2021). In addition, there are few studies that attempt to examine the coupled effects of hydrology and *in situ* microbial response.

One reason for this is because imaging microorganisms and enzymes *in situ* has been, and still is, a great challenge (Védère et al., 2022). Traditional methods to examine enzyme activity require destructive sampling and homogenization (Nannipieri et al., 2018) offering little information on the spatial distribution of enzymes. However, with the advent of *in situ* zymography (Spohn et al., 2013; Vandooren et al., 2013) various enzymes can now be visualized within the 2D structural make-up of the soil.

Although, even with this new methodology most studies do not examine enzyme response in relation to flow. Rather, most studies have focused on examining enzymes in relation to structures. For example, Kravchenko et al. (2019; 2019b) coupled x-ray computed tomography with zymography, relating pore sizes 30-150  $\mu\text{m}$  with enzyme activity. Many others have examined worm burrows and root channels using zymography (Hoang et al., 2016; Razavi et al., 2017; Tian et al., 2020; Zhang et al., 2019). Macropores from argillic horizons have been examined using zymography (Leue et al., 2021) and others performed zymography on undisturbed samples of

descending depth of a soil profile even suggesting that preferential flow may be the reason for subsoil enzymatic hotspots (Heitkötter and Marschner, 2018). In either case the influence of flow itself was not examined and only one study we are aware of has examined both flow and enzyme activity using zymography (Krueger et al., 2018). On the other hand many studies have examined the transport of DOM, but have not looked at *in situ* microbial response (Leinemann et al., 2018, 2016; Rieckh et al., 2015; Tiefenbacher et al., 2021).

Therefore, in this chapter the *in situ* response of soil enzymes to saturated flow events in three model systems was examined. The objectives of this study were to i) conduct tracer breakthrough experiments to describe the flow regime and estimate relevant flow parameters ii) perform time-lapse zymography to examine the spatial distribution of  $\beta$ -glucosidase and phosphatase activities and iii) to evaluate if 2D models of solute transport could also provide insight into the intensity and spatial distribution of  $\beta$ -glucosidase and phosphatase activity in three model systems.

### **A.3 Materials and Methods**

#### **A.3.1 Soil Sampling**

Soil was sampled from the top 15 cm at the University of Delaware Webb Farm. The loamy soil was taken back to the lab and laid out to air dry under the fume hood for 48

h. Following drying the soil was triple sieved through 500  $\mu\text{m}$  to remove any visible organic matter. The soil was then stored at 4°C.

### **A.3.2 Column Preparation**

The three model systems include homogenous, one path, and two paths. For the homogenous treatment, the sieved soil was weighed out to 105.2 g and was then mixed with 49.5 g of 250-400  $\mu\text{m}$  particle size sand to achieve a desired texture. Before packing the soil mixture into the columns, a thin layer of sand was added to the base to facilitate drainage and minimize clogging. Once the sand was added to the base the soil mixture was then poured into the columns. The soil was packed with a custom-made slide hammer to a bulk density of 1.4  $\text{g}/\text{cm}^3$ . The final dimensions of the soil were 10 cm height by 3.75 cm diameter. For the one and two path treatments the same soil mixture was made and packed around 0.5 cm diameter tubes. However, to ensure the same soil bulk density of 1.4  $\text{g}/\text{cm}^3$ , 1.96 g or 3.92 g was removed to correct for volume filled by tubes. For the one path treatment the tube was placed in the center. For the two path treatment the tubes were 0.975 cm from the wall with a 0.8 cm distance between the two tubes. The packing methodology can be seen in Figure A.1.

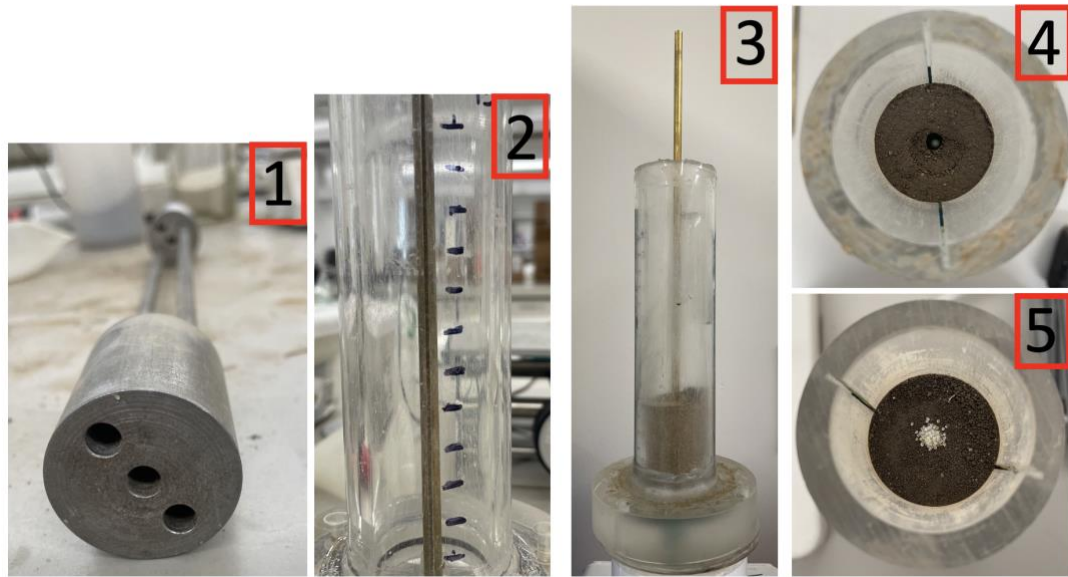


Figure A.1. Packing methodology used in the study. Image 1 shows the slide hammer used to pack the soil around tubes. Image 2 shows centering of the tube. Image 3 shows soil beginning to be packed around the tube. Image 4 shows the removal of the tube leaving an empty hole. Image 5 shows the empty hole filled with sand.

To saturate the soil, columns were flushed with CO<sub>2</sub>, which is water soluble, for 1 h to minimize air entrapment during saturation (Klute, 1986). The soil was then saturated from bottom of the column with a 10 mM CaCl<sub>2</sub> solution to avoid dispersion of clays. In the case of the one and two path treatments the soil was saturated to the surface and then disconnected from water. Then the tubes were removed, and the holes were filled with 3.3 g of 250-400 μm particle size sand. Once the sand was added the columns were again attached to water and slowly saturated. The saturation was done until the columns were overflowing from the top and left overnight.

### A.3.3 Conservative Tracer

Breakthrough curves of conservative tracers are commonly measured to describe the hydrology of the soil system and estimate relevant flow parameters for modeling (Dousset et al., 2007; Köhne et al., 2011; Steenhuis et al., 1994). In this study, once the column's saturation was complete, they were connected to fraction collectors. The CaCl<sub>2</sub> solution was allowed to drain to level with the soil surface and then the drainage tube was clipped to pause flow. While paused a 3 cm pond of 150 ppm KBr<sup>-</sup> solution was added. The KBr<sup>-</sup> solution was added manually for the duration of 1 pore volume, maintaining the 3 cm pond. Then a Mariotte bottle containing 10 mM CaCl<sub>2</sub> solution was connected to the 3 cm pond to flush out the KBr<sup>-</sup> remaining in the soil. The effluent samples were collected from the columns using a fraction collector in 15 mL tubes; each tube was weighed to record the flow rate. Once weighed, the samples were vacuum filtered through 0.2 μm in preparation for measurement of Br<sup>-</sup> concentration on a Metrohm IC Pro.

#### A.3.4 Modeling

When considering modeling approaches in HYDRUS (Šimůnek and Genuchten, 2008). Solute transport in homogenous porous media can be described using the convection-dispersion equation (Eq. 1).

$$\frac{\partial C_l}{\partial t} = D \frac{\partial^2 C_l}{\partial z^2} - V \frac{\partial C_l}{\partial z} \quad \text{where } D = D_e/\theta \quad (\text{Eq. 1})$$

$C_l$  is the dissolved chemical concentration,  $t$  is time,  $D$  is dispersion coefficient,  $z$  is distance, and  $V$  is velocity.  $D_e$  is effective diffusion-dispersion coefficient comprised of hydrodynamic dispersion and liquid diffusion, and  $\theta$  is water content. For the flow path treatments, there are two approaches used in HYDRUS to quantify preferential flow, those being dual-porosity and dual-permeability. Here the dual-permeability model is a more appropriate choice. The dual-porosity model assumes that there is a mobile and immobile region of water flow. This would assume convective flow taking place through the sand paths while the immobile water in the soil matrix would only contribute to flow by diffusional exchange with the mobile region. However, in this experiment the soil matrix was conductive making the dual-permeability approach more applicable. The dual-permeability model allows for water flow in the matrix and sand paths or macropores, as well as exchange between the two regions (Gerke and van Genuchten, 1993). Equation 2a is representative of the fracture or sand flow path region while equation 2b is that of the matrix, denoted with subscripts  $f$  and  $m$  respectively. Equation 2c describes the mass transfer between the two regions.

$$\frac{\partial \theta_f c_f}{\partial t} = \frac{\partial}{\partial z} \left( \theta_f D_f \frac{\partial c_f}{\partial z} \right) - \frac{\partial q_f c_f}{\partial z} - \phi_f - \frac{\Gamma_s}{w} \quad (\text{Eq. 2a})$$

$$\frac{\partial \theta_m c_m}{\partial t} = \frac{\partial}{\partial z} \left( \theta_m D_m \frac{\partial c_m}{\partial z} \right) - \frac{\partial q_m c_m}{\partial z} - \phi_m + \frac{\Gamma_s}{1-w} \quad (\text{Eq. 2b})$$

$$\Gamma_s = \omega_{dp}(1 - w)\theta_m(c_f - c_m) + \Gamma_w c^* \quad (\text{Eq. 2c})$$

$\Gamma_s$  is mass transfer of solutes between fast and slow regions,  $\phi$  is porosity,  $q$  is Darcy velocity,  $w$  is the ratio of the volume of the sand to the total domain,  $\omega_{dp}$  is mass transfer coefficient,  $\Gamma_w$  is the transfer rate of water between sand and matrix, and  $c^*$  is equal to  $c_f$  for  $\Gamma_w > 0$  and  $c_m$  for  $\Gamma_w < 0$ . To apply these models, mass transfer coefficient between fast and slow regions and dispersion coefficients need to be estimated by performing inverse modeling from the measured breakthrough curves. In addition to modeling of solute transport, the decay of substrate can also be expressed by equation 3.

$$\theta \frac{\partial c_l}{\partial t} = D_e \frac{\partial^2 c_l}{\partial z^2} - q \frac{\partial c_l}{\partial z} - \mu \theta c_l \quad (\text{Eq. 3})$$

Equation 3 resembles equation 1 very closely but includes the addition of a sink term with the first-order decay rate constant  $\mu$ . Therefore, it can be expected to see a relationship between dispersion and microbial utilization.

### A.3.5 Time-Lapse Zymography

Following the flush of  $\text{KBr}^-$ , a 250 mL solution (~5 pore volumes) containing 2 mg  $\text{mL}^{-1}$  glucose and 1.52 mg  $\text{mL}^{-1}$   $(\text{NH}_4)_2\text{SO}_4$  was added at the same 3 cm ponded head. After the glucose addition was complete the columns were allowed to drain freely by gravity for 48 h, which served as an incubation period for microbial growth. Following the 48-h incubation period, time-lapse zymography was performed (Guber et al.,

2021). To perform time-lapse zymography, enzyme specific substrates were prepared. Here phosphatase and  $\beta$ -glucosidase were examined, thus 4-methylumbelliferyl phosphate and 4-methylumbelliferyl  $\beta$ -D-glucopyranoside substrates were used. First the substrates were made to 10 mM concentration by dissolving 0.0338 g and 0.0256 g in 300  $\mu$ l dimethyl sulfoxide, and then 9.7 ml of DI water. The 10 mM solution was then diluted to 6 mM and prior to use. In addition to the enzymatic substrate, a standard curve was prepared so that fluorescent signal strength could be related to activity. For this, a 10 mM 4-methylumbelliferyl solution was made by dissolving 0.1762 g of 4-methylumbelliferyl in 50 mL of methanol. This was then added to 50 mL of DI. The 4-methylumbelliferyl was diluted to 0.1, 0.25, 0.5, 1.0, and 2.0 mM for the standard curve (Figure A.2). The 10 mM 4-methylumbelliferyl solution was diluted using 0.1 2-(N-morpholino)ethanesulfonic acid buffer solution. Once the dilution was completed 5  $\mu$ l of each concentration was applied to a hydrophilic 0.45  $\mu$ m polyamide filter paper and imaged under UV light. The camera was set to an aperture of f/5.6 and exposure time of 1/125 s. After these steps were completed time-lapse zymography was performed under the same camera settings.

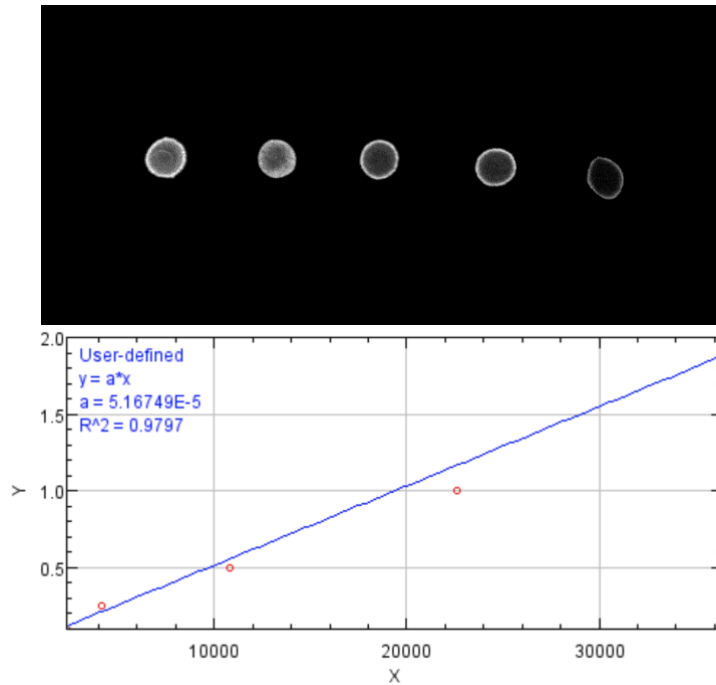


Figure A.2 Standard curve of 4-methylumbelliferyl solution at concentrations of 2.0, 1.0, 0.5, 0.25, 0.1 mM with the linear regression generated in ImageJ.

Based on the results found in Chapter 2, showing oxygen depletion along macropore linings, the macropore linings were again of significant interest here to monitor for enzyme activity. Thus, to examine the macropore linings the soil was cut in half along the sand flow paths. To do this the base of the soil columns were removed and then placed on top of a circular disk matching the diameter of the cylinder. The column was secured in place and the disk was slowly pushed upwards via a thread rod. Once 5 cm of soil protruded (half the total soil height), it was cut vertically. The 5 cm piece of soil was collected, and the other 5 cm piece was then pushed out. This process can be seen in Figure A.3. To minimize the amount of soil that would become stuck to the cutting blade, the soil was placed into a freezer for 10 min prior to cutting. This allowed the soil to become firm, but not frozen. Once the two halves were cut, they

were given time to return to room temperature before beginning time-lapse zymography. Fully cut soil halves for each treatment can be seen in Figure A.4.

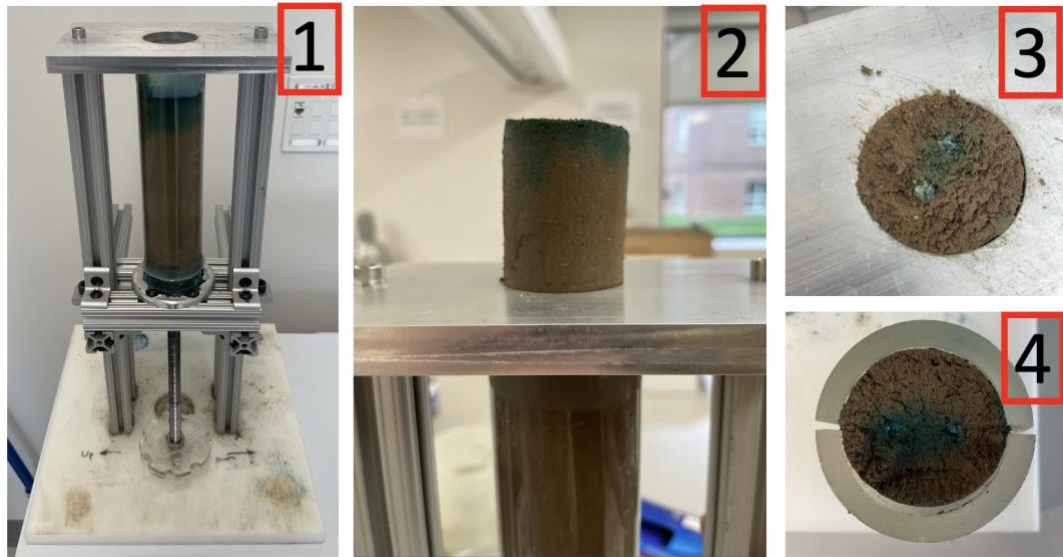


Figure A.3. Cutting method to achieve two halves for time-lapse zymography. Image 1 shows the secured column on top of the circular disk. Image 2 shows the cutting platform. Image 3 shows the base of a horizontal cut. Image 4 shows the soil surrounded by two cylinder halves which served as guide rails for the vertical cut along the sand flow paths.

To perform time-lapse zymography a soil half was placed into a custom made zymography frame (Figure A.5). The base is half of a cylinder of the same 3.75 cm diameter as the soil was packed into. The rest of the frame was made using a laser cutter and glued together. Threaded studs were glued on so that an attachment made using the laser cutter could press down onto the 3D printed frame which clipped over a substrate-soaked polyamide membrane, which would be in direct contact with the soil surface. On top of the substrate-soaked membrane was a non-reflective plastic film

that served to limit evaporation from the membrane over the duration of image capture.

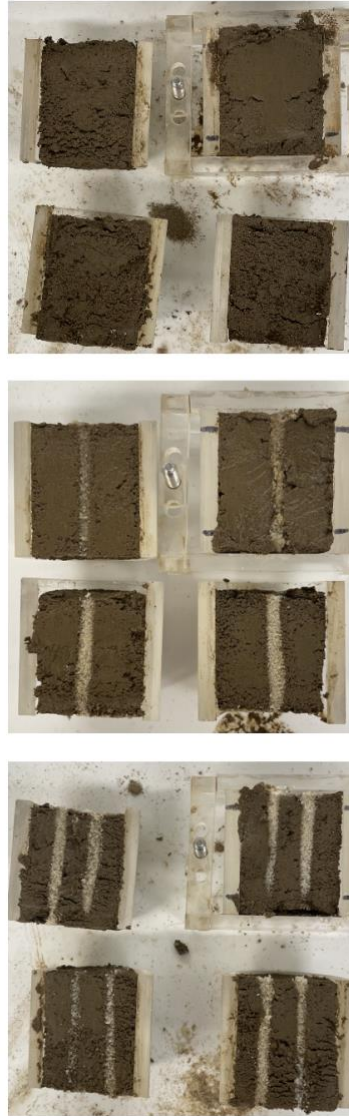


Figure A.4. Soil halves after the cutting process for each of the three treatments.

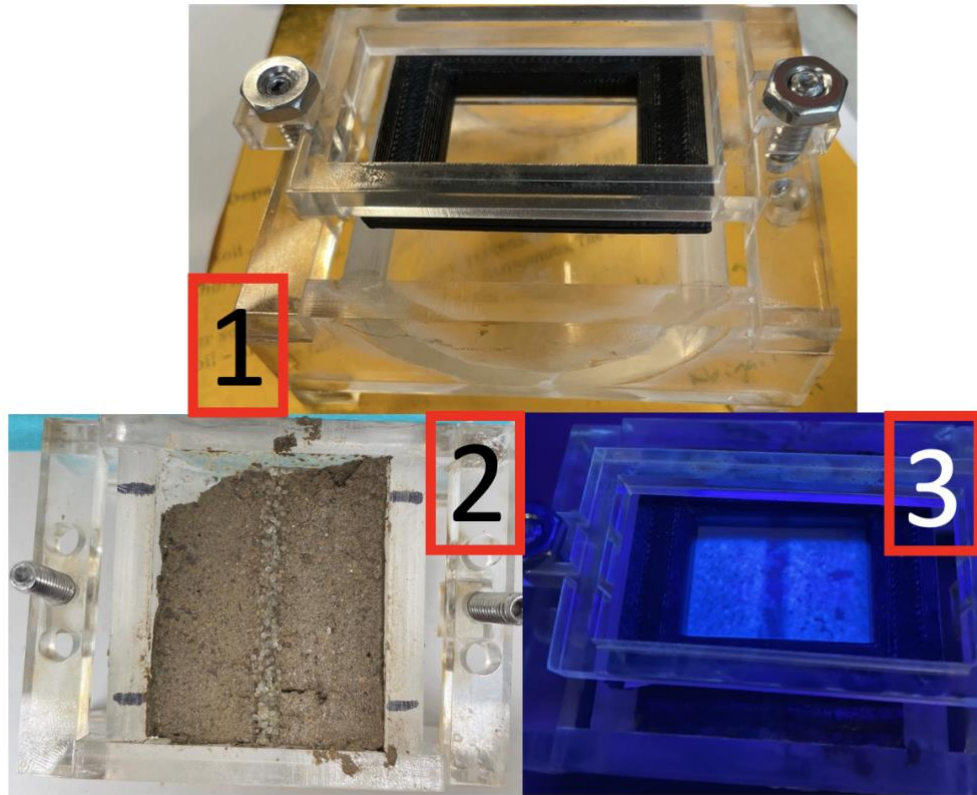


Figure A.5. Image 1 shows the empty zymography frame. Image 2 shows one path soil half in the zymography frame. Image 3 shows a  $\beta$ -glucosidase soaked membrane, being pressed firmly onto the soil surface with the screwed on attachment, during 45-minute incubation.

## A.4 Results and Discussion

### A.4.1 Breakthrough Curves

The outflow rates of the three columns can be seen in Figure A.6. The average volume flux from the homogenous, 1 path, and 2 path treatments was 1.26, 4.56, and 7.96 ml  $\text{min}^{-1}$ . These correspond to a Darcy velocity of 6.85, 24.78, and 43.26  $\text{cm hr}^{-1}$ ,

respectively, when converting using the surface area of the column. The saturated conductivity ( $K_s$ ) of the soil based on a calculation from the outflow rate of the homogenous column is  $5.27 \text{ cm hr}^{-1}$  while the measured ( $K_s$ ) of the sand was  $213.2 \text{ cm hr}^{-1}$ .

The breakthrough curves are shown in Figure A.7. In the one path system the  $C/C_0$  value reaches a maximum value of 0.63. For the two path treatment  $C/C_0$  never reaches 1, reaching its highest value at 0.80. Both cases reflect dispersion. As dispersion increases breakthrough curves become more spread out and the peak decreases. Preferential flow can be confirmed in the one and two path treatment due to early arrival time and long tailing expressed in the shape of the curve indicative of extended hydrodynamic dispersion in the flow path systems. The tailing indicates that there are some immobile or pockets of slowly mixing water in the system. This contrasts with the homogenous treatment which expresses gradual breakthrough and flushing of  $\text{Br}^-$  resulting in a symmetrical curve. In addition, after 4 pore volumes,  $\text{Br}^-$  could no longer be detected in the effluent coming from the homogenous column, while one and two path systems contained  $\text{Br}^-$  for over 10 pore volumes.

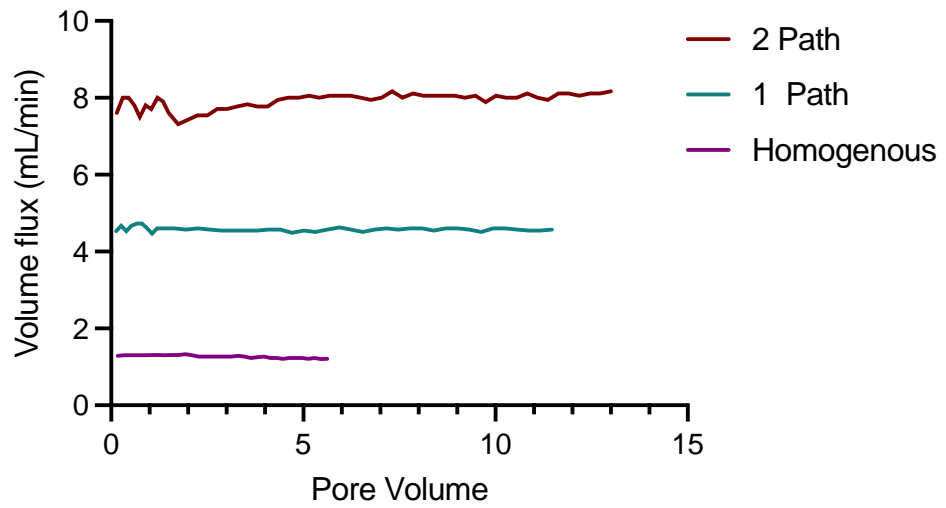


Figure A.6. Measured outflow through time from all treatments. Time 0 marks the start of  $\text{Br}^-$  addition.

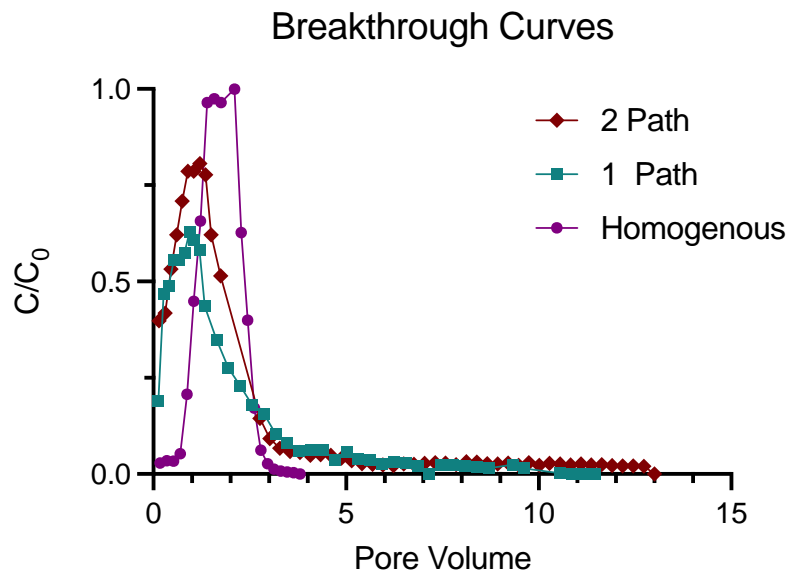


Figure A.7. Breakthrough curves of  $\text{Br}^-$  for the three treatments.

#### A.4.2 Enzyme Activity

In each treatment there are clear differences in both the distribution of enzyme activity as well as the intensity of it. The results show that in the flow path treatments phosphatase activity is greatest near the macropore linings while the flow path effect on  $\beta$ -glucosidase is less apparent. In the homogenous treatment the spatial distribution of both enzymes is more evenly spread (Figure A.8). In all cases phosphatase activity decreased with depth and  $\beta$ -glucosidase activity increased with depth with phosphatase activity being greater overall. The function of phosphatase is to catalyze the hydrolysis of ester-phosphate bonds releasing phosphate into the environment (Margalef et al., 2021; Nannipieri et al., 2011) while  $\beta$ -glucosidase degrades cellulose releasing glucose (Turner et al., 2002). Glucose has been shown to have inhibitory effects on  $\beta$ -glucosidase, termed end product inhibition (Xiao et al., 2004) explaining the lack of  $\beta$ -glucosidase activity. On the other hand, while glucose was ample for microbial utilization, phosphorus is a limiting nutrient. Therefore high levels of phosphate enzymes were produced to acquire the growth limiting phosphate (Allison and Vitousek, 2005). The phosphatase activity was highest in the top 5 cm. This is because the top 5 cm has the first opportunity to use the added nutrients leaving less available at depth. This also explains why  $\beta$ -glucosidase activity is greater at depth. Due to their being less glucose at depth there would have been less end product inhibition. Additionally, there may be an effect of time on the enzyme activity. In

Figure A.5, image 3,  $\beta$ -glucosidase activity is resemblant of phosphatase activity with activity greatest along the macropore lining. The difference here is that zymography here was performed after 3 days rather than 24 hours. With the extended period glucose may have been largely exhausted from the system causing the expression of  $\beta$ -glucosidase enzymes to acquire carbon.

The highest activity for phosphatase was reached in the one path system followed by the two path and homogenous respectively. This was true even at the furthest distances from the flow path indicating an effect of higher hydrodynamic dispersion due to higher flow velocities (Bear, 1972) and increased oxygen availability. The results indicate that macroscopic residence time of glucose is less important than flow velocity and residence time in localized pore space. For example, in the homogenous system, the 250 mL pulse of glucose ran through the soil for 3.3 hours. However, most of the pore water in homogenous repacked soil is moving at all times (Jury and Horton, 2004). In contrast, the one and two path systems had total glucose flow times of 0.913 hours and 0.52 hours respectively. Despite rapid loss through the flow paths and less total exposure glucose was trapped in the matrix as reflected by the long tailing in the breakthrough curves. Therefore, while macroscopic residence time was less, residence time in some pores was greater allowing the glucose to be utilized more effectively. Convection allows DOM to travel distances much further than diffusion alone as microbial utilization is oftentimes greater than diffusional speed limiting the sphere of influence via diffusion to only a few mm (Don et al., 2008; Kuzyakov et al.,

2003; Smercina et al., 2021; Védère et al., 2020). These results are in agreement with previous work that shows that biodegradation rates are higher along macropore channels (Pivetz et al., 1996; Pivetz and Steenhuis, 1995). Additionally, enzymatic hotspots have also been found at textural interfaces by Krueger et al. (2018). In this experiment had the texture of soil matrix been more clayey, it could be expected that the enzyme activity would be more localized at the wall due to less interaction with the soil matrix (Chenu et al., 2001) suggesting that the amount of interaction the macropore has with the soil matrix will change the overall outcome (Weiler and Flühler, 2004). This has implications for overall carbon gains and losses at the pedon scale.

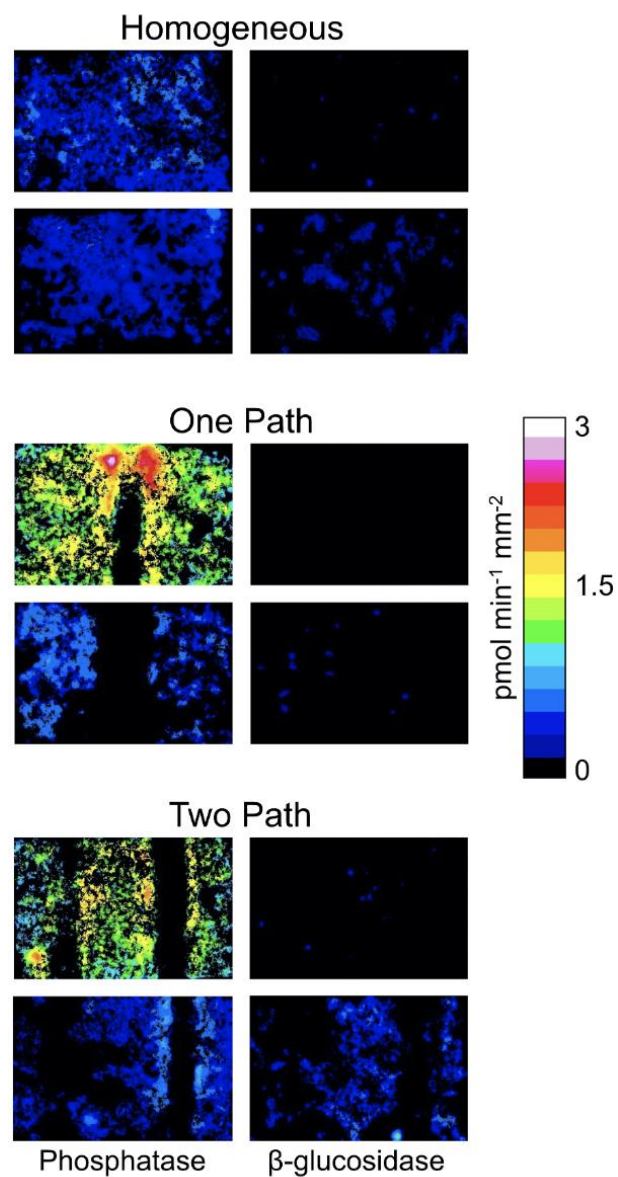


Figure A.8. Zymograms of phosphatase and  $\beta$ -glucosidase for each of the soil treatments. The images shown are from the center of of the upper 5 cm and lower 5 cm of the soil cores. Each image shows the measured 2.5 x 2 cm area from the center each soil half.

Recent findings suggest that microbial activity over spatially larger areas of the pore system promote carbon gains (Kravchenko et al., 2019). This is counterintuitive, but it is because microbial necromass in soil accretes constituting considerable portions of soil organic matter (Angst et al., 2021; Anthony et al., 2020; Liang et al., 2019). Furthermore, the organic matter that is processed will result in slower cycling forms (Lange et al., 2015) that quickly become mineral bound (Cotrufo et al., 2013). In this study enzyme activity was greatest in the flow path systems in comparison to the homogenous system. In the field flow paths are often reported to contain greater bacterial biomass and function as hotspots (Bak et al., 2019; Bundt et al., 2001b; Fuhrmann et al., 2019). This would seemingly suggest that the flow path systems would be more advantageous for carbon gains than the homogenous system. However, preferential flow through macropores has been shown to limit sequestration of DOC (Jardine et al., 2006) due to reduced contact time between DOM and mineral surfaces (Kaiser and Kalbitz, 2012). This is further expressed by Chabbi et al. (2009) who show that while flow paths contain greater contents of carbon than the soil matrix, the soil matrix shows greater affinity for the carbon to form mineral associations and long term storage. Furthermore, macropore flow can lead to additional carbon losses due to priming of soil carbon stored at depth (Fontaine et al., 2007).

Preferential flow effectively shrinks the microbial spatial footprint macroscopically by bypassing large portions of the soil matrix. The carbon gains found in Kravchenko et al. (2019) study have been associated with an increase in total porosity and greater

presence of pores 30-150  $\mu\text{m}$  where enzyme activity is most abundant (Kravchenko et al., 2019). Elsewhere, soil sampled along a natural carbon gradient showed that higher carbon contents led to soil with greater presence of 200-600  $\mu\text{m}$  pores (Larsbo et al., 2016). The increased abundance of pores of these sizes significantly reduced the amount of preferential flow through larger macropores. Therefore, when defining management strategies aimed at carbon gains, understanding the manipulation of the pore system is imperative. The key appears to be well structured soil with an abundance of well-connected transmission pores that allow for flow, nutrient delivery, and processing of new carbon which can then diffuse into smaller pores in close association with the transmission pores.

## **A.5 Conclusions**

The breakthrough curves and flow rates express the expected trends. The results time-lapse zymography express the expected trends, showing activity greatest along the macropore linings and a more even distribution in the homogenous case. Phosphatase activity was greater than  $\beta$ -glucosidase. This can be concluded to be because of  $\beta$ -glucosidase experiencing end product inhibition while phosphatase was expressed to acquire growth limiting P. It may be interesting going forward to examine other enzymes or create a nutrient solution containing other compounds coming from root exudates. In addition, the time zymography is performed may influence enzyme expression. While the model systems used in this experiment cannot be considered

real soil structure it can provide insight into how flow and enzyme activity may be related. There is a clear relationship between heightened activity and dispersion. This is because dispersion cause some nutrients to become stuck in pore space for long periods of time making it easily accessible to microbes. Higher flow velocities limit microbial utilization. The results suggest a well-balanced pore structure with well-connected transmission pores are key for carbon gains.

A flaw of the study was the soil saturation technique that used for the one and two path systems. They were saturated, disconnected from water, holes filled with sand, and then re-saturated. Even with the overnight flush, air bubbles remain. While steady-state flow rates were achieved, it is hard to say if the soil was truly fully saturated. Continued work is now being performed by using tubes with a larger internal diameter that allow the tube to be filled with sand while the soil is packed around it. Once the soil is packed the tube can be removed and the sand can fall into the void space. The columns can then be flushed with CO<sub>2</sub> and saturated in a single step. Additionally, the enzyme activities will be higher when improving the resolution of image capture, however the trends are correct.

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Franklin, S.M., Kravchenko, A.N., Vargas, R., Vasilas, B., Fuhrmann, J.J., Jin, Y., 2021. The unexplored role of preferential flow in soil carbon dynamics. *Soil Biology and Biochemistry* 161, 108398. Copyright © 2021 by Elsevier Ltd.