

AN ABSTRACT OF THE THESIS OF

Thomas Wanzek for the degree of Master of Science in Soil Science presented on October 31, 2017

Title: Measuring Electron Activity to Constrain the Role of Soil Structure in the Formation of Biogeochemical Heterogeneity

Abstract approved:

Markus Kleber

Soils have a critical role in global carbon (C) cycling, containing one of the largest fast-cycling carbon stocks on earth. Robust representation of soil organic matter dynamics in Earth System Models is critical for future climate prediction. Current C cycling models assume that all C cycling in non-hydric (i.e. ‘upland’) soils occurs solely via aerobic microbial metabolism. However for at least 30 years, it has been known that what may be a seemingly aerobic soil environment can in fact contain pockets of both aerobic and anaerobic metabolic processes. None of the modeling efforts to date have the capacity to estimate or include the spatial abundance of or contribution to C cycling from anaerobic microbial metabolic processes. What is missing is quantitative information detailing the division of ‘upland’ soils into aerobic or anaerobic environments on seasonal time scales. This thesis addresses the general question of how the division of an ‘upland’ soil into aerobic or anaerobic environments can be quantified and the driving mechanisms identified and parameterized. Platinum-based electrodes were used to measure changes in electron activities (reported as electromotive potential) as a method for distinguishing

biogeochemically distinct soil environments. The first chapter details long-term field measurements of aerobic and anaerobic environments in three ‘upland’ Mollisols, all in close geographical proximity, but which formed a hydrologic gradient. The extent to which each of the soils was divided into biogeochemically distinct environments was measured using two-dimensional grids of Pt-electrodes. Variation in electron activity as a function of depth, horizontal position within the soil profile, and seasonal climatic drivers was recorded. The second chapter establishes a first-order mechanistic relationship between the volume, connectivity, and general shape of the soil pore system and the metabolic status of the soil (measured as electron activity) as a function of pore network architecture. X-ray computed tomography was used to parameterize three different pore network architectures; one native and two artificially generated using one of the soils from chapter one. The strength of the relationships between the resulting pore network metrics and electron activity dynamics were then established. This work demonstrates that diverse biogeochemical conditions can not only simultaneously coexist in ‘upland’ soils, but that the extent to which a soil is divided into heterogeneous environments is more a function of seasonal precipitation events than seasonal temperature changes. Furthermore, the pore network characteristics related to the formation of anaerobic environments are a function of the scale of observation as well as aggregate size.

©Copyright by Thomas Wanzek
October 31, 2017
All Rights Reserved

Measuring Electron Activity to Constrain the Role of Soil Structure in the Formation
of Biogeochemical Heterogeneity

by
Thomas Wanzek

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented October 31, 2017
Commencement June 2018

Master of Science thesis of Thomas Wanzek presented on October 31, 2017

APPROVED:

Major Professor, representing Soil Science

Head of the Department Crop and Soil Science

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Thomas Wanzek, Author

ACKNOWLEDGEMENTS

I am most grateful for and thankful to my advisor, Markus Kleber, for his seemingly endless reserves of guidance, input, and support over my graduate school experience. I would also like to acknowledge all of the research opportunities and outlets that he provided in an effort to produce meaningful scientific results.

I would also like to express my gratitude toward my committee, Mark Johnson, Maria Dragila, and Jeff Jenkins. Thank you for always being available whenever there seemed to be an unsolvable hitch in my research efforts. There are also many of my fellow graduate students that I would like to thank here, but there is not enough space to list all of you. Thank you for all of the impromptu, and sometimes meandering, conversations about research, school, and life in general. Without these outlets, I would likely not have made it through.

Finally, thank you to my family, both nuclear and extended. Without your encouragement and unending love and support, this would have been a much more arduous adventure.

CONTRIBUTION OF AUTHORS

Wanzek and Markus Kleber (Oregon State University, Department of Crop and Soil Science) were responsible for the synthesis of the manuscript entitled “Quantifying biogeochemical heterogeneity in soil systems” and all authors discussed the results and provided editorial comments. Wanzek, Marco Keiluweit (Stockbridge School of Agriculture and School of Earth and Sustainability, University of Massachusetts), and Kleber prepared field sites, collected the data, and synthesized the results. Maria Dragila (Oregon State University, Department of Crop and Soil Science) and Sabine Fiedler (Geographisches Institut, Johannes Gutenberg-Universität Mainz) provided field equipment and sensors. John Baham (Oregon State University, Department of Crop and Soil Science) provided advice and schematics for Pt-electrode construction. Peter S. Nico (Lawrence Berkeley National Laboratory), Scott Fendorf (Stanford University), and Keiluweit provided data interpretation support and manuscript synthesis guidance.

For the manuscript “The ability of pore network metrics to predict soil redox status is scale dependent” Wanzek was responsible for data collection and along with Kleber for synthesis of the manuscript. Tamas Varga (Environmental Molecular Sciences Laboratory, PNNL) generated the x-ray scans and trained Wanzek on the Nikon (Metris) X-ray computed tomography system. Adam Lindsley (Oregon State University, Department of Crop and Soil Science) provided assistance and direction in data visualization. Keiluweit, Fendorf, and Nico contributed to the overall development of the experimental design and discussion of results.

TABLE OF CONTENTS

	<u>Page</u>
1. General Introduction	1
2. Quantifying biogeochemical heterogeneity in soil systems (Chapter One)	6
2.1. Abstract.....	7
2.2. Introduction	8
2.3. Methods	15
2.4. Results	20
2.5. Discussion.....	35
2.6. Conclusions	38
2.7. Acknowledgments	39
3. The ability of pore network metrics to predict soil redox status is scale dependent (Chapter Two)	40
3.1. Abstract.....	41
3.2 Introduction	42
3.3 Methods	50
3.4 Results and Discussion	65
3.5 Conclusions	88
3.6 Acknowledgments	92
4. Summary and Conclusions	93
5. Bibliography	95
6. Appendices	106

TABLE OF CONTENTS (Continued)

6.1 Appendix A (Supplementary Information for Chapter One).....	106
6.2 Appendix B (Supplementary Information for Chapter Two)	112
6.3 Appendix C (All E_{Pt} curves)	121
6.4 Appendix D ($VoI_{0.2}$ XCT images)	123
6.5 Appendix E (VoI_{25} XCT images)	125
6.6 Appendix F (VoI_{100} XCT images)	126
6.7 Appendix G (Definitions of terms).....	128

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
 Chapter One:	
Figure 1. Disparity between specific redox potentials for common redox couples and the experimentally observed range for the first appearance of the reduced species in soils as a function of increasing electron activity following inundation.	13
Figure 2. Time series of precipitation (panel a); and water filled pore space (WFPS) at the Woodburn (Panel b) and Amity (Panel c) soils for the observation period.	22
Figure 3. Time series of platinum probe potentials within the linear array of n=5 probes installed at a depth of 20 cm in the Amity soil.	25
Figure 4. Time series of platinum electrode potentials (E_{Pt}) across the drainage gradient and for 4 depths (10 cm, 20 cm, 50 cm and 100 cm).	27
Figure 5. Time series of metabolic heterogeneity index across the drainage gradient and for 4 depths (10 cm, 20 cm, 50 cm and 100 cm).	30
Figure 6. Variation of metabolic heterogeneity index across the drainage gradient. .	33
Figure 7. Variation of metabolic heterogeneity index as a function of intensity of the wet season.	34
 Chapter Two:	
Figure 1. E_{Pt} curves for all three pore network architectures.	53
Figure 2. Division of the E_{Pt} curve into electron activity metrics.	55
Figure 3. Location three of volume of interest (VoI) sub-samples.	59
Figure 4. Examples of XCT images generated in the course of this work.	60
Figure 5. Illustrations depicting each pore network metric.	64
Figure 6. Significant correlations between the electron activity metrics and pore network metrics.	77
Figure 7. Frequency of correlation with electron activity metrics. Frequency of correlations with electron activity metrics as a function of pore network metrics (a), skeleton-based pore network metrics (b), and void-based pore network metrics (c). .	79

Figure 8. Incidence of correlations between electron activity metrics and pore network metrics as a function of pore network architecture (a), and volume of soil observed (i.e. Vol; b). 81

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Chapter One:	
Table 1. Symbols, definitions and associated equations	31
Chapter Two:	
Table 1. Pore network metrics and associated descriptions.....	62
Table 2. Variation in Pore Network Metric 11 "Average Void Surface Area" across Pore Network Architectures and Volumes of Interest	68
Table 3. Overview of container volume, volume of soil subsection for X-ray computed tomography analysis, and minimum diameter of soil pores analyzed.	71
Table 4. Significant differences in Electron Activity metrics across Pore Network Architectures and Volumes of Interest	74

LIST OF APPENDIX FIGURES

<u>Figure</u>	<u>Page</u>
Chapter One:	
Figure A.1. Willamette, Woodburn and Amity sites and position of weather station at the Hyslop Crop Science Field Research Laboratory.....	106
Figure A.2. Schematic for the resistance amplifier used to increase the input resistance of a hand held digital multimeter (DMM).....	107
Figure A.3. Time series of precipitation (panel a); and soil temperature per depth for Woodburn (panel b) and Amity (panel b) soils.	108
Figure A.4. Size comparison between Pt-electrodes used in Amity and Woodburn soils.	109
Chapter Two:	
Figure B1. Experimental layout of PVC rings.....	113
Figure B2. Visual timeline of experimental steps and water saturation conditions..	114
Figure B3. Example images of PVC cylinder sub-sectioning.	115

LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
Chapter One:	
Table A.1. Redox couples, associated reduction half – reactions, and specific redox potentials.	110
Table A2. Soil descriptions, profile information, and site locations	111
Table A.3. Monthly precipitation totals for December 2014 through April 2016. ...	112
Chapter Two:	
Table B1. Woodburn series characteristics for A _p horizon, 0 – 20 cm.....	116
Table B2. Significant differences between electron activity metrics within the pore network architectures.	117
Table B3. Significant differences in pore network metrics across pore network architectures for Vol ₁₀₀	118
Table B4. Significant differences in pore network metrics across pore network architectures for Vol ₂₅	119
Table B5. Significant differences in pore network metrics across pore network architectures for Vol _{0.2}	120

1. General Introduction

Upland soils, or soils not falling within the definition of a hydric soil (Staff, 2015), tend to be treated as completely aerobic. However, since at least the seminal work of Sexstone et al. (1985), it has been known that a soil can encompass heterogeneous environments that foster microbially mediated biogeochemical processes ranging from unrestricted aerobic metabolism to green house gas production (von Fischer et al., 2009). This invalidates the assumption that all reaction spaces within soil system can be treated as entirely aerobic. The presence of various biogeochemical processes functioning simultaneously in the soil pore network (Kuzyakov and Blagodatskaya, 2015) shifts the understanding of how soils process (or store) carbon and nutrients (Ball, 2013), and has yet to be integrated fully into the understanding of soil biological functioning in general (Nunan, 2017).

Specifically, global carbon (C) cycling models are still designed around the assumption that C cycling in upland soils is entirely driven by oxygen-consuming aerobic respiration (Parton et al., 1998; Wieder et al., 2013). This becomes an issue when considering the fact that soils contain one of the largest fast-cycling C stocks on earth, about 3300 Pg is stored in the top 3 m, which is three to four times the C stored in the atmosphere (Batjes, 1996; Jobbagy and Jackson, 2000; Tarnocai et al., 2009). Despite mounting evidence in support of the formation and impact that less than well-oxygenated pore spaces have on soil C cycling in seemingly aerobic soils (Ball, 2013; Keiluweit et al., 2016; Kleber, 2010; Kleber et al., 2011; Riley et al., 2011), it has gone mostly unaccounted for in existing C cycling models (Keiluweit et al., 2017). For example, the current Department of Energy sponsored Community Land Model

includes one scaling factor to retard aerobic C mineralization as a function of percent saturation of the pore network (Koven et al., 2013).

Under conditions that may restrict oxygen resupply from the surface, such as partial to full saturation of the pore network (Chadwick and Chorover, 2001), microbes are required to switch from aerobic heterotrophic respiration to a diversity of less efficient anaerobic metabolic pathways (Arndt et al., 2013; Hansel et al., 2008; Wania et al., 2009). With oxygen lacking, these pathways utilize alternative terminal electron acceptors (aTEAs) and include, in order of decreasing energetic return, manganese (III and IV), iron (III), sulfate, or carbon dioxide (James and Brose, 2011). Carbon mineralization rates in anaerobic environments, as a function of aTEA utilization have been shown to dramatically decline compared to aerobic environments, frequently by 60-95% (Wania et al., 2009).

Microsites can be delineated on a functional basis from the surrounding soil environment by the intensity (McClain et al., 2003) and type (Keiluweit et al., 2016; Sexstone et al., 1985) of the microbially mediated geochemical reactions they harbor. The vast majority of the geochemical reactions occurring in environmental systems such as soils are oxidation – reduction (redox) reactions, i.e., processes that involve the transfer of electrons from an electron donor (such as reduced soil organic matter) to an electron acceptor (such as ferric iron, Fe^{3+}). Electrons can thus be treated as essential reactants in redox reactions (Bohn, 1971). A given redox couple has an inherent potential associated with the transfer of electrons (i.e. electromotive potential) from the reduced partner to the oxidized. The potential for this exchange to occur can be measured using a volt meter (Fiedler et al., 2007), and expressed as

electron activity (pe). The electron activity measurement functions similarly to that of the pH measurement (Stumm, 1966) (Appendix C). Following the IUPAC definition of reduction-oxidation reactions (variable = Eh), in order to be called a ‘redox potential’ it is assumed the reaction between an oxidized and reduced couple (i) is in a pure, pH balanced environment, (ii) that there is a single redox couple active, and (iii) that the reaction is at equilibrium. Such environments do not tend to exist in soils systems due to their complexity and inherent impurity (Young and Crawford, 2004).

In a strict sense then, soils do not have ‘redox potentials’ and such specific measurements have been called into question (Bartlett and James, 1995; Peiffer, 1992; Peiffer, 1999; Sposito, 2016), especially when used to predict the speciation and quantity of given redox couples in soil systems (Chapelle et al., 1996; Mansfeldt, 2004; Stumm, 1966). Instead of the strict ‘redox’ measurement, the term platinum-probe potentials (E_{Pt}) (Bartlett, 1998) has been proposed with the understanding that a specific redox couple is not being measured and no specific inference can be made to abundances of oxidized or reduced forms of said couple.

If the division of ‘upland’ soils into aerobic and anaerobic volumes is to be included in larger scale nutrient and C cycling models, then (i) quantitative information about what part(s) of the soil are aerobic or anaerobic and when, and (ii) information about the ‘severity’ of the anaerobic conditions (i.e. which aTEAs are being utilized and when?) must be generated. Interpreting changes in electron activity in soils as E_{Pt} (i.e. no direct association with a single redox-active couple or equilibrium status) as opposed to Eh (single redox-active couple in equilibrium) should allow for the (i) robust delineation of biogeochemically distinct microsites

over seasonal time scales and (ii) estimation of the dominant active terminal electron acceptor and the likely byproducts of said microbial metabolism (e.g. formation of ferrous iron or NO_x gases).

The overarching question that is addressed in this thesis is: How can the division of a soil into aerobic and anaerobic volumes be quantified, and the driving factors behind the formation of anaerobic conditions be parameterized? This thesis explores the use of Pt-based electrodes as an affordable, high-resolution method to distinguish biogeochemical heterogeneity in soils via measurements of electron activity. The driving objective was to elucidate the process chain leading from (i) variation in climatic parameters (e.g. temperature or precipitation), to (ii) the regulatory effect(s) of the soil pore network architecture, and (iii) to the resulting variation in metabolic performance of the microbiota.

Chapter one of this thesis aims to develop a quantitative means to describe the extent to which a given soil may be subdivided into biogeochemically distinct microsites as a function of seasonal climatic events and position along the hydrologic gradient. It was assumed that the existence of said microsites would be expressed as differences in absolute magnitude of electron activity within a given volume of soil. It was also assumed that the degree to which the same volume of soil was divided into biogeochemically distinct microenvironments would be captured by variability in electron activity and captured with a geometrically fixed array of multiple Pt-electrodes per depth.

To constrain the effect of different climate parameters (i.e. precipitation and temperature), three geographically close Mollisols were monitored for changes in

electron activity over a period of 18 months. What must be considered here is that, though these soils had morphological differences, the texture profiles (percent sand/silt/clay) were similar from top to bottom (~100 cm). The similarity in texture and climate parameters, but differences in biogeochemical heterogeneity across the drainage gradient, prompted the consideration of what must be one of the main differences between all three soils: the structure of the pore network itself.

The role of the pore network in regulation of water and gas movement is not a novel concept (e.g. Kravchenko, et al., 2017). Similarly, the pore network has been shown to have a tight regulation on organic matter cycling (Kravchenko et al., 2015) and the overall composition of the microbial community (Negassa et al., 2015). The focus of the second chapter however is a synthesis of these functions. The main question addressed is what is the role of the pore network architecture in the formation of anaerobic conditions? To address this question three different types of pore network architectures were created by sieving soil from a Mollisol A horizon to ≤ 1 cm and ≤ 2 mm and comparing them to a control with intact soil structure. Three replicate mesocosms from each structural treatment were instrumented with three Pt-electrodes each to record variations in electron activity while being subjected to varying saturation conditions (full saturation, drainage, and partial rewetting).

A first-order analysis was made to establish a mechanistic relationship between the pore network architecture (parameterized using X-ray computed tomography), changes in pore network saturation status, and the resulting variations in electron activity.

2. Quantifying biogeochemical heterogeneity in soil systems

Authors: Thomas Wanzek^{1*}, Marco Keiluweit², John Baham¹, Maria I. Dragila¹, Scott Fendorf³, Sabine Fiedler⁴, Peter S. Nico⁵ and Markus Kleber¹

¹ *Department of Crop and Soil Science, Oregon State University, 3017 ALS Building, Corvallis, OR, USA*

² *Stockbridge School of Agriculture and School of Earth and Sustainability, University of Massachusetts, 411 Paige Lab, Amherst, MA, USA*

³ *Earth System Science Dept., Stanford University, Via Ortega 473, Stanford, CA, USA*

⁴ *Geographisches Institut, Johannes Gutenberg-Universität Mainz, Johann-Joachim-Becher-Weg 21, 55099 Mainz, Germany*

⁵ *Earth and Environmental Sciences Area, Lawrence Berkeley National Laboratory, Building 85B, Berkeley, CA, USA*

*Corresponding author: wanzekt@oregonstate.edu

Submitted to Geoderma
Elsevier Inc.
1600 John F Kennedy Boulevard Suite 1800
Philadelphia PA 19103-2879, USA

2.1. Abstract

Soils are increasingly perceived as complex systems with properties and biogeochemical functions that vary on millimeter scales. Quantitative information about the resulting biogeochemical heterogeneity is needed to improve process knowledge and to render biogeochemical models more mechanistic. Here we demonstrate how standardized arrays of Pt-electrodes can be used to quantify biogeochemical or ‘functional’ soil heterogeneity, defined as the extent to which the soil is subdivided into microenvironments. Our case study confirmed the validity of this approach for a soil sequence consisting of a well drained, a moderately well drained and a poorly drained Mollisol. We found that (i) variations in soil moisture content are the immediate cause for variations in functional heterogeneity, with (ii) soil porosity influencing rates and the magnitude of change. We posit that the deployment of standardized arrays of Pt-electrodes will offer an affordable option to monitor the general metabolic state of the soil system and simultaneously quantify the functional heterogeneity of underlying processes at any point in time. Such information should be useful to improve quantitative estimates of processes as diverse as trace gas emissions, trace gas consumption, reductive dehalogenation and mobilization of metals in the subsurface biosphere. We recommend that parameterization of functional soil heterogeneity be included in long-term soil monitoring programs such as Superfund Sites, Critical Zone Observatories, Long Term Ecological Research (LTER) and National Ecological Observatory Network (NEON) sites.

2.2. Introduction

2.2.1. *Soils are assemblies of functionally dissimilar microenvironments*

Soil is a porous medium necessary to support plant and animal life in the terrestrial biosphere. Soil borne fauna and flora of all sizes and lifestyles continuously rework the mineral-organic soil matrix, rendering its pore network highly random and inherently transitory. Because habitat form (pore size and morphology) affects (microbial) function, the resulting functional heterogeneity within "the most complicated biomaterial on the planet" (Young and Crawford, 2004) poses a problem when it becomes necessary to assess the direction and intensity of the processes that cycle biologically important elements such as C, N, P and S at a given site. Variations in soil structure induce variations in system behavior, with the consequence that a given biogeochemical reaction may be logistically and energetically possible in one pore while inhibited in the pore right next to it. For instance, Kravchenko et al. (2015) show that losses of particulate organic matter were 3–15 times higher from atmosphere-connected soil pores than in isolated pores, while Negassa et al. (2015) demonstrated a dependence of microbial community composition on physical pore characteristics. Recognition of functional heterogeneity has given rise to the concept of biogeochemical hot spots and hot moments (Kuzakov and Blagodatskaya, 2015; McClain et al., 2003) and has prompted calls to develop means for the robust quantification of soil heterogeneity across scales (Groffman et al., 2009; Vereecken et al., 2016).

Microenvironments can be functionally distinguished from the surrounding environment by the intensity (McClain et al., 2003) and type (Keiluweit et al., 2016;

Sexstone et al., 1985) of the microbially mediated geochemical reactions they harbor. Here it is useful to remember that the vast majority of the chemical reactions occurring in environmental systems are oxidation - reduction reactions, i.e., processes that involve the transfer of electrons from an electron donor (such as reduced organic matter) to an electron acceptor (such as ferric iron, Fe^{3+}). Electrons can thus be considered as essential reactants in oxidation-reduction reactions (Bohn, 1971). Here we propose to quantify functional heterogeneity in soil environments by leveraging information on the electrochemical potential of a given microenvironment.

Our overarching goal is to develop a quantitative means to describe the extent to which a given soil volume may be subdivided into biogeochemically distinct microsites. We assumed that functional heterogeneity would express itself as a variation in the electron activity within a given soil volume that can be captured with a geometrically fixed array of multiple electrodes. We then develop a numerical indicator value for the extent to which a given soil volume is subdivided into biogeochemically distinct microsites. Our objectives were to demonstrate the validity of our assumptions for a set of hydrologically different soils and to explore the importance of external controls (precipitation, temperature and height of the water table) for the formation of functionally distinct microenvironments. To this end, we examined functional heterogeneity along a gradient extending from well drained over moderately well drained to poorly drained soils.

Specifically, we hypothesized that

(i) functional heterogeneity increases along a drainage gradient with increasingly wetter soil moisture regime,

- (ii) functional heterogeneity decreases with soil depth and
- (iii) functional heterogeneity in soil systems is a function of external environmental drivers and hence not constant over time.

2.2.2 Measuring electron activities: the Pt-electrode approach

Contemporary methods to characterize soil three-dimensional structure are increasingly allowing us to observe variations in microbial metabolism at the submicron scale, but there is a lack of robust, quantitative information about the spatial and temporal distribution of physiologically diverse microenvironments at the mm to cm (meso-)scale. A microscale observation requiring that a sample be taken to an elaborate research facility such as a synchrotron and there examined for several hours cannot easily be replicated at the frequency necessary to make valid inference about a watershed or a landscape. While we can observe processes at the submicron scale as well as at the much larger watershed or landscape scale, we still lack the ability to express the latter as a function of the former, mainly because we do not yet have a practical means to enter a parameter "soil heterogeneity" into testable scaling laws that would allow us to bridge the scale gap.

Our suggestion to use Pt-electrode potentials for the identification of functionally different soil microsites stems from early observations that replicate Pt-electrodes deployed in the same soil horizon do not typically return the same electromotive potential but may deviate by more than 300 mV (McKeague, 1965). While sometimes attributed to technical deficiencies of the Pt-electrode as discussed below, the phenomenon was further examined by Cogger et al. (1992) and Fiedler

(1999 Figure 7.1 therein). Fiedler installed replicate Pt-probes at a distance of 10 mm in the topsoil (5cm depth) of a Humaquept and found the potentials returned to be close during summer but to deviate up to 800 mV during periods of high rainfall and snowmelt. Corroborating the assessment of Cogger et al. (1992), Fiedler (1999) concluded that soil microsites are the primary source of variability among properly constructed and maintained electrodes. The operational consequence of these findings was the practice of subsequent workers to install multiple electrodes per same depth, thereby obtaining a more robust indicator for the general redox state of the soil horizon or soil depth of interest (Austin and Huddleston, 1999; Dorau and Mansfeldt, 2016; Dwire et al., 2006; Faulkner and Patrick, 1992; Faulkner et al., 1989; Fiedler and Kalbitz, 2003; Mansfeldt, 2003; Mansfeldt, 2004; Megonigal et al., 1993; Seybold et al., 2002; Vepraskas and Faulkner, 2001; Yu et al., 2006).

With the work presented here, we take the insight that Pt-electrodes are able to sense the existence of functionally different soil microsites as an opportunity to quantitatively assess the biogeochemical or ‘functional’ heterogeneity of soil. We argue that, if related to an array of geometrically related observation points, the heterogeneity of metabolic conditions within the soil volume defined by such an array can be conveniently expressed using descriptive statistics, effectively providing us with a metric for functional diversity within that soil volume.

It must be emphasized that the electromotive potentials thus obtained are not accurate predictors for the speciation of given redox couples in the soil system (Chapelle et al., 1996; Mansfeldt, 2004; Stumm, 1966). This limitation arises from both, (i) systemic issues related to the chemical complexity of the soil system and (ii)

restrictions resulting from the properties of the electrodes used to measure the potential. Briefly, measurements of electromotive potentials in soils are complicated by the simultaneous presence of multiple redox couples in the soil system giving rise to 'mixed potentials', by a dependence on soil pH, by slow kinetics of important potential-determining redox couples at the electrode and by the lack of chemical equilibrium among various redox couples (Sigg, 1999; Stumm, 1966). These systemic constraints are further complicated by the propensity of the Pt-electrode to chemically react with oxygen, to exhibit voltage drift and to experience fouling under conditions as reviewed by James and Brose (2011). As a result, a comparison of published data for natural environments (Figure 1) suggests measurements of electromotive potentials in soil can certainly indicate a general disposition of a soil system towards given redox reactions but no clear delineations between different soil redox states. For instance (Figure 1), the first appearance of Mn^{2+} in a cohort of 9 independent investigations carried out in different soils and settings occurred at an apparent Pt-probe potential of +700 mV in one study but ranged all the way down to +200 mV in another report.

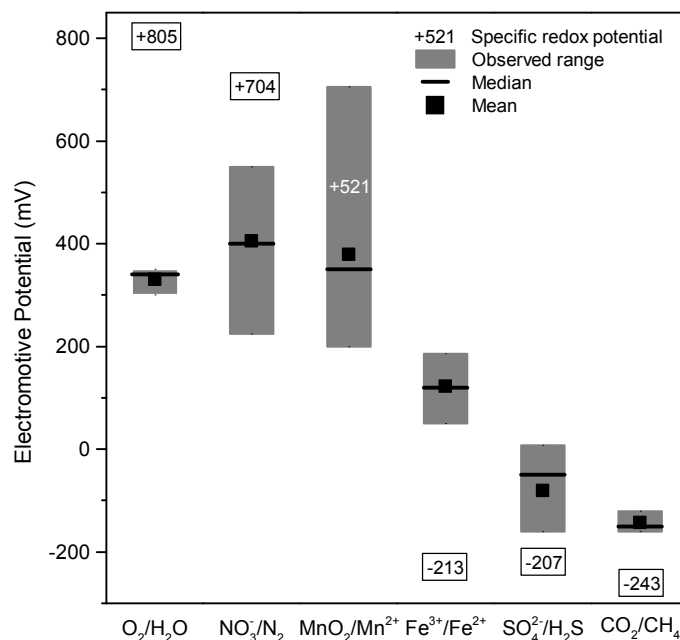


Figure 1. Disparity between specific redox potentials for common redox couples and the experimentally observed range for the first appearance of the reduced species in soils as a function of increasing electron activity following inundation. N = 9 independent studies all using platinum electrodes (Brümmer, 1974; Gotoh and Yamashita, 1966; Kofod, 1999; Mansfeldt 2004; Patrick and Jugsujinda, 1992; Patrick and Mahapatra, 1968; Patrick and Turner, 1968; Wang et al., 1993; Yu et al., 2007). For the oxygen/water couple, the range of values below which O_2 was no longer detectable is given and n = 3). Oxygen data from Patrick and Turner (1968), Patrick and Jugsujinda (1992), and Yu et al. (2006). All studies were either conducted at pH values close to 7 or the data were published including a correction to pH 7. Specific redox potentials from James and Brose (2011) representing conditions of pH=7, $[red] = [ox] = 10^{-4}$ M, 0.21 atm for O_2 , 0.78 atm for N_2 , and 0.00032 atm for CO_2 .

Clearly, this prevents the user from making robust predictions about the speciation of Mn in soils returning potentials above +200 mV. The scientific community has long been engaged in a debate as to the value of such information. While some question the usefulness of Pt-electrodes for electromotive potential measurements in soil altogether (Bartlett and James, 1995; Peiffer, 1992; Peiffer, 1999; Sposito, 2016), others point at obvious correlations of Pt-electrode derived electromotive potentials with soil morphology (Chadwick and Chorover, 2001; Fiedler and Sommer, 2004; Patrick et al., 1996), element distributions (Fiedler and Sommer, 2004; Mansfeldt, 2004; Masscheleyn et al., 1990) pedogenic thresholds of Fe, P and S (Chacon et al., 2006; Dorau and Mansfeldt, 2016; Gomez et al., 1999), methane production (Fiedler and Sommer, 2000; Wang et al., 1993; Yu et al., 2007) and even suggest the use of Pt-probe potentials as agricultural planning tools (Husson, 2012; Husson et al., 2016). For the purpose of this study, we refrain from any specific inference regarding the abundance of certain chemical species or from inference for microbial ecology in the sense of Baas Becking et al. (1960), but use electromotive potentials solely as unspecific proxies to establish the existence and the magnitude of functional differences between soil microenvironments.

To parameterize functional heterogeneity, we installed linear arrays of five equidistant (10 cm) Pt-electrodes at four depths (10, 20, 50 and 100 cm) in each soil. The magnitude of the standard deviation of the mean ($n=5$) electromotive potential among the electrodes at a given observation instance then represents the biogeochemical heterogeneity of the soil along a horizontal distance of 40 cm. The electrode tips (Figure A.4) were hypothesized to probe a microenvironment of the

size of a few mm³ (Fiedler et al., 2007). To capture seasonal variations, electromotive potentials were recorded several times a week for a period of 18 months, including two wet winter seasons.

2.3. Methods

2.3.1. Site and soil properties

Our conceptual approach consisted of selecting three soils in a way that they were derived from the same parent material, belonged to the same taxonomic soil order (Mollisols), were spatially close enough to be considered as being under the exact same climate regime but represented a gradient in soil moisture regime between soils. The drainage classes used by NRCS-USDA (Schoeneberger et al., 2012) refer to the frequency and duration of wet periods under conditions similar to those under which the soil developed. A soil is classified as **well drained** when water is removed readily and the soil is free of redoximorphic features. In a **moderately well drained soil**, water is removed somewhat slowly during some periods of the year, allowing for weak hydromorphic features to develop. Hydraulic conductivity is lower than in a well-drained soil. As a result of very low saturated hydraulic conductivity, **poorly drained soils** are wet at shallow depths periodically or for extended periods, allowing for the development of prominent hydromorphic features.

The three research sites were located in the central part of the Willamette Valley, 7 miles north east of the Oregon State University campus in Corvallis, Oregon (Figure A.1). The Willamette Valley is a broad level plain in western Oregon that lies to the east of the Coast Range Mountains. Complex soil stratigraphy related to interfingering of Late Pleistocene glacial flood deposits (Balster and Parsons,

1968) combined with postglacial pedogenic clay translocation results in physical discontinuities within the solum that strongly influence transmission or lack of transmission of water (Austin and Huddleston, 1999; Boersma et al., 1972). The rain shadow of the Coast Range allows for an average of 900 mm of precipitation between October and May (Fall/Winter/Spring), and only 80 mm during June, July, August, and September. Mean air temperature averages 4°C in January and 19°C in July.

Two of the sites (Willamette and Amity) were on a USDA managed research farm and the third immediately adjacent on Hyslop Research Farm (Woodburn), which is part of the Oregon State University College of Agricultural Sciences – Corvallis Farm Unit. The Willamette soil is a well-drained Pachic Ultic Argixeroll, the Woodburn is a moderately well drained Aquultic Argixeroll and the Amity is classified as a poorly drained Argiaquic Xeric Argialboll (Soil Survey Staff, 2015). The three sites were about one and a half miles apart, were situated on spots adjacent to but outside of active research fields, had been unmanaged for more than 10 years with the exception of biannual mowing, and all had a largely constant grassland vegetation mix dominated by *agrostis capillaris*, *agrostis stolonifera* and *hypochaeris radicata*. Soil reaction across the profiles was similar with a gradient from pH 5.5 ± 0.07 in the topsoil to pH 6.3 ± 0.28 at 100 cm depth (mean $\pm$ SD from all three soils). All three series share a similar overall texture class (silt-loam), but bulk density was variable with a trend towards denser horizons from Willamette over Woodburn towards Amity. Basic soil properties are given in Table A.2.

2.3.2. *Measurement of electromotive potentials*

Two types of Pt-electrodes were used in this study. Woodburn and Amity were instrumented first, with electrodes similar to those described by Fiedler et al. (2007). The need to obtain 20 additional probes for the monitoring of the Willamette soil was taken as an opportunity to miniaturize the Pt-probes for the purpose of lesser soil disturbance while maintaining the same functionality and durability as the larger electrodes. Briefly, 1.5 cm lengths of 16 ga. 99.95% pure platinum wire (Surepure Chemetals, Florham Park, NJ USA) were soldered, using light duty rosin core solder (Radio Shack, Fort Worth, TX USA), to two meter lengths of insulated 16 ga. copper wire. The Pt – Cu junction was then encased in low conductivity, clear epoxy casting resin (Alumilite Corp., Kalamazoo, MI USA) for increased stability and protection. Prior to installation in the field, each Pt-electrode was tested against a quinhydrone solution (0.1 g quinhydrone per 50 ml deionized water) buffered to pH 7. If the measured potential was more than ± 10 mV outside of the ideal potential at 20 °C (92 mV), the probe was cleaned and retested (Austin and Huddleston, 1999; Jones, 1966). After successful testing, Pt-electrodes were installed at depths of 10, 20, 50 and 100 cm in linear arrays of 5 Pt-electrodes each. Electrode arrays had a horizontal extension of 40 cm and a distance between individual Pt-electrodes of 10 cm. The tip of each Pt-electrode was inserted horizontally into the pit wall to a distance of 10 cm from the wall face. Once installed in the field, the electrode leads were gathered and protected in a closable junction box. All electrodes were given two weeks to equilibrate *in situ* prior to the start of data collection. E_{Pt} data was collected manually every other day beginning in January 2015 through April 2016.

Potentials were collected manually using a Fluke 27 digital multi-meter (DMM, Fluke Corporation, Everett, WA USA), a custom-built resistance amplifier (detailed below), and a 3M Ag/AgCl InLab reference electrode (Mettler and Toledo, Columbus, OH USA). Field measurements were normalized relative to the standard hydrogen electrode by adding a correction factor calculated for each measurement and depth using daily soil temperature data (Nordstrom and Wilde, 2005). E_{Pt} data are reported based on actual soil pH as reported in Table A.2. Inexpensive DMMs have low input resistance ($\sim 10^6$ ohms), which makes measurements of electromotive potentials in low voltage systems such as soils unreliable (Fiedler et al., 2007; Rabenhorst, 2009) as an unpredictable drop in voltage can occur when current flows from the soil microenvironment through the DMM. To remedy this issue, Wanzek built a one-off resistance amplifier to increase the input resistance of the hand held DMM from 10^6 ohms to $>10^{13}$ ohms (Figure A.2) following the design proposed by Rabenhorst (2009). The resistance amplifier was designed to be self-contained and utilized a TLE2426 precision virtual ground operational amplifier from Texas Instruments (Dallas, TX USA), one 9V battery, and an on/off switch (to preserve battery life). Functionality of the resistance amplifier was tested and asserted in a 6-week test campaign by plotting corrected (plus amplifier) versus raw (minus amplifier) data to find the same magnitude of deviations as in Rabenhorst (2009; data not shown)

2.3.3. Soil moisture content and soil temperature

Volumetric water content and soil temperature was monitored at each depth in the Woodburn and Amity soils using Decagon 5TE soil moisture sensors (n=1, with the exception of Woodburn 10, Woodburn 20, Amity 20 and Amity 50, where n=2). Data were recorded every six hours on Decagon EM50 data-loggers (Decagon Devices, Pullman, WA USA). Water filled pore space was then calculated for each depth in the Woodburn and Amity soils using volumetric water content (VWC) and percent porosity, which was calculated using bulk density data.

Pt-electrodes and Decagon sensors were installed in the Woodburn and Amity soils in January 2015. A total absence of any redoximorphic features in the Willamette soil prompted us to initially focus our resources on the wetter soils of our sequence. Hence instrumentation of the Willamette soil was delayed until the beginning of the 2015/16 wet season.

2.3.4. Climate data

Climate data were recorded daily by a weather station installed at Hyslop Farm and managed by the National Oceanic and Atmospheric Administration (NOAA) as a part of their Global Historical Climatology Network (Menne et al., 2012). Data were downloaded through the National Climatic Data Center's Climate Data Online tool. The depth to standing water in the Amity soil pit was measured from ground level at each E_{Pt} data collection event. There was no standing water at the level of the electrodes in the Woodburn and Willamette at any point in time.

2.3.5. Soil pH

Soil samples for pH measurement were collected once per month from each soil and depth from January 2015 through May 2015. Using a 5 cm by 10 cm bucket auger, about 200 cm³ of soil was removed at each sampling event (n=5 sampling events for the Woodburn and Amity and n=4 for the Willamette). Samples were manually homogenized and large root matter was removed. A slurry was prepared at a 2:1 water/soil ratio and pH was measured using an Accumet AB15 pH meter and gel probe (Fisher Scientific, Waltham, MA USA). Monthly pH measurements were discontinued when no significant change over time was observed.

2.4. Results

2.4.1. Environmental drivers and electron activities

2.4.1.1. Precipitation

Cumulative rainfall data comparing the wet season of 2015 with the wet season of 2016 is given in Table A.3, showing that winter 2015 was much drier (378 mm for the period from January - April) than winter 2016 (601 mm for the same period). With a total pore space in the order of 500 L m⁻² m⁻¹ (Table A.2), the precipitation values indicate a potential for whole profile saturation in the year 2016 but not for the winter of 2015. The highest monthly precipitation total was observed in December 2015 (354.9 mm) and the lowest in July 2015 (0 mm). While there were fewer precipitation events in winter 2015 than in 2016, the proportion of intense rainfall events (> 30 l m⁻²) was greater. Overall, on six occasions (Figure 2, panel a), daily rainfall reached or exceeded 30 liters per m². Two of these events occurred in the first wet season: one in mid January 2015 (41.1 mm) and one in mid March 2015

(44.5 mm). The remaining four events preceded the second wet season: one occurred in late October 2015 (30.5 mm) and three in December 2015 (30.2 mm, 42.4 mm, and 49.8 mm).

2.4.1.2. Water Filled Pore Space

The fluctuation of water filled pore space (WFPS, given as % of total pore space) over time is presented in Figure 2 panels b (Woodburn) and c (Amity) together with precipitation (Figure 2 panel a). Panels b and c have a horizontal dashed line to indicate 60% water filled pore space, a value considered as optimal for aerobic microbial activity, with higher WFPS values indicative of restricted oxygen supply (Linn and Doran, 1984). During the dry season, WFPS dropped well below the 60% mark in all soils and at all depths. During wet seasons WFPS exceeded this 60% optimum threshold very briefly at the 10 and 20 cm depths of the Woodburn and extended periods of time in the Amity, reaching full saturation in the Amity during the second wet season. At the 50 and 100 cm depths, the 60% WFPS mark was exceeded throughout the wet seasons, with the distinction that saturation was reached in the Amity but not in the Woodburn.

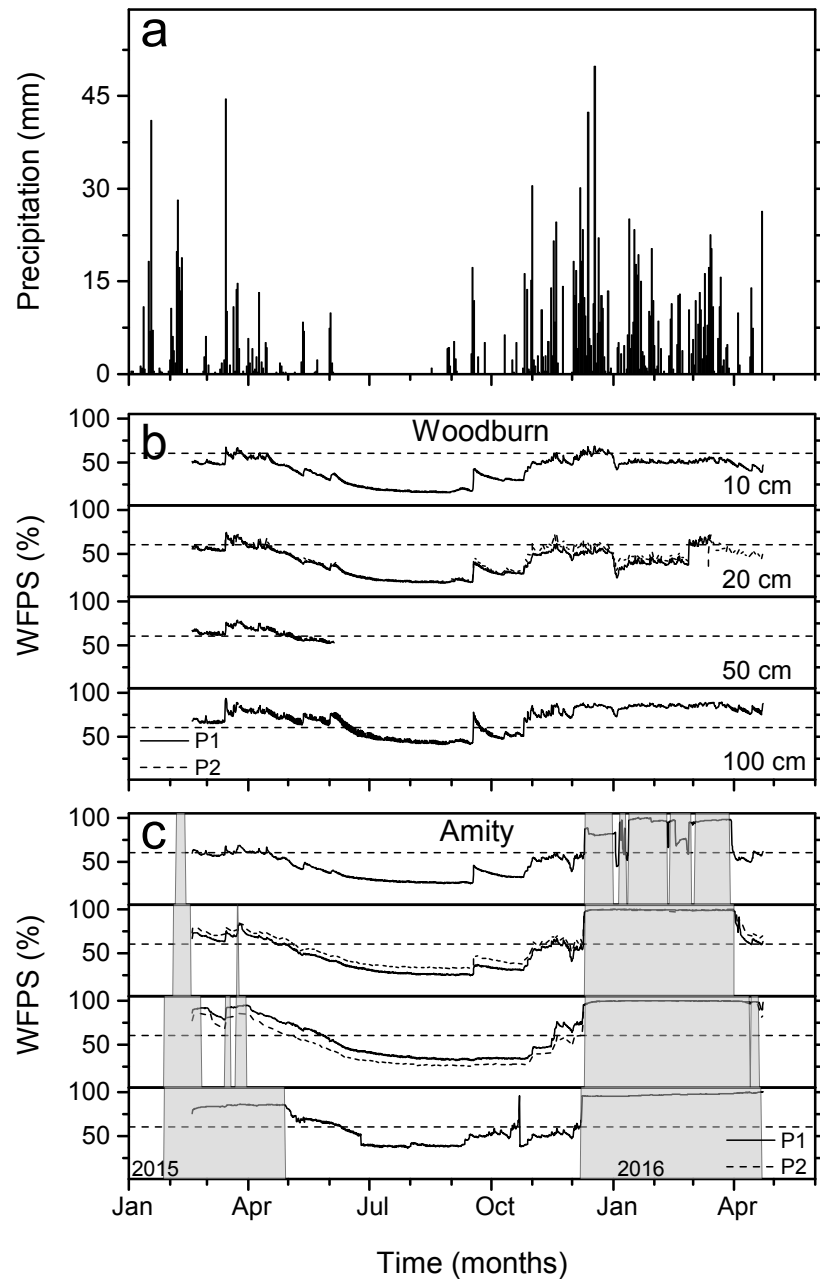


Figure 2. Time series of precipitation (panel a); and water filled pore space (WFPS) at the Woodburn (Panel b) and Amity (Panel c) soils for the observation period. Shaded area indicates periods where electrodes were submerged. Depths labeled in the Woodburn panel are the same for the Amity panel. Percent water filled pore space was calculated using volumetric water content data (measured using Decagon 5TE electrodes and recorded every six hours) and bulk density for each depth. Replicate volumetric water content measurements and subsequent WFPS calculations were made at the Woodburn 10 and 20 cm depths and Amity 20 and 50 cm depths: WFPS calculated from probe 1 = solid and probe 2 = dashed. The horizontal dashed line in each WFPS panel indicates 60% water filled pore space.

2.4.1.3. Soil Temperature

Soil temperature for the Woodburn and Amity soils is given in Figure A.3 b and c as a function of time on the same timescale as precipitation (Figure A.3 a). In both the Woodburn and Amity, soil temperature increased from February to maximum values in mid-July with a subsequent decline towards seasonal minima in early December, coincident with peaks in precipitation. Changes in temperature from day to day were similar between the two soils, but during periods when the Amity soil was inundated with water, the soil temperature oscillated much less compared to the non-saturated Woodburn, likely owing to the high heat capacity of H₂O. During the relatively dry 2015 winter season, topsoil (10 - 50 cm) temperatures in the Amity were about 2 degrees C lower than in the Woodburn. Both soils were at the same 10.4 degrees C at 100 cm. During the much wetter 2016 winter season, both soils had nearly identical and slightly warmer temperatures than in 2015 at all depths.

2.4.2. Variation of electromotive potentials within one 5-electrode array

Figure 3 documents the response of individual Pt-probe potentials to variations in water filled pore space (WFPS). This is illustrated using data from the Pt-probe array installed at the 20 cm depth in the poorly drained Amity soil. During the 2015 dry season (Figure 3), with WFPS well below the 60% optimum value for aerobic microbial activity (dashed line in panel 2), the five probes of the array all show near identical potentials close to the theoretical value for the O₂/H₂O couple (805 mV for prevailing conditions, Figure 1). With the onset of sporadic rainfall events and the concomitant rise of water filled pore space towards the 60% optimum

level, potentials begin to drop slightly below 800 mV, but remain close together until the end of November. As the soil pore volume fills with water in early December of 2015, water filled pore space rapidly rises towards full saturation and potentials start to move towards the 0 to -50 mV region (Figure 3 panels a, b). Reflecting the conditions in the microenvironments probed by individual electrode tips, individual probes follow separate trajectories, indicating that biogeochemical conditions at the probe tip became increasingly diverse among the 5 electrodes during this period. The biggest spread is reached after the soil begins to dry out again in early April, when two probes (P2 and P4, 20 cm apart) are still at negative potentials (-152 and -18 mV) while the three others (P1, P3, P5) are consistent with full aeration (+786, +695 and +706 mV). As WFPS returns to the 60% optimum value by the end of April 2016 (Figure 3 panel b), the potentials at all 5 electrodes of the array reconverge towards a range between +700 and +800 mV.

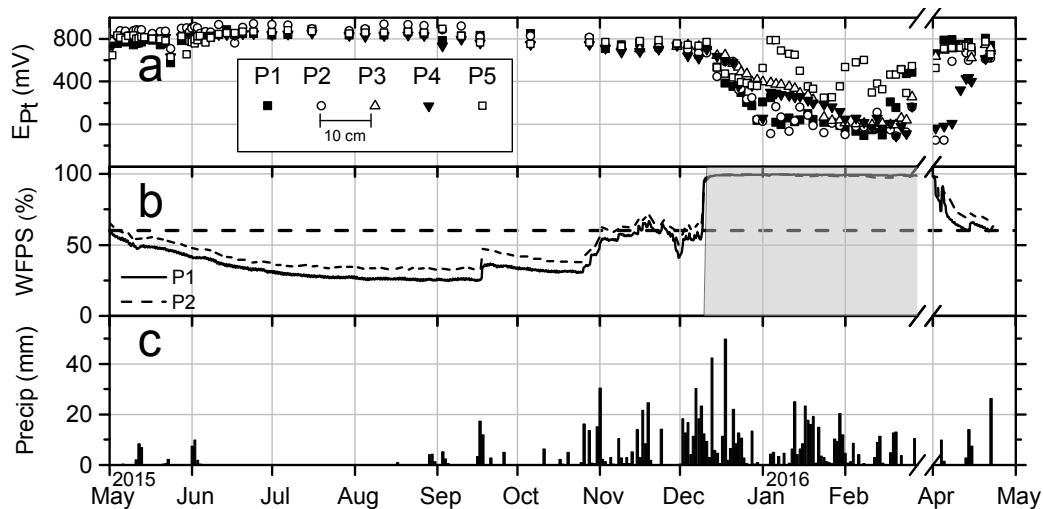


Figure 3. Time series of platinum probe potentials within the linear array of $n=5$ probes installed at a depth of 20 cm in the Amity soil. Panel a gives E_{Pt} data for each electrode. Inset shows position of electrodes within the array. Panel b gives water filled pore space as a fraction of total pore space (% WFPS). Continuous and dotted line are replicate Decagon 5TE moisture sensors (D1 and D2) as primary moisture data sources. The horizontal dashed line indicates optimum WFPS for microbial activity according to Linn and Doran (1984; 60% WFPS). Shaded area indicates time period where electrodes were submerged. Panel c presents daily precipitation.

2.4.3. General patterns of electromotive potentials

Plotting time series of electromotive potentials (mean potential per array and observation point; $J=164$ observations for Woodburn and Amity; $J=37$ observations for Willamette; Figure 4) reveals unique patterns for each of the three soils. At all depths of the well-drained **Willamette** soil (Figure 4, panel a), mean potentials remained close to the value for the O_2/H_2O couple throughout the wet season of 2016. Neither temperature nor precipitation events were able to generate significant oscillations of the mean. In the moderately well drained **Woodburn** soil, negative spikes in electromotive potentials occurred during April 2015 and in January 2016 (Figure 4, panel b) in the topsoil (10 and 20 cm depth), both following periods of high rainfall. Mean potentials stayed above +400 mV at all depths, although individual probes in the topsoil registered values as low as 137 mV (10 cm) and 224 mV (20 cm).

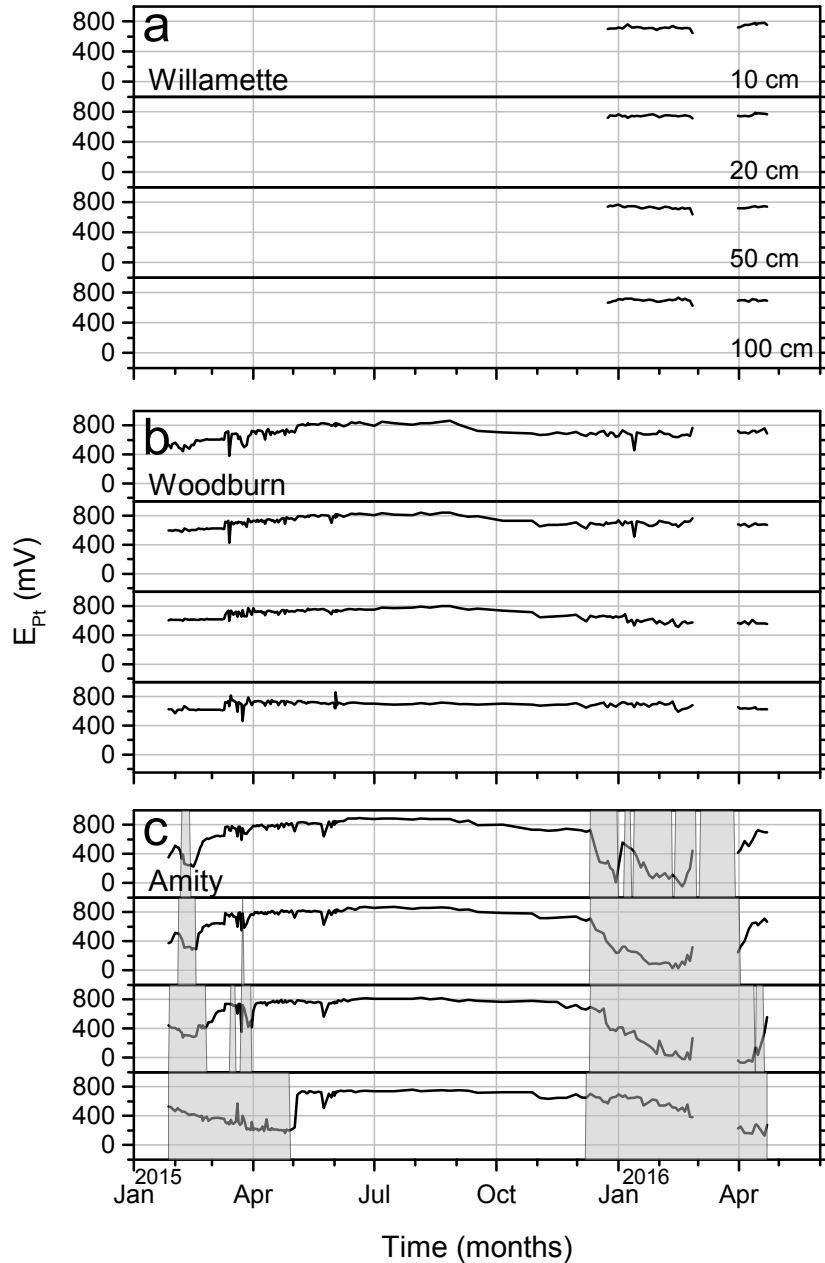


Figure 4. Time series of platinum electrode potentials (E_{Pt}) across the drainage gradient and for 4 depths (10 cm, 20 cm, 50 cm and 100 cm, labels are not repeated in panels b and c for clarity). Values are means per array ($n=5$ electrodes) and observation time point (Table 1). Panel a: well-drained Willamette soil series, Panel b: moderately well drained Woodburn soil, Panel c: poorly drained Amity soil. Shaded area indicates time period where electrodes were submerged.

The largest dynamic of mean electromotive potentials was observed in the poorly drained **Amity** soil, which filled up with water for less than 2 weeks in the 2014/15 wet season but was constantly inundated for a period of almost 4 months in the 2015/16 wet season (Figure 4, panel c, periods of water saturation are indicated by gray shading). In the 2015/16 wet season, mean electromotive potential decreased with depth and the lowest mean value was recorded at the 50 cm depth. To our surprise, the lowest electromotive potentials recorded by individual electrodes were not observed in the deepest depth (100 cm: -47 mV), but at a soil depth of 20 cm (-152 mV), followed by the 10 cm depth (-141 mV) and the 50 cm depth (-129 mV). Similar observations were made by Hall et al. (2016), who report more intense microbial Fe reduction in tropical surface soil microsites compared to lesser Fe reduction at greater depths. The time series for the 10, 20 and 50 cm depths resemble each other in their general shape, while the potentials at 100 cm exhibit a distinctly different dynamic, indicating decoupling from any controls acting on the horizons above.

2.4.4. Trends in functional heterogeneity

Biogeochemical or ‘functional’ heterogeneity was parameterized as the standard deviation of the mean potential within each five-electrode array at each observation time point and plotted for all soils and depths in Figure 4. In the well drained **Willamette** soil (receiving the same amount of precipitation as the other two soils), functional heterogeneity was close to zero across all depths and throughout the 2015/2016 wet season (Figure 5, panel a), despite the fact that rainfall (956 liters m⁻², Table A.3) during this period amounted to about 1.9 times the volume of estimated

total porosity (500 liters $\text{m}^{-2} \text{m}^{-1}$ of soil depth, Table A.2). In the **Woodburn** soil, heterogeneity within individual 5-probe arrays was near zero during the dry season, but reached peak values approaching $\pm 231 \text{ mV}$ at the end of March 2015 in the topsoil (10 cm depth) and in mid February 2016 at a depth of 50cm ($\pm 265 \text{ mV}$). Short term changes in soil heterogeneity in the Woodburn topsoil tended to be larger when rain events were more episodic as in the drier wet season of 2014/2015 compared to dampened amplitudes during the much wetter winter of 2015/2016. Heterogeneity in the Woodburn soil declined with depth during the "drier" 2014/2015 wet season, while it increased with depth down to 50 cm during the "wetter" 2015/2016 wet season (Figure 5, panel b).

The largest measured functional heterogeneity was observed at the 20 cm depth of the poorly drained **Amity** soil, reaching a SD of $\pm 450 \text{ mV}$ ($n = 5$) in early April 2016 with a low extreme of -152 mV and a high extreme of $+ 786 \text{ mV}$ measured simultaneously by two electrodes only 10 cm apart. Heterogeneity in the Amity exhibited different patterns compared to the Woodburn between the 'dry' 2014/2015 winter and the wetter 2015/2016 winter season. During the 2015 wet season, heterogeneity oscillated around a value of $\pm 70 \text{ mV}$ with occasional peaks and an isolated maximum near 300 mV at the 50 cm depth in late March 2015. There was no obvious trend with depth during that period. This was much different during the 2016 wet season, when the Amity topsoil (10 and 20 cm) exhibited large functional heterogeneity over a period of more than five months (Figure 5, panel c). Both extreme values and amplitudes of heterogeneity declined with depth during that time.

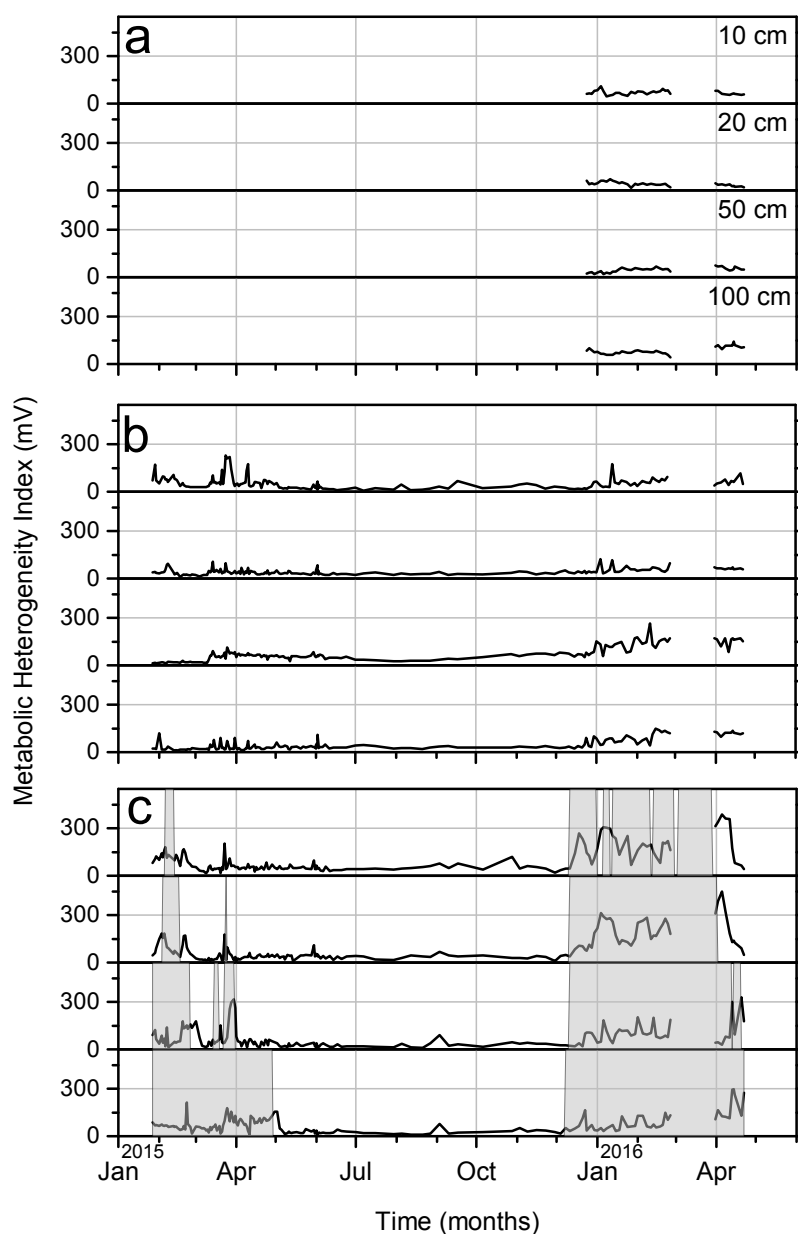


Figure 5. Time series of metabolic heterogeneity index across the drainage gradient and for 4 depths (10 cm, 20 cm, 50 cm and 100 cm, labels are not repeated in panels b and c for clarity). Values are standard deviations per array ($n=5$ electrodes) and observation time point (Table 1). Panel a: well-drained Willamette soil series, Panel b: moderately well drained Woodburn soil, Panel c: poorly drained Amity soil. Shaded area indicates full water saturation with electrodes submerged.

Table 1. Symbols, definitions and associated equations

Symbol	Definition	Equation/Unit
x_{ij}	Single data point; value returned by electrode i on day j	mV
a	Number of electrodes per individual linear array.	$a = 5 = \text{constant, distance between electrodes} = 10 \text{ cm}$
J	Number of observations time points	$J = \# \text{ of days and observations per observation period}$

1.) Variability within one array, single observation point j

$\mu_{(aj)}$ (Figure 5)	Mean electromotive potential among 5 electrodes within one array on day j	$\mu_{(aj)} = \frac{\sum x_{ij}}{a}$
$S_{(aj)}$ (Figure 5)	Standard deviation of the mean within one electrode array on day j	$S_{(aj)} = \sqrt{\frac{1}{a} \sum_{i=1}^a (x_{(ij)} - \mu_{(aj)})^2}$

2.) Variability within one array and depth

$\mu_{(D)}$ (Figure 6)	Mean electromotive potential at depth D for J = 37 days	$\mu_{(D)} = \frac{\sum x_{ij}}{J}$
$S_{(D)}$ (Figure 6)	Standard deviation of the mean at depth D for J = 37 days	$S_{(D)} = \sqrt{\frac{1}{J} \sum_{i=1}^J (x_{(ij)} - \mu_{(D)})^2}$

3.) Dimension of and changes in variability

$\mu_{[S_{(aj)}]}$ (Figure 7)	Mean variability at a given depth D for J = 18 days	$\mu_{[S_{(aj)}]} = \frac{\sum S_{(aj)}}{J}$
$s_{[S_{(aj)}]}$ (Figure 7)	Standard deviation of the variability at depth D for J = 18 days	$s_{[S_{(aj)}]} = \sqrt{\frac{1}{J} \sum_{i=1}^J (S_{(aj)} - \mu_{[S_{(aj)}]})^2}$

A direct comparison of functional heterogeneity across the three soils is possible by plotting the Metabolic Heterogeneity Index (MHI) as a function of drainage gradient (Figure 6). This representation emphasizes the correlation between drainage regime and the extent to which soils are structured into biogeochemically distinct microsites. The transient nature of microsites is revealed by comparing metabolic heterogeneity between two wet seasons of different intensity. In the 2015 wet season, MHI decreased with depth in the Woodburn soil (Figure 7, WO '15) and remained constant with depth in the Amity soil (Figure 7, AM '15). A completely different picture emerged for the much wetter 2016 winter: heterogeneity increased to a maximum at 50 cm depth in the Woodburn (Figure 7, WO '16) but had its maximum at the 10 and 20 cm depths in the Amity (Figure 7, AM '16).

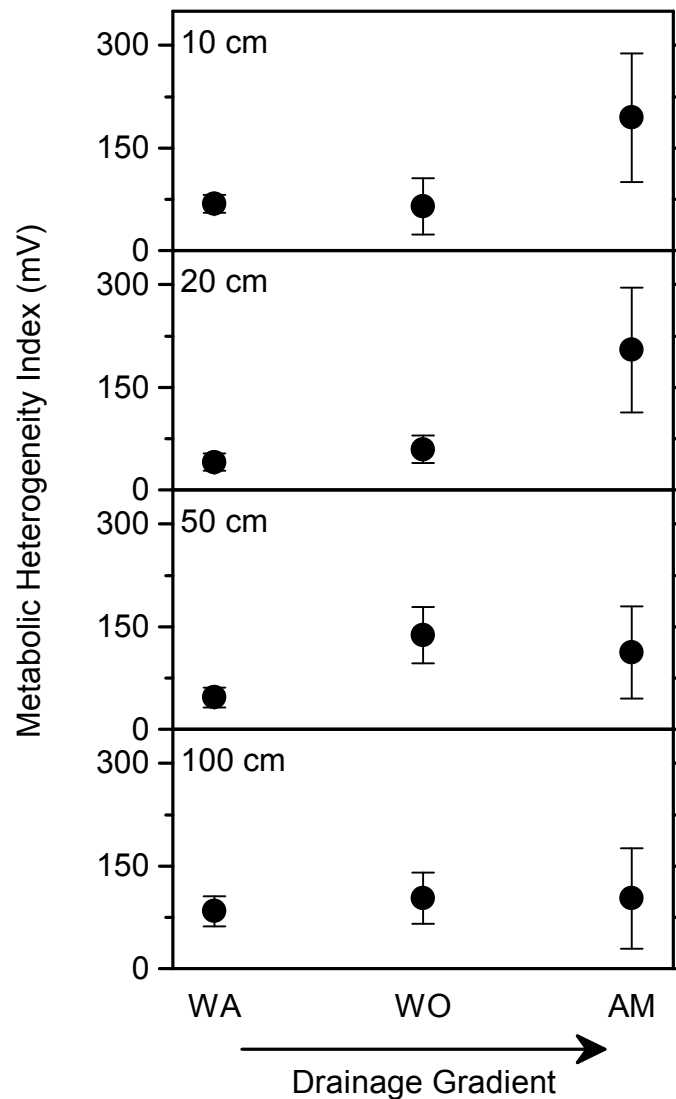


Figure 6. Variation of metabolic heterogeneity index across the drainage gradient. Values are standard deviations per array ($n=5$ electrodes) and observation time point. Dots represent mean variability at a given depth for the same subset of matching observation time points from the 2016 wet season ($J = 37$ observations) across all three soils. Error bars represent the standard deviation of the mean (Table 1). Data are arranged going from well drained (WA) to poorly drained (AM): WA = Willamette, WO = Woodburn, and AM = Amity.

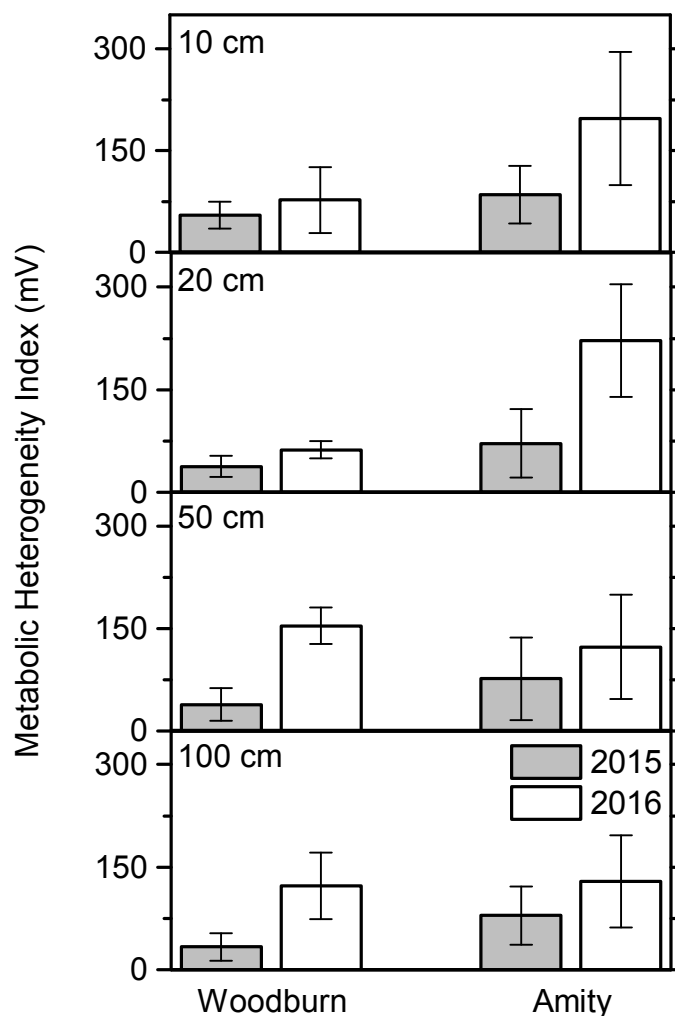


Figure 7. Variation of metabolic heterogeneity index as a function of intensity of the wet season. Bars represent the mean of the variability at a given depth for the same subset of matching observation time points ($J = 18$ observations, Table 1) across the 2015 (gray bars) and 2016 (white bars) wet seasons. The error bars are one standard deviation of the mean variability at the given depth for the observation period J (Table 1).

2.5. Discussion

2.5.1. Extent and origin of functional heterogeneity in soils

Prompted by the seminal work of Sexstone et al. (1985), and seconded by the growing availability of two- and three-dimensional imaging techniques (Tippkötter et al., 2009; Vereecken et al., 2016), the scientific community has increasingly become mindful of the fact that biogeochemical conditions in soil vary on very small spatial scales. Being able to parameterize the extent of such variation has implications for matter transformations including the biodegradation of carbon substrates (Keiluweit et al., 2016; Keiluweit et al., 2017; Vogel et al., 2015) as it may hold the key to explaining the experimental variability and observed non-linearities associated with these processes (Falconer et al., 2015; Ruamps et al., 2013). Consequently, quantitative information on functional soil heterogeneity, i.e. on the extent to which ongoing metabolic processes differ among microsites within the same soil horizon, is needed to achieve full mechanistic representation of biogeochemical cycling in soil. The observations we were able to make in the course of this research are in line with previous reports of increasing variability in electromotive potentials across transitions from dry to wet meadow soils, soils in alluvial planes, and soils on pond margins (Dwire et al., 2006; Fiedler and Sommer, 2004; Mansfeldt, 2003). Our observations also concur with previous communications reporting variations in electromotive potentials as a function of soil depth (Dorau and Mansfeldt, 2016; Fiedler and Sommer, 2004). Yang et al. (2006) recorded increasing heterogeneity in electromotive potentials as depth increased in a well-drained silt loam. Thomas et al. (2009) measured electromotive potentials in six different plots on Everglade wetland

soils. In all locations monitored, variability in electromotive potentials decreased with depth and they determined that most of the variability could be attributed to fluctuating water tables. Seybold et al. (2002) and Thomas et al. (2009) recorded rapid changes in redox potentials in response to changes in water-table levels in tidal marshes and Everglade wetland soils. However, previous studies did not deploy standardized electrode arrays and hence do not permit a direct parameterization of soil heterogeneity. Our work shows that, through the utilization of geometrically fixed arrays, heterogeneity can be parameterized for the soil volumes defined by the geometry of the array. Deployment of fixed electrode arrays allows the researcher to make comparisons between soil types, soil horizons and assess soil response over time. Doing so allowed us to make the following inference.

- a) The degree to which a soil system is divided into biogeochemically distinct microenvironments is not constant over time. The same soil horizon will exhibit vastly different patterns of biogeochemically distinct microsites in two consecutive seasons as a function of changing meteorological conditions.
- b) Functional heterogeneity increases with the decreasing hydraulic conductivity associated with the structural impediments to drainage as reflected in the taxonomic soil drainage class, allowing us to accept the hypothesis that functional soil heterogeneity follows the taxonomic drainage class.
- c) There is a strong causal link between soil moisture status and the occurrence, abundance and spatial distribution of biogeochemically distinct microenvironments and consequently, the occurrence of biogeochemical hot spots and hot moments.

- d) In addition to depending on micro-pore structure and moisture, functional heterogeneity shows a threshold around 60 % water filled pore space, likely associated with the maximum of aerobic microbial activity often observed in that moisture range (Linn and Doran, 1984). Below that threshold moisture value, heterogeneity does not vary much regardless of soil texture or precipitation events.
- e) Pedological theory dictates that porosity will change with depth, but can be assumed to be constant over the 18-month time frame of our study. This means soil porosity plays an important, yet secondary role for the development of functional heterogeneity in soil.
- f) In the case of our soils and dissimilar to the observations of Seybold et al. (2002), variations in temperature did not seem to be as consequential for fluctuations in functional heterogeneity as were changes in water filled pore space.

2.5.2. A role for Pt-probe arrays in monitoring metabolic heterogeneity

Conventional Pt-electrode tips probe the electron activity resulting from chemical reactions and associated microbial activity within a sphere of a volume of just a few mm³ (Fiedler et al., 2007). Compared to lysimeters, trace gas measurement systems, or x-ray tomographic equipment, such Pt-based sensors are cheap, easy to assemble and able to survive deployment in challenging environments for extended times to provide real time information about the status of the soil system and any change thereof.

It is important to realize that the parameter chosen as an index of heterogeneity, the standard deviation of the mean of the probes deployed or Metabolic Heterogeneity Index, will depend on the geometric outline of the electrode array. In the case presented here, we arbitrarily chose a linear, horizontal arrangement of five electrodes spanning a distance of 40 cm, with 10 cm between each electrode tip. This geometric design was kept constant across all soils and depths. Installation will disturb the probed environment, however, interference can be contained by miniaturization of the sensors such as demonstrated in the course of this work (Figure A.4). Through the utilization of geometrically fixed arrays, heterogeneity can be parameterized for the soil volumes defined by the geometry of the array.

2.6. Conclusions

With the work presented here, we establish the "Pt-probe array approach" as a practical means to pursue and further develop the science of biogeochemical soil heterogeneity. To this end, we demonstrate how fixed arrays of sensors capable of probing the electron activity in very small individual volumes can return quantitative information on the extent to which the soil is subdivided in functionally different microsites. The parameter "Metabolic Heterogeneity Index = the standard deviation of the mean electromotive potential within a fixed array of 5 probes" allowed us to obtain several insights. Within a horizontal distance of 40 cm and among five microenvironments probed, the functional state of an 'upland' soil may simultaneously include conditions of unrestricted aerobiosis as well as conditions allowing for sulfate reduction and even methane production (von Fischer et al., 2009). Functional soil heterogeneity is not constant over time, i.e., it cannot be predicted based on soil pore

metrics alone (which we assume to remain constant over the observation period). Maximum biogeochemical diversity is most likely to be encountered in organic matter rich topsoils (Hall et al., 2016) subject to large fluctuations in soil moisture. Rapid draining followed by the gradual drying out of the soil will generate greater functional heterogeneity than rapid rewetting, and will be more sensitive to moisture fluctuations than to temperature variations overall. This latter finding could mean that moisture changes associated with climate change could be much more consequential for the broader scale intensity of biogeochemical soil processes than temperature changes. Given the importance of soil heterogeneity for matter transformations in soil systems and the relative ease of technical implementation, we recommend that the standard deviation of mean electromotive Pt-probe potentials within fixed electrode arrays be further developed as a routine parameter for the characterization of soils in long-term monitoring programs such as Critical Zone Observatories, LTER and NEON sites. This may require standardization of electrode number and the geometry of arrays utilized.

2.7. Acknowledgments

This work was supported by the US Department of Energy, Office of Biological and Environmental Research, Terrestrial Ecosystem Program (Award Number DE-FG02-13ER65542).

3. The ability of pore network metrics to predict soil redox status is scale dependent

Authors: Thomas Wanzek^{1*}, Marco Keiluweit², Tamas Varga³, Adam Lindsley¹, Peter S. Nico⁴, Scott Fendorf⁵, and Markus Kleber¹

¹ *Department of Crop and Soil Science, Oregon State University, 3017 ALS Building, Corvallis, OR, USA*

² *Stockbridge School of Agriculture and School of Earth and Sustainability, University of Massachusetts, 411 Paige Lab, Amherst, MA, USA*

³ *Environmental Molecular Sciences Lab, Pacific Northwest National Laboratory, Richland, WA, USA*

⁴ *Earth and Environmental Sciences Area, Lawrence Berkeley National Laboratory, Building 85B, Berkeley, CA, USA*

⁵ *Earth System Science Dept., Stanford University, Via Ortega 473, Stanford, CA, USA*

*Corresponding author: wanzekt@oregonstate.edu

Manuscript in preparation for submission to Water Resources Research

American Geophysical Union
2000 Florida Ave. NW
Washington D.C., 20009

3.1. Abstract

Variations in microbial lifestyle and metabolic efficiency are governed by the presence or absence of water and oxygen, for this reason, the volume, connectivity and general shape of the soil pore system can be expected to be a relevant factor in the development of spatial metabolic diversity in soil. Here we show how sets of quantitative pore network metrics (PNM) derived from X-ray computed tomography (XCT) can be combined with measurements of electron activity (EA) to assess how the metabolic status of soil depends on variations of the overall pore network architecture (PNA). Three types of PNAs were created by sieving soil from a Mollisol – A horizon to ≤ 1 cm and ≤ 2 mm and comparing them to a control with intact soil structure. Three replicate mesocosms from each structural treatment were instrumented with three Pt-electrodes each to record variations in electron activity while subjected to a regimen of full saturation, drainage and rewetting. We found that void-based XCT – metrics were more frequently correlated with metrics describing changes in electron activity than skeleton based XCT – metrics. The abundance of significant correlations between PNMs and electron activity parameters was not only a function of pore network architecture, but also of the volume of interest (VoI) chosen for XCT analysis. PNMs had the greatest power to statistically explain changes in electron activities in the smallest VoI, corroborating previous assumptions that Pt-electrode tips probe a very limited soil volume immediately surrounding the probe tip. Our work underscores the importance of scale in observations of natural systems.

3.2 Introduction

Reducing subsurface environments are important for the functioning of the biosphere on many levels, examples including processes as diverse as soil carbon and nitrogen mineralization, trace metal mobility and remediation of contamination with organo-chemicals. For instance, reducing conditions are among the most significant factors in the retardation of soil carbon decomposition, because anaerobic carbon cycling can be 60 to 95% slower than aerobic respiration (Wania et al., 2009) as a result of lower energy yields associated with the use of terminal electron acceptors other than oxygen (e.g. Fe^{3+} or CO_2) (Arndt et al., 2013). Reducing conditions can further contribute to the contamination of groundwater systems with naturally occurring metalloids such as arsenic. Arsenic conversion from primary mineral sources to secondary forms that can contaminate ground water is the result of two reduction-oxidation dependent processes: 1) oxidative weathering causes As-containing primary minerals (such as pyrite) to transition to secondary Fe-oxyhydroxides with the ability to strongly adsorb co-weathered As^{5+} , and 2) the microbial reduction of these Fe-oxides under anaerobic conditions causes arsenic to become vulnerable to microbial reduction to water soluble forms ($\text{As}^{5+} \rightarrow \text{As}^{3+}$) (Fendorf et al., 2010; Kumpiene et al., 2008; Polizzotto et al., 2008; Zheng et al., 2004). Affected populations in East and Southeast Asia alone are estimated to be 100 million and rising (Fendorf et al., 2010). An upside of reducing conditions in the subsurface biosphere is the facilitation of molecular transformations involved in the decontamination of toxic halogenated organic compounds. Polychlorinated biphenyls (PCBs) are an example of a widely used organic molecule turned subsurface

contaminant (Borja et al., 2005). PCBs are a synthetic family of lipid-like compounds consisting of two joined aromatic carbon rings and 10 locations for potential chlorine attachment. PCBs have a wide variety of industrial applications due to their low vapor pressures, low solubility in water, and excellent dielectric properties. Commonly believed to be “indestructible”, PCBs have been shown to be biodegradable via reductive dechlorination (Abramowicz, 1990). Reductive dechlorination is a naturally occurring process that can detoxify and completely destroy PCBs (Bedard, 2008; Borja et al., 2005). Under such anaerobic conditions where Mn^{4+} and Fe^{3+} are the primary terminal electron acceptors (Christ et al., 2004), detoxification is most efficient and the chlorine atoms are removed. The aromatic rings however, are left intact. If the PCBs are subsequently subjected to aerobic oxidation, the remaining benzene rings can be broken open (Abramowicz, 1990), thereby completing the detoxification process. These examples illustrate the importance of a robust representation of soil redox state in reactive transport models as well as in earth system models, yet large uncertainties exist regarding how, and to what extent, the potentially relevant mechanisms should be included in such models (Riley et al., 2014).

The historic approach to the problem of representing reducing conditions in models has been centered on the fact that O_2 diffusion rates in liquid water are reduced by up to 4 orders of magnitude compared to diffusion rates in the atmosphere (Cussler, 1997) and led to the notion that the development of reducing conditions in soil can be understood based on three major assumptions (for critical discussion and pertinent references compare Keiluweit et al., 2016):

1. Oxygen diffusion within aggregate domains can be estimated based on porosity alone
2. There is a single critical oxygen concentration at which heterotrophic respiration (the major energy yielding process in soil) ceases in all organisms
3. Oxygen consumption is constant throughout "aerobic" aggregate domains

But, despite a longstanding recognition of metabolic "hot spots" and "hot moments" in soils (Kuzyakov and Blagodatskaya, 2015; McClain et al., 2003), none of the modeling strategies explored to date have the capacity to isolate the effect of either diffusive domain size or spatial void pattern on O₂ availability and, by extension, on the development of reducing conditions. The DOE sponsored Community Land Model (CLM), for instance, lacks features such as subgrid-scale hydrology, anaerobic microbial carbon metabolism, and aqueous chemistry (Riley et al., 2011). Current biogeochemical models (Davidson et al., 2012; Koven et al., 2013; Kuka et al., 2007) are thus resorting to rather general approaches to account for the formation and relative quantitative importance of (i) aerobic pore volumes with particularly efficient aerobic (O₂-dependend) respiration versus (ii) anaerobic pore volumes with specific functionality (metal reduction, CH₄ and N₂O production). None of the existing models have provisions to estimate the spatial abundance of the metabolically diverse microenvironments that are increasingly observed in soil (Keiluweit et al., 2017). Yet we know now that form and function of the soil microbial community involved in transformations of matter and energy is tightly regulated by the pore network architecture (Negassa et al., 2015). The composition of the microbial community that resides in soil aggregates is more related to the characteristics of the intra-aggregate

pore structure (Kravchenko et al., 2014) than to the type of organic substrate available as an electron donor (Ruamps et al., 2011). These findings coincide with insights that, at the global scale, soil carbon stocks can be modeled with much greater confidence when the model includes a representation of microbial metabolic performance (Wieder et al., 2015; Wieder et al., 2013) and the postulate that ‘microbial metabolism is a less significant regulator of soil organic decomposition than are microbial habitat properties’ (quoted from Ruamps et al., 2013).

At this point, the community has progressed to a state where the importance of reducing conditions for soil biogeochemistry has been recognized, but modeling concepts do not extend beyond a provision to limit oxygen availability. An example is the recent version of the Community Land Model (CLM4, Koven et al., 2013) where oxygen limitation r_O is introduced as a scaling factor for the intrinsic turnover rate of each carbon pool:

$$k_i = k_{0,i} r_T r_W r_O r_Z \dots \dots \dots (1)$$

where $k_{0,i}$ is the intrinsic (= substrate specific) turnover time for each pool (yr^{-1}), r_T is the temperature modifier, r_W is the moisture modifier and r_Z is a depth modifier. Current models are thus unable to account for the formation, spatial abundance and relative quantitative importance of aerobic pore volumes with particularly efficient aerobic (O_2 -dependent) respiration or anaerobic pore volumes with specific functionality (e.g. Fe^{3+} reduction, CH_4 and N_2O production).

Here we assess that the greatest obstacle towards a robust implementation of soil redox state in models is a lacking capability to fully represent the process chain leading from (i) variation in climatic parameters (e.g. temperature, precipitation), to (ii) soil structure, to (iii) variations in soil redox state, and (iv) to the resulting physiological disposition and subsequent metabolic performance of the microbiota. Consequently, the purpose of this manuscript is to offer an initial answer to the overarching question "How can pore network structure contribute to the propensity of soil systems to become reducing environments?". To achieve this goal, we pursued two major objectives:

- 1.) Find quantitative, numerical indices of soil structure that can be used to test assumptions about causality regarding soil structure - electron activity relationships
- 2.) Obtain robust information about the electron activity (i.e. redox state) within soil microenvironments

Our conceptual approach consisted of comparing changes in electron activity within the pore network architecture of an intact, well-aggregated topsoil to the dynamics of electron activities in two artificially modified pore network architectures derived from the same soil material. In the resulting model systems, we focused on three aspects:

1. Parameterization of soil structure using computed tomography. Diffusive domains and the surrounding spatial void pattern within a given soil volume (i.e. soil structure) are considered as quantifiable through X-ray Computed Tomography (XCT, Luo et al., 2008). Because we wanted to interrogate soil volumes of a physical size

(cylinders of 24 cm width x 18 cm height) that can be considered relevant for upscaling to the pedon and ultimately, landscape scale, we used a Nikon (Metris) XTH 320/225 kV X-ray Tomography (XCT) System available through a user proposal at the Environmental Molecular Science Laboratory (PNNL-EMSL). Nimmo and Perkins (2008) hypothesized that as the manipulation of a pore network increased, macroporosity would decrease. We assumed that, by manipulating saturation level and manipulating the geometry of the pore network while measuring concomitant changes in electron activity in multiple microenvironments, relationships between the XCT quantified pore network and electron activity could be determined. In doing so, we aimed to contribute to the development of parameters, procedures and concepts for the application of computed tomography to the investigation of structure - functionality relations in soil systems.

2. Variation of electron activity in soil microenvironments. Our decision to use Pt-electrode potentials for the identification of biogeochemically distinct soil microsites was based on previous reports that Pt-electrodes are capable of probing the electron activity in very small individual volumes in the order of few cubic millimeters (Cogger et al., 1992; Fiedler, 1999; Fiedler et al., 2007). To address uncertainties regarding the soil volume "seen" by the Pt-electrode tip, the relationships between strategically sub-sampled portions of the pore network (Volumes of Interest, VoI) and electron activity were examined.

3. Variation in moisture content. To elucidate the relationship(s) between wet up and dry down events and the formation of reducing conditions we decided to focus on short-term time brackets where moisture conditions change how the resulting

variations in redox state are modulated/alterd/controlled by XCT derived pore network metrics.

Our experimental approach involved testing the following hypotheses:

H1: Electromotive potentials sensed by platinum electrodes respond to changes in water saturation level in a predictable, non-random fashion. We hypothesize that for all electrodes probing the same network architecture type (PNA), the associated electron activity (EA) metrics (ΔE_{Pt} per time interval) = $\pm$ constant

Null: EA metrics compared between PVC rings ($n = 3$ per PNA) within a PNA are significantly different

H2: EA metrics are specific to pore network architectures. When subjected to the same moisture change, changes in EA metrics should be significantly different between pore network architectures. This hypothesis can be accepted if ΔE_{Pt} per time interval $\neq \Delta E_{Pt}$ between pore network types

Null: EA metrics are not significantly different for any two PNA comparisons

H3: The ability of XCT-derived pore network metrics to predict EA metrics improves with decreasing average pore size, or $\Delta E_{Pt}(a...j) = f(PNA)$, (PNM 1....18), where PNA = pore network architecture, (a...j) are a set of electron activity metrics, and (1...18) is a set of pore network metrics

Null: The number of significant correlations between EA metrics and PNMs does not increase as aggregate size decreases

- H4: The ability of XCT-derived pore network metrics to predict EA metrics improves when the volume of the observed pore network is small and immediately surrounds the platinum electrode tip, compared to larger soil volumes or “the power of XCT derived network metrics to predict change in redox state = $f(\text{VoI})$ ”
- Null: The number of significant correlations between EA metrics and PNMs does not increase as VoI decreases.

3.3 Methods

3.3.1 Experimental approach

3.3.1.1 Soil description and sample collection

Soil was collected from the Ap horizon of a moderately well drained Woodburn soil series (Argixeroll) at Hyslop Farms in the Willamette Valley, Oregon. Basic soil characteristics were measured following protocols detailed in the Kellog Soil Survey Laboratory Methods Manual (Staff, 2014) and are provided in Table B1. The site had been unmanaged for more than 10 years with the exception of biannual mowing and had a grassland vegetation mix dominated by *agrostis capillaris*, *agrostis stolonifera* and *hypochaeris radicata*. Three intact, cylindrical cores with dimensions of 18 cm (height) by 25 cm (diameter) were taken by inserting PVC rings down to a depth of 20 cm (Figure B2). Bulk soil to create the two manipulated pore network architectures was removed from the same depth and sieved to ≤ 1 cm and to ≤ 2 mm. Sieved soils were filled into $n = 3$ cores for each treatment, to yield a total of 9 cores representing 3 different pore network architectures (PNAs): “Random” PNA; “Large aggregate” PNA (sieved to ≤ 1 cm), and “Small aggregate” PNA (sieved to ≤ 2 mm).

3.3.1.2 Set up and instrumentation

A fine plastic mesh (~ 1 mm openings) was glued to the bottom of each core to retain the soil and allow for water infiltration and drainage. Cores were stored at 10 °C for seven days prior to the start of the experiment. A large fiberglass tub (125 cm x 98 cm x 23 cm, Figure B1) was used to house all nine cores for simultaneous

manipulations of the water saturation level. The bottom of the tub was filled with 2 cm of coarse, autoclaved sand to allow for unrestricted water infiltration in to and drainage out of the soil cores. To monitor structure dependent variations in electron activity, each core was equipped with an array of three Pt-electrodes (width = 5 mm; Pt wire tip length = 10 mm, overall electrode length including tip = 25 mm), each installed in one quadrant of the PVC ring such that the electrode tip was 9 cm above the base of the PVC ring. Electrodes were connected to a 3M Ag/AgCl InLab reference electrode (Mettler and Toledo, Columbus, OH USA) installed at the surface of the fourth ring quadrant (Figure B1). The electrodes were installed in a strict geometric array (one per quadrant) to ensure that each Pt-electrode accessed the same volume of soil. Prior to installation, Pt-electrodes were tested against a quinhydrone solution (0.1 g quinhydrone per 50 ml deionized water buffered to pH 7 (Austin and Huddleston, 1999; Jones, 1966). If the measured potential was more than ± 10 mV outside of the ideal potential at 20 °C (92 mV), the probe was cleaned and retested. Electromotive potential data were measured each minute and an average value was recorded every hour using a Campbell Scientific CR800 data logger and AM16/32B multiplexer (Campbell Scientific, Logan, UT, USA). The data logger and multiplexer were assembled by Dynamax Inc. (Houston, TX, USA). Electromotive potentials were normalized relative to the standard hydrogen electrode by adding an individualized, temperature adjusted correction factor for each measurement (Nordstrom and Wilde, 2005).

3.3.1.3 Experimental conditions

To investigate the effects of variations in pore network architecture on changes in electron activity, we subjected the cores to variations in water saturation levels. The first such event occurred on day 1.5 and was a rapid transition from near field capacity to full inundation (simulating phreatic conditions) of the electrode tips by flooding the tub to a water level just below the rim of the PVC cores (Figure A.2). Full saturation was maintained for 12.5 days until electron activity had reached a near equilibrium state in all PVC rings (Figure 1). Following day 14, the PVC rings were allowed to drain for 8 days until electron activities returned to values similar to those at the start of the experiment. At day 22, water was added to a level of 4.5 cm below the electrode tip to create conditions similar to partial saturation in a simulated vadose environment. These conditions were maintained for another 5.5 days, when the water was drained and the PVC rings monitored for another 3 days until electron activities returned again to levels close to starting conditions. Overall duration of the experiment was 30.5 days. During this time, the cores were kept in a greenhouse equipped with a climate control system allowing us to exclude precipitation while maintaining ambient diurnal temperature variation.

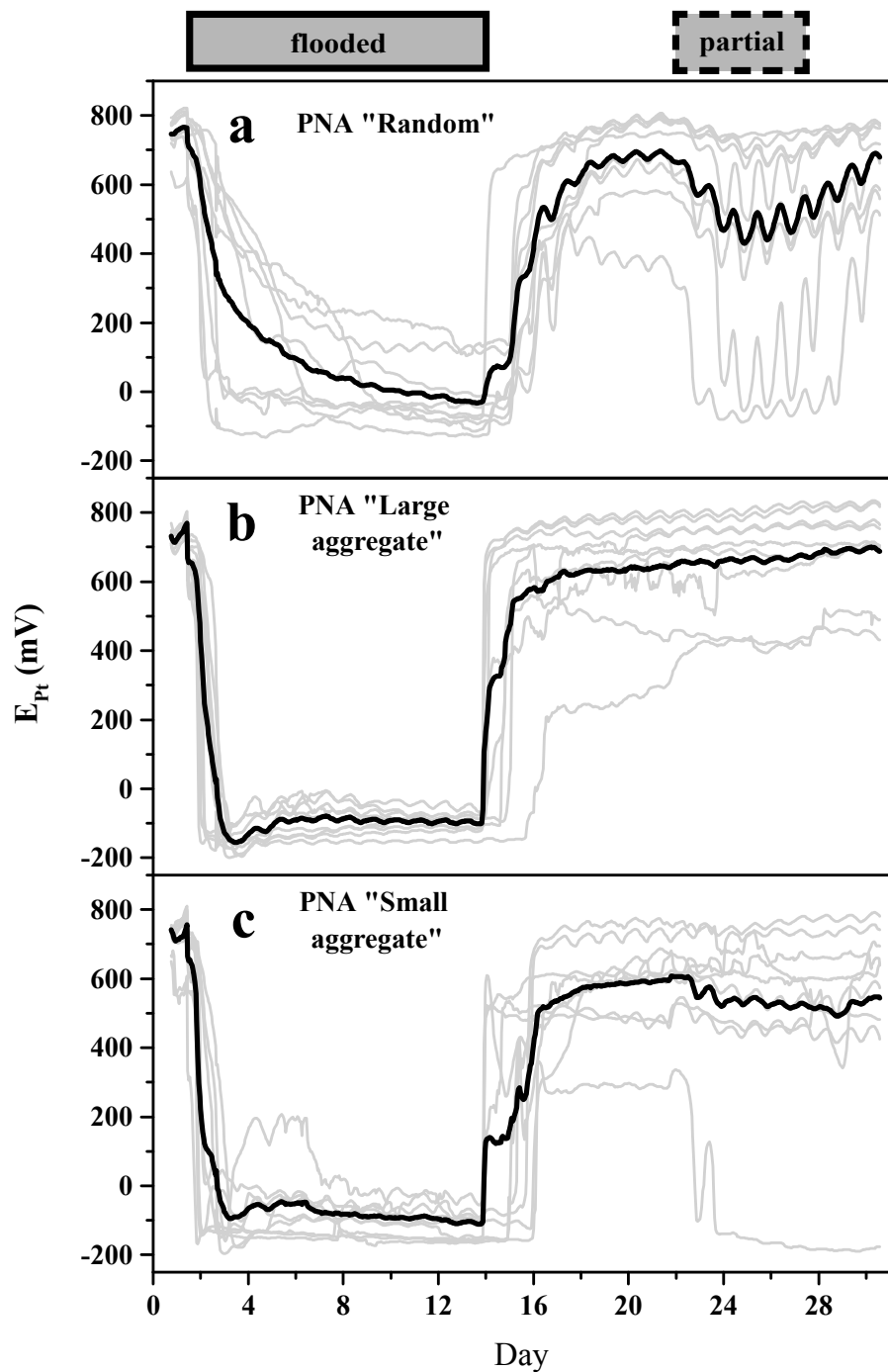


Figure 1. E_{Pt} curves for all three pore network architectures. Mean E_{Pt} curves (black) are shown for each pore network architecture (Random, Large aggregate, and Small aggregate). E_{Pt} data from each individual electrode per pore network architecture ($n=9$ per pore network architecture) are also shown (gray). Timing and duration of complete saturation with water is represented by solid gray box “flooded” (day 1.5 to 14) and partial saturation is represented by dashed gray box “partial” (day 22 to 27.5).

A set of ten parameters (Electron Activity metrics, EA) was developed to enable a numerical description of the response of electron activities to manipulations of soil moisture status. These metrics are illustrated and defined in Figure 2 (panels b and c). After samples were drained for the last time, they remained in the fiberglass tub for two more days until further processing. They were then carefully removed and packaged in individual containers in preparation for transport to the X-ray computed tomography (XCT) facility in the Environmental and Molecular Science (EMSL) division at the Pacific Northwest National Laboratory (PNNL; Richland, WA, USA).

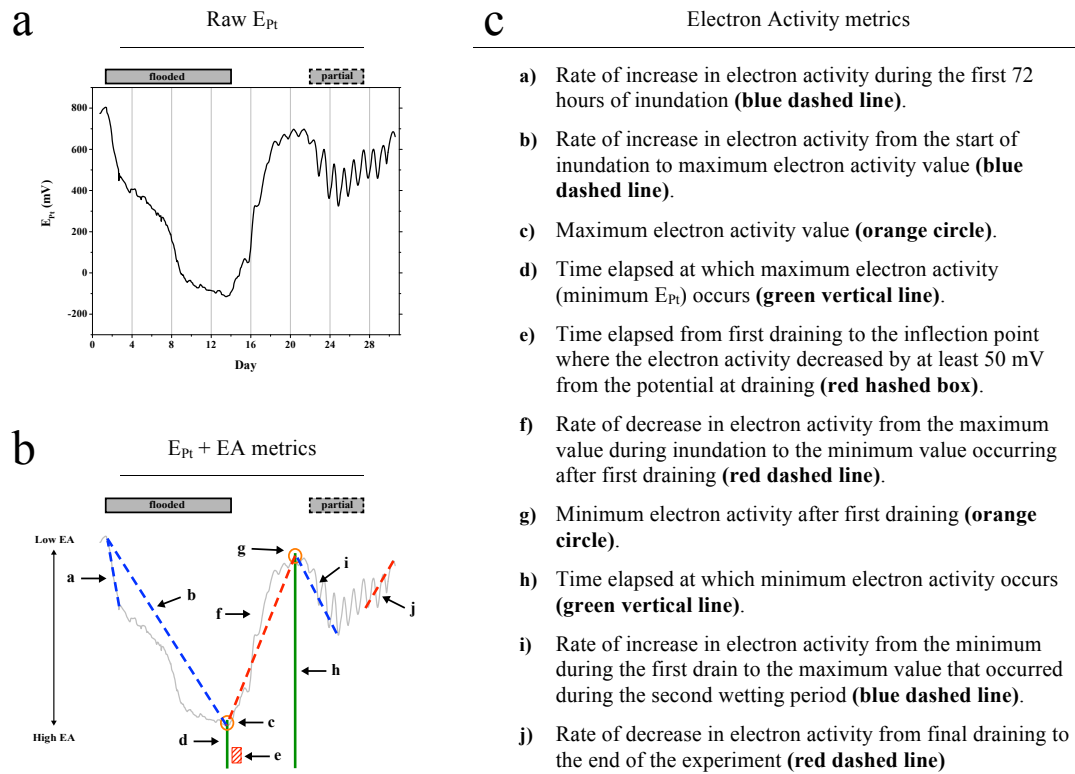


Figure 2. Division of the E_{Pt} curve into electron activity metrics. The raw E_{Pt} curve (panel a) was divided into 10 different portions (panel b), defined as electron activity metrics (EA metrics; panel c). Panel c contains the description of each metric and its corresponding location on the curve in panel b. Timing and duration of complete saturation with water is represented by solid gray box “flooded” and partial saturation is represented by dashed gray box “partial”.

3.2.2 Pore network quantification using X-ray computed tomography

3.2.2.1. XCT theory and scan conditions

X-ray computed tomography (XCT) is a non-destructive technique for determining the internal structure of an object (Taina et al., 2008) and has been used for nearly two decades (Tippkötter et al., 2009) to quantify the 3D architecture of pore spaces in natural soils (Perret et al., 1999; Pierret et al., 2002). XCT techniques are based on deriving an image using the quantity of applied x-rays that pass or do not pass through the object of interest. The effective atomic number and the density of the sample material being x-rayed determine the linear attenuation coefficient representing the quantity of x-rays that pass through the sample. The denser the sample material, the fewer x-rays can penetrate and the higher its linear attenuation coefficient (Taina et al., 2008; Tippkötter et al., 2009). When using conventional two-dimensional x-ray techniques, depth information is lost, but when x-ray attenuation information is obtained from multiple radiographic images, scanned at different angles, called projections, a complete three-dimensional image can be constructed (Wildenschild et al., 2002).

Each sample was scanned on the Nikon (Metris) XTH 320/225 kV X-ray Computed Tomography (XCT) System at PNNL's EMSL division. This equipment allows for the investigation of large samples of up to bucket-size. However, proof of concept work we carried out using the same soil and sample containers, revealed that the entire PVC rings would absorb too much radiation to achieve satisfactory image quality. We thus decided to sub-section the PVC to allow for sufficient beam penetration. Following the original layout of Pt-electrodes, each PVC core was

physically divided into four quadrants (Figure B3), with the Pt-electrode at the center; and additional acrylic glass panels added to contain individual sub-samples. With 3 Pt-electrodes deployed per PVC ring, this amounted to $n = 3$ quadrants per individual ring and a total of $N = 27$ samples for CT-image analysis. The poly-chromatic beam conditions were set at 130 kV and 200 μA , and a Mo target with a 0.25mm Al filter were used to reduce beam hardening. A total of 2146 projections, with four frames per projection, were taken of each PVC quadrant. Together with the reduced specimen size (now 18 x 12.5 cm), these settings allowed us to achieve a final image resolution of 110 μm .

3.2.2.2. *Image pre-processing*

The pre-processing workflow was the same for all analyzed image files. The entire 16-bit raw image file was imported in to the open-source image analysis software, Fiji (Schindelin et al., 2012), and converted to an 8-bit image stack. For image analysis, we identified volumes of interest (VoI, Figure 3) to test three assumptions:

a) the electron activity sensed by the Pt-electrode tip represents the state of the soil solution in the pore system connecting the soil surface and the electrode tip. The resulting Volume of Interest (VoI_{100}) was of cylindrical shape centered around the electrode with a height of approximately 8 cm (minor variations between individual cylinders), a diameter of 4 cm and an average volume of 100 ml

b) the potential sensed represents a more constrained but still sizable region right below the electrode tip. This VoI had a diameter of 4 cm and extended 2 cm

down from the bottom of the probe tip, resulting in a volume of approximately 25ml (VoI₂₅).

c) testing the suggestion of Fiedler (1999) that Pt-electrodes are only sensitive to the conditions in a space of few cubic mm immediately surrounding and connected to the platinum tip, we finally selected a volume of interest surrounding the platinum wire in the fashion of a cylindrical sleeve with a height of 7 mm, an inner diameter of 5 mm and a wall thickness of 0.84 mm, yielding a volume of 190 mm³ or approximately 0.2 ml (VoI_{0.2}). The dimensions of the inner core were chosen to avoid image artifacts created by the metal of the probe tip. Figure 4 demonstrates how the respective images varied as a function of pore network structure. Representative curves are added to reiterate significant differences in electron activity dynamics. For each sub-sampled VoI the contrast was set using Fiji's auto brightness/contrast setting. The binary threshold was then set manually by comparing pore edges in four different images to the same pore edges in the corresponding images from the 8-bit image stack prior to thresholding. A 3D median filter of dimension 2 x 2 x 2 pixels was then applied to each binary stack which reduced noise, but preserved pore edges (Jassogne et al., 2007). Because each of these processing steps were destructive processes, a version of the image stack was saved prior to each subsequent step should a reprocessing be necessary.

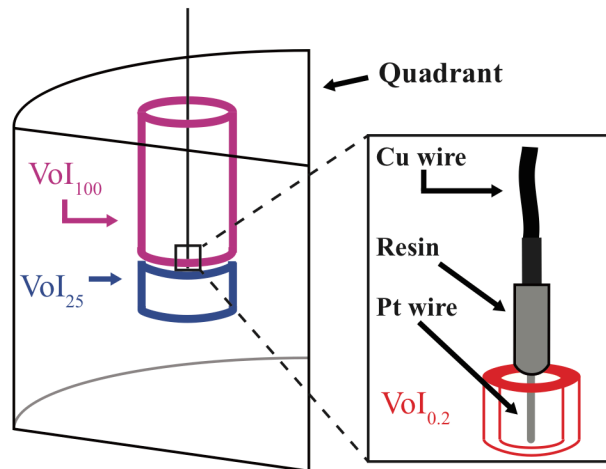


Figure 3. Location three of volume of interest (VoI) sub-samples. Three digital sub samples ($n = 81$) were created from the image file for each soil sample ($N = 27$) using Fiji image analysis software. In relation to the location of the Pt-electrode the locations of the VoI sub-samples are as follows: the VoI₁₀₀ (purple, $r = 2$ cm, $h = \sim 8$ cm) sample extended from the electrode tip to the soil surface, the VoI₂₅ sample (blue, $r = 2$ cm, $h = 2$ cm) extended from just below, but not including the electrode tip downward, and the VoI_{0.2} sample (red, $r_1 = 5$ mm, $r_2 = 5.84$ mm, $h = 7$ mm) immediately surrounded the Pt-tip it self. The PVC quadrant (black) was 18 cm high and the Pt-electrode was inserted to the halfway point (9 cm; black line through VoIs).

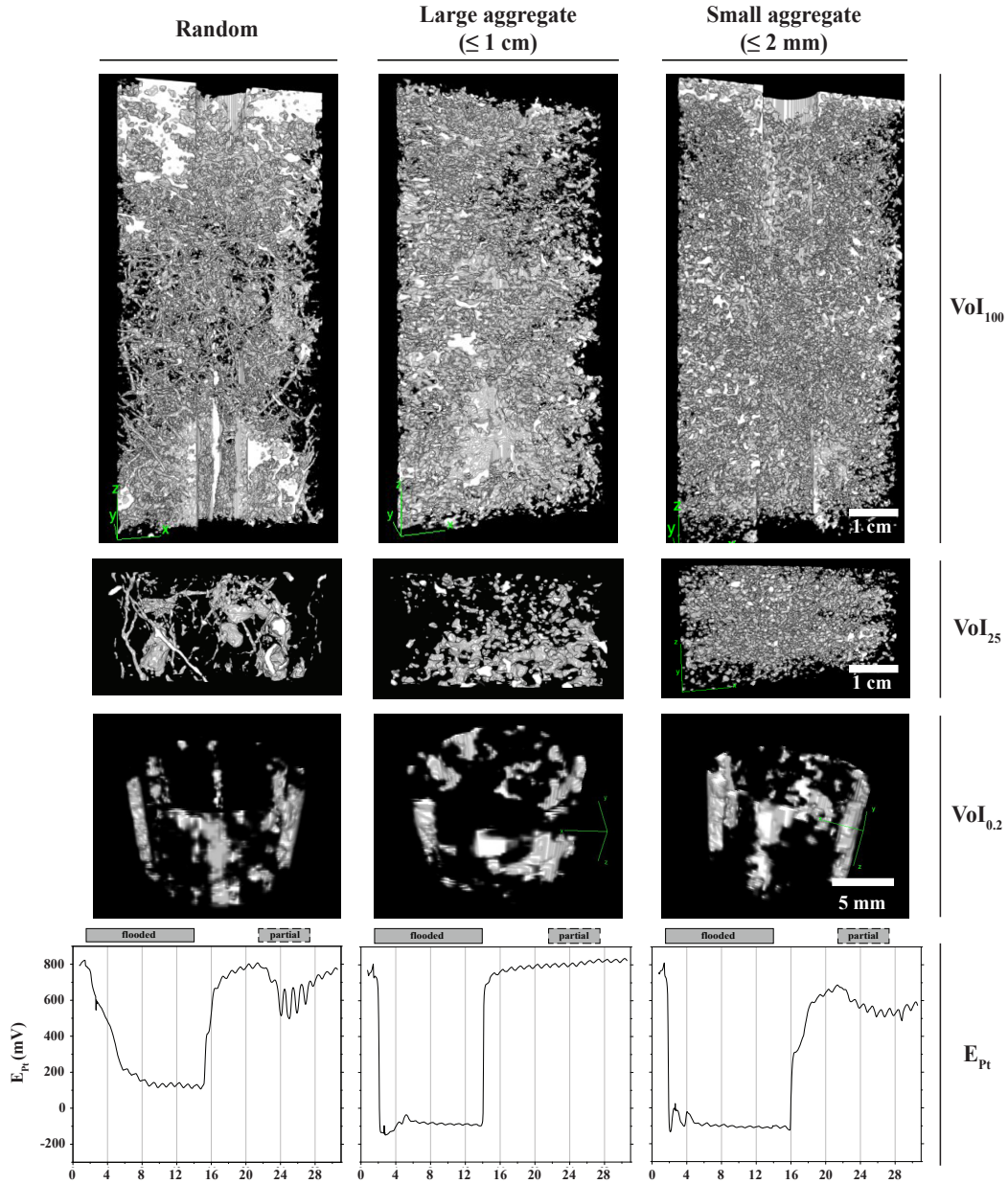


Figure 4. Examples of XCT images generated in the course of this work. Each column represents a quadrant with a single electrode from a given pore network architecture: “Random” (left column) is the undisturbed soil, “Large aggregate” (center column) represents the treatment that was sieved to ≤ 1 cm, while “Small aggregate” (right column) shows the pore network architecture created by sieving to ≤ 2 mm. Rows represent the three different volumes of interest. Pore space is realized in gray (intact pore) and white (pore that has been sliced). The associated electromotive potential curves from the Pt-electrode specific to the pictured PNA are in the bottom row. All PNA images are oriented such that the Pt-electrode is at the bottom with the Cu wire extending out the top.

3.2.2.3 Image analyses

To quantify the pore space architecture, each thresholded sub-sample was analyzed with the help of two sets of metrics (pore network metrics, PNM). One set of metrics (skeleton-based metrics, # 1-9 in Table 1; Figure 3) was used to describe the interconnectedness and complexity of the pore network in the VoI, while a second set (void-based metrics, # 10-18 in Table 1; Figure 3) was used to characterize the three-dimensional reaction space that the pores occupied. The skeleton network of the pore space is generated by eroding the voxels defined as pores in each image down to their single voxel width medial axes. A skeleton network is defined as all interconnected medial axes, and a single image stack can be comprised of multiple skeleton networks. The medial axes for each image stack were generated using the Skeletonize3D plugin (Arganda-Carreras, 2014; Lee et al., 1994). Analysis of the medial axes of each sample using the AnalyzeSkeleton plugin (Arganda-Carreras et al., 2010) generated the skeleton-based pore network metrics (Table 1; Figure 5). The void-based metrics (Table 1; Figure 5) were calculated using the Particle Analyzer function of the BoneJ plugin as well as the Bone Volume to Total Volume plugin to calculate the image based percent porosity (Doubé et al., 2010). All pore network metrics generated by the above plugins as well as the metrics calculated using the output from the above plugins are visualized in Figure 5.

Table 1. Pore network metrics and associated descriptions.

Metric number	Pore network metric (PNM)	Unit	Metric description
1	Number of branches	Count	The number of slab segments (composites of slab voxels) in a VoI
2	Total number of junctions	Count	The total number of voxels in the VoI with more than two neighbor voxels
3	Mean branch length	mm	Average length of a branch in the VoI; calculated using all branches in the VoI
4	Maximum branch length	mm	Length of the longest branch in the VoI
5	Number of triple points	Count	The number of junctions in the VoI with exactly three branches
6	Number of quadruple points	Count	The number of junctions in the VoI with exactly four branches
7	Total number of skeletons	Count	Number of individual (non-connected) skeleton (centerline) networks in the VoI
8	Number of skeletons with branches>1	Count	The number of skeleton networks that contain at least one junction and branch
9	Mean tortuosity	n/a	Mean convolution of all pores in the VoI. Calculated as the sum of all total branch lengths in the sample divided by the sum of the straight-line distances of all branches in the VoI. (Luo et al, 2010a)
10	Image based void volume	mm ³	Volume occupied by an individual pore. Reported as average pore volume for each sample. Calculated by counting the number of voxels contained within a given void
11	Void surface area	mm ²	Calculated by fitting a triangular surface mesh (via marching cubes) to the interior of each individual void
12	Enclosed void volume	mm ³	Volume of an individual void enclosed by triangular surface mesh (0 if no mesh could be fit)
13	Mean pore diameter	mm	Calculated at several points as the diameter of the greatest sphere that fits within the void and which contains the point
14	Standard deviation of mean pore diameter	mm	Standard deviation of sphere diameters used in mean pore diameter calculation
15	Surface area to volume ratio	mm ⁻¹	Surface area divided by image based void volume
16	Total number of individual voids	Count	Number of individual voids identified in the VoI
17	Number of individual voids with enclosed volume>0	Count	The number of voids to which a triangular surface mesh was fit in the VoI
18	Image based porosity	%	Number of void voxels in the VoI divided by the total number of voxels in the VoI

3.2.3 Statistics

Statistics were performed using OriginPro (Version X, OriginLab, Northampton, MA) or RStudio for Mac version 1.0.136 (RStudio, 2015). All PNMs were normalized to a unit per mm^3 basis. Linear regression was carried out between the mean value for each pore network metric (PNM; explanatory) and the value for each electron activity metric (EA metric; response). A relationship between a PNM and an EA metric was deemed significant at the $p < 0.05$ level when the r^2 value from a given regression was greater than or equal to 0.44 ($n = 9$ Vol's, each representing one quadrant as illustrated in Figure B3, d.f. = 8, f-value = 5.59). Tukey's honest significant difference test in RStudio was used to determine if there were significant differences between the mean values ($n = 9$) from each PNA for (i) the electron activity metrics and (ii) the pore network metrics for each volume of interest. A two-tailed t-test was used to determine if there were significant differences between electron activity metrics within a given pore network architecture.

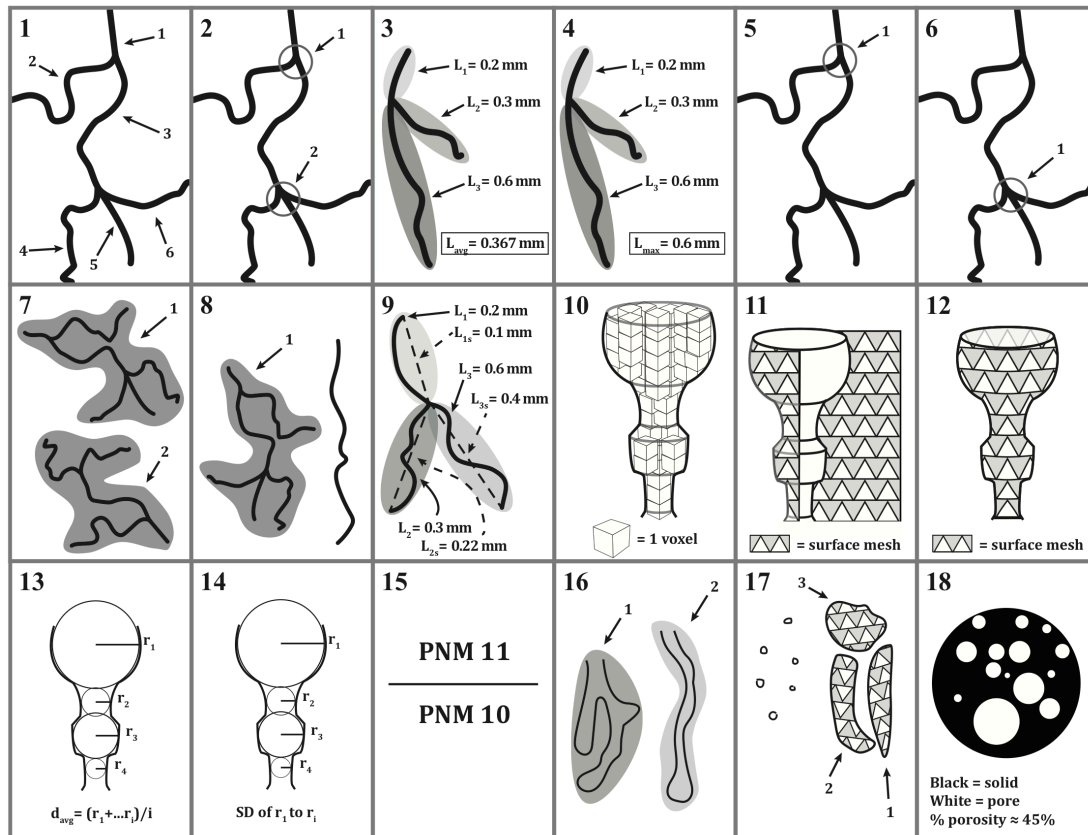


Figure 5. Illustrations depicting each pore network metric. Generalized versions of pore network metrics 1 through 18 are shown. Descriptions are in Table 1.

3.4 Results and Discussion

3.4.1 *Pt-electrodes provide robust and reliable information about electron activity*

There have been concerns about the ability of platinum electrodes to describe the electron activity (pe) status of soils (Bartlett, 1998; Bartlett and James, 1995). These concerns are based on limitations of the Pt-electrode system and on the multispecies, non-equilibrium nature of the soil solution (James and Brose, 2011). Thus, when early researchers (McKeague, 1965) noted deviations of more than 300 mV among replicate Pt-electrodes installed in the same soil horizon, they attributed such variability to the technical deficiencies of the Pt-electrode. An alternative explanation for variation among replicate electrodes installed in the same soil horizon would be that they are probing biogeochemically distinct microenvironments (Cogger et al., 1992; Fiedler, 1999). In our setting, 27 soil-electrode systems were subjected to the same changes in moisture content and hence, to a very similar biogeochemical stimulus. All electrodes showed the same general response to changes in moisture content, with modifications specific to the respective pore network architectures (Figure 1). For instance, in the PNA "Large Aggregate" (Figure 1, panel b), all 9 electrodes registered the same rapid initial increase in electron activity (= EA metric a , Figure 2). This response is nearly identical in the PNA "Small Aggregate" (Figure 1, panel c) while the 9 electrodes installed in the PNA "Random" with its native soil structure showed a much wider range of trajectories and associated slopes of the EA parameter " a " (Figure 1, panel a). We take these observations as supporting the view of Cogger et al. (1992) and Fiedler (1999) that variations among the potentials registered by multiple, well calibrated Pt-electrodes installed in the same soil horizon

indicate corresponding variations in the biogeochemical states of the microenvironments probed by the respective electrodes. Figure 1 further suggests the existence of a complex link between pore network architecture and moisture state. During the first inundation phase and while completely saturated, the potential registered by the $n = 9$ electrodes in the “Large Aggregate” and “Small Aggregate” PNAs behave very similarly in terms of timing of and absolute value of the mean minimum electromotive potentials achieved (about -150 mV). (Figure 1, panels b and c). However the mean minimum electromotive potential in the “Random” PNA continued to decrease over the entire period of complete inundation, only reaching a minimum value near 0 mV (Figure 1, panel a). Following the second, partial inundation (days 22 – 27.5, Figure 1) however, biogeochemical conditions in the PNA “Random” (Figure 1, panel a) begin to diverge widely while they stay relatively similar (with outliers) in the PNA’s “Large Aggregate” and “Small Aggregate” (Figure 1, Panels b and c). These findings allow us to accept hypothesis 1 (electron activity metrics are $\pm$ constant within the same pore network) with the qualification that over time, the “Random” PNA (Figure 1, panel a; Table A.2) offer a greater opportunity for diverse biogeochemical conditions to evolve independently in individual microenvironments.

3.4.2 The absolute magnitude of pore network metrics (PNMs) depends on the observed soil volume

The values of the metrics (PNM) chosen to quantify variations in pore network architecture varied as a function of both network architecture and volume of

interest. This is illustrated in Table 2 using PNM # 11: "Average Void Surface Area", as an example (for definition and illustration of PNM 11 see Table 1 and Figure 5). Depending on the VoI considered, PNM values can be near identical across PNAs (VoI_{0.2}, Table 2), or significantly different (VoI₂₅, Table 2).

Table 2. Variation in Pore Network Metric 11 "Average Void Surface Area" across Pore Network Architectures and Volumes of Interest. Unit: mm^2 , values in brackets are coefficients of variation with $n = 9$

	PNA type			Significant differences between PNAs		
	"Random" (R)	"Large Aggregate" (LA)	"Small Aggregate" (SA)	R : LA	R : SA	SA : LA
VoI ₁₀₀	5.1 (57)	6.3 (89)	2.4 (130)	---	---	---
VoI ₂₅	9.2 (35)	5.0 (52)	0.9 (70)	$p < 0.01$	$p < 0.001$	$p < 0.01$
VoI _{0.2}	1.0 (82)	1.4 (63)	1.3 (92)	---	---	---

Tukey's honest significant difference test ($n=9$ per PNA; p-values adjusted for multiple comparisons) was used to determine significant differences between electron activity metrics. Random = Random PNA, LA = Large aggregate PNA (≤ 1 cm) and SA = Small aggregate PNA (≤ 2 mm). Significance levels * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

An analysis including all 18 PNMs (Tables A.3 - A.5) reveals that the occurrence of significant differences between PNAs is greatest in the 25 ml VoI (30 significant differences), followed by the 100 ml VoI (15 significant differences), while PNMs in the 0.2 ml

VoIs were largely constant across PNAs with only 2 significant differences observed. This trend is consistent with the fact that the selection of a small VoI will necessarily limit the abundance of larger sized, or complex pores within the observed VoI. To the best of our knowledge, a systematic study of different volumes of interest within the same sample has so far not been reported for soils investigated with CT-base methods. The choice of VoI in CT-based work so far is typically based on aggregate or container size, with authors typically virtually sub sampling down to a smaller region of interest (RoI) or VoI simply to exclude edge effects (e.g. Katuwal et al., 2015b; Luo et al., 2010a). Table 3 compiles the container volumes, volumes of soil analyzed, and minimum pore diameters measured in related work. Of the six other studies cited, our research falls roughly in the middle in terms of volume of soil analyzed and minimum pore diameter measured. The closest scaled XCT analysis of the soil pore network to this work was carried out by (Köhne et al., 2011), who sampled two soils of contrasting texture using three different sample container sizes. Where possible, the resulting images were merged using a concept called ‘Scale fusion’ in an effort to capture both the larger interaggregate pores and the smaller intraaggregate pores. Though assessing the role of the pore network in regulating contaminant movement in soils as a function of scale of observation, their results

suggest an intimate relationship between the pore network structure and function for physical transport processes.

Table 3. Overview of container volume, volume of soil subsection for X-ray computed tomography analysis, and minimum diameter of soil pores analyzed.

Author	Minimum pore diameter	Volume analyzed	Container volume
	μm	(mL)	(L)
Peth et al., 2008	≥ 6	Between 0.00213 and 0.01	Variable (d = ~5 mm)
Köhne et al., 2011	> 10	Not specified	0.8 to 2
This work	≥ 220	0.2, 25, 100	8.8 (one quadrant)
Luo et al., 2008	≥ 750	Not specified	2.4
Luo et al., 2010a	≥ 750	2400	2.7
Katuwal et al., 2015a	≥ 1200	4325	5.7
Sammartino et al., 2012	≥ 1500	Variable	2.0

3.4.3 Pore network architecture modifies electron activities

The pore network has been thought to play a role in redox dynamics in soils for nearly 70 years (Grable and Siemer, 1968; Quispel, 1947; Vepraskas and Wilding, 1983). The extent to which the mechanisms relating the redox status of the soil and the pore network however usually ends at differentiating between macro and micro pores (e.g. Horn and Smucker, 2005) or changes in porosity overall (e.g. Zimmermann et al., 2016). Most major models of aerobic or anaerobic microsite formation depend on estimating water-filled porosity or air-filled porosity to calculate diffusing coefficients (of gases or dissolved organic carbon) and generally ignore pore network structure (Keiluweit et al., 2016). X-ray computed tomography provides a method to more directly measure the structure of the pore network and its impacts on soil processes. However, most applications focus on quantification of water fluxes (Or et al., 2007), saturated hydraulic conductivity (Luo et al., 2010b; Paradelo et al., 2016), air fluxes (Katuwal et al., 2015b; Naveed et al., 2013; Rappoldt and Crawford, 1999), or pore space genesis as a function of microbial activity (Helliwell et al., 2014), and not directly on the formation (or location) of microbial ‘hotspots’ (i.e. anaerobic soil volumes) (Kuzakov and Blagodatskaya, 2015). Part of this may be due to the complex relationship between the pore network and microbial activity; even studies specifically designed to generate contrasting results, have not necessarily produced conclusive results (Negassa et al., 2015). To the best of our knowledge, very little attention has been paid to purposefully manipulating the pore network and quantifying the resulting changes in electromotive potential dynamics (e.g. Keller et al., 2017).

To test the null-hypothesis "the dynamics of electron activities are independent of pore network architecture", we evaluated the extent to which electron activity (EA) metrics (Figure 2) differed between individual PNAs. To do so, Tukey's honest significant differences test (Tukey's HSD) was used to compare the means of $n = 9$ EA metrics from each PNA. This was done for all of the 10 EA metrics developed to describe the dynamics of electron activities during the duration of the experiment (Table 4). The greatest number of significant differences, six in total, was registered comparing the PNA "Random" with the PNA "Large Aggregate". The significant differences occurred between EA metrics a, b, c, d, f, and i (Table 4). Only one EA metric had a borderline significant p-value ($p = 0.073$) in the Random – Large Aggregate comparison (EA metric j). There were two significant differences and one borderline significant difference in EA metrics when the Random and Small Aggregate PNAs were compared (significant: c and d; borderline b, $p\text{-value} = 0.053$). The EA metrics for the Large and Small Aggregate PNAs were very similar, with only one borderline significant difference identified between the EA metrics (EA metric d, $p\text{-value} = 0.066$). We refute the null hypothesis that electron activities are independent of pore network architecture based on the clear differences noted in the comparison between the random (or native soil structure) and artificially created structures. To our surprise, variations in electron activity were not significantly different between the two artificially created PNAs, indicating that aggregate size is not likely to be the sole controlling factor in the dynamics of electron activities.

Table 4. Significant differences in Electron Activity metrics across Pore Network Architectures and Volumes of Interest, values are mean and (coefficients of variation) with n = 9

EA metric	Parameter	Unit	Pore network architecture (PNA)			Significant differences between PNAs		
			R	LA	SA	R : LA	R : SA	SA : LA
			$\bar{x}$ (CV)	$\bar{x}$ (CV)	$\bar{x}$ (CV)			
a	- slope	mV/hr	-10.4 (42)	-18.0 (18)	-14.5 (33)	**	---	---
b	- slope	mV/hr	-3.5 (66)	-24.8 (53)	-18.3 (96)	**	(p = 0.053)	---
c	min	mV	-48.0 (195)	-166.1 (17)	-156.6 (17)	***	**	---
d	time	hr	292.4 (26)	59.4 (22)	154.6 (82)	***	**	(p = 0.066)
e	elapsed time	hr	22.8 (51)	12.0 (154)	22.6 (98)	---	---	---
f	+ slope	mV/hr	4.2 (24)	2.3 (25)	3.5 (61)	*	---	---
g	max	mV	709.3 (16)	669.4 (23)	614.4 (21)	---	---	---
h	elapsed time	hr	480.8 (6)	437.1 (14)	440.2 (17)	---	---	---
i	- slope	mV/hr	-2.8 (76)	-0.5 (242)	-1.1 (93)	*	---	---
j	+ slope	mV/hr	2.3 (133)	0.2 (148)	0.6 (196)	(p = 0.073)	---	---

Tukey's honest significant difference test (n = 9 per pore network architecture) was used to determine significant differences between curve part means. P-values were adjusted for multiple comparisons. R = Random PNA, LA = Large aggregate PNA (≤ 1 cm) and SA = Small aggregate PNA (≤ 2 mm). Significance levels: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

3.4.4 Pore network metrics have differential power to explain electron activity metrics

The pore network architecture is traditionally parameterized by generating moisture release functions, a method that has been extensively used in the soil physics community. However, these functions are typically obtained using soil samples in the 3 to 10 cm size range and the resulting information provided on pore characteristics is an average across the entire sample (Kravchenko and Guber, 2017). For example, basic physical soil properties were not significant predictors of saturated hydraulic conductivity when compared to XCT derived macropore characteristics (Luo et al., 2010b).

The use of 3D X-ray computed tomography to quantify the structure of the soil pore network has become almost ubiquitous in recent years. Because of this, the associated parameters have become equally broad ranging. These parameters can be broken down in to two major categories: 1) parameters that describe the reaction space (i.e. the 3D pore space it self) and 2) parameters that describe the complexity of the pore network (i.e. the number of pore connections per unit volume).

Variables that belong to the first category are: total porosity (Kravchenko et al., 2015; Larsbo et al., 2016; Negassa et al., 2015; Paradelo et al., 2016; Rabbi et al., 2016; Toosi et al., 2017), pore size distribution (Kravchenko et al., 2015; Luo et al., 2010a; Peth et al., 2008; Toosi et al., 2017), pore surface area (Li et al., 2016; Naveed et al., 2016; Sammartino et al., 2015), pore thickness (i.e. diameter) (Jarvis et al., 2017; Larsbo et al., 2016; Naveed et al., 2016), and total pore volume (Dong and Blunt, 2009; Luo et al., 2010a; Perret et al., 1999).

Variables that fall in the second category rely largely on what is referred to as the skeleton, or single voxel width medial axes, of the pore network (Arganda-Carreras, 2014; Lee et al., 1994). Examples here include: tortuosity calculated as total pore length/Euclidean distance (Jassogne et al., 2007; Katuwal et al., 2015a; Luo et al., 2010a; Perret et al., 1999) or using the Euler number (Köhne et al., 2011; Larsbo et al., 2014; Peth et al., 2008), pore connectivity calculated using (i) Euler number (Rabot et al., 2015; Sammartino et al., 2015), or (ii) node density (Katuwal et al., 2015b; Luo et al., 2010a), and macropore length (Luo et al., 2010a; Perret et al., 1999).

Nine correlation matrices (three pore network architectures versus three volumes of interest, Figure 6) were constructed to explore the existence of linear correlations between electron activity metrics (EA, dependent variable) and pore network metrics (PNMs, predictor variable). In these matrices (Figure 6), the ability of each pore network metric (1.....18) to predict any of the electron activity metrics (a...j) is represented by either a plus (positive correlation) or a minus symbol (negative correlation). The existence of a significant correlation was assumed if the coefficient of determination (r^2) for the relationship between the respective PNM and a given EA metric ($n = 9$ for each combination of PNA and VoI) was ≥ 0.44 (f-value = 5.59, d.f. = 8).

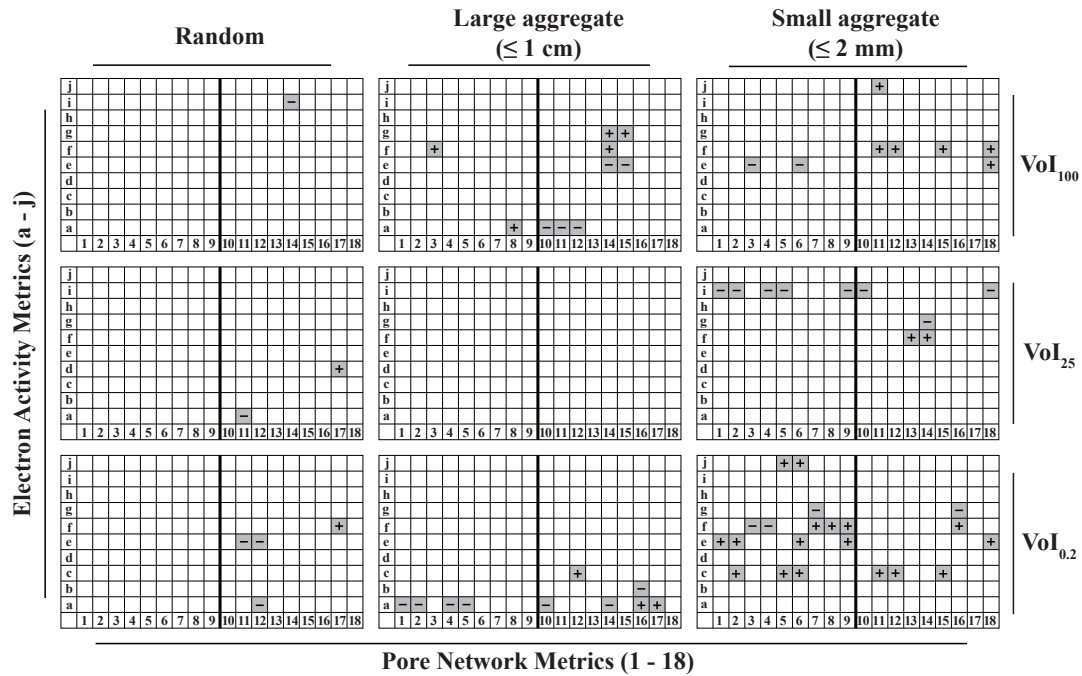


Figure 6. Significant correlations between the electron activity metrics (Figure 2; a – j; vertical axis) and pore network metrics (Figure 3, 1 – 18; horizontal axis) are shown for each treatment and volume of interest (VoI) ($p < 0.05$, d.f. = 8). Significant correlations are marked by a grey box; + indicates a positive relationship between the pore network metric and electron activity metric, and – indicates a negative relationship. The vertical black line in each panel indicates the transition from skeleton based metrics to void based metrics.

Examination of the entire cohort of nine correlation matrices allows the assessment of the ability of somewhat arbitrarily chosen metrics to quantify the relationship between soil structure and electron activity dynamics. Figure 7a compares the 'efficiency' of individual EA metrics as descriptor variables and shows that variables f, a, and e were much more often correlated with a pore network metric than any of the other EA metrics. Figure 7a also illustrates the abundance of positive versus negative correlations. For instance, the rate of E_{Pt} increase (i.e. electron activity decrease) after the first drainage (EA metric "f") was mainly positively correlated with pore network metrics (PNMs), while the subsequent decline in E_{Pt} (i.e. electron activity increase) following partial inundation (EA metric "i") was always negatively correlated with PNMs. A similar analysis can be conducted for the PNMs. Here we find that void-base metrics (Figure 7c) tend to appear more frequently in significant correlations with EA metrics than skeleton-based metrics (Figure 7b), with particularly high scores noted for PNMs 14 (seven instances), 11, and 12 (five and six instances respectively). Overall, void-based pore network metrics correlate on a total of 38 occasions compared to 28 total correlations observed for skeleton-based metrics (Figure 7b and c). Considering subtotals for the three different PNAs we find a trend of increasing number of correlations going from PNA "Random" with 7 total correlations, 20 correlations for the PNA "Large Aggregate", to 39 correlations in the PNA "Small Aggregate" (Figure 8a).

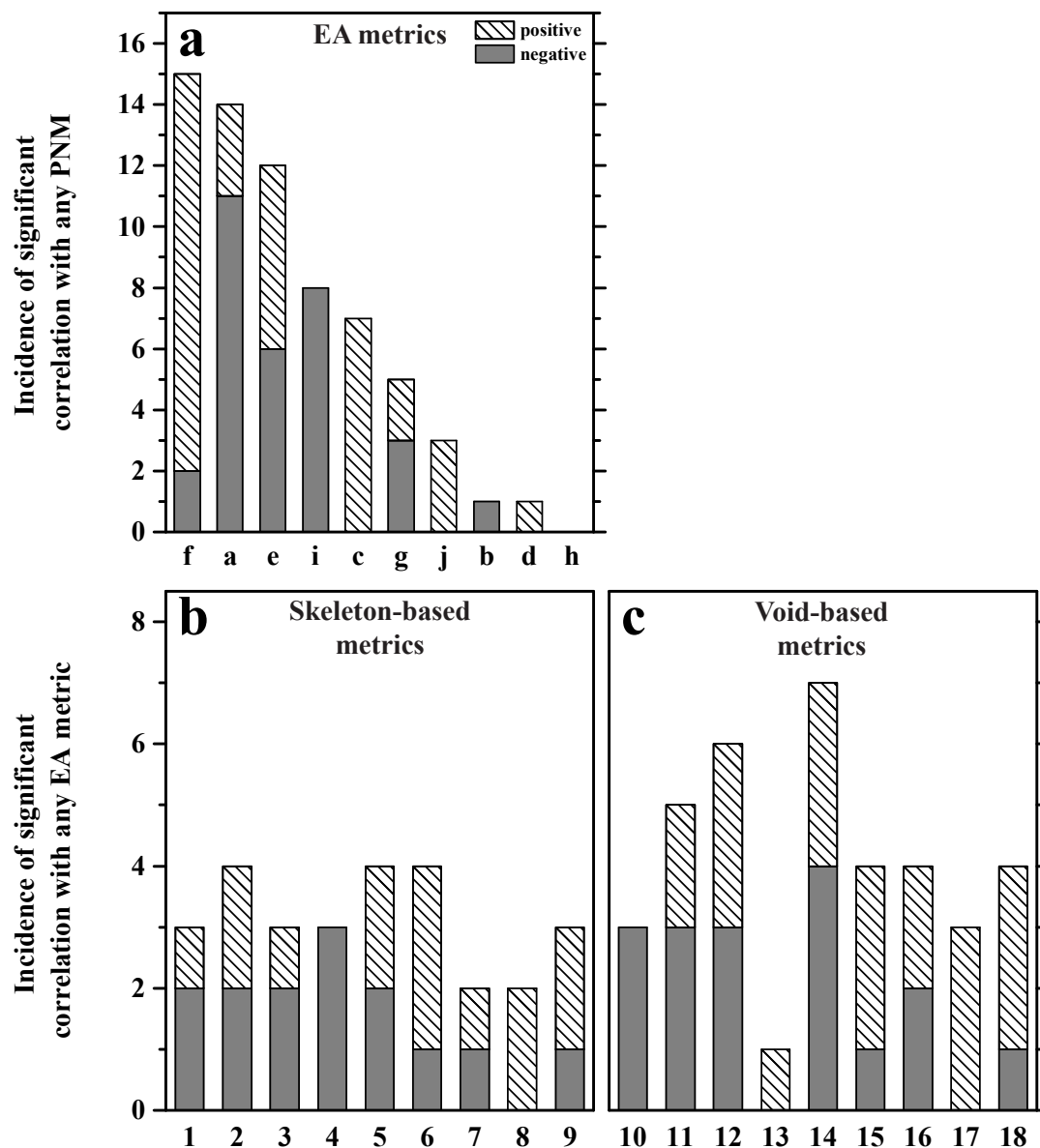


Figure 7. Frequency of correlation with electron activity metrics. Frequency of correlations with electron activity metrics as a function of pore network metrics (a), skeleton-based pore network metrics (b), and void-based pore network metrics (c). Positive correlations are diagonal-hatched, negative correlations in gray. PNA = pore network architecture, EA = electron activity.

We found that the void-based pore network metrics tended to have statistically stronger relationships with EA metrics, evidenced by way of a greater total number of significant correlations compared to the skeleton-based metrics.

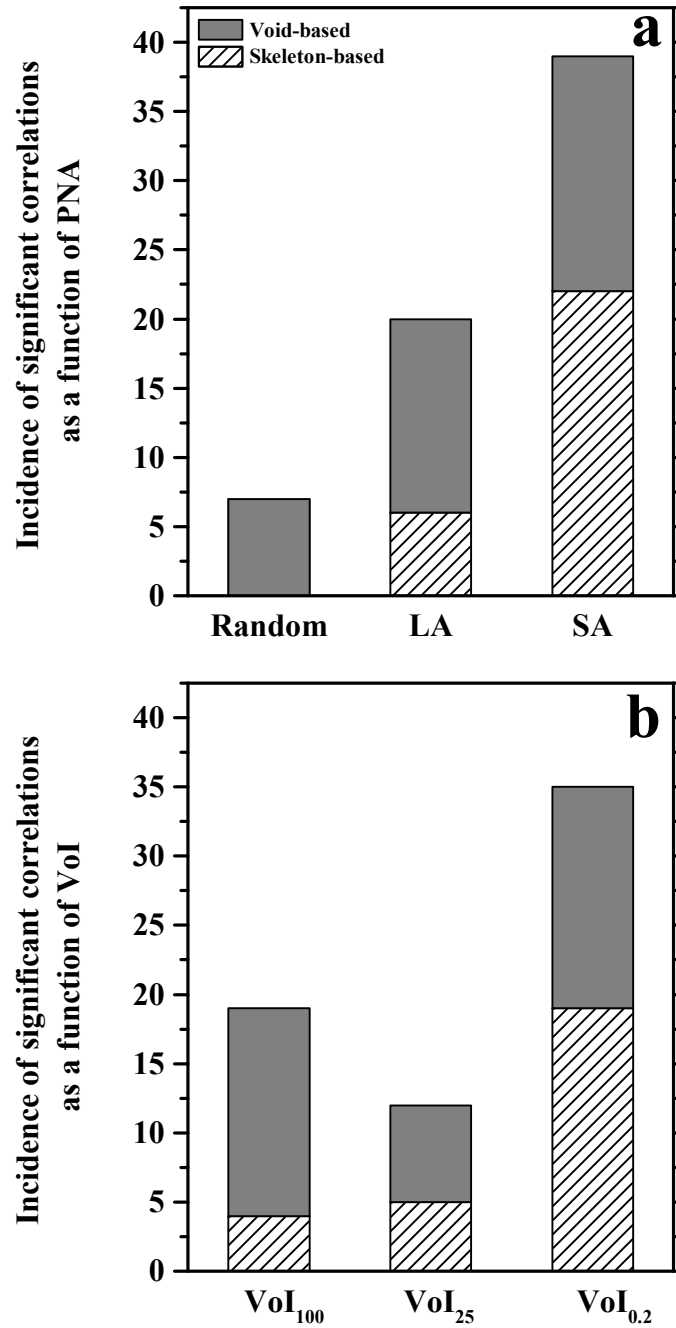


Figure 8. Incidence of correlations between electron activity metrics and pore network metrics as a function of pore network architecture (a), and volume of soil observed (i.e. Vol; b). Skeleton-based metrics are diagonal-hatched, void-based metrics are in gray. PNA = pore network architecture.

As the shape, size, or layout of the pore network changes, so must the relationship with electron activity, and subsequently the existence or lack of statistically significant correlations. We decided to investigate four categories of EA metrics, each representing different functionalities of the pore network: (i) Decreasing E_{Pt} (negative slopes) – represents water movement into the pore network, an increasing restriction on the resupply of atmospheric oxygen, and a facilitation of nutrient diffusion and advection; (ii) Extreme E_{Pt} values (maximums or minimums) – are seen as capacitive indicators, potentially useful to parameterize boundary conditions for the system studied. A soil not returning to fully aerobic state (high E_{Pt}) after rewetting may have a pore system with poor connectivity, while a soil unable to achieve high electron activities (low E_{Pt}) may lack electron donors such as reduced organic matter; (iii) Timing of minimum or maximum potentials – along with the pore characteristics necessary for point (ii) above, the timing of the extreme values depends on the rate at which water can flow in or out as well as the rate at which nutrients or gases can move through the pore network; (iv) Increasing E_{Pt} – requires that water flow out of the pore network in an unrestricted fashion, but more importantly the uninhibited reentry of oxygen in to the pore network.

The relationship between the void-based and skeleton-based metrics and the electron activity metrics is scale dependent. At larger scales void-based metrics are likely to be more important in regulating water infiltration, nutrient, and gas movement (e.g. increasing electron activity), while skeleton-based metrics are likely to be more important at smaller scales, influencing nutrient and gas diffusion, as well as water retention in the pores immediately the Pt-electrode (e.g. timing of extreme

values). Void-based metrics are more likely to capture the surface topology (or roughness) of the pores. As pore roughness increases, the ability (and likelihood) of pockets of water storage on the pore face it self increases, maintaining optimal air/water conditions (Linn and Doran, 1984) for longer periods of time (Kravchenko and Guber, 2017).

Though the void-based PNMs incurred a higher total number of correlations compared to the skeleton-based PNMs, the correlation of a void-based or skeleton-based PNM with an EA metric was always a function of VoI and PNA. In the “Small Aggregate” PNA, as the VoI decreased the number of significant correlations with skeleton-based metrics increased while they decreased in the void-based metrics. This suggests that the reactions occurring in immediate proximity to the Pt-electrode tip are more dependent on the connectivity of the pore network as opposed to the reaction space provided by the pores. The formation of anaerobic conditions (or ‘hotspots’) at scales beyond the immediate vicinity of the Pt-electrode tip is likely more dependent on advective movement of gases (e.g. oxygen) and solutes (e.g. nutrients and DOC) through macropores (Kuziyakov and Blagodatskaya, 2015) as opposed to diffusion-based transport, which has been thought to be the dominant mechanism in micropores (Keiluweit et al., 2016; Negassa et al., 2015). We summarize that our exploratory analysis of the relationship between pore network parameters and parameters across different pore network architectures and volumes of interest suggests the existence of a set of parameters with the potential to be particularly useful for the investigation of structure – functionality relationships in soils.

3.4.5 *The explanatory power of PNMs depends on pore network architecture*

This picture becomes more differentiated when correlations between PNMs and EA metrics are examined separately for each individual pore network architecture (PNA) and volume of interest (Figure 6 and Figure 8a and b). Within the native pore structure (aka PNA "Random") none of the skeleton-based pore network metrics correlate with any of the EA metrics (Figure 6). The void-based metrics show one, two, and four correlations going from VoI_{100} , to VoI_{25} , to $VoI_{0.2}$, respectively. Of the three PNAs and across all VoIs, the PNA "Random" had the fewest total number of correlations (7; Figure 8a).

The total number of correlations increased to 20 for the PNA "Large Aggregate", with 6 contributed by skeleton-based metrics and 14 involving void-based metrics (Figure 8a). In this PNA there was no trend with VoI: 10 correlations were found for the large VoI_{100} , zero for the intermediate VoI_{25} , and 10 for the small $VoI_{0.2}$ (Figure 6 and 8b). The correlations observed for the VoI_{100} were generally not the same as the ones observed for the $VoI_{0.2}$. There was one combination of metrics (EA "a" as a function of PNM "10") that showed a significant negative correlation in both the VoI_{100} and $VoI_{0.2}$ (Figure 6).

In the "Small Aggregate" pore network architecture (PNA), where the soil had been sieved to ≤ 2 mm, 39 correlations between EA metrics and PNMs were found. Within this PNA, correlations involving skeleton-based metrics increased from 2, to 5, to 15 with decreasing VoI size (Figure 6), while correlations involving void-based metrics remained nearly constant as the VoI changed. Considering both skeleton and void-based metrics together, we observed a progression from 8, to 10, to 21

correlations going down in VoI size from VoI₁₀₀, to VoI₂₅, to VoI_{0.2}, respectively (Figure 6). Out of 39 correlations observed for PNA "Small Aggregate", only 2 combinations occurred in more than one of the VoIs, and both involved electron activity metric "e": this metric correlated negatively with PNM 6 in VoI₁₀₀ and positively in VoI_{0.2}. It also correlated positively with PNM 18 in both, VoI₁₀₀ and VoI_{0.2}. We observed only one incidence of correlation occurring between the same parameter pair across pore network architectures: EA "c" was negatively correlated with PNM "12" in PNA "Large Aggregate" and in the PNA "Small Aggregate" (Figure 6).

The observed effects of manipulating the pore network varied as a function of VoI. We assumed that the pore network would become more homogeneous in terms of both void and skeleton-based metrics as aggregate size decreased (reflected as smaller coefficients of variation). While this trend was somewhat evident in VoI_{0.2} (coefficient of variation is similar across all PNMs, Table A.5), most of the pore network metrics from the "Large Aggregate" PNA tended to be more variable than those from the "Random" or "Small Aggregate" pore network architectures in the two larger VoIs (coefficients of variation in Tables A.3 and A.4). What we ended up doing by sieving the soil to ≤ 1 cm for the "Large Aggregate" PNA was to effectively increase the pore network heterogeneity per unit volume. However, following Nimmo and Perkins (2008), the average void volume (i.e. macroporosity) did decrease as aggregate size decreased in the 100 ml and 25 ml VoIs, but stayed statistically the same in the 0.2 ml VoI (Tables A.3, A.4, and A.5)

An outcome of manipulating the pore network architecture (PNA) and varying the scale of observation were contrasting results in terms of the types of relationships between EA metrics and PNMs. An example of VoI changing the relationship was in the “Small Aggregate” PNA. EA metric “e” (Figure 2) correlated negatively with PNM 6 (number of junctions with four branches; Figure 4 and Table 1) in VoI₁₀₀ and positively in VoI_{0.2} (Figure 6). In VoI₁₀₀ as the number of junctions with four branches increased, the lag time from the first drain to an E_{Pt} increase of at least 50 mV became shorter. Because the 100 ml VoI was connected to the surface, as the number of pore connections from the electrode tip to the soil surface increased, oxygen was able to reach the electrode more quickly (Kawamoto et al., 2006; Moldrup et al., 2001; Tuli and Hopmans, 2004) thus decreasing the time required for the electromotive potential to change. In VoI_{0.2} the opposite occurred, as the number of quadruple points increased, the lag time also increased. A large number of connections implies that there are many branches/pores in the immediate vicinity of the Pt-electrode. As the number of branches/pores increases the pore volume able to be occupied with water increases, it would then take longer for the pore network to drain and oxygen to reach the electrode tip (Tracy et al., 2015).

An example of the type of a change in relationship between EA metrics as a function of PNA occurred in the “Random” and “Large Aggregate” PNAs and pore network metric “12” (Figure 5 and Table 1; average enclosed void volume). In the same VoI (VoI_{0.2}) PNM “12” correlated negatively with EA metric “a” (Figure 2; rate of electron activity increase over the first 72 hours after inundation) in the “Random” PNA, while the same PNM correlated positively with EA metric “c”

(Figure 2; maximum electron activity during pore network inundation) in the “Large Aggregate” PNA. In the “Random” PNA, as the enclosed void volume increased, the rate at which electron activity increased over the first 72 hours after flooding became increasingly negative (the rate of change became larger). In the “Large Aggregate” PNA, as the enclosed void volume increased, the maximum electron activity decreased (E_{Pt} became less negative). These results suggest that the relationship between pore network metrics and electron activity dynamics is a function of aggregate size, and by extension the pore network architecture of the system as a whole. Therefore the establishment and scaling of such a relationship must take in to account the pore network architecture across a sufficiently sized representative elementary volume so as to capture the majority of aggregate sizes.

3.4.6 Utility of electron activity and pore network metrics

Not all electron activity or pore network metrics were equally useful in describing the electron activity dynamics over the course of this work. Of the 66 total correlations between EA metrics and PNMs, Figure 7a shows that three out of the ten EA metrics accounted for over half of those correlations (41 in total). Those EA metrics (defined in Figure 2) were: “a” (rate of increase in electron activity during the first 72 hours after inundation), “e” (time elapsed from first draining to the inflection point where the electron activity decreased by at least 50 mV from the potential at draining), and “f” (rate of decrease in electron activity from the maximum value during inundation to the minimum value occurring after first draining).

Similarly, there were certain pore network metrics (described in Table 1 and Figure 5) that correlated more often with EA metrics than others. The top three skeleton-based metrics (Figure 7b) were all concerning the number of pore junctions: “2” (total number of junctions), “5” (the number of junctions with exactly three branches), and “6” (the number of junctions with exactly four branches). The top three void-based metrics (Figure 7c) were: “11” (void surface area), “12” (enclosed void volume), and “14” (the standard deviation of the mean pore diameter).

These results indicate that specific portions of electron activity dynamics are likely more closely regulated by the pore network compared to others. Similarly the pore network metrics that were most often correlated with changes in electron activity were those that have been directly tied to gas (Katuwal et al., 2015a; Katuwal et al., 2015b) and water movement (Larsbo et al., 2014; Sammartino et al., 2015) through the pore network.

3.4.7 The explanatory power of PNMs is greatest for a small soil volume immediately surrounding the electrode tip

Size and placement of the soil volume observed were found to have a profound influence on the ability of PNM's to statistically explain variations in electron activity metrics (Figure 8b). In Vol₁₀₀, pore network metrics were mostly correlated with EA parameters "f" (6 incidences, all positive) and "e" (5 incidences, 4 negative, 1 positive). Out of 19 total correlations observed for this volume, only one occurred in the PNA "Random" (Figure 6). In Vol₂₅, 7 PNM's correlated with EA metric "i", all of these correlations occurred in PNA "Small Aggregate" and all of

them were negative. More than half (35 out of 66 total) of all observed correlations occurred in VoI_{0.2}; with EA variable "a" showing 9 correlations while variables "c", "e" and "f" had 7 correlations each. Correlations with EA variable "a" occurred overwhelmingly in PNA "Large Aggregate", while correlations with variables "c", "e" and "f" mostly occurred in the PNA "Small Aggregate". This finding can be seen as supportive of the hypothesis that the Pt-electrode is most sensitive to the electrochemical conditions immediately surrounding the platinum wire tip, as proposed by Fiedler (1999).

EA metric "i" is the rate at which electron activity increases during the second, partial fill, with water. Water movement to the electrode tip during this portion of the experiment is dependent entirely on capillary rise. It stands to reason then that the portion of the pore network that is most related to these dynamics would be the portion of the pore network immediately below the Pt tip. This assessment partially breaks down on one other occasion in the "Random" PNA where EA metric "i" significantly correlates (again in a negative fashion) with PNM 14 (SD of mean pore diameter), but this time in VoI₁₀₀. Though the 100 ml VoI does not quantify the pores directly responsible for capillary movement of water, it does capture the pores surrounding the electrode tip. These pores will still be responsible for movement of water from the 'water table' (water level during the second, partial inundation) to the electrode tip.

Significant differences in EA metrics between pore network architectures do not always mean that there will be significant correlations between those EA metrics and pore network metrics. For example, EA metrics "c" and "d" (the time elapsed at

which the maximum electron activity occurs) were significantly different between the “Random” and “Large Aggregate” ($p < 0.001$), and “Random” and “Small Aggregate” ($p < 0.01$) pore network architectures Table 4). But EA metric “d” only had one total significant correlation across all VoIs and pore network architectures (Random VoI₂₅, PNM = 16) where EA metric “c” had a total of seven: “Large Aggregate” VoI_{0.2}, PNM = 12 (positive) and “Small Aggregate” VoI_{0.2}, PNMs = 2, 5, 6, 11, 12, 15 (all positive) (Figure 6).

EA metric “b” only had one significant correlation in all VoIs and pore network architectures (VoI_{0.2}, PNA = “Large Aggregate”, PNM = 16) (Figure 6). The lack of correlations could be explained by the fact that we completely flooded the pore networks, so the electromotive potential was left no choice but to decrease, and the pore network had little to no opportunity to influence the decrease. If this were the explanation, then there should have been no significant difference in rate of electromotive potential decline. However, EA metric “b” was significantly different between the “Random” and “Large Aggregate” pore network architectures ($p < 0.05$) and nearly significant between the “Random” and “Small Aggregate” pore network architectures ($p = 0.053$; Table 4).

We measured the strongest statistical relationship between electron activity and the pore network when examining the pores immediately surrounding the Pt-electrode. One possible explanation for the increased number of significant correlations between EA metrics and PNMs in the smallest VoI and PNA with the smallest pores is: As aggregate size decreased, large biopores, with smooth, potentially hydrophobic surfaces (Bachmann et al., 2008) were increasingly

destroyed. This lead to redistributed (i.e. more easily accessible) electron sources (Negassa et al., 2015) and the generation of rough pore surfaces (Ananyeva et al., 2013; Kravchenko et al., 2015; San José Martínez et al., 2015). Rough pore surfaces have been shown to collect small pools of water, provide more attachment points and habitable niches for microbes (Massol-Deya et al., 1995; Or et al., 2007; Vandevivere and Baveye, 1992), along with increased diffusion of nutrients in the pore water (Or and Tuller, 2000). This result is also inline with the assessment made by Fiedler (1999) that the Pt-electrode is most sensitive to the soil volumes and biogeochemical processes that are in immediate, direct contact.

We summarize that the mechanisms that regulate gas and nutrient transport (which ultimately control electron activity) are different across scales. At larger scales water, gases, and nutrients are dominantly moved by advection, while at smaller scales diffusive movement dominates. The PNMs that more tightly regulate diffusion (e.g. pore tortuosity or the number/class of pore connections) will correlate at the small VoI, while the PNMs that are more involved in advective movement (e.g. SD of mean pore diameter or void surface area) will correlate more often at larger VoIs, or in the pore network architectures with larger aggregates/pores.

3.5 Conclusions

To the best of our knowledge, there has so far not been a report of an attempt to investigate variations in electron activity or redox status of soil environments with the help of metrics from three-dimensional imaging methods such as computed tomography. Similarly, we are not aware of investigations that would have manipulated soil structure with the intent to explore resulting effects on the dynamics

of soil redox status. We were able to demonstrate that the pore network and the dynamics of electron activities in soils are indeed correlated, but we did not expect the extent to which these correlations depended on both, (i) the type of pore network architecture and (ii) the soil volume chosen for imaging analysis. We recognize an overall trend of increasing predictability as both, aggregate sizes and volumes of interest get smaller, but there were exceptions to the trend that prevent us from making generalizing statements at this time. But our work showed: the fact that a given CT-derived pore network metric does not have much predictive power in a certain type of structural environment and at a certain scale of observation does not necessarily mean that this would be the same in a different environment and at another scale of observation. Our findings suggest that future investigations of physiological processes in porous soil and subsurface systems should involve preliminary activities to determine the scale of observation (volume of interest) and the associated kind of pore network metric best suited to generate the answer sought.

3.6 Acknowledgments

This work was supported by the US Department of Energy, Office of Biological and Environmental Research, Terrestrial Ecosystem Program (Award Number DE-FG02-13ER65542). We were also supported through instrument time grants provided by the Environmental Molecular Sciences Laboratory (EMSL), a user facility at the Pacific Northwest National Laboratory at Richland, WA through proposals ,48704 (Kleber); 48750 (Kleber) and 48910 (Fendorf).

4. Summary and Conclusions

This thesis highlighted a robust method for the quantification of biogeochemical heterogeneity, measured as electron activity, in a non-hydric soil and the existence of a mechanistic relationship between electron activity and soil structure.

The first chapter showed that Pt-based electrodes can return robust, quantitative information concerning the division of three non-hydric Mollisols in to biogeochemically distinct microsites. The utilization of a fixed grid of Pt-electrodes also allowed for the establishment of a metric, termed the Metabolic Heterogeneity Index, capable of quantifying the degree to which a soil was divided in to biogeochemically distinct microsites on seasonal time scales. These soils contained microenvironments that included conditions of (i) unrestricted aerobiosis immediately adjacent to (ii) conditions allowing for sulfate reduction or methane production. The variation in electron activities was mainly a function of changes in precipitation patterns as opposed to seasonal fluctuations in temperature. Interestingly, variability in the measured electron activities was insensitive to precipitation events until the water filled pore space exceeded 60%. Finally, saturation of the pore network does not immediately equate the existence of reducing conditions.

The second chapter demonstrated that the pore network and the dynamics of electron activities in soils are indeed correlated. A linear relationship between electron activity and pore network architecture of a soil as measured using Pt-based electrodes and x-ray computed tomography (XCT) was established. The statistical strength of the relationship between the pore network architecture and electron

activity is a function of (i) aggregate size and (ii) the volume of interest investigated using XCT. As aggregate size decreased the linear relationship between the electron activity and pore network architecture became stronger.

Taken together, the results of this thesis allowed the following insights to be drawn. (i) The writing of models to describe the formation of biogeochemical heterogeneity at cm to m scales could be made more robust by including void-based metrics to parameterize the pore network. With the impetus on more accurately representing the reaction space the pore network provides (e.g. pore volume and pore surface area) and the shape of the pores overall (e.g. variability of pore diameter). (ii) Changes in precipitation patterns or sub-surface water flow should be weighted more heavily compared to shifts in temperature. (iii) The influence of thick films on rough pore surfaces should likely be accounted for when considering gas and solute (re)distribution and impacts on biogeochemical heterogeneity, especially in soils that have undergone recent disturbance or destruction of carbon-coated biopores.

5. Bibliography

- Abramowicz, D., 1990. Aerobic and Anaerobic Biodegradation of PCBs: A Review. *Critical Reviews in Biotechnology* 10(3), 241-251.
- Ananyeva, K., Wang, W., Smucker, A.J.M., Rivers, M.L., Kravchenko, A.N., 2013. Can intra-aggregate pore structures affect the aggregate's effectiveness in protecting carbon? *Soil Biology and Biochemistry* 57, 868-875.
- Arganda-Carreras, I., 2014. ImageJ software Plug-in, Skeletonize, 3D
- Arganda-Carreras, I., Fernandez-Gonzalez, R., Munoz-Barrutia, A., Ortiz-De-Solorzano, C., 2010. 3D reconstruction of histological sections: Application to mammary gland tissue. *Microsc Res Tech* 73(11), 1019-1029.
- Arndt, S., Jørgensen, B.B., LaRowe, D.E., Middelburg, J.J., Pancost, R.D., Regnier, P., 2013. Quantifying the degradation of organic matter in marine sediments: A review and synthesis. *Earth-Science Reviews* 123, 53-86.
- Austin, W.E., Huddleston, J.H., 1999. Viability of permanently installed platinum redox electrodes. *Soil Science Society of America Journal* 63(6), 1757-1762.
- Baas Becking, L.G., Kaplan, I.R., Moore, D., 1960. Limits of the Natural Environment in terms of pH and Oxidation-Reduction potentials. *The Journal of Geology* 68(3), 243-288.
- Bachmann, J., Guggenberger, G., Baumgartl, T., Ellerbrock, R.H., Urbanek, E., Goebel, M.-O., Kaiser, K., Horn, R., Fischer, W.R., 2008. Physical carbon-sequestration mechanisms under special consideration of soil wettability. *Journal of Plant Nutrition and Soil Science* 171(1), 14-26.
- Ball, B.C., 2013. Soil structure and greenhouse gas emissions: a synthesis of 20 years of experimentation. *European Journal of Soil Science* 64(3), 357-373.
- Balster, C.A., Parsons, R.B., 1968. *Geomorphology and Soils Willamette Valley*, Oregon, Oregon State University.
- Bartlett, R.J., 1998. Characterizing soil redox behavior. In: D.L. Sparks (Ed.), *Soil physical chemistry*. CRC Press, Boca Raton, FL., pp. 371-397.
- Bartlett, R.J., James, B.R., 1995. System for Categorizing Soil Redox Status by Chemical Field Testing. *Geoderma* 68(3), 211-218.
- Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 47(2), 151-163.
- Bedard, D.L., 2008. A case study for microbial biodegradation: anaerobic bacterial reductive dechlorination of polychlorinated biphenyls-from sediment to defined medium. *Annu Rev Microbiol* 62, 253-270.
- Boersma, L., Watts, D.G., Simonson, G.H., 1972. Soil morphology and water table relations. 1. Annual water table fluctuations. *Soil Science Society of America Proceedings* (36), 644-648.
- Bohn, H.L., 1971. Redox Potentials. *Soil Science* 112(1), 39 - 45.
- Borja, J., Taleon, D.M., Auresenia, J., Gallardo, S., 2005. Polychlorinated biphenyls and their biodegradation. *Process Biochemistry* 40(6), 1999-2013.
- Chacon, N., Silver, W.L., Dubinsky, E.A., Cusack, D.F., 2006. Iron Reduction and Soil Phosphorus Solubilization in Humid Tropical Forests Soils: The Roles of Labile Carbon Pools and an Electron Shuttle Compound. *Biogeochemistry* 78(1), 67-84.

- Chadwick, O.A., Chorover, J., 2001. The chemistry of pedogenic thresholds. *Geoderma* 100(3-4), 321-353.
- Chapelle, F.H., Haack, S.K., Adriaens, P., Henry, M.A., Bradley, P.M., 1996. Comparison of E(h) and H-2 measurements for delineating redox processes in a contaminated aquifer. *Environmental science & technology* 30(12), 3565-3569.
- Christ, J.A., Ramsburg, C.A., Abriola, L.M., Pennell, K.D., Löffler, F.E., 2004. Coupling Aggressive Mass Removal with Microbial Reductive Dechlorination for Remediation of DNAPL Source Zones: A Review and Assessment. *Environmental Health Perspectives* 113(4), 465-477.
- Cogger, C.G., Kennedy, P.E., Carlson, D., 1992. Seasonally Saturated Soils in the Puget Lowland II. Measuring and Interpreting Redox Potentials. *Soil Science* 154(1), 50-58.
- Cussler, E.L., 1997. *Diffusion: Mass Transfer in Fluid Systems*. 2nd ed. Cambridge University Press, New York.
- Davidson, E.A., Samanta, S., Caramori, S.S., Savage, K., 2012. The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. *Global change biology* 18(1), 371-384.
- Dong, H., Blunt, M.J., 2009. Pore-network extraction from micro-computerized-tomography images. *Phys Rev E Stat Nonlin Soft Matter Phys* 80(3 Pt 2), 036307.
- Dorau, K., Mansfeldt, T., 2016. Comparison of redox potential dynamics in a diked marsh soil: 1990 to 1993 versus 2011 to 2014. *Journal of Plant Nutrition and Soil Science* 179(5), 641-651.
- Doube, M., Klosowski, M.M., Arganda-Carreras, I., Cordelieres, F.P., Dougherty, R.P., Jackson, J.S., Schmid, B., Hutchinson, J.R., Shefelbine, S.J., 2010. BoneJ: Free and extensible bone image analysis in ImageJ. *Bone* 47(6), 1076-1079.
- Dwire, K.A., Kauffman, J.B., Baham, J.E., 2006. Plant species distribution in relation to water-table depth and soil redox potential in Montane riparian meadows. *Wetlands* 26(1), 131-146.
- Falconer, R.E., Battaia, G., Schmidt, S., Baveye, P., Chenu, C., Otten, W., 2015. Microscale heterogeneity explains experimental variability and non-linearity in soil organic matter mineralisation. *PloS one* 10(5), e0123774.
- Faulkner, S.P., Patrick, W.H., 1992. Redox Processes and Diagnostic Wetland Soil Indicators In Bottomland Hardwood Forests. *Soil Science Society of America Journal* 56, 856-865.
- Faulkner, S.P., Patrick, W.H., Gambrell, R.P., 1989. Field Techniques for Measuring Wetland Soil Parameters. *Soil Science Society of America Journal* 55, 883-890.
- Fendorf, S., Michael, H.A., van Geen, A., 2010. Spatial and temporal variations of groundwater arsenic in South and Southeast Asia. *Science* 328(5982), 1123-1127.
- Fiedler, S., 1999. In-situ longterm measurements of redox potential in hydromorphic soils, in *Redox Fundamentals*. In: J. Schüring, H.D. Schulz, W.R. Fischer, J.

- Böttcher, W.H.M. Duijnisveld (Eds.), Redox Fundamentals, Processes and Measuring Techniques. Springer-Verla, New York, pp. 81-94.
- Fiedler, S., Kalbitz, K., 2003. Concentrations and Properties of Dissolved Organic Matter in Forest Soils as Affected by the Redox Regime. *Soil Science* 168(11), 793-801.
- Fiedler, S., Sommer, M., 2000. Methane emissions, groundwater levels and redox potentials of common wetland soils in a temperate-humid climate. *Global Biogeochemical Cycles* 14(4), 1081-1093.
- Fiedler, S., Sommer, M., 2004. Water and redox conditions in wetland soils - Their influence on pedogenic oxides and morphology. *Soil Science Society of America Journal* 68(1), 326-335.
- Fiedler, S., Vepraskas, M.J., Richardson, J.L., 2007. Soil Redox Potential: Importance, Field Measurements, and Observations. 94, 1-54.
- Gomez, E., Durillon, C., Rofes, G., Picot, B., 1999. Phosphate adsorption and release from sediments of brackish lagoons: pH, O₂ and loading influence. *Water Research* 33(10), 2437-2447.
- Grable, A.R., Siemer, E.G., 1968. Effects of bulk density, aggregate size, and soil water suction on oxygen diffusion, redox potentials, and elongation of corn roots. *Soil Science Society of America Journal* 32(2), 180-186.
- Groffman, P.M., Butterbach-Bahl, K., Fulweiler, R.W., Gold, A.J., Morse, J.L., Stander, E.K., Tague, C., Tonitto, C., Vidon, P., 2009. Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* 93(1-2), 49-77.
- Hall, S.J., Liptzin, D., Buss, H.L., DeAngelis, K., Silver, W.L., 2016. Drivers and patterns of iron redox cycling from surface to bedrock in a deep tropical forest soil: a new conceptual model. *Biogeochemistry* 130(1-2), 177-190.
- Hansel, C.M., Fendorf, S., Jardine, P.M., Francis, C.A., 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. *Applied and environmental microbiology* 74(5), 1620-1633.
- Helliwell, J.R., Miller, A.J., Whalley, W.R., Mooney, S.J., Sturrock, C.J., 2014. Quantifying the impact of microbes on soil structural development and behaviour in wet soils. *Soil Biology and Biochemistry* 74, 138-147.
- Horn, R., Smucker, A., 2005. Structure formation and its consequences for gas and water transport in unsaturated arable and forest soils. *Soil and Tillage Research* 82(1), 5-14.
- Husson, O., 2012. Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: a transdisciplinary overview pointing to integrative opportunities for agronomy. *Plant and Soil* 362(1-2), 389-417.
- Husson, O., Husson, B., Brunet, A., Babre, D., Alary, K., Sarthou, J.P., Charpentier, H., Durand, M., Benada, J., Henry, M., 2016. Practical improvements in soil redox potential (Eh) measurement for characterisation of soil properties. Application for comparison of conventional and conservation agriculture cropping systems. *Anal Chim Acta* 906, 98-109.

- James, B.R., Brose, D.A., 2011. Oxidation Reduciont Phenomena. In: Y.L. Pan Ming Huang , and Malcolm E. Sumner (Ed.), Handbook of Soil Sciences: Properties and Processes. CRC Press, pp. 1 - 24.
- Jarvis, N., Larsbo, M., Koestel, J., 2017. Connectivity and percolation of structural pore networks in a cultivated silt loam soil quantified by X-ray tomography. *Geoderma* 287, 71-79.
- Jassogne, L., McNeill, A., Chittleborough, D., 2007. 3D-visualization and analysis of macro- and meso-porosity of the upper horizons of a sodic, texture-contrast soil. *European Journal of Soil Science* 58(3), 589-598.
- Jobbagy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10(2), 423-436.
- Jones, R.H., 1966. Oxidation-Reduction Potential Measurement. *Isa Journal* 13(11), 40-&.
- Katuwal, S., Arthur, E., Tuller, M., Moldrup, P., de Jonge, L.W., 2015a. Quantification of Soil Pore Network Complexity with X-ray Computed Tomography and Gas Transport Measurements. *Soil Science Society of America Journal* 79(6), 1577.
- Katuwal, S., Norgaard, T., Moldrup, P., Lamandé, M., Wildenschild, D., de Jonge, L.W., 2015b. Linking air and water transport in intact soils to macropore characteristics inferred from X-ray computed tomography. *Geoderma* 237-238, 9-20.
- Kawamoto, K., Moldrup, P., Schjønning, P., Iversen, B.V., Komatsu, T., Rolston, D.E., 2006. Gas Transport Parameters in the Vadose Zone: Development and Tests of Power-Law Models for Air Permeability. *Vadose Zone Journal* 5(4), 1205.
- Keiluweit, M., Nico, P.S., Kleber, M., Fendorf, S., 2016. Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? *Biogeochemistry* 127(2-3), 157-171.
- Keiluweit, M., Wanzek, T., Kleber, M., Nico, P.S., Fendorf, S., 2017. Anaerobic Microsites have an Unaccounted Role in Soil Carbon Stabilization. *Nature Communications* (in press).
- Keller, T., Colombi, T., Ruiz, S., Manalili, M.P., Rek, J., Stadelmann, V., Wunderli, H., Breitenstein, D., Reiser, R., Oberholzer, H., Schymanski, S., Romero-Ruiz, A., Linde, N., Weisskopf, P., Walter, A., Or, D., 2017. Long-Term Soil Structure Observatory for Monitoring Post-Compaction Evolution of Soil Structure. *Vadose Zone Journal* 16(4), 0.
- Kleber, M., 2010. What is recalcitrant soil organic matter? *Environmental Chemistry* 7(4), 320.
- Kleber, M., Nico, P.S., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2011. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. *Global change biology* 17(2), 1097-1107.
- Kofod, M., 1999. Variance of the Redox Potential Value in Two Anoxic Groundwater Systems. In: J. Schüring, H.D. Schulz, W.R. Fischer, J. Böttcher, W.H.M.

- Duijnisveld (Eds.), Redox: Fundamentals, Processes and Applications. Springer-Verlag, pp. 120-134.
- Köhne, J.M., Schlüter, S., Vogel, H.-J., 2011. Predicting Solute Transport in Structured Soil Using Pore Network Models. *Vadose Zone Journal* 10(3), 1082.
- Koven, C.D., Riley, W.J., Subin, Z.M., Tang, J.Y., Torn, M.S., Collins, W.D., Bonan, G.B., Lawrence, D.M., Swenson, S.C., 2013. The effect of vertically-resolved soil biogeochemistry and alternate soil C and N models on C dynamics of CLM4. *Biogeosciences Discussions* 10(4), 7201-7256.
- Kravchenko, A.N., Guber, A.K., 2017. Soil pores and their contributions to soil carbon processes. *Geoderma* 287, 31-39.
- Kravchenko, A.N., Negassa, W.C., Guber, A.K., Hildebrandt, B., Marsh, T.L., Rivers, M.L., 2014. Intra-aggregate Pore Structure Influences Phylogenetic Composition of Bacterial Community in Macroaggregates. *Soil Science Society of America Journal* 78(6), 1924.
- Kravchenko, A.N., Negassa, W.C., Guber, A.K., Rivers, M.L., 2015. Protection of soil carbon within macro-aggregates depends on intra-aggregate pore characteristics. *Sci Rep* 5, 16261.
- Kuka, K., Franko, U., Rühlmann, J., 2007. Modelling the impact of pore space distribution on carbon turnover. *Ecological Modelling* 208(2-4), 295-306.
- Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments--a review. *Waste Manag* 28(1), 215-225.
- Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review. *Soil Biology and Biochemistry* 83, 184-199.
- Larsbo, M., Koestel, J., Jarvis, N., 2014. Relations between macropore network characteristics and the degree of preferential solute transport. *Hydrology and Earth System Sciences* 18(12), 5255-5269.
- Larsbo, M., Koestel, J., Kätterer, T., Jarvis, N., 2016. Preferential Transport in Macropores is Reduced by Soil Organic Carbon. *Vadose Zone Journal* 15(9), 0.
- Lee, T.C., Kashyap, R.L., Chu, C.N., 1994. Building Skeleton Models Via 3-D Medial Surface Axis Thinning Algorithms. *Cvgip-Graphical Models and Image Processing* 56(6), 462-478.
- Li, T.C., Shao, M.A., Jia, Y.H., 2016. Application of X-ray tomography to quantify macropore characteristics of loess soil under two perennial plants. *European Journal of Soil Science* 67(3), 266-275.
- Linn, D.M., Doran, J.W., 1984. Effect of Water-Filled Pore-Space on Carbon-Dioxide and Nitrous-Oxide Production in Tilled and Nontilled Soils. *Soil Science Society of America Journal* 48(6), 1267-1272.
- Luo, L., Lin, H., Halleck, P., 2008. Quantifying Soil Structure and Preferential Flow in Intact Soil Using X-ray Computed Tomography. *Soil Science Society of America Journal* 72(4), 1058.
- Luo, L., Lin, H., Li, S., 2010a. Quantification of 3-D soil macropore networks in different soil types and land uses using computed tomography. *Journal of Hydrology* 393(1-2), 53-64.

- Luo, L., Lin, H., Schmidt, J., 2010b. Quantitative Relationships between Soil Macropore Characteristics and Preferential Flow and Transport. *Soil Science Society of America Journal* 74(6), 1929.
- Mansfeldt, T., 2003. In situ long-term redox potential measurements in a dyked marsh soil. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 166(2), 210-219.
- Mansfeldt, T., 2004. Redox potential of bulk soil and soil solution concentration of nitrate, manganese, iron, and sulfate in two Gleysols. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 167(1), 7-16.
- Masscheleyn, P.H., Delaune, R.D., Patrick, W.H., 1990. Transformations of Selenium as Affected by Sediment Oxidation Reduction Potential and Ph. *Environmental science & technology* 24(1), 91-96.
- Massol-Deya, A.A., Whallon, J., Hickey, R.F., Tiedje, J.M., 1995. Channel structures in aerobic biofilms of fixed-film reactors treating contaminated groundwater. *Applied and environmental microbiology* 61(2), 769-777.
- McClain, M.E., Boyer, E.W., Dent, C.L., Gergel, S.E., Grimm, N.B., Groffman, P.M., Hart, S.C., Harvey, J.W., Johnston, C.A., Mayorga, E., McDowell, W.H., Pinay, G., 2003. Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems. *Ecosystems* 6(4), 301-312.
- McKeague, J.A., 1965. Relationship of water table and Eh to Properties of three clay soils in the Ottawa Valley. *Canadian Journal of Soil Science* 45, 49-62.
- Megonigal, J.P., Patrick, W.H., Faulkner, S.P., 1993. Wetland Identification in Seasonally Flooded Forest Soils - Soil Morphology and Redox Dynamics. *Soil Science Society of America Journal* 57(1), 140-149.
- Menne, M.J., Durre, I., Vose, R.S., Gleason, B.E., Houston, T.G., 2012. An Overview of the Global Historical Climatology Network-Daily Database. *Journal of Atmospheric and Oceanic Technology* 29(7), 897-910.
- Moldrup, P., Olesen, T., Komatsu, T., Schønning, P., Rolston, D.E., 2001. Tortuosity, Diffusivity, and Permeability in the Soil Liquid and Gaseous Phases. *Soil Science Society of America Journal* 65, 613-623.
- Naveed, M., Moldrup, P., Arthur, E., Wildenschild, D., Eden, M., Lamandé, M., Vogel, H.-J., de Jonge, L.W., 2013. Revealing Soil Structure and Functional Macroporosity along a Clay Gradient Using X-ray Computed Tomography. *Soil Science Society of America Journal* 77(2), 403.
- Naveed, M., Moldrup, P., Schaap, M.G., Tuller, M., Kulkarni, R., Vogel, H.-J., Wollesen de Jonge, L., 2016. Prediction of biopore- and matrix-dominated flow from X-ray CT-derived macropore network characteristics. *Hydrology and Earth System Sciences* 20(10), 4017-4030.
- Negassa, W.C., Guber, A.K., Kravchenko, A.N., Marsh, T.L., Hildebrandt, B., Rivers, M.L., 2015. Properties of soil pore space regulate pathways of plant residue decomposition and community structure of associated bacteria. *PloS one* 10(4), e0123999.
- Nimmo, J.R., Perkins, K.S., 2008. Effect of soil disturbance on recharging fluxes: case study on the Snake River Plain, Idaho National Laboratory, USA. *Hydrogeology Journal* 16(5), 829-844.

- Nordstrom, D.K., Wilde, F.D., 2005. Chapter A6 Field Measurements: Section 6.5 Reduction- Oxidation Potential (Electrode Method), NATIONAL Field Manual for the Collection of Water- Quality Data. U.S. Geological Survey Office, Reston, VA
- Nunan, N., 2017. The microbial habitat in soil: Scale, heterogeneity and functional consequences. *Journal of Plant Nutrition and Soil Science* 180(4), 425-429.
- Or, D., Smets, B.F., Wraith, J.M., Dechesne, A., Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. *Advances in Water Resources* 30(6-7), 1505-1527.
- Or, D., Tuller, M., 2000. Flow in unsaturated fractured porous media: Hydraulic conductivity of rough surfaces. *Water Resources Research* 36(5), 1165-1177.
- Paradelo, M., Katuwal, S., Moldrup, P., Norgaard, T., Herath, L., de Jonge, L.W., 2016. X-ray CT-Derived Soil Characteristics Explain Varying Air, Water, and Solute Transport Properties across a Loamy Field. *Vadose Zone Journal* 15(4), 0.
- Parton, W.J., Hartman, M., Ojima, D., Schimel, D., 1998. DAYCENT and its land surface submodel: description and testing. *Global and Planetary Change* 19(1-4), 35-48.
- Patrick, W.H., Gambrell, R.P., Faulkner, S.P., 1996. Redox Measurements of Soils. In: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner (Eds.), *Methods of Soil Analysis. Part 3. Chemical Methods*. Soil Science Society of America Inc., Madison, USA, pp. 1255 - 1273.
- Peiffer, S., 1992. Redox measurements in aqueous solutions - A theoretical approach to data interpretation, based on electrode kinetics. *Journal of Contaminant Hydrology* 10(1), 1-18.
- Peiffer, S., 1999. Characterisation of the Redox State of Aqueous Systems: Towards a Problem-Oriented Approach. In: J. Schüring, H.D. Schulz, W.R. Fischer, J. Böttcher, W.H.M. Duijnvisveld (Eds.), *Redox Fundamentals, Processes and Measuring Techniques*. Springer-Verla, New York, pp. 24-39.
- Perret, J., Prasher, S.O., Kantzas, A., Langford, C., 1999. Three-Dimensional Quantification of Macropore Networks in Undisturbed Soil Cores. *Soil Science Society of America Journal* 63(6), 1530.
- Peth, S., Horn, R., Beckmann, F., Donath, T., Fischer, J., Smucker, A.J.M., 2008. Three-Dimensional Quantification of Intra-Aggregate Pore-Space Features using Synchrotron-Radiation-Based Microtomography. *Soil Science Society of America Journal* 72(4), 897.
- Pierret, A., Capowiez, Y., Belzunces, L., Moran, C.J., 2002. 3D reconstruction and quantification of macropores using X-ray computed tomography and image analysis. *Geoderma* 106(3-4), 247-271.
- Polizzotto, M.L., Kocar, B.D., Benner, S.G., Sampson, M., Fendorf, S., 2008. Near-surface wetland sediments as a source of arsenic release to ground water in Asia. *Nature* 454(7203), 505-508.
- Quispel, A., 1947. Measurement of the oxidation-reduction potentials of normal and inundated soils. *Soil Science* 101, 265-275.

- Rabbi, S.M., Daniel, H., Lockwood, P.V., Macdonald, C., Pereg, L., Tighe, M., Wilson, B.R., Young, I.M., 2016. Physical soil architectural traits are functionally linked to carbon decomposition and bacterial diversity. *Sci Rep* 6, 33012.
- Rabenhorst, M.C., 2009. Making Soil Oxidation–Reduction Potential Measurements using Multimeters. *Soil Science Society of America Journal* 73(6), 2198.
- Rabot, E., Lacoste, M., Hénault, C., Cousin, I., 2015. Using X-ray Computed Tomography to Describe the Dynamics of Nitrous Oxide Emissions during Soil Drying. *Vadose Zone Journal* 14(8), 0.
- Rappoldt, C., Crawford, J.W., 1999. The distribution of anoxic volume in a fractal model of soil. *Geoderma* 88(3-4), 329-347.
- Riley, W.J., Maggi, F., Kleber, M., Torn, M.S., Tang, J.Y., Dwivedi, D., Guerry, N., 2014. Long residence times of rapidly decomposable soil organic matter: application of a multi-phase, multi-component, and vertically resolved model (BAMS1) to soil carbon dynamics. *Geoscientific Model Development* 7(4), 1335-1355.
- Riley, W.J., Subin, Z.M., Lawrence, D.M., Swenson, S.C., Torn, M.S., Meng, L., Mahowald, N.M., Hess, P., 2011. Barriers to predicting changes in global terrestrial methane fluxes: analyses using CLM4Me, a methane biogeochemistry model integrated in CESM. *Biogeosciences* 8(7), 1925-1953.
- RStudio, T., 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA
- Ruamps, L.S., Nunan, N., Chenu, C., 2011. Microbial biogeography at the soil pore scale. *Soil Biology and Biochemistry* 43(2), 280-286.
- Ruamps, L.S., Nunan, N., Pouteau, V., Leloup, J., Raynaud, X., Roy, V., Chenu, C., 2013. Regulation of soil organic C mineralisation at the pore scale. *FEMS Microbiol Ecol* 86(1), 26-35.
- Sammartino, S., Lissy, A.-S., Bogner, C., Van Den Bogaert, R., Capowicz, Y., Ruy, S., Cornu, S., 2015. Identifying the Functional Macropore Network Related to Preferential Flow in Structured Soils. *Vadose Zone Journal* 14(10), 0.
- San José Martínez, F., Muñoz Ortega, F.J., Caniego Monreal, F.J., Kravchenko, A.N., Wang, W., 2015. Soil aggregate geometry: Measurements and morphology. *Geoderma* 237-238, 36-48.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9(7), 676-682.
- Schoeneberger, P.J., Wysocki, D.A., Benham, E.C., Staff, S.S., 2012. Field book for describing and sampling soils, Version 3.0. In: N.R.C. Service (Ed.). National Soil Survey Center, Lincoln, NE.
- Sexstone, A.J., Revsbech, N.P., Parkin, T.B., Tiedje, J.M., 1985. Direct Measurement of Oxygen Profiles and Denitrification Rates in Soil Aggregates. *Soil Science Society of America Journal* 49(3), 645-651.
- Seybold, C.A., Mersie, W., Huang, J.Y., McNamee, C., 2002. Soil redox, pH, temperature, and water-table patterns of a freshwater tidal wetland. *Wetlands* 22(1), 149-158.

- Sigg, L., 1999. Redox Potential Measurements in Natural Waters: Significance: Concepts and Problems. In: J. Schüring, H.D. Schulz, W.R. Fischer, J. Böttcher, W.H.M. Duijnsveld (Eds.), Redox Fundamentals, Processes and Measuring Techniques. Springer-Verlag, New York, pp. 1-11.
- Sposito, G., 2016. The Chemistry of Soils. 3rd ed. Oxford University Press, New York.
- Staff, S.S., 2014. Kellogg Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report No. 42. In: R. Burt, S.S. Staff (Eds.). U.S. Department of Agriculture, Natural Resources Conservation Service.
- Staff, S.S., 2015. Examination and Description of Soils. In: C. Ditzler, L.T. West (Eds.), Soil Survey Manual. Natrual Resources Conservation Service, U.S. Department of Agriculture.
- Stumm, W., 1966. Redox Potential as an Environmental Parameter - Conceptual Significance and Operational Limitation. Journal Water Pollution Control Federation 38(3p1), 333-&.
- Taina, I.A., Heck, R.J., Elliot, T.R., 2008. Application of X-ray computed tomography to soil science: A literature review. Canadian Journal of Soil Science 88(1), 1-20.
- Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochemical Cycles 23(2), n/a-n/a.
- Thomas, C.R., Miao, S., Sindhoj, E., 2009. Environmental factors affecting temporal and spatial patterns of soil redox potential in Florida Everglades wetlands. Wetlands 29(4), 1133-1145.
- Tippkötter, R., Eickhorst, T., Taubner, H., Gredner, B., Rademaker, G., 2009. Detection of soil water in macropores of undisturbed soil using microfocus X-ray tube computerized tomography (μ CT). Soil and Tillage Research 105(1), 12-20.
- Toosi, E.R., Kravchenko, A.N., Mao, J., Quigley, M.Y., Rivers, M.L., 2017. Effects of management and pore characteristics on organic matter composition of macroaggregates: evidence from characterization of organic matter and imaging. European Journal of Soil Science 68(2), 200-211.
- Tracy, S.R., Daly, K.R., Sturrock, C.J., Crout, N.M.J., Mooney, S.J., Roose, T., 2015. Three-dimensional quantification of soil hydraulic properties using X-ray Computed Tomography and image-based modeling. Water Resources Research 51(2), 1006-1022.
- Tuli, A., Hopmans, J.W., 2004. Effect of degree of fluid saturation on transport coefficients in disturbed soils. European Journal of Soil Science 55(1), 147-164.
- Vandevivere, P., Baveye, P.C., 1992. Saturated hydraulic conductivity reduction caused by aerobic bacteria in sand columns. Soil Science Society of America Journal 56(1), 1-12.
- Vepraskas, M.J., Faulkner, S.P., 2001. Morphological features of seasonally reduced soils. Wetland Soils—Genesis, Hydrology, Landscapes and Classification. Lewis Publishers, Boca Raton, FL.

- Vepraskas, M.J., Wilding, L.P., 1983. Aquic Moisture Regimes in Soils with and without Low Chroma Colors. *Soil Science Society of America Journal* 47(2), 280-285.
- Vereecken, H., Schnepf, A., Hopmans, J.W., Javaux, M., Or, D., Roose, D.O.T., Vanderborght, J., Young, M.H., Amelung, W., Aitkenhead, M., Allison, S.D., Assouline, S., Baveye, P., Berli, M., Bruggemann, N., Finke, P., Flury, M., Gaiser, T., Govers, G., Ghezzehei, T., Hallett, P., Franssen, H.J.H., Heppell, J., Horn, R., Huisman, J.A., Jacques, D., Jonard, F., Kollet, S., Lafolie, F., Lamorski, K., Leitner, D., McBratney, A., Minasny, B., Montzka, C., Nowak, W., Pachepsky, Y., Padarian, J., Romano, N., Roth, K., Rothfuss, Y., Rowe, E.C., Schwen, A., Simunek, J., Tiktak, A., Van Dam, J., van der Zee, S.E.A.T.M., Vogel, H.J., Vrugt, J.A., Wohling, T., Young, I.M., 2016. Modeling Soil Processes: Review, Key Challenges, and New Perspectives. *Vadose Zone Journal* 15(5), 0.
- Vogel, L.E., Makowski, D., Garnier, P., Vieublé-Gonod, L., Coquet, Y., Raynaud, X., Nunan, N., Chenu, C., Falconer, R., Pot, V., 2015. Modeling the effect of soil meso- and macropores topology on the biodegradation of a soluble carbon substrate. *Advances in Water Resources* 83, 123-136.
- von Fischer, J.C., Butters, G., Duchateau, P.C., Thelwell, R.J., Siller, R., 2009. In situ measures of methanotroph activity in upland soils: A reaction-diffusion model and field observation of water stress. *Journal of Geophysical Research* 114(G1).
- Wang, Z.P., DeLaune, R.D., Patrick, W.H., Masscheleyn, P.H., 1993. Soil Redox and pH Effects on Methane Production in a Flooded Rice Soil. *Soil Science Society of America Journal* 57(2), 382.
- Wania, R., Ross, I., Prentice, I.C., 2009. Integrating peatlands and permafrost into a dynamic global vegetation model: 2. Evaluation and sensitivity of vegetation and carbon cycle processes. *Global Biogeochemical Cycles* 23(3).
- Wieder, W.R., Allison, S.D., Davidson, E.A., Georgiou, K., Hararuk, O., He, Y.J., Hopkins, F., Luo, Y.Q., Smith, M.J., Sulman, B., Todd-Brown, K., Wang, Y.P., Xia, J.Y., Xu, X.F., 2015. Explicitly representing soil microbial processes in Earth system models. *Global Biogeochemical Cycles* 29(10), 1782-1800.
- Wieder, W.R., Bonan, G.B., Allison, S.D., 2013. Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change* 3(10), 909-912.
- Wildenschild, D., Hopmans, J.W., Vaz, C.M.P., Rivers, M.L., Rikard, D., Christensen, B.S.B., 2002. Using X-ray computed tomography in hydrology: systems, resolutions, and limitations. *Journal of Hydrology* 267(3-4), 285-297.
- Yang, J., Hu, Y., Bu, R., 2006. Microscale Spatial Variability of Redox Potential in Surface Soil. *Soil Science* 171(10), 747-753.
- Young, I.M., Crawford, J.W., 2004. Interactions and self-organization in the soil-microbe complex. *Science* 304(5677), 1634-1637.
- Yu, K., Böhme, F., Rinklebe, J., Neue, H.-U., DeLaune, R.D., 2007. Major Biogeochemical Processes in Soils-A Microcosm Incubation from Reducing

- to Oxidizing Conditions. *Soil Science Society of America Journal* 71(4), 1406.
- Yu, K., Faulkner, S.P., Patrick, W.H., Jr., 2006. Redox potential characterization and soil greenhouse gas concentration across a hydrological gradient in a Gulf coast forest. *Chemosphere* 62(6), 905-914.
- Zheng, Y., Stute, M., van Geen, A., Gavrieli, I., Dhar, R., Simpson, H.J., Schlosser, P., Ahmed, K.M., 2004. Redox control of arsenic mobilization in Bangladesh groundwater. *Applied Geochemistry* 19(2), 201-214.
- Zimmermann, I., Fleige, H., Horn, R., 2016. Soil structure amelioration with quicklime and irrigation experiments in earth graves. *Journal of Soils and Sediments* 16(11), 2514-2522.

6. Appendices

6.1 Appendix A (Supplementary Information for Chapter One)

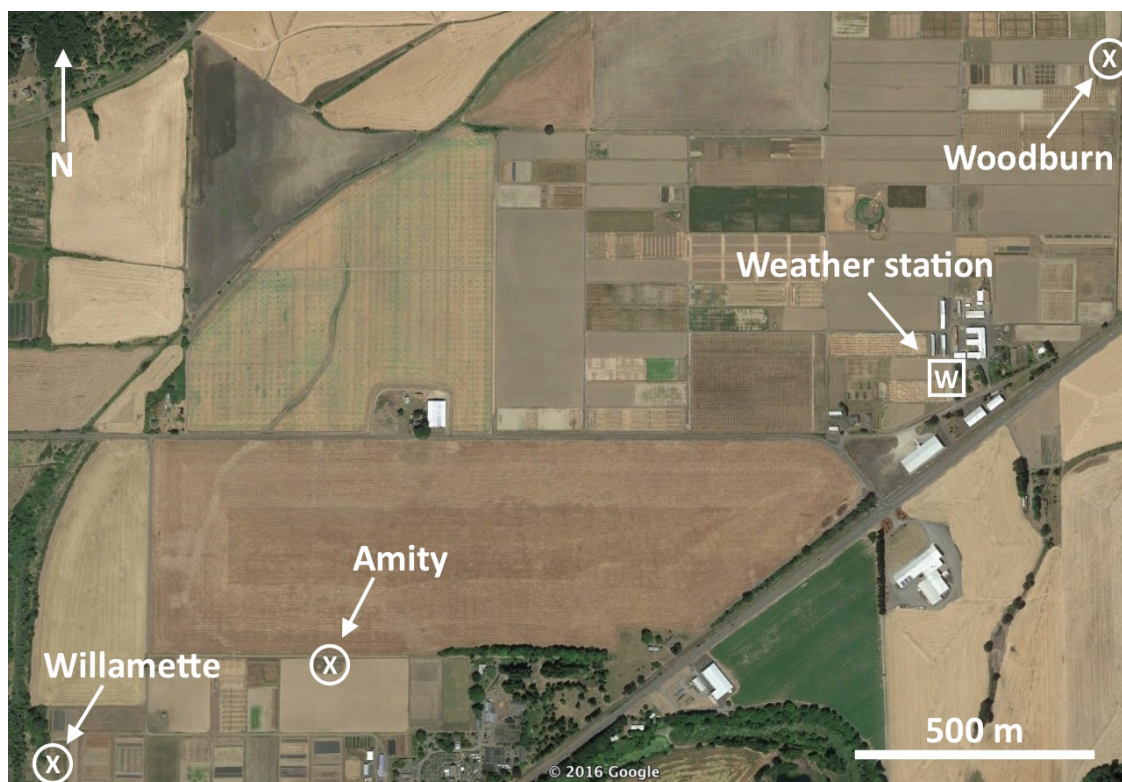


Figure A.1. Willamette, Woodburn and Amity sites and position of weather station at the Hyslop Crop Science Field Research Laboratory, 3455 NE Granger Rd., Corvallis, OR 97330. Oregon Agricultural Experiment Station and College of Agricultural Sciences, Oregon State University.

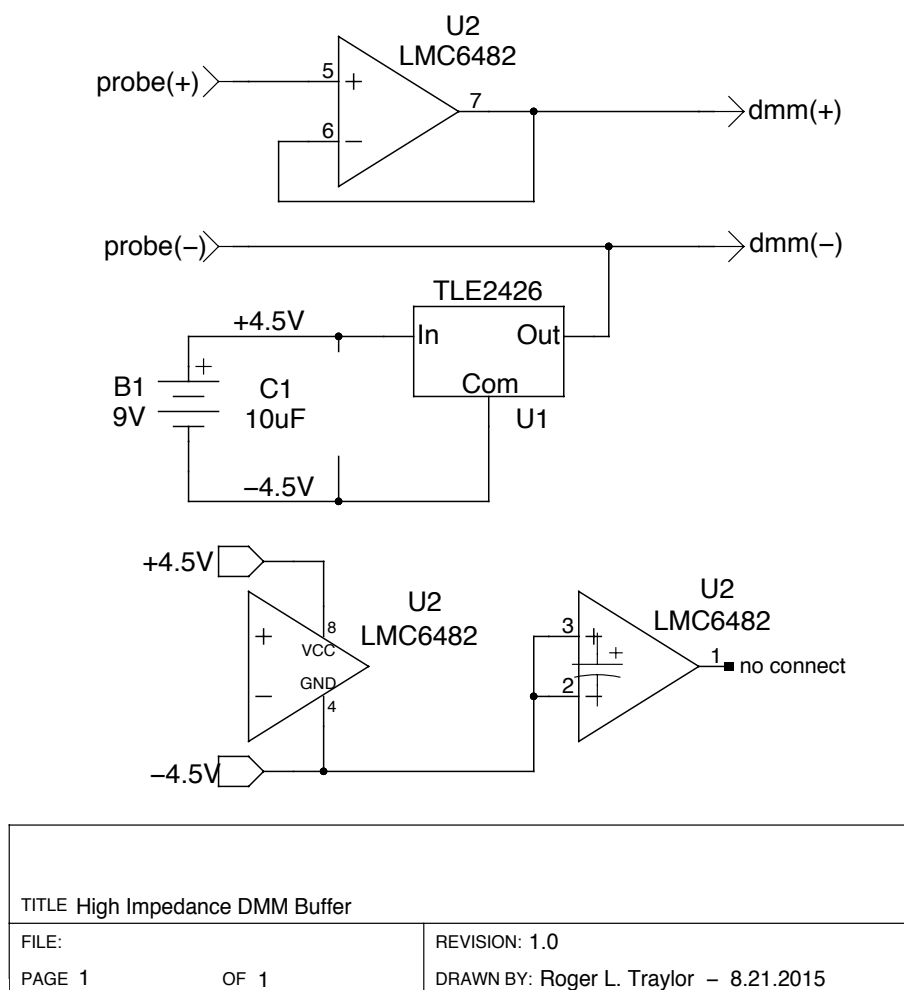


Figure A.2. Schematic for the resistance amplifier used to increase the input resistance of a hand held digital multimeter (DMM). The effective input resistance was increased from 10^6 ohms to $>10^{13}$ ohms using a TLE2426 precision virtual ground operational amplifier from Texas Instruments and a single 9V battery. The amplifier was packaged in a watertight enclosure and designed to plug directly in to the positive and negative ports of the DMM. The corresponding polarity leads would then connect directly to the amplifier.

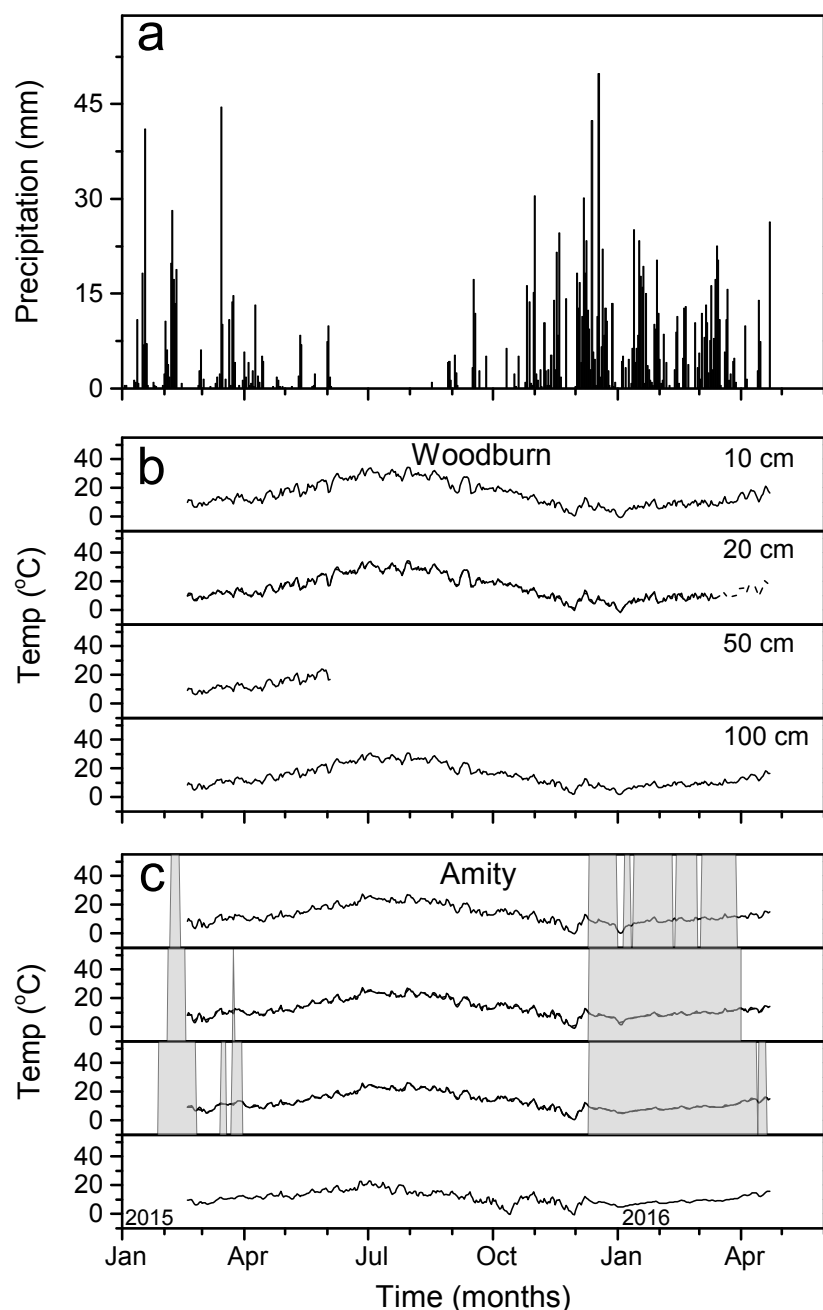


Figure A.3. Time series of precipitation (panel a); and soil temperature per depth for Woodburn (panel b) and Amity (panel b) soils. Shaded area indicates full water saturation with electrodes submerged. Soil temperature (b and c) was measured using Decagon 5TE electrodes at 10, 20, 50, and 100 cm depths in the Woodburn and Amity soils ($n=1$, with the exception of Woodburn 10, Woodburn 20, Amity 20 and Amity 50, where $n=2$). Data from replicate electrodes are indicated by a solid (probe 1) and dashed (probe 2) lines. Depths labeled in the Woodburn panel are the same for the Amity panel. Temperature data were recorded every six hours and then the average temperature per day was calculated and shown here.

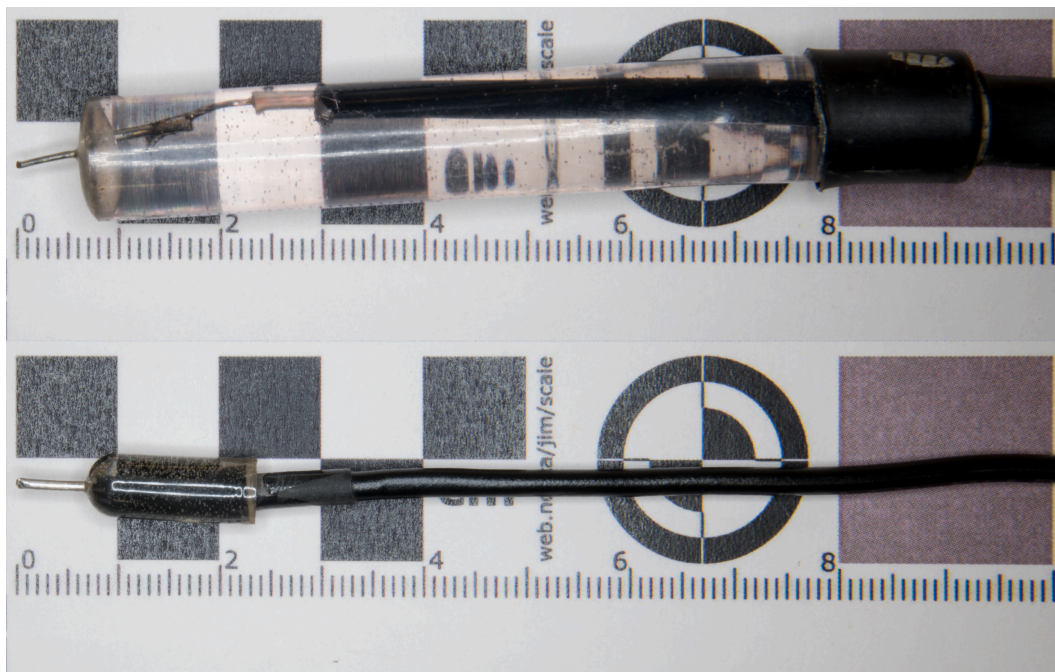


Figure A.4. Size comparison between Pt-electrodes used in Amity and Woodburn soils. The Fiedler model is at the top and was installed at the Woodburn and Amity soils. The miniaturized Baham/Wanzek model is at the bottom and was deployed at the Willamette site. The larger electrodes were roughly 2 cm in diameter and 10 cm in length (Pt tip to end of epoxy body). Contrastingly the smaller electrodes were roughly 1 cm in diameter and 2 cm in length (Pt tip to end of epoxy body).

Table A.1. Redox couples, associated reduction half – reactions, and specific redox potentials (Eh) for pH = 7; [red] = [ox] = 10^{-4} M, 0.21 atm for O₂, 0.78 atm for N₂ and 0.00032 atm for CO₂.

Couple	Reaction	Eh (mV)
O ₂ /H ₂ O	$\frac{1}{4} \text{O}_2 + \text{e}^- = \frac{1}{2} \text{H}_2\text{O}$	805
NO ₃ ⁻ /N ₂	$\frac{1}{5} \text{NO}_3^- + \text{e}^- + \frac{6}{5} \text{H}^+ = \frac{1}{10} \text{N}_2 + \frac{3}{5} \text{H}_2\text{O}$	704
Mn ⁴⁺ /Mn ²⁺	$\frac{1}{2} \text{MnO}_2 + \text{e}^- + 2\text{H}^+ = \frac{1}{2} \text{Mn}^{2+} + \text{H}_2\text{O}$	521
SO ₄ ²⁻ /H ₂ S	$\frac{1}{8} \text{SO}_4^{2-} + \text{e}^- + \frac{5}{4} \text{H}^+ = \frac{1}{8} \text{H}_2\text{S} + \frac{1}{2} \text{H}_2\text{O}$	-207
Fe ³⁺ /Fe ²⁺	$\frac{1}{2} \text{Fe}_2\text{O}_3 + \text{e}^- + 3\text{H}^+ = \text{Fe}^{2+} + \frac{3}{2} \text{H}_2\text{O}$	-213
Fe ³⁺ /Fe ²⁺	$\text{FeOOH} + \text{e}^- + 3\text{H}^+ = \text{Fe}^{2+} + 2\text{H}_2\text{O}$	-236
CO ₂ /CH ₄	$\frac{1}{8} \text{CO}_2 + \text{e}^- + \text{H}^+ = \frac{1}{8} \text{CH}_4 + \frac{1}{8} \text{CO}_2$	-243

Table A.2. Soil descriptions, profile information and site locations.

Drainage class ^a	Series	Classification ^a	Texture ^b	Layer (cm)	Porosity (%)	Pore volume (L m ⁻²) ^c	Mean pH ± SD ^d	Db (g/cm ³) ^e	Site location
Well drained	Willamette	Fine-silty, mixed, superactive, mesic Pacific Ultic Argixeroll	Silt loam	0 - 10	52	52	5.59 ± 0.4	1.3	44° 37' 31" N 123° 13' 06" W
				10 - 20	52	52	5.84 ± 0.4	1.3	
				20 - 50	47	141	6.08 ± 0.3	1.4	
				50 - 100	51	255	6.26 ± 0.2	1.3	
						Σ 500			
Moderately well drained	Woodburn	Fine-silty, mixed, superactive, mesic Aquific Argixeroll	Silt loam	0 - 10	51	51	5.47 ± 0.4	1.3	44° 38' 16" N 123° 11' 27" W
				10 - 20	45	45	5.79 ± 0.2	1.5	
				20 - 50	45	135	6.02 ± 0.1	1.5	
				50 - 100	50	250	5.97 ± 0.3	1.3	
						Σ 481			
Poorly drained	Amity	Fine-silty, mixed, superactive, mesic Argiaquic Xeric Argialboll	Silt loam	0 - 10	55	55	5.47 ± 0.3	1.2	44° 37' 38" N 123° 12' 36" W
				10 - 20	49	49	5.68 ± 0.2	1.4	
				20 - 50	50	150	6.08 ± 0.1	1.5	
				50 - 100	54	270	6.53 ± 0.2	1.2	
						Σ 524			

^aDrainage class according to Schoeneberger et al. (2012)^bSoil classification and texture follow the US Taxonomy data (Soil Survey Staff, 2015).^cPore volume was calculated by multiplying the % porosity and the thickness of each layer.^dMean pH $\pm$ the standard deviation of n=5 sampling events for the Woodburn and Amity and n=4 for the Willamette at each depth from January 2015 through May 2015 (1 sample per depth per month).^efield estimates

Table A.3. Monthly precipitation totals for December 2014 through April 2016^a, four-month totals for each wet season (December through April), and mean daily precipitation by month are provided to illustrate differences in precipitation amounts between wet seasons.

Year	Month	Σ (mm)	Daily Mean (mm/day)
2014	December	200.7	6.5
2015	January	93.5	3.0
	February	130.2	4.7
	March	114.4	3.7
	April	39.9	1.3
<i>Σ Dec – Apr</i>		<i>578.7</i>	
	May	28.1	0.9
	June	12.0	0.4
	July	0.0	0.0
	August	10.7	0.3
	September	48.7	1.6
	October	92.1	3.0
	November	117.3	3.9
2015	December	354.9	11.4
2016	January	226.6	7.3
	February	93.0	3.2
	March	200.1	6.5
	April	81.0	2.7
<i>Σ Dec – Apr</i>		<i>955.6</i>	

^a Daily precipitation amounts were summed for each month. Precipitation data collected daily from a National Oceanic and Atmospheric Administration Global Historical Climatology Network weather station on Hyslop Research Farm, Corvallis, Oregon.

6.2 Appendix B (Supplementary Information for Chapter Two)

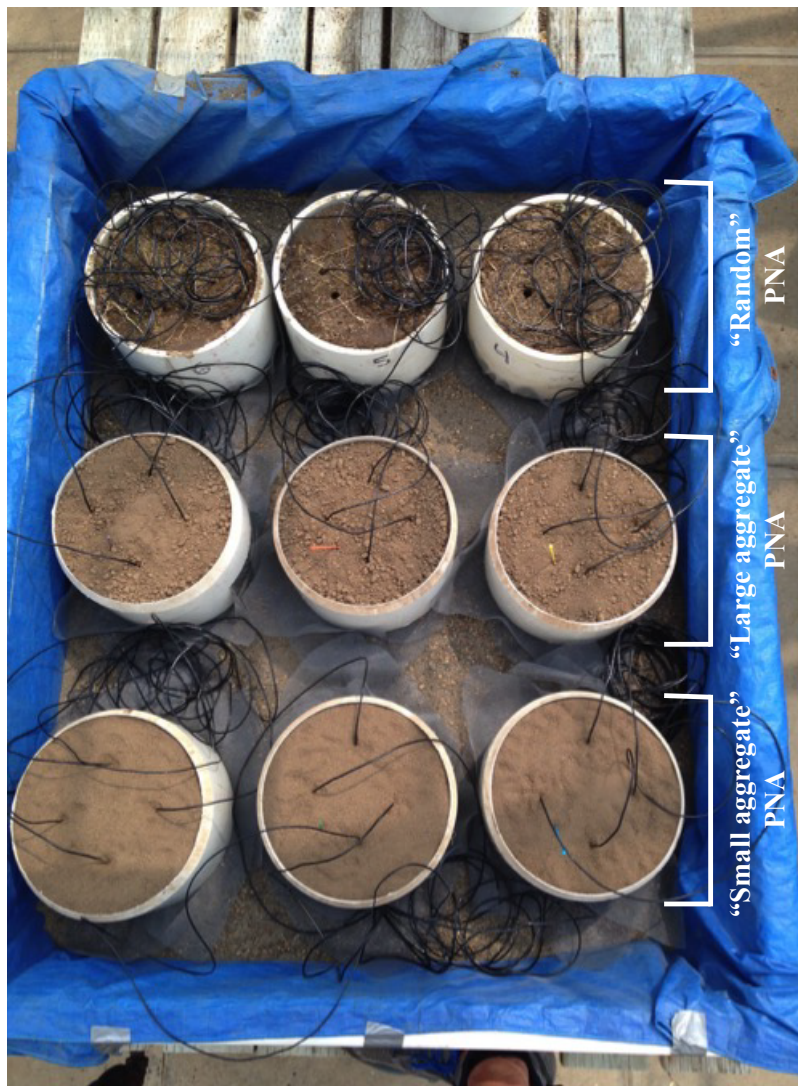


Figure B1. Experimental layout of PVC rings. All nine PVC rings were in the same fiberglass tub for the duration of the experiment. Each pore network architecture occupies one row: “Random” (top row), “Large aggregate” (middle), and “Small aggregate” (bottom). The tub was lined with a polyurethane tarp (blue) and filled with ~ 2 cm of coarse, autoclaved sand to allow for water infiltration into and exfiltration out of the soil samples.

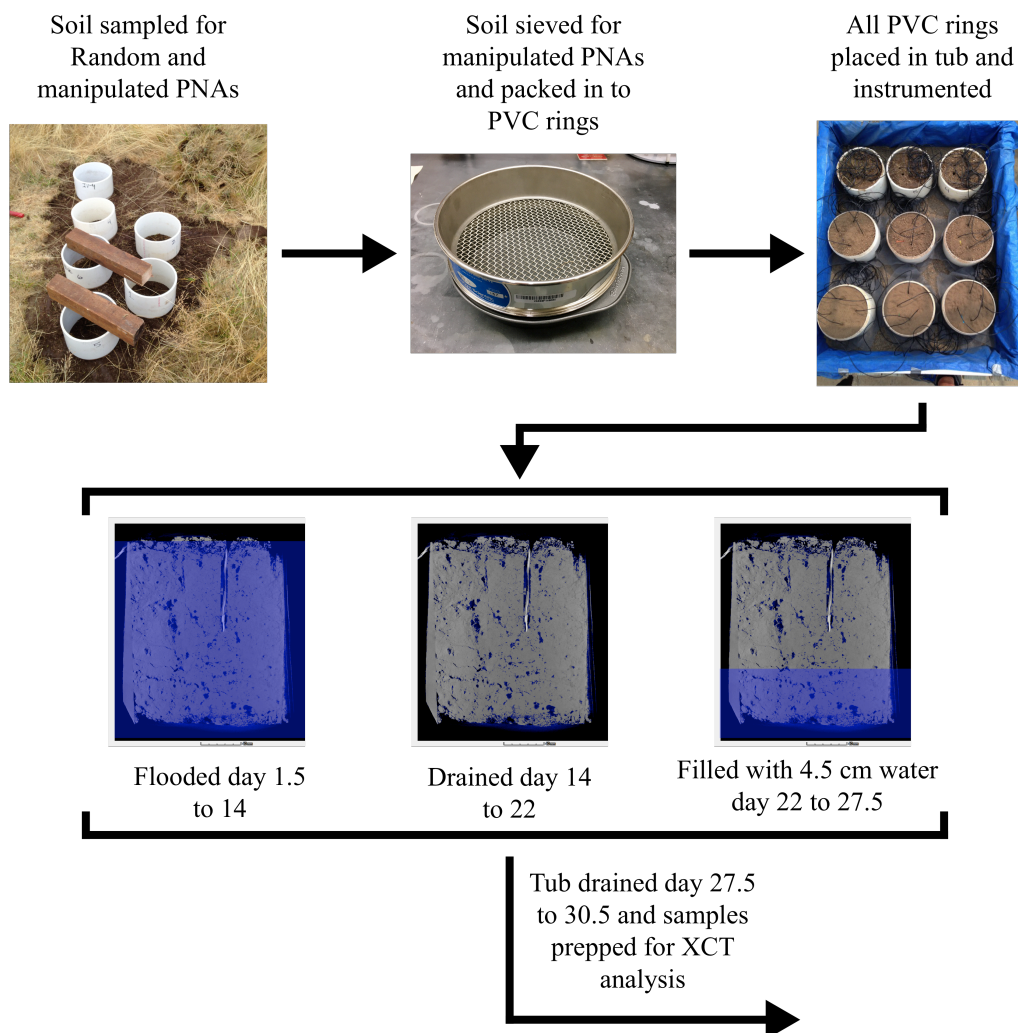


Figure B2. Visual timeline of experimental steps and water saturation conditions. Using an example XCT image, a time line of saturation conditions is shown (blue shading). Timing of changes in and duration of conditions are indicated.

a



b

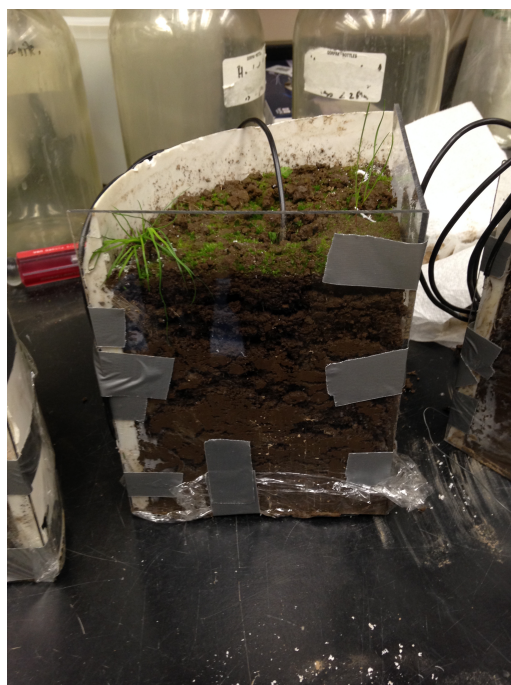


Figure B3. Example images of PVC cylinder sub-sectioning. To achieve the sufficient penetration of the x-ray beams, each PVC cylinder was sub-sectioned into four equally sized quadrants. Wanzek (pictured) cut the PVC cylinder and used acrylic glass sheets to create each sub-sample container.

Table B1. Woodburn series characteristics for A_p horizon, 0 – 20 cm

Classification ^a	Texture ^a	sand/silt/clay ^c	Porosity ^b	Pore volume ^c	pH ^d	C _{org} ^d	N _{org} ^d	CEC _{eff} ^d	Db ^e
		%	%	L m ⁻²		%	%	mmol _c kg ⁻¹	g cm ⁻³
Fine-silty, mixed, superactive, mesic Aquatic Argixeroll	Silt loam	5.7/79.5/15.3	48	96	5.6	1.3	0.2	237	1.3

^aSoil classification and texture follow the US Taxonomy data [Staff, 2015].

^bPercent porosity was calculated as $100 - \left[\left(\frac{D_b}{D_p} \right) * 100 \right]$, where $D_p = 2.65 \text{ g/cm}^3$

^cPore volume was calculated as % porosity * horizon thickness (in dm)

^dSoil characteristic data was collected following methods detailed in Kellogg Soil Survey Laboratory Methods Manual (Staff, 2014).

^eBulk density was estimated in the field

Table B2. Significant differences between electron activity metrics within the pore network architectures.

EA metric	R1 and R2	R1 and R3	R2 and R3	LA1 and LA2	LA1 and LA3	LA2 and LA3	SA 1 and SA2	SA1 and SA3	SA2 and SA3
a	---	---	---	---	---	---	---	---	*
b	---	---	---	---	---	---	---	---	***
c	---	---	---	---	---	**	---	---	---
d	---	---	---	---	---	---	---	---	**
e	---	---	---	---	---	---	---	---	*
f	---	---	---	---	---	---	---	---	---
g	---	---	---	*	---	---	---	---	---
h	---	---	---	---	---	---	---	---	---
i	---	---	---	---	---	---	---	---	---
j	---	---	---	---	---	---	---	---	---

A two-tailed t-test ($n = 3$ per sample) was used to test for significant differences in mean values for EA metrics within a pore network architecture (PNA). R1,2,3 = “Random” PNA with 3 PVC rings containing 3 Pt-electrodes each. LA1,2,3 = “Large aggregate” PNA with 3 PVC rings containing 3 Pt-electrodes each. SA1,2,3 = “Small aggregate” PNA with 3 PVC rings containing 3 Pt-electrodes each. Significance levels: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table B3. Significant differences in pore network metrics across pore network architectures for **VoI₁₀₀**, values are mean and (coefficients of variation) with n = 9

PNM	Units	Pore network architecture			Significant differences between PNAs		
		"Random" (R)	"Large aggregate" (LA)	"Small aggregate" (SA)	R : LA	R : SA	SA : LA
		$\bar{x}$ (CV)	$\bar{x}$ (CV)	$\bar{x}$ (CV)			
1	count	3.8 (31)	5.0 (87)	3.9 (37)	---	---	---
2	count	1.5 (36)	2.0 (101)	1.5 (45)	---	---	---
3	mm	0.03 (20)	0.04 (21)	0.04 (13)	**	**	---
4	mm	1.0 (41)	1.4 (92)	1.0 (40)	---	---	---
5	count	0.3 (33)	0.5 (116)	0.4 (53)	---	---	---
6	count	0.03 (22)	0.04 (20)	0.04 (14)	*	**	---
7	count	7987.8 (37)	10199.5 (25)	16586.7 (25)	---	***	***
8	count	357.9 (30)	656.5 (34)	1158.4 (24)	*	***	***
9	---	1.3 (10)	1.4 (26)	1.4 (14)	---	---	---
10	mm ³	1.6 (67)	1.6 (93)	0.8 (54)	---	---	---
11	mm ²	5.1 (57)	6.3 (89)	2.4 (130)	---	---	---
12	mm ³	1.5 (69)	1.4 (106)	0.3 (164)	---	(p = 0.076)	---
13	mm	0.3 (11)	0.3 (23)	0.3 (13)	(p = 0.053)	---	---
14	mm	0.014 (29)	0.022 (19)	0.023 (18)	**	***	---
15	mm ⁻¹	3.2 (21)	4.5 (35)	2.3 (70)	---	---	**
16	count	9015.9 (37)	12702.3 (31)	18581.7 (17)	---	***	**
17	count	4414.5 (33)	8188.3 (34)	12215.3 (19)	**	***	**
18	%	12.8 (37)	13.6 (47)	12.7 (31)	---	---	---

PNM = pore network metric. Tukey's honest significant difference test (n=9 per PNA; p-values adjusted for multiple comparisons) was used to determine significant differences between electron activity metrics. Random = Random PNA, LA = Large aggregate PNA (≤ 1 cm) and SA = Small aggregate PNA (≤ 2 mm). Significance levels * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table B4. Significant differences in pore network metrics across pore network architectures for **Vol₂₅**, values are mean and (coefficients of variation) with n = 9

PNM	Units	Pore network architecture			Significant differences between PNAs		
		"Random" (R)	"Large aggregate" (LA)	"Small aggregate" (SA)	R : LA	R : SA	SA : LA
		$\bar{x}$ (CV)	$\bar{x}$ (CV)	$\bar{x}$ (CV)			
1	count	4.2 (38)	2.7 (48)	2.2 (74)	---	*	---
2	count	1.7 (46)	1.0 (66)	0.7 (106)	---	*	---
3	mm	0.04 (17)	0.04 (18)	0.03 (13)	---	(p = 0.056)	**
4	mm	1.2 (44)	0.7 (61)	0.5 (94)	---	*	---
5	count	0.3 (54)	0.2 (79)	0.1 (126)	---	*	---
6	count	0.05 (23)	0.05 (19)	0.04 (15)	---	*	*
7	count	825.7 (37)	1786.3 (32)	4695.4 (26)	*	***	***
8	count	43.3 (45)	143.4 (43)	313.7 (37)	*	***	***
9	---	1.3 (3)	1.3 (1)	1.3 (2)	---	---	---
10	mm ³	3.1 (46)	1.1 (71)	0.5 (84)	***	***	---
11	mm ²	9.2 (35)	5.0 (52)	0.9 (70)	**	***	**
12	mm ³	3.0 (48)	1.0 (76)	0.1 (110)	***	***	---
13	mm	0.3 (12)	0.4 (12)	0.3 (8)	*	---	*
14	mm	0.022 (36)	0.030 (22)	0.023 (24)	*	---	(p = 0.078)
15	mm ⁻¹	3.2 (18)	4.7 (14)	2.3 (77)	*	---	***
16	count	1018.8 (53)	2095.6 (36)	5243.6 (27)	(p = 0.067)	***	***
17	count	566.5 (36)	1555.4 (32)	3457.9 (29)	**	***	***
18	%	9.8 (0.5)	8.7 (0.5)	8.7 (0.7)	---	---	---

PNM = pore network metric. Tukey's honest significant difference test (n=9 per PNA; p-values adjusted for multiple comparisons) was used to determine significant differences between electron activity metrics. Random = Random PNA, LA = Large aggregate PNA (≤ 1 cm) and SA = Small aggregate PNA (≤ 2 mm). Significance levels * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

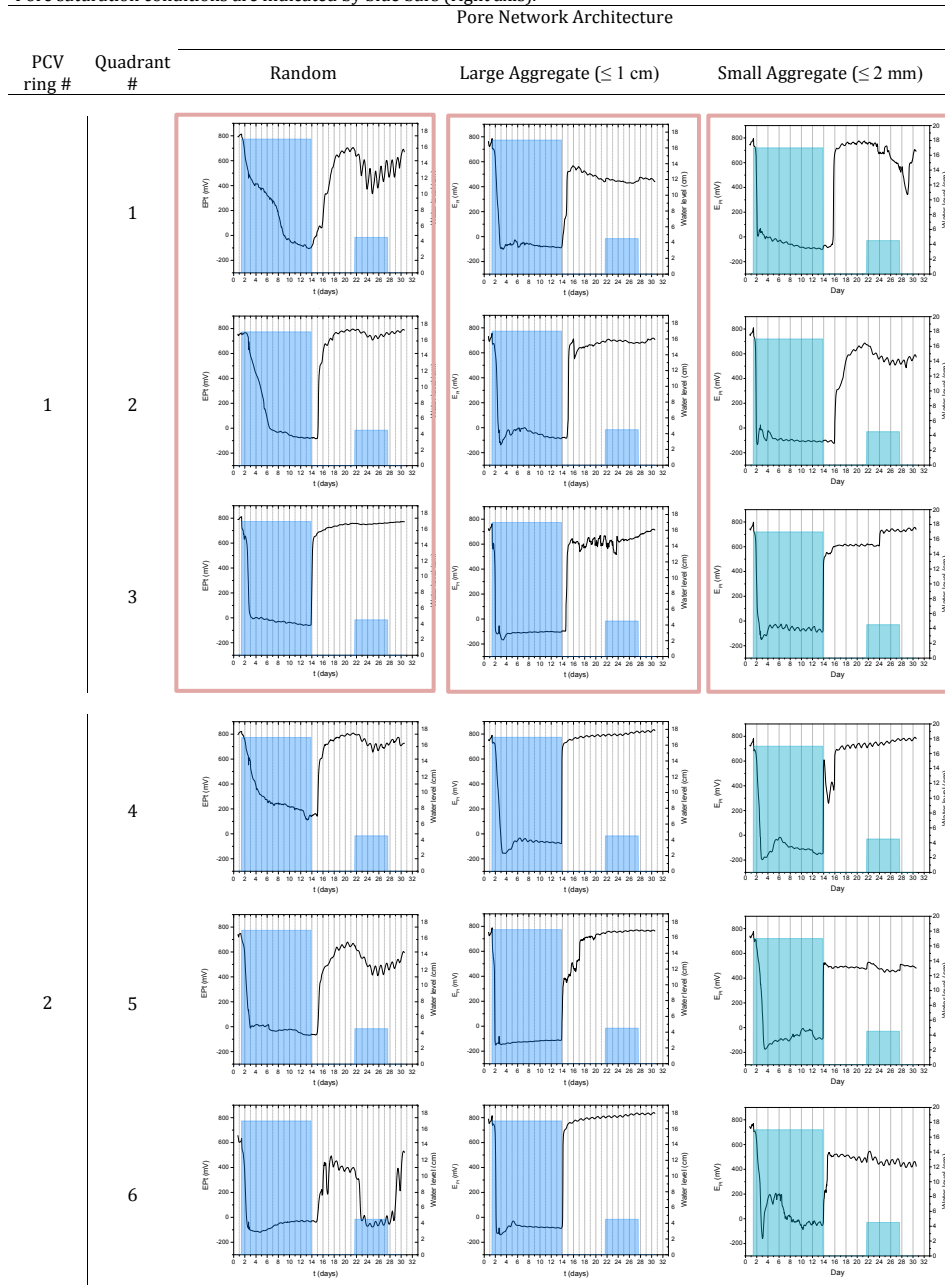
Table B5. Significant differences in pore network metrics across pore network architectures for $\text{Vol}_{0.2}$, values are mean and (coefficients of variation) with $n = 9$

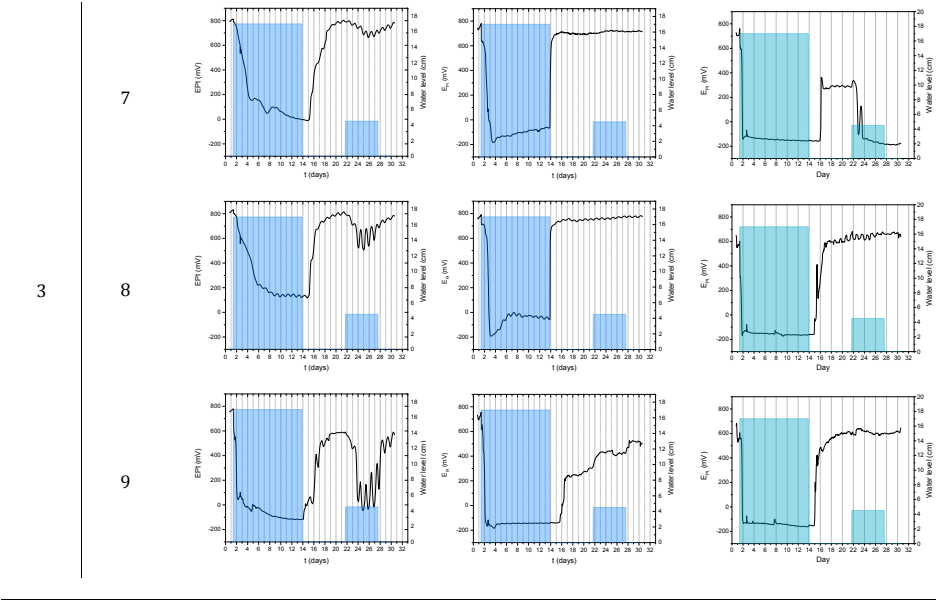
PNM	Units	Pore network architecture (PNA)			Significant differences between PNAs		
		"Random" (R)	"Large aggregate" (LA)	"Small aggregate" (SA)	R : LA	R : SA	SA : LA
		$\bar{x}$ (CV)	$\bar{x}$ (CV)	$\bar{x}$ (CV)			
1	count	2.1 (45)	2.4 (43)	1.6 (43)	---	---	---
2	count	0.7 (58)	0.8 (61)	0.4 (70)	---	---	---
3	mm	0.3 (31)	0.4 (32)	0.4 (29)	*	---	---
4	mm	0.5 (27)	0.7 (35)	0.6 (32)	(p = 0.065)	---	---
5	count	0.4 (82)	0.6 (68)	0.3 (61)	---	---	(p = 0.075)
6	count	0.2 (81)	0.1 (55)	0.1 (110)	---	---	---
7	count	60.6 (32)	64.2 (69)	69 (67)	---	---	---
8	count	8.4 (29)	8.6 (58)	7.4 (58)	---	---	---
9	---	1.3 (8)	1.3 (7)	1.2 (6)	---	---	---
10	mm ³	0.4 (99)	0.6 (70)	0.5 (81)	---	---	---
11	mm ²	1.0 (82)	1.4 (63)	1.3 (92)	---	---	---
12	mm ³	0.1 (111)	0.2 (91)	0.2 (125)	---	---	---
13	mm	0.2 (23)	0.3 (12)	0.3 (23)	*	---	---
14	mm	0.03 (38)	0.03 (30)	0.03 (38)	---	---	---
15	mm ⁻¹	2.8 (73)	3.0 (75)	2.7 (74)	---	---	---
16	count	68.4 (30)	60.5 (55)	75.9 (61)	---	---	---
17	count	28.5 (21)	30.2 (41)	37.8 (21)	---	---	---
18	%	10.6 (61)	14.2 (34)	11.7 (49)	---	---	---

PNM = pore network metric. Tukey's honest significant difference test ($n=9$ per PNA; p-values adjusted for multiple comparisons) was used to determine significant differences between electron activity metrics. Random = Random PNA, LA = Large aggregate PNA (≤ 1 cm) and SA = Small aggregate PNA (≤ 2 mm). Significance levels * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

6.3 Appendix C (All E_{Pt} curves)

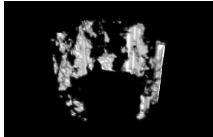
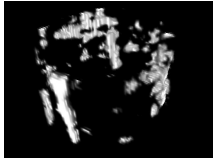
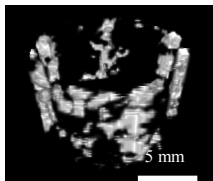
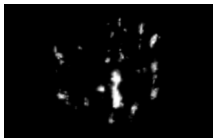
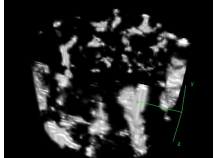
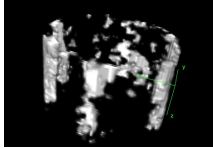
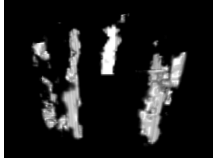
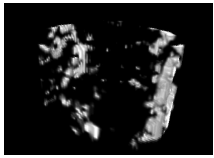
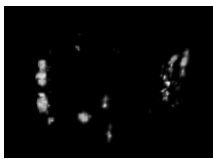
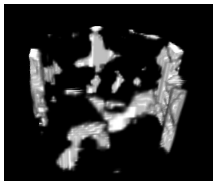
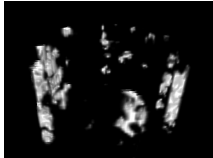
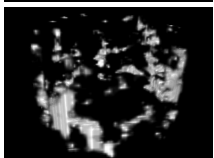
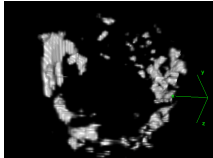
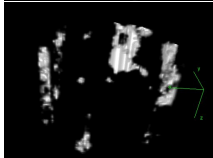
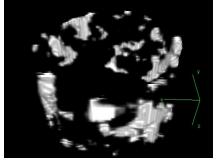
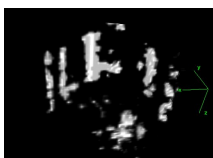
Appendix C. Electromotive potential (mV; left axis) curves for all PVC rings and quadrants. Samples are grouped by PVC ring (1 – 3) and quadrants per ring (grouped 1 – 3, 4 – 6, and 7 – 9). Example groupings are outlined in red boxes. Pore saturation conditions are indicated by blue bars (right axis).

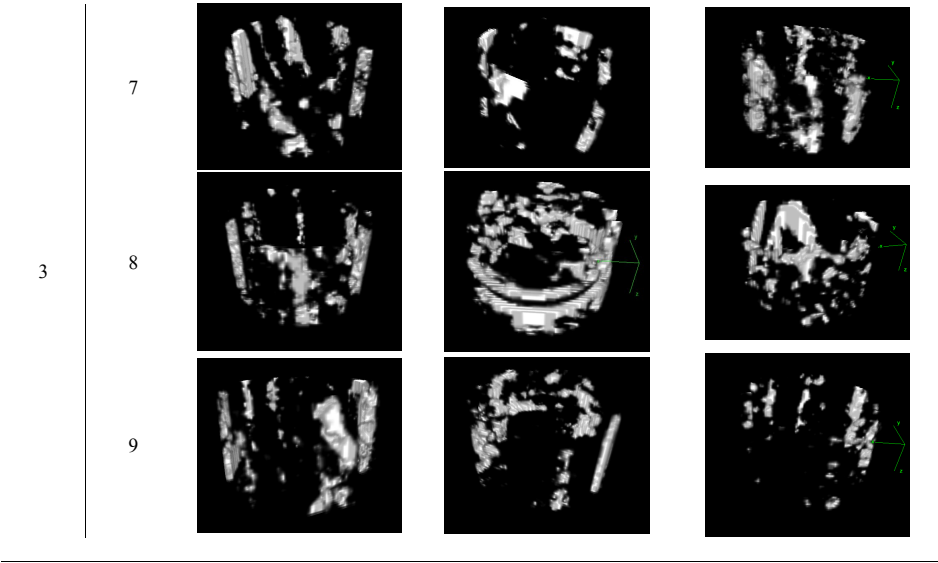




6.4 Appendix D (VoI_{0.2} XCT images)

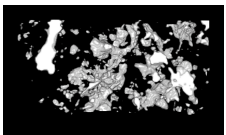
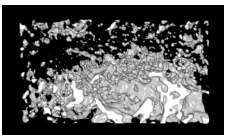
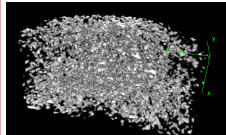
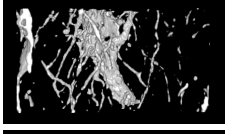
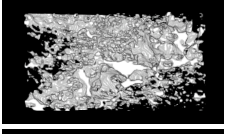
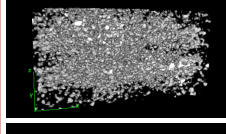
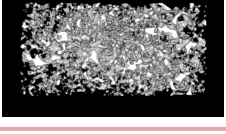
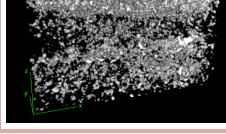
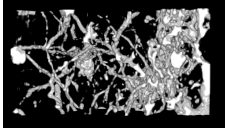
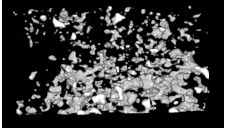
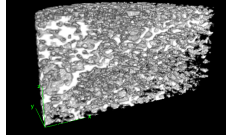
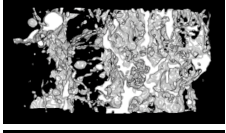
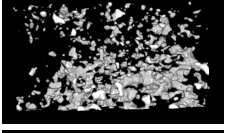
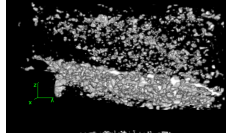
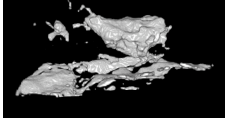
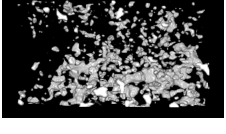
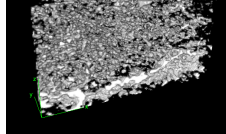
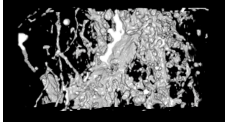
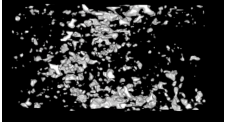
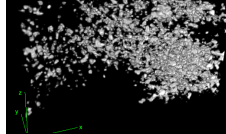
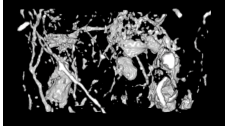
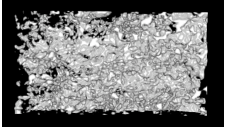
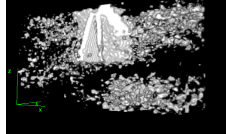
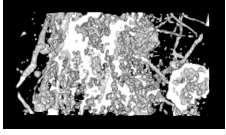
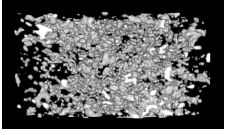
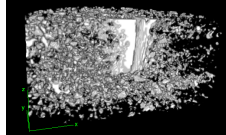
Appendix D. Images from all quadrants and pore network architectures (PNAs) for VoI_{0.2}. Three quadrants (grouped 1 – 3, 4 – 6, and 7 – 9) were imaged for each PVC ring (1 – 3) and PNA. Example groupings are outlined in red boxes. All images are oriented such that the Pt-electrode tip is in the center and the Cu wire is extending out of the top of the image. Dimensions: $r_1 = 5\text{ mm}$, $r_2 = 5.84\text{ mm}$, $h = 7\text{ mm}$.

		Pore Network Architecture		
PCV ring #	Quadrant #	Random	Large Aggregate ($\leq 1\text{ cm}$)	Small Aggregate ($\leq 2\text{ mm}$)
1	1			
	2			
	3			
2	4			
	5			
	6			



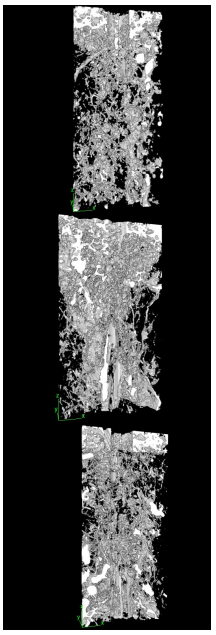
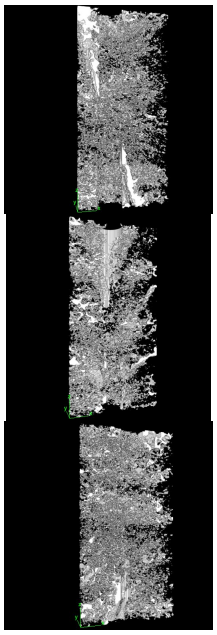
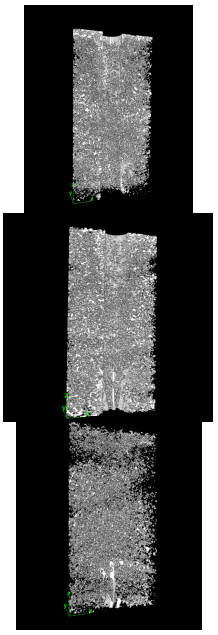
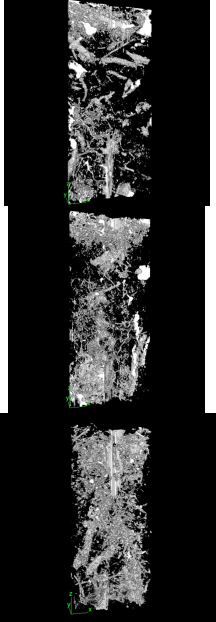
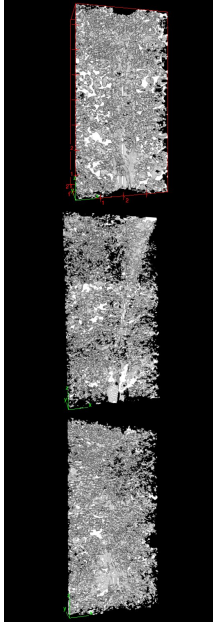
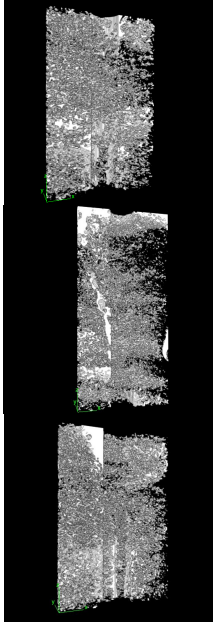
6.5 Appendix E (VoI₂₅ XCT images)

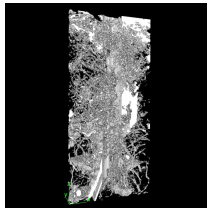
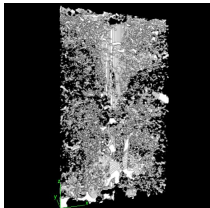
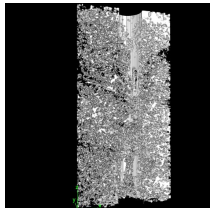
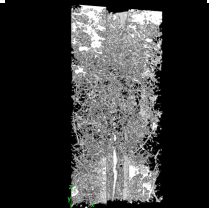
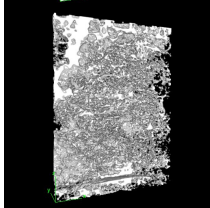
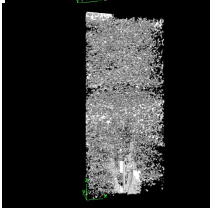
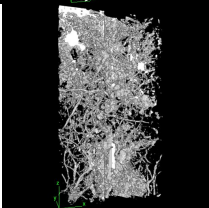
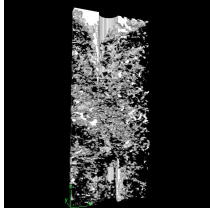
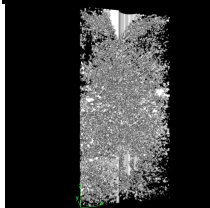
Appendix E. Images from all quadrants and pore network architectures (PNAs) for VoI₂₅. Three quadrants (grouped 1 – 3, 4 – 6, and 7 – 9) were imaged for each PVC ring (1 – 3) and PNA. Example groupings are outlined in red boxes. All images are oriented such that the Pt-electrode tip is in the center, just out of the image frame, and the Cu wire is extending upward. Dimensions: r = 2 cm, h = 2 cm.

		Pore Network Architecture		
PCV ring #	Quadrant #	Random	Large Aggregate (≤ 1 cm)	Small Aggregate (≤ 2 mm)
1	1			
	2			
	3			
2	4			
	5			
	6			
3	7			
	8			
	9			

6.6 Appendix F (Vol_{I100} XCT images)

Appendix F. Images from all quadrants and pore network architectures (PNAs) for Vol_{I100}. Three quadrants (grouped 1 – 3, 4 – 6, and 7 – 9) were imaged for each PVC ring (1 – 3) and PNA. Example groupings are outlined in red boxes. All images are oriented such that the Pt-electrode tip is in the bottom-center of the image, and the Cu wire is extending upward out the top. Dimensions: $r = 2\text{ cm}$, $h = \sim 8\text{ cm}$.

		Pore Network Architecture		
PCV ring #	Quadrant #	Random	Large Aggregate ($\leq 1\text{ cm}$)	Small Aggregate ($\leq 2\text{ mm}$)
1	1			
	2			
	3			
2	4			
	5			
	6			

3	7			
	8			
	9			

6.7 Appendix G (Definitions of terms)

- 1) A soil system is defined as a natural environment showing evidence of pedogenic processes such as accumulation of organic carbon, mineral alteration, translocation of organic and mineral matter and the subsequent development of mineral-organic colloids, aggregates and associated pore systems
- 2) We consider a soil system to be a reducing environment when the electron acceptor O_2 is absent from the majority of the pore system.
- 3) Electron activity (EA) is defined according to Stumm (1966) as $pe = -\log [e^-]$ analogous to H^+ activity ($pH = -\log [H^+]$)
- 4) E_{Pt} is the electromotive potential (measured in mV or V) returned by a platinum electrode connected to a reference electrode and a Voltmeter
- 5) pe and E_{Pt} are related by the expression: $\frac{E_{Pt} (V)}{0.059} = pe$ (James and Brose, 2011)
- 6) Soil structure – Soil structure is defined as the physical arrangement of solids and voids in the soil system. At a bulk density of 1.3 kg L^{-1} , a soil with standard mineralogy (containing feldspar and quartz as major mineral phases) will have a solid to void ratio of 1:1 (50%).
- 7) Pore network architecture (PNA) – the term used to describe and delineate the physical arrangement of solids and voids in each structure type: 1) no-manipulation (“Random” PNA), 2) soil sieved to $\leq 1 \text{ cm}$ (“Large Aggregate” PNA), and 3) soil sieved to $\leq 2 \text{ mm}$ (“Small Aggregate” PNA).
- 8) Volume of Interest (VoI) – Operationally defined as the volume to which each XCT image file was cropped before the pore network contained therein was analyzed.
- 9) Pore network metric (PNM) – A variable describing a characteristic of the pore network architecture, derived using X-ray computed tomography and image analysis software (Fiji).

