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Examining Soil Microbial Diversity in Transition Zones between Corn Fields and Restored Prairie in the Upper Midwest

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Preface

This work is the second of two theses that I wrote about the Liberty Prairie Reserve during the 2021-2022 academic year. I published the first under the name "Footprints on the prairie: Examining the interlocking land histories of the Liberty Prairie Reserve, Illinois." While this thesis falls under the disciplines of ecology and soil microbiology, "Footprints on the prairie" is primarily a history and ecology text, in which I examine the Indigenous and settler histories of what is today the Liberty Prairie Reserve, the broader contexts of U.S. agriculture, an overview of prairie ecology, and the history and theory of prairie restoration. If you want to learn more about the Liberty Prairie Reserve, you can find "Footprints on the prairie" at Scholarship@Claremont (https://scholarship.claremont.edu/).

Acknowledgements

This thesis has been a labor of love for the past year and a half, and it would not have been possible without the support and encouragement of my community. First, I want to thank my parents, Tamara and Patrick, and Donovan, for their unconditional love as I have navigated not only this thesis, but college as a whole. Thank you to my friends for getting excited about prairies with me. Thank you to the Scripps Environmental Analysis Award and the Pomona Environmental Analysis Department for financially supporting my research. And, last but absolutely not least, thank you to the professors who have encouraged me from the start of this ambitious project – Dr. Char Miller, Dr. Marc Los Huertos, Dr. Charlotte Chang, Dr. Colin Robins, and Dr. Kevin Vennemann. I appreciate your support more than words can say.

1. Introduction

Liberty Prairie is located at the intersections of rural and urban, residential and agricultural, preservation and renewal. A 5,000-acre protected area in Lake County, Illinois, the Liberty Prairie Preserve attempts to "create a model Reserve of exceptional land, water, and biodiversity health where public and private landowners manage their land in ways that sustain people, plants, and wildlife" (Conserve Lake County, 2019, 6). This plan reflects a patchwork of private and public lands, all stewarded under the requirements of the Reserve as Lake County residents use them for housing, agriculture and conservation. The result is a multitude of border ecosystems between agriculture and preserved prairies and wetlands. These borders are sites of exchange between lands under extremely different management regimes. While the agricultural fields are corn-soy rotations that are regularly plowed, tilled, fertilized, and sprayed with pesticides, conservationists manage the restored habitats in attempts to recreate the tallgrass prairies and wetlands the Bodwéwadmi stewarded. These edge communities sparked my curiosity about restoration beyond the aesthetics of prairie flora; did the replanting of prairie grasses and forbs restore the ecosystem functions of grassland soil? Further, did the Liberty Prairie's management strategies re-establish keystone prairie bacterial phyla abundances?

The soil microbiome includes any microscopic component of an ecosystem's soil biota, including archaea, bacteria, fungi, and soil fauna (Paul, 2015). The microbiome is an important component of soil organic carbon, with an estimated global biomass pool of 14.6 petagrams of microbial carbon (Serna-Chavez, Fierer, & van Bodegom, 2013, 1167). Moisture availability is positively correlated with microbial biomass, because soil hydration is a limiting factor for microbes; accordingly, temperature has a negative correlation with microbial biomass because of its impact on evapotranspiration rates (Serna-Chavez, Fierer & van Bodegom, 2013, 1167).

Additionally, high soil carbon-to-nitrogen ratios or low pH from debris accumulation reduce substrate quality and are negatively correlated with microbial biomass (Serna-Chavez, Fierer & van Bodegom, 2013, 1169). In microbial biomass distribution across biomes, temperate grasslands rank fourth with a mean of 131 g biomass C/m², following tropical forests (203 g biomass C/m²), temperate coniferous forests (175) and tundra (136) (Fierer *et al.*, 2009, 1241). The microbes in these systems have functional roles regulating energy flow (largely through decomposition of debris and waste), carbon flux, and nitrogen fixation and mineralization (Morris & Blackwood, 2015). These primary functions systemically scale to impact ecosystem processes such as soil aggregate stability and decomposition rates; the broader impact of healthy microbial communities is soil ecosystem resilience, a desirable outcome for restoration projects.

Ecologists first studied prairie microbial composition and function in the 1980s (Anderson, 2009, 6). Microbes are important to soil carbon cycling due to their role in organic debris decomposition (Horwath, 2015), and institutional interest in prairie restoration often focuses on the high belowground carbon storage potential of grasslands compared to other biomes (Janowiak et al., 2017). Further, prairie management impacts soil carbon sequestration ability. A single-site analysis in Wisconsin found that carbon content in the top 100 cm of remnant prairie soil was nearly double that in the restored prairie (Kucharik, Fayram & Cahill, 2006, 131); a single-site study in Illinois found that soil organic carbon content increases with forb abundance in replanted vegetation and decreases with grass abundance (Ampleman, Crawford & Fike, 2014, 911).

Because human activity has directly altered between 80-99.9% of all tallgrass prairies in North America (Docherty & Gutknecht, 2019, 2), a consequence detailed in Part I of my thesis, efforts to study the prairie microbiome largely center around effectively restoring it. Recent

developments in this discipline include a Wang *et al.* (2019) study linking soil microbe biodiversity to plant productivity and biodiversity in Kansas and Missouri. Zhu *et al.* (2020) compared burned, mowed, and undisturbed prairie plot management techniques in Nebraska, finding that burned plots had higher proportions of microbes beneficial for plant growth than mowed plots. Finally, Reynolds *et al.* (2020) found potential benefits in introducing known prairie fungi populations to Kansas restorations, documenting increased survival of two late succession prairie species, showy partridge pea (*Chamaecrista fasciculata*) and plains coreopsis (*Coreopsis tinctoria*).

One of my primary research interests in evaluating the microbiome of restored prairies is Verrucomicrobia re-establishment. Verrucomicrobia is a free-living bacteria phylum that is closely related to the Planctomycetes and Chlamydia phyla (Fuerst, 2019). The phylum is globally common in soils and is also found in freshwater, oceans, and human gut microbiomes (Fuerst, 2019). Verrucomicrobia is typically studied in-situ rather than in a lab, because cultivation is typically difficult (Sangwan *et al*., 2005). Bergmann *et al.* (2011) found that grasslands and prairies had the highest average relative abundance of verrucomicrobia at 35% (compared to an average across 181 Northern Hemisphere soils between 0.9-23.5%). Shortly after, Fierer *et al*. (2013) found that the phylum was dominant across 31 North American tallgrass prairie remnants, representing >50% of the bacteria genomic reads in mid-latitude prairies. Their reconstruction of tallgrass prairie microbial communities via spatial modeling demonstrated that verrucomicrobia abundance in Illinois prairies varies from 30% in the northwest to 15% in the central region (Fierer *et al.*, 2013, 623). The high proportions of verrucomicrobia in prairie microbiomes suggests that the abundance of this phylum could be indicative of whether restoration projects successfully restored functional prairie soils.

My thesis examines microbial diversity in edge environments between restored prairies and agricultural fields on the former Casey Farm in the Liberty Prairie Reserve, Illinois. Because narrow prairie strips and patches are common methods for incorporating prairie ecosystems within agricultural settings (Docherty & Gutknecht, 2019, 2), understanding the microbial biodiversity gradients of edge zones could improve the efficacy of prairie restoration efforts. I hypothesize that both bacterial diversity and verrucomicrobia abundance will increase with distance into restored prairie habitats, and decrease with distance into the agricultural fields. I hope that my senior thesis will deepen understandings of restoration impacts on prairie soil dynamics, and ultimately will advocate for implementing microbially-conscious prairie restoration (Docherty & Gutknecht, 2019) to improve restored prairie resilience and ecosystem functioning.

2. Methods

a. Soil sample collection

I collected soil samples to analyze microbial and physical soil characteristics at the Liberty Prairie Reserve in Lake County, Illinois on July 7, 2021. The average July temperature at the Reserve is 73.9°F, with 3.5 inches of precipitation and 68% humidity; annual precipitation is 39.2 inches (Climate-Data.org). I collected the samples from three fields, A, B, and E. In each field, I replicated the sampling procedure, so that there were paired A-1/A-2, B-1/B-2, and E-1/E-2 replicate samples approximately 25 meters apart from one another (for a total of six sites across three fields). I selected the sample sites qualitatively based on the length of the edge between the agricultural field and the prairie, and on the fragments' distances from infrastructure (e.g. roads and bike paths). Each site was at least 10 meters away from such infrastructure to minimize confounding anthropogenic factors influencing soil properties.

Field A was the northwestern-most field and had corn that was four feet in height, in the n-th leaf vegetative stage of corn growth (e.g. pre-reproduction, Licht). Using the University of California Davis SoilWeb tool (University of California Davis), I determined that Field A exhibited a 531B soil type, a Markham series silt loam on 2 to 4 percent slopes (Caslyn, 2005, 112). Field A's 531B type is a well-drained prime farmland soil formed of moderately eroded moraine till and loess materials. The Markham series are mesic Mollic Oxyaquic Hapludalfs (111). This taxonomy indicates a soil typical of a deciduous forest, well-drained and well-developed (Morris, 2017, 3) with a thick and organically rich mollic A-horizon, located in a cool temperate environment with enough water to support plants year round.

Field B was the northeastern-most field and had corn that was three feet in height in the n-th leaf vegetative stage. Using the same methods, I determined that Field B had a 989B soil type, a Mundelein and Elliot series silt loam on 2 to 4 percent slopes (Caslyn, 2005, 139). The Mundelein and Elliot series are mesic Aquic Argiudolls, indicating a water-saturated clay-dominant grassland soil (134). This soil's parent materials are loess and moraine till. Once again, this series is categorized as prime farmland, but with moderately poor drainage.

Finally, Field E was the southeastern-most field, and was covered in 2-3 feet tall row corn crops in the n-th leaf vegetative stage. Most of this field had a 223C2 Varna silt loam series on 4 to 6 percent slopes, which is a moderately well-drained and highly eroded moraine soil, with glacial till and loess parent materials (Caslyn, 2005, 191). However, the samples from the agricultural field were in the 223B Varna series. The only difference between the sites within Field E was that the 223C2 soil type had a surface layer thinned by erosion that is not present in the 223B soils. This could be due to a longer agricultural history on the 223C2 sites, or a more intensive tilling or cultivation method that could break down soil structures and cause faster

erosion. The Varna series are mesic Oxyaquic Argiudolls (189), which is very similar to the Mundelein and Elliot series, with the Varna series being further developed (as indicated by the Oxy- prefix).

All three soil series have a well-defined plow layer, which is reflective of the 150-year history of conventional agriculture in the Liberty Prairie Reserve. The edge of the plow line is evident, which guided where I took edge measurements. Two of the three soils (in Fields B and E) are mollisols, which are typical for grassland regions. Notably, Field A has an alfisol soil, which is characteristic of deciduous forests. Initially, I expected this to be due to reforestation after anthropogenic burning ceased post-colonization, and forests grew where many prairies had once been (as discussed in Chapter 3). However, the time-scale of 200 years since colonization did not support the time required to develop a haplic (e.g. simple but well-developed) soil; the Lake County Soil Survey suggests that the soils in the region began forming with the glacial retreat, which took place over 12,000 years ago (Caslyn, 2005, 14). Instead, the combination of alfisol (with mollic characteristics) and mollisol soils, all of which have aquic properties, reaffirms Government Land Office maps from the 1830s detailing the mixed wetlands, prairies and forests which constituted the land in Lake County, Illinois prior to Euro-American colonization (4).

All three fields were in the corn phase of the corn/soy rotations that is typical across the Corn Belt of the Midwestern United States. The Liberty Prairie Reserve suggests that farmers adopt "biological farming" practices, which includes applying fertilizers "which do the least damage to soil life," and minimizing the use of pesticides, nitrogen fertilizers, and herbicides (Conserve Lake County, 2013, 64). Additionally, the Reserve suggests that farmers on reserve lands use "combinations of stone tillage, shallow incorporation of residues, and deep tillage" to prevent

soil degradation. The scope of my research did not allow me to evaluate how closely these suggestions are followed, but the Liberty Prairie Reserve Master Plan details neither enforcement nor monitoring strategies for these guidelines.

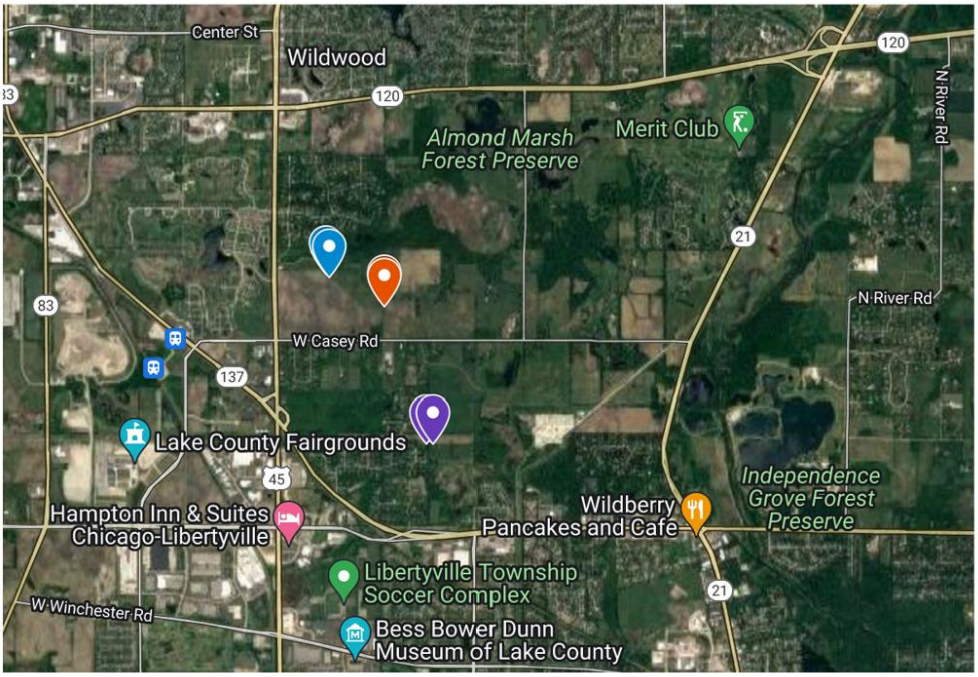


Figure 2.1. Approximate positions of the six sample sites in the Liberty Prairie Reserve, Illinois. Field A is blue, B is red, and E is purple. Map created by Anna Burns using Google Maps.

To analyze the change in soil properties between restored prairies and agricultural fields, at each site I collected five samples from the: inner prairie (depth of 6m), outer prairie (depth of 3m), edge (along the outer plow-line between the prairie and the agricultural field), outer corn field (depth of 3m), and inner corn field (depth of 6m) (Figure 2.2).

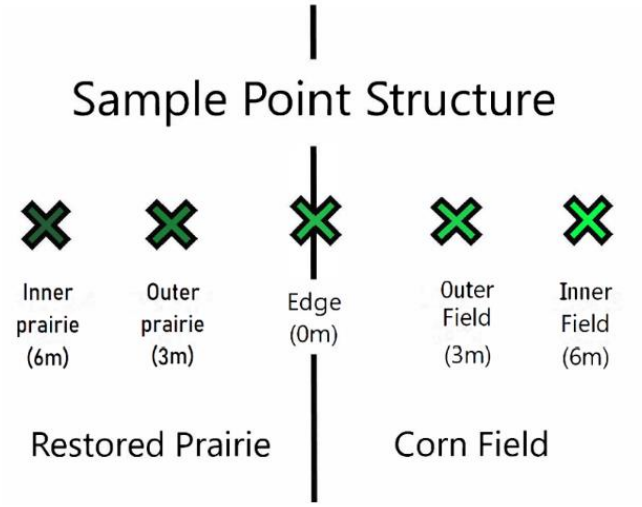


Figure 2.2. Sample structure plan, with samples from the restored prairie patch, the adjacent agricultural field, and the edge between the two habitats.

For each soil sample, I used a soil probe to collect 2 cm of top-soil, following the Fierer *et al.* (2013) method (622). In each sample, I aggregated 5 collections from within a 15-centimeter radius, and filtered the aggregated sample through a 2mm mesh sieve into a plastic bucket. I then stored each sample in a polyethylene bag (Sheppard & Addison, 2008, 44). I immediately placed the bags in a cooler with a block of dry ice to preserve the microbial populations present in the samples at time of collection (44). I shipped the samples on dry ice to Pomona College, where they were stored at field moisture content in a 4°C freezer for six weeks before processing.

In addition to the microbial samples, I also collected a sample from each point in fields A-1, B-1, and E-1 to use in analyses of soil physical properties. I limited collection to one replicate in each field due to shipping and processing requirements. At each sample point in the three fields, I used a spade to collect 300 mL of soil that I kept at field temperature and moisture for the duration of the field work, before storing it at 4°C for two days. I then removed rocks

(>25 mm) and organic materials including sticks and leaves from the samples, before drying them in an oven at 105°C for 20 minutes as a proxy for air-drying due to weather constraints (Sheppard & Addison, 2008, 40). After drying, the samples were stored at room temperature until further processing.

Figure 2.3. (top left). Field A-1, with collection bucket and cooler at the edge sample point.
Figure 2.4. (top right). Field B-1.
Figure 2.5.(below). Field C-1; the proximity to a deciduous tree stand is visible in the background.

b. Microbial analysis

Before I sent the soil samples for genetic analysis of microbial biodiversity, I extracted the DNA associated with the microbe from each sample. I initially attempted to do this with the Soil Genomic DNA Isolation Kit from LSBio, which uses bead-beating homogenization and buffer

treatments to remove impurities (LSBio). After extracting the DNA, I quantified the nucleic acid yields in each of the 30 samples using the NanoPhotometer, a protein UV spectrophotometer from Implen (Implen, n.d.). The mean nucleic acid yield of the samples was 14.6 ± 18.3 ng/uL of nucleic acid content, which did not meet the 20 ng/uL threshold for 16S rDNA analysis at the DNA analysis vendor, LC Sciences. Due to the low quality of the samples, I opted to use a new kit and start the DNA extraction again. For three months between the two extraction attempts, I stored the soil samples in a -20°C freezer.

I began the process of DNA extraction again in early February of 2021 with the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, n.d.). Using the kit, I homogenized the soil samples with a bead-beating technique. I then removed inhibitors from the homogenized soil product via aluminum chloride solution and bound the DNA to a guanidine thiocyanate solution. Next, I purified the DNA by washing it with a solution of ethanol, guanidine, hydrochloride, and isopropanol, and then with a second ethanol-based solution. Finally, I eluted the DNA in a spin column, resulting in an extracted DNA solution suitable for downstream PCR applications (Qiagen, 2021). I then quantified the DNA using UV absorbance ratios, following the same method as with the LSBio extractions. Ultimately, these extractions had an average nucleic acid content of 89.3 ± 61.4 ng/uL. This satisfied the requirements of LC Sciences' initial quality control of 20 ng/mL, so I was able to use these samples for 16S rDNA analysis.

Once I had extracted the DNA from the soil, I sent my samples to the LC Sciences Lab for 16S rDNA microbiome sequencing (LC Sciences [a]). I stored the eluted DNA in a -20°C freezer until I packaged them as per the instruction of the LC Sciences lab, cushioning the samples with packing peanuts and keeping them frozen between two blocks of dry ice to prevent sample degradation (LC Sciences [b]). 16S rDNA sequencing is the standard method for bacterial

identification because genes in the region tend to be species-specific (i.e. have limited inter-species horizontal gene transfer) and evolve relatively slowly (i.e. have stable identification markers) (He, 2019). LC Sciences' 16S rDNA sequencing service amplifies the V3 and V4 regions of the 16S region using primers 341F and 805R, resulting in an amplicon of a 465 base pairs length. The company then sequences this amplified library on an Illumina NovaSeq platform using the 2x250 base paired-end mode. LC Sciences also provided quality control and output-cleaning, using the DADA2 (Divisive Amplicon Denoising Algorithm) to derive taxonomical information on the extracted rDNA, which was then ready for biodiversity analyses.

c. Physical soil characteristic tests

I conducted both the organic matter and acidity tests according to protocols in the NRCS' Kellogg Soil Survey Laboratory (2014) *Soil Survey Laboratory Methods Manual*, using the soil samples I collected from each sample point in field replicates A-1, B-1, and E-1. I previously oven-dried the samples to a stable moisture content for storage, as described in the soil sample collection methods. I calculated organic matter content from my soil samples according to the Mineral Content protocol (495-497). First, I weighed 10 grams of each sample into a tared tin. To ensure that water content was removed from the soil, I heated the samples at 105°C for 16 hours. I then reweighed the samples, before heating them again at 400°C for 16 hours. Finally, I calculated the mineral and organic content as a ratio of the soil weight after the 105°C and 400°C heating periods. I calculated soil acidity from the 15 samples according to the Hydrogen-Ion Activity protocol (269-271). I made a saturated soil paste by saturating 50g of the soil with water and soaking it overnight. Then, I used a calibrated Accumet XL500 pH meter with a glass electrode and automatic temperature correction to measure the pH of the saturated paste.

In addition, I measured soil texture according to the hydrometer method (Gee & Bauder, 1986). Using the same 50g of saturated soil paste from the pH test, I added 100 mL of distilled water and 100 mL of 5% sodium hexametaphosphate solution. I then mixed this solution for 2 minutes using a blender, before pouring it into a 1L graduated cylinder. I filled the remaining volume in the graduated cylinder with water up to the 1L mark, and then used a hydrometer to take readings at 44 seconds to calculate sand volume. I also measured the temperature of the solution at this time. After 7 hours and 20 minutes, I took a third reading with the hydrometer and thermometer to calculate clay volume. Finally, I used these values to calculate the settling times with Stoke's Law, which I used as a proxy for soil texture.

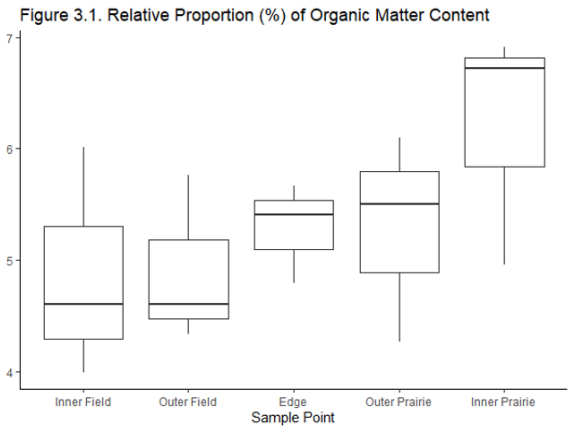
3. Results

a. Physical characteristics

Sample Point	Mean % organic matter content ± s.d.	Mean pH ± s.d.
Inner prairie	6.20 ± 1.08	6.06 ± 0.88
Outer prairie	5.29 ± 0.93	6.06 ± 0.98
Edge	5.29 ± 0.45	6.41 ± 1.22
Outer field	4.90 ± 0.76	5.92 ± 1.30
Inner field	4.87 ± 1.04	5.60 ± 1.28

Table 3.1. the pH and mean proportions of organic matter content per sample weight (n = 15, n = 3 per sample point, n = 5 per field).

I analyzed sample organic matter and acidity for variation between prairie and field sample points (Table 3.1). The relative organic matter in the soil samples generally increased with proximity to (and distance within) the prairie (fig. 3.1). Using an Analysis of



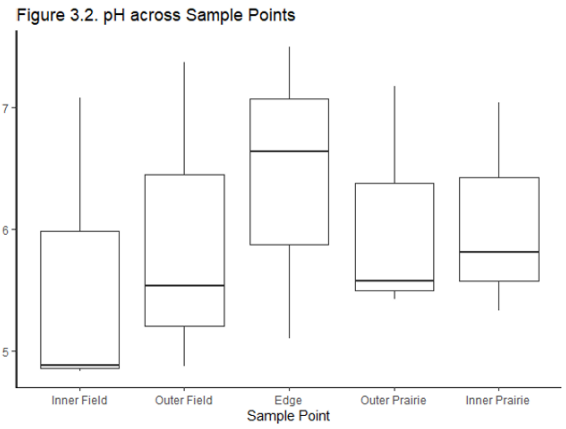


Figure 3.1. (left). A boxplot showing the distribution of relative organic matter content across sample points (n = 15, n = 3 per sample point).
Figure 3.2. (right). A boxplot showing the distribution of soil pH across sample points (n=15, n = 3 per sample point).

Variance (ANOVA) test (Schéffe, 1959), I determined that this trend was not significant ($p = 0.40$). Although the trend is noteworthy within the scope of the thesis, the test power was 0.79 at the 0.05 significance level, indicating that the true significance rate is nearly at the standard 80 percent threshold (Cohen, 1969). Therefore, the scope of the experiment is likely not the cause for low correlation rates between organic matter and sample point location.

Further, there was minimal correlation between sample point location and pH (ANOVA, $p = 0.93$, fig. 3.2). Unlike with organic matter, these results are limited by the experimental design, with the test power at 0.07 for the 0.05 significance level ($F = 0.20$). The general trend demonstrates a slightly more neutral soil directly along the plow line (edge sample points), with the rest of the point locations trending towards acidic.

Although neither soil organic matter nor pH varied significantly with sample point location, there is significant variation in these properties between fields (ANOVA, $p = 0.02$ and $p < 0.001$ respectively). Using a Tukey's Range Test for pairwise comparison (Tukey, 1949), I determined that Field B-1 samples had 1.5% higher organic matter relative abundance than those from Field A-1 ($p = 0.02$). Additionally, Field E-1 was significantly more basic than both Field A-1

(average difference in pH of 1.68, $p < 0.001$) and Field B-1 (average difference of 1.99, $p < 0.001$).

Similar to organic matter and pH, there was no evidence that texture varied between sample point locations (ANOVA, $p > 0.75$). However, testing power was less than 20% ($F < 0.312$) for all three soil texture classes (e.g. sand particles between 0.05-2mm in diameter, silt between 0.002-0.05mm, and sand >0.002mm, Weil & Brady, 2017, 39), indicating that experimental design limited the significance of the results. Although texture did not vary significantly between sample point locations, Field E-1 had significantly higher clay content than Field A-1 (Tukey HSD, mean difference of 4.98%, $p = 0.03$). Additionally, Fields A-1 and E-1 were much sandier than Field B-1 (with mean respective differences of 9.90% with $p = 0.02$, and 8.04% with $p = 0.07$). Accordingly, field B-1 tended to be much siltier than either Field E-1 (mean difference of 8.39% with $p = 0.05$) or A-1 (mean difference of 11.50% with $p = 0.01$). All samples displayed high proportions of silts and clays (figs. 3.3 and 3.4) (National Resources Conservation Service, Soils, n.d.). Field A-1 was the most diverse, with samples falling in the clay, silty clay, and silty clay loam texture classes. Field B-1 samples fell entirely under the silty clay class, while Field E-1 samples fell between clay and silty clay.

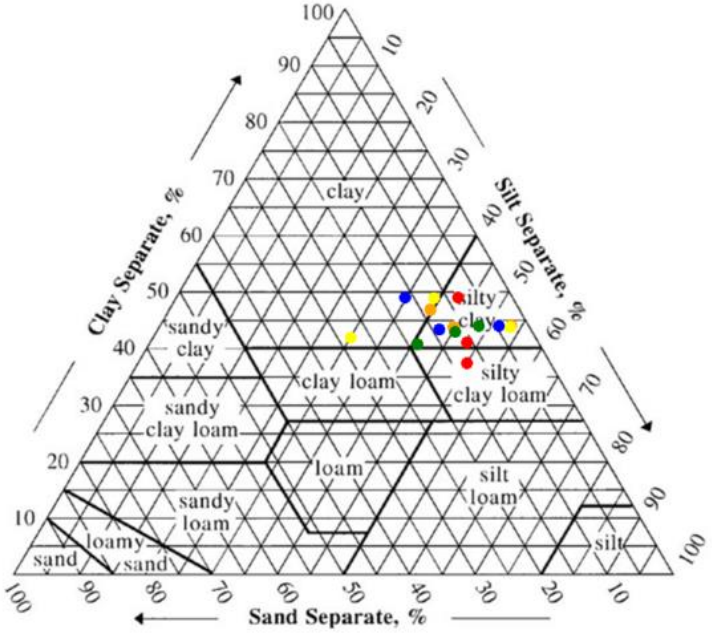


Figure 3.3.. Soil texture pyramid representing the proportions of sand, clay and silt present at each sample point. Inner prairie sample points are red, outer prairies are orange, edges are yellow, outer fields are blue, and inner fields are green.

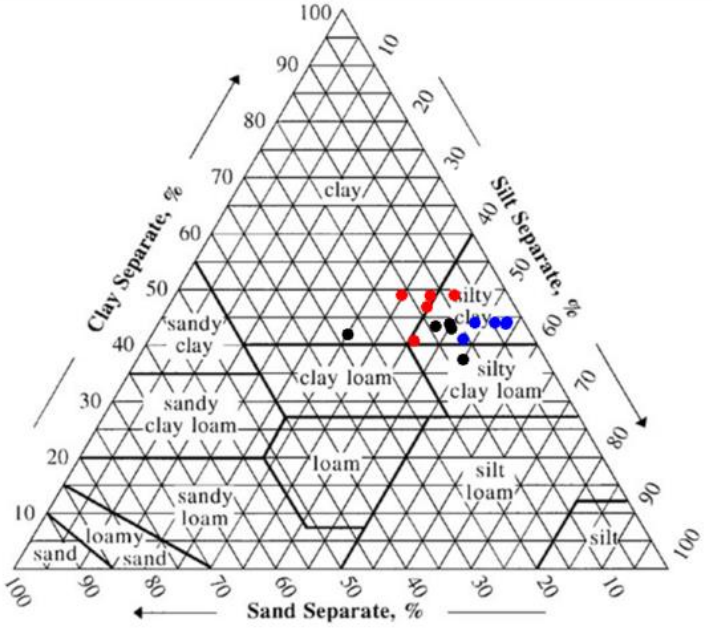


Figure 3.4. Soil texture pyramid (similar to Figure 3.5) by field; samples from Field A-1 are black, Field B-1 are blue, and Field E-1 are red.

b. Evaluating extraction kit success

The Qiagen DNeasy PowerSoil Pro Kit was more successful at extracting high quality DNA from the soil samples than the LSBio Soil Genomic DNA Isolation Kit. The minimum concentration necessary for the LS Sciences 16s rDNA analysis is 20 ng/uL nucleic acid concentration; the Qiagen kit produced an average of 89.3 ± 61.4 ng/uL, while the LSBio kit produced an average of 14.6 ± 18.3 ng/uL. All samples from the Qiagen kit were within the nucleic acid concentration range necessary for downstream processing (Tables 3.2 and 3.3).

	Mean nucleic acid concentration (ng/mL ± s.d.)	
Field	**LSBio**	**Qiagen**
A-1	12.22 ± 17.57	61.98 ± 46.73
A-2	16.80 ± 14.07	71.62 ± 47.94
B-1	9.42 ± 3.81	66.12 ± 39.87
B-2	20.14 ± 9.47	113.50 ± 50.76
E-1	5.13 ± 0.49	82.72 ± 37.95
E-2	24.16 ± 38.84	139.96 ± 106.03

	Mean nucleic acid concentration (ng/mL ± s.d.)	
Site	**LSBio**	**Qiagen**
Inner field	9.21 ± 6.38	43.62 ± 26.15
Outer field	8.48 ± 6.94	57.52 ± 23.41
Edge	13.17 ± 14.01	76.30 ± 41.12
Outer prairie	17.75 ± 15.90	122.13 ± 57.19
Inner prairie	24.62 ± 34.53	147.02 ± 80.33

Tables 3.2 (left).Mean nucleic acid concentrations (ng/uL) at the field level in the elutions for the LSBio and Qiagen extraction kits.
Table 3.3 (right). Mean nucleic acid concentrations (ng/uL) at the sample point level in the elutions for the LSBio and Qiagen extraction kits.

Further, the Qiagen kit extractions had insignificant differences in nucleic acid concentrations between fields (p=0.27). The least similar field was field E-2, which had an average absolute difference in concentration of 60.77 ng/uL from the other five fields (Tukey HSD). Field E-2 also displayed the largest range of nucleic acid concentrations (36.7 - 280.7 ng/uL, fig. 3.5).

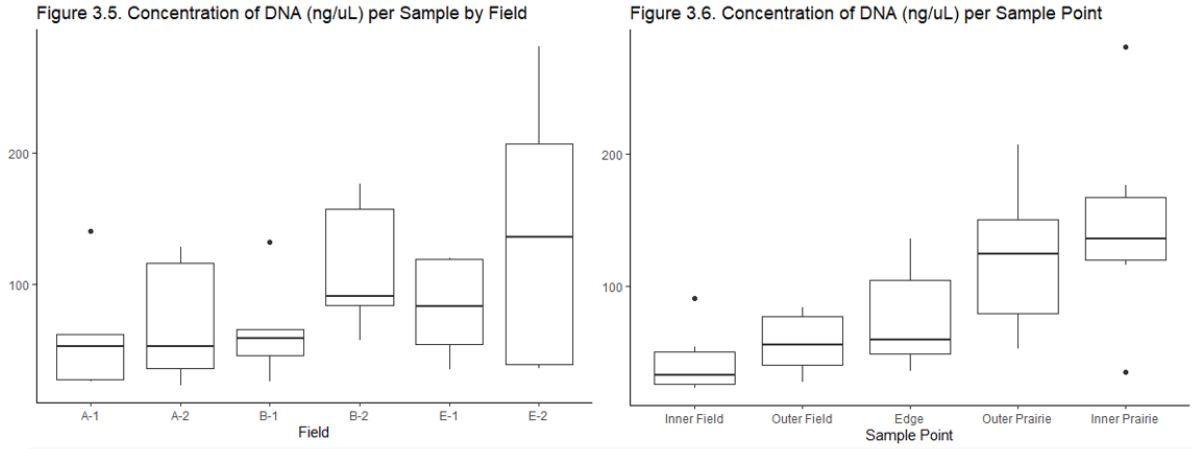


Figure 3.5 (left). A boxplot displaying the distribution of nucleic acid concentration (ng/uL) per field in the Qiagen kit extractions (n = 5).
Figure 3.6 (right). A boxplot displaying the distribution of nucleic acid concentration (ng/uL) per sample point in the Qiagen kit extractions (n = 6).

Finally, the extractions displayed significantly variable nucleic acid concentrations along the gradient between corn field to restored prairie (ANOVA, $p < 0.01$). The mean extraction concentration was lower at the inner field sample points, and increased with proximity to the restored prairie, peaking at the inner prairie. The inner prairie and inner field sample points had an average difference in concentration of 103.40 ng/uL (Tukey HSD, p=0.01), suggesting either that the Qiagen kit was more effective at extracting DNA from sample points in the prairie compared to the agricultural sample sites, or that there was more extractable DNA present at the prairie sample points.

Twenty-six of the thirty Qiagen kit extractions passed quality control at LC Sciences for downstream 16s rDNA processing. The unsuccessful samples were the edge and inner field sample points of Field B-2, and the inner field and inner prairie sample points of Field E-1.

c. Alpha biodiversity

	Mean OTUs ± s.d. per field		
Sample point	**A**	**B**	**E**
Inner Prairie	1991.5 ± 44.6	2077.0 ± 121.6	2127.0 ± NA
Outer Prairie	2081.0 ± 45.3	2144.5 ± 6.4	1701.0 ± 49.5
Edge	1770.0 ± 401.6	2031.0 ± NA	2210.5 ± 142.1
Outer Field	2226.5 ± 44.6	2130.0 ± 67.9	2130.0 ± 67.9
Inner Field	1904.0 ± 2.8	2077.0 ± 121.6	1864.0 ± NA

Table 3.4. The distribution of observed taxonomic units (OTUs) per sampling point in each field (n = 2). Missing values are due to the four samples that did not pass 16S rDNA quality control.

Alpha biodiversity is a measure of community structure, including species richness and/or evenness (Willis, 2019). The first metric I used was an operational taxonomical unit (OTU) measure for species richness, which compares observed rDNA sequences from 16S methods to a reference database of known sequences (i.e. reference-based alpha diversity, Edgar, 2017, 3889). I also analyzed species evenness using the Shannon Index for population evenness between samples (Shannon & Weaver, 1949).

I determined that there was no significant difference in OTUs between replicates within fields A, B, and E (Tukey HSD, $p > 0.98$). Therefore, I was able to consider both field replicates (A-1/A-2, B-1/B-2, and E-1/E-2) together, to have a sample size per field of $n = 10$, with $n = 2$ per sample point in each field (Table 3.4).

Considering the fields with larger sample sizes enabled me to use an interaction Multivariate Analysis of Variance test (MANOVA, Barker & Barker, 1984). Neither the relationship between OTUs and field (ANOVA, $p = 0.99$, $F = 0.01$, power = 0.05) nor OTUs and sample point ($p = 0.48$, $F = 0.90$, power = 0.96) were significant. However, there was significant variance between field and sample point locations with OTUs (MANOVA, $p = 0.03$). This suggests that species richness per sample is highly heterogenous for the combination of sample points and field.

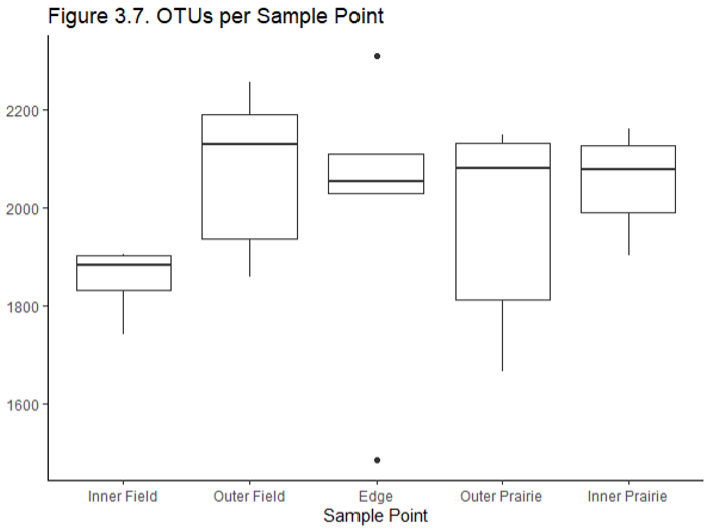


Figure 3.7. A boxplot depicting the distribution of observed taxonomic units (OTUs) between sample points (n=6). Because the variation in OTUs between field replicates was insignificant ($p > 0.98$), both replicates for fields are considered together.

Further, there was no significant difference in OTUs across sample points (ANOVA, $p = 0.48$), the sample points with the greatest mean absolute difference were the average decrease of 223.42 OTUs between the inner and outer field points (Tukey HSD, 223.42 OTUs, $p = 0.42$), and the average decrease of 199.05 OTUs between the inner prairie and inner field sample points ($p = 0.57$). Although neither of these differences were statistically significant at the 0.05 level, and the experimental design had a high true significance rate (power = 0.96), the pattern in decreased species richness with distance from the prairie into the corn field is notable.

Next, I analyzed the variance in Shannon Indices between sample points and fields. Similar to my prior analysis with OTUs, there was no significant difference between the two replicates within each field (Tukey HSD, $p > 0.99$), so I was able to consider the replicates as one sample field ($n = 10$ per field, and $n = 2$ per sample point in each field, Table 3.5).

	Mean Shannon Index ± s.d. per field		
Sample point	**A**	**B**	**E**
Inner Prairie	10.12 ± 0.12	10.16 ± 0.10	10.09 ± NA
Outer Prairie	10.15 ± 0.02	10.22 ± 0.01	9.79 ± 0.12
Edge	9.84 ± 0.32	10.17 ± NA	10.22 ± 0.07
Outer Field	10.22 ± 0.10	10.22 ± 0.10	10.15 ± 0.06
Inner Field	9.94 ± 0.04	9.97 ± NA	10.04 ± NA

Table 3.5. The distribution of Shannon Indices by sample point per field (n = 2). Similar to Table 3.4, there is no standard deviation for the edge and inner field of B, or for the inner prairie and field of E, due to failures in 16S rDNA processing (n = 1).

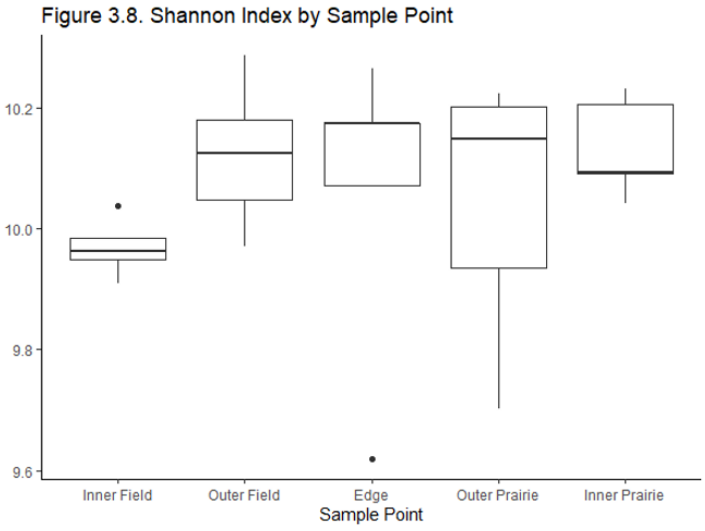


Figure 3.8. A boxplot demonstrating the variance in Shannon Index distribution for each sample point (n=6).

Shannon index values were also highly heterogenous between sample points and field (MANOVA, p = 0.03). Similar to species richness measured by OTUs, neither the variance of sample point (ANOVA, p = 0.60) nor field (p = 0.71) alone was statistically significant, although this could be influenced by the lower true significance probabilities of the experimental design (F < 0.70, power < 0.40). However, once again, the greatest absolute mean differences in Shannon

Indices between sample points were between the inner prairie and inner field at 0.17 (ANOVA, $p = 0.60$), and between the inner field and outer field at 0.15 ($p = 0.63$). This supports the OTU trends in species richness; community species evenness also tends to decrease with distance from the prairie into the corn field.

d. Verrucomicrobia abundance and presence

The final variable I investigated for this thesis was verrucomicrobia abundance, which is represented by the proportion of species in each sample that is in the verrucomicrobia phylum. There was no significant difference in verrucomicrobia abundance between replicates A-1/A-2 and B-1/B-2 (Tukey HSD, $p > 0.99$). Thus, as before, I was able to consider these replicates as field A and B, increasing my available degrees of freedom from $n = 5$ per field to $n = 10$. Although Fields E-1 and E-2 showed slightly more variance ($p = 0.47$), because it is not significant at a 0.05 level, I also combined these fields together as Field E (Table 3.6).

	Mean % Verrucomicrobia abundance ± s.d. per field		
Sample point	**A**	**B**	**E**
Inner Prairie	2.28 ± 0.32	2.87 ± 0.82	3.82 ± NA
Outer Prairie	2.29 ± 0.50	2.94 ± 0.19	3.97 ± 2.32
Edge	2.10 ± 1.01	3.82 ± NA	2.59 ± 0.30
Outer Field	1.29 ± 0.01	1.52 ± 0.80	3.56 ± 1.08
Inner Field	1.15 ± 0.14	2.83 ± NA	2.90 ± NA

Table 3.6. The distribution of Verrucomicrobia relative abundances by sample point per field. The missing values are due to failure of four samples to pass quality control for 16S rDNA analysis.

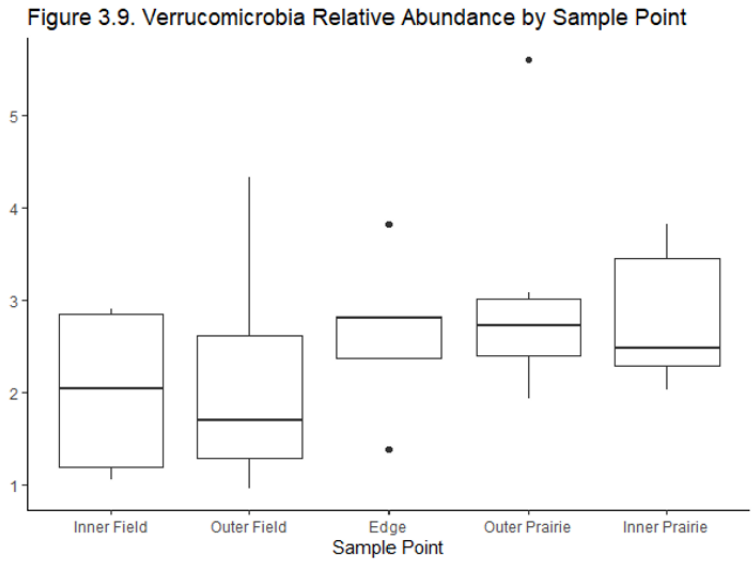


Figure 3.9. A boxplot demonstrating the variation in Verrucomicrobia relative abundance (%) by sample point (n = 6).

There was no significant correlation between sample point location and verrucomicrobia relative abundance (ANOVA, $p = 0.49$, $F = 0.89$, power = 0.96). The sample points which displayed the greatest mean difference in verrucomicrobia relative abundance were between sample points P1 and F2 with a mean difference of 1.06% abundance (Tukey HSD, $p = 0.57$), and between P1 and F2 with a mean difference of 0.94% ($p = 0.58$). Unlike in the alpha diversity analysis, there was no significant interaction between sample point, field, and verrucomicrobia abundance (MANOVA, $p = 0.65$). However, verrucomicrobia relative abundance differed significantly across the fields (ANOVA, $p < 0.01$). In particular, mean relative abundance difference was 1.55% higher in Field E than in Field A (Tukey HSD, $p < 0.01$).

4. Discussion

One of the salient results from this thesis was the correlation between verrucomicrobia abundance and soil texture. Verrucomicrobia relative abundance was greatest in Field E, which had the highest clay texture proportions of the three fields analyzed for this thesis. This led me to consider the relationship between soil clay content and verrucomicrobia abundance (fig. 4.1).

Figure 4.1. Trends in abundance of Verrucomicrobia with soil clay content

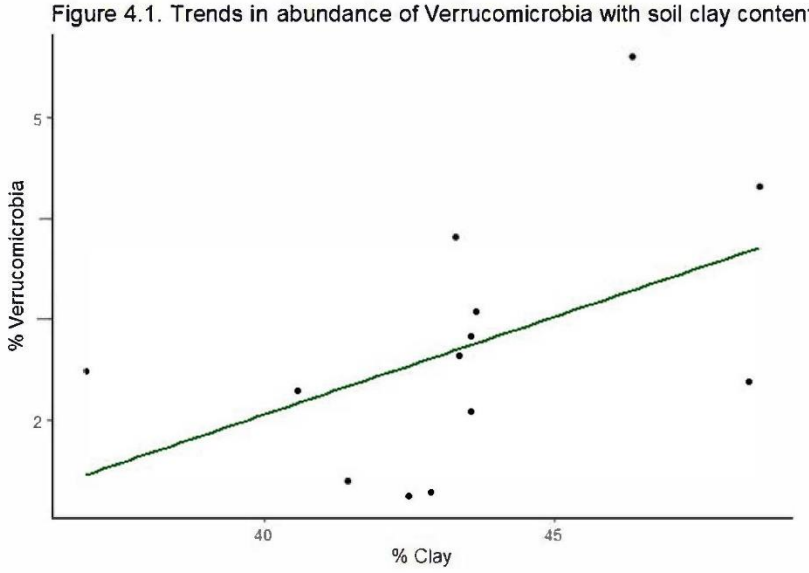


Fig 4.1. A scatter plot demonstrating the correlation between clay content and verrucomicrobia phylum abundance in the Liberty Prairie soil samples. $y = -5.70 + 0.19x$, $R^2 = 0.23$, and $df = 11$.

The linear correlation between clay content and verrucomicrobia relative abundance was significant at the 0.1 level ($p = 0.10$). This finding supports a Biesgen *et al*. (2020) study that found a positive correlation between clay content and distinct bacterial communities in agricultural soil, which they attributed to higher clay content influencing increased aggregate stability. A similar impact could be occurring in these soils in northern Illinois, which would be an interesting topic of further study.

Additionally, verrucomicrobia abundance was positively correlated with soil alkalinity (LR, $p = 0.14$, fig. 4.2).

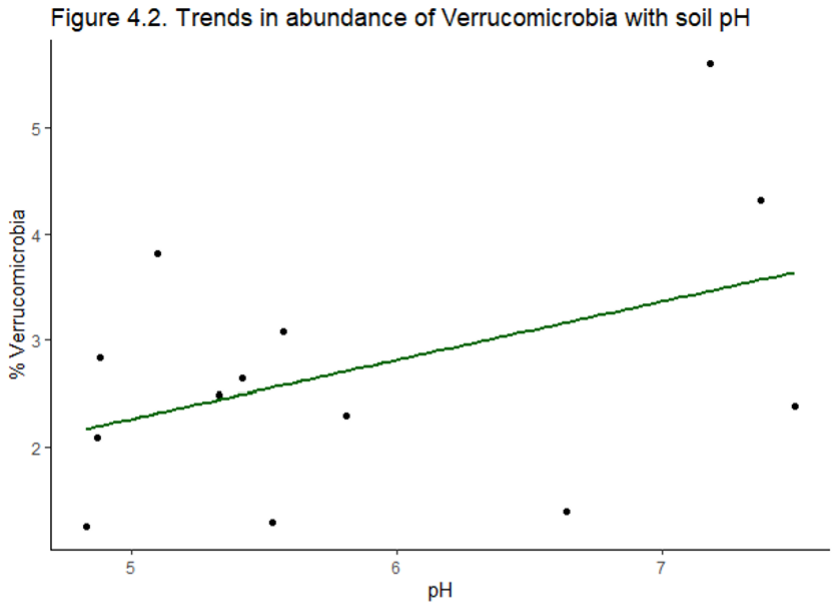


Figure 4.2. A scatter plot visualizing the linear correlation between verrucomicrobia abundance and pH in soil samples from the Liberty Prairie. y = 0.55x – 0.50, R-squared = 0.19, p = 0.14.

This positive correlation could be related to the correlation between clay abundance and verrucomicrobia presence discussed above, because clay minerals can bind to hydrogen molecules and decrease acidification rates (Voroney & Heck, 2015, 28). My field-level data supports this phenomenon, as Field E was significantly more alkaline than Fields A (Tukey HSD average difference in pH of 1.68, $p < 0.001$) or B (average difference of 1.99, $p < 0.001$). Field E also had the highest average abundance of clay particles, with an average clay abundance 5.0% greater than Field A (Tukey HSD, $p = 0.03$) and 3.5% greater than Field B ($p = 0.14$). Besides its interactions with clay, pH can also influence the solubility and ionization of soil solution constituents, which would impact nutrient (and potentially toxin) availability to microbes, thus limiting their biomass (Voroney & Heck, 2015, 29).

Sample point	Verrucomicrobia relative abundance ± s.d.
Inner prairie	2.81 ± 0.77
Outer prairie	3.07 ± 1.31
Edge	2.64 ± 0.88
Outer field	2.12 ± 1.27
Inner field	2.01 ± 0.99

Table 4.1. The relative abundance of verrucomicrobia across sample points in three Liberty Prairie fields.

Finally, the range in verrucomicrobia abundance in the restored prairie soils was much lower than expected based on Fierer *et al.* (2013)'s study on microbial communities in prairie remnants. This study found that verrucomicrobia represented >50% of 16s rDNA bacterial sequences in mid-latitude prairies (generally from Oklahoma to southern Minnesota), and <15% of the sequences on the edges of the prairie range (623). The reconstructions of pre-agricultural prairie microbiomes predicted ~30% verrucomicrobia abundance in northwestern Illinois, and ~15% abundance in central Illinois. Therefore, I hypothesized that the verrucomicrobia abundance in Liberty Prairie would fall within that range. However, the verrucomicrobia abundance in the Liberty Prairie samples were much lower than this expected range, with the furthest prairie sites averaging 2.81% ± 0.77% verrucomicrobia abundance. Ramirez, Crane & Fierer (2012) corroborate these findings, concluding that verrucomicrobia abundance tended to decrease with nitrogen fertilization across a range of North American regions. This suggests that in the restoration sites at Liberty Prairie, verrucomicrobia populations have not recovered from the pre-restoration conventional agricultural practice of nitrogen addition. This is troubling considering the relatively high abundance of verrucomicrobia in remnant soils and could suggest that the restored prairie soils at Liberty Prairie do not have the same microbial niches as prairie remnants in other regions of Illinois.

I was able to compare the physical characteristics of my soil samples to another study by Kucharik et al. (2006) at the University of Wisconsin Arboretum (Kucharik, Fayram, & Cahill,

2006). Although this study takes place approximately 100 miles northwest of the Liberty Prairie Reserve, its analysis of soil physical characteristics between restored and remnant prairie sites is comparable to the Liberty Prairie restoration (Table 4.2).

Characteristic	Prairie remnant, Wisconsin	Restored prairie, Wisconsin	Inner prairie sample, Liberty Prairie	Outer prairie sample, Liberty Prairie
pH	6.65	5.19	6.06	6.06
Clay %	14	9	41.99	44.45
Silt %	49	58	46.36	45.28
Sand %	34	33	11.65	10.27
Series	Silty clay loam	Silty clay loam	Silty clay	Silty clay

Table 4.2. Comparison between prairie remnant and restoration in south-central Wisconsin (Kucharik et al., 2006) with physical soil characteristic data from the Liberty Prairie, northeastern Illinois.

There are some notable differences between the sites in southern-central Wisconsin and the site at Liberty Prairie that likely impact the physical soil characteristics at each site. First, while the Liberty Prairie conservation program has been in place since 1991 (Conserve Lake County, 2013, 8), the University of Wisconsin's Curtis Prairie restoration project began in 1934 and is notable as the oldest prairie restoration in the United States (Kucharik, Fayram & Cahill, 2006, 122). Therefore, the University of Wisconsin has managed the Curtis Prairie site for sixty years longer than Conserve Lake County has managed the Liberty Prairie, potentially influencing stages of restoration succession. Second, the soil at the University of Wisconsin sites is part of a different soil series, with silty clay loam compared to the silty clay at the Liberty Prairie. Accordingly, the textures of the soils between the two regions are slightly different (Table 4.2), with the Liberty Prairie sites having higher clay and lower sand proportions than the University of Wisconsin sites. The relatively shorter restoration timeline or differences in parent material could influence these soil texture differences. While the soils in both regions are the result of glacial deposits, variations in glacial retreat between northern Illinois and southern-central

Wisconsin could cause this difference in soil texture. Finally, the pH of the soil at the Liberty Prairie is between the remnant and restored prairie pHs at the University of Wisconsin (Table 4.2). Management choices or pedogenic processes could cause these differences in pH, and there is a clear trend that soils across all three prairies are slightly acidic.

Another compelling trend is that both alpha diversity richness and evenness tended to be lower in the inner field sample points (6m into the corn field) than in sample points in closer proximity to restored prairie habitats. This supports the substantial body of research that suggests conventional agriculture methods (heavy tilling, pesticide and insecticide application, and nutrient fertilization) have negative impacts on the health of the soil microbiome. Carbonetto *et al.* (2014) found that microbes in tilled systems in Argentina shifted life strategies and became less resilient than in untilled systems, making them more vulnerable to perturbation. Additionally, although several herbicides have been found to have slightly bacteriotoxic effects, very little is understood about how common herbicides, pesticides, and insecticides impact the soil microbiome in agricultural settings (Sanchez-Barrios, Sahib, & DeBolt, 2017, 90). My data demonstrated a trend of lower species richness and evenness at the inner field sample point (depth of 6m) than at sample points with closer proximity to the prairie. This suggests that the benefits of having restored prairie habitats bordering agricultural fields could extend beyond the edge of the prairie, positively impacting the field-edge microbiome with heightened alpha diversity. To further verify this observed trend, I suggest future work might compare baseline measurements of corn field microbiome alpha diversity to that at the edge of the field, near restored prairies.

Overall, the bulk of the analyses for this thesis was statistically insignificant, although qualitative trends fit my hypotheses. This is likely due to limitations in sample size (n = 26

successful 16s rDNA sequences, and n = 15 samples for physical soil tests). Due to the high granularity of soil microclimates, as demonstrated by the significant ($p < 0.05$) heterogeneity of alpha diversity between fields and sample locations, it is vital to have a robust sample structure with multiple replicates, which was not possible within the scope of this project. Secondly, the samples I collected for DNA extraction and downstream 16s rDNA sequencing were in a 4°C fridge for two months. While it is standard practice to snap freeze soil samples intended for DNA extraction with liquid nitrogen and store them at -80°C (Pavlovska *et al.*, 2021), recent studies have found that samples stored at 4°C for one month have performed well in measurements of quantity and integrity of extracted DNA (Pavlovska et al., 2021 & Delavaux *et al.*, 2020). Because my samples were in a 4°C environment for over twice that time, I am unsure of how storage temperature impacted downstream 16s rDNA sequencing. However, based on the studies from Pavlovska *et al.* (2021) and Delavaux *et al.* (2020), I anticipate that the impacts were minimal in proportion to the scope of the project. Notably, the Pavlovska *et al*. (2021) study found that samples stored in non-ideal conditions (i.e. not snap-frozen in situ and stored at a temperature greater than -80°C) had improved quality when treated with a DNA-preserving solution. For future research, I suggest using such a preservation solution when possible, to minimize impacts of sample degradation during shipping and storage.

5. Conclusion

Preliminary analysis of the microbial diversity on the edges of restored prairies and corn fields at the Liberty Prairie Reserve yielded mixed results for the success of the restoration effort in building robust microbial diversity that are similar to that of prairie remnants. Correlations between clay content, pH, and verrucomicrobia relative abundance suggested that restored agricultural soils with enhanced aggregate stability could benefit the re-establishment of

verrucomicrobia in amended soils. Additionally, the low relative abundance of verrucomicrobia in the restored prairie soils compared to expected levels suggests that the phylum's abundance may not have recovered from pre-restoration nitrogen additions to the soil for agricultural purposes. Finally, alpha diversity (both richness and evenness) tended to decrease with distance into the corn field, suggesting that the benefit of prairie restoration in heightening alpha diversity extended past the edge of the restoration plots. While the Liberty Prairie restoration project appears to have been successful in restoring the alpha diversity of former agricultural soils, evidence suggests that the assemblages of prairie bacterial communities (such as verrucomicrobia) have not returned to their pre-agricultural state.

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Appendix: Data access

The data and code I used for this thesis are available at https://github.com/aburns2293/BurnsThesis2022.