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Comparison of Urban and Agricultural Soil Microbial Diversity and Assessing the Potential for Remediation Using a BEAM Composting Bioreactor

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Abstract

Urban areas play an increasingly important role in human society with more than half of the global population living in urban areas and increasing to 68% by 2050. Urban soils are considered highly disturbed, anthropogenic in origin, and often contaminated and significantly altered by human activity. However, they are of increasing interest as a resource for food production, for mitigating heat island impacts, and for population health. Soil microbial diversity is the functional foundation of terrestrial ecosystems. This project compared soil microbial diversity of urban soil, a commercial farm, and a high intensity, small acreage regenerative farm. Also investigated was microbial diversity generated by organic composting as a means of developing a high microbial resource for regenerating soils. Methods included assessing soil and compost samples for environmental parameters, fungal and bacterial diversity using agar smear plates, PCR amplification, and DNA genomic sequencing. Loss-On-Ignition identified patterns in organic matter content and soil moisture with the regenerative farm soil highest in each. Visual analysis of agar plate colonies revealed similarities and stark differences in microbial abundance and diversity. These patterns are supported by the 16S rRNA gene analysis results which identified the diversity and abundance of bacteria highest in the compost and regenerative soils. The compost has exponentially more microbial diversity when compared to soil from commercial and urban lots. An unexpected result was the degree of impoverishment of the commercial farm soil. Soil microbial composition from a variety of settings provides critical information for regenerating soils and potential urban food production.

Keywords

Soil Microbial Diversity, Commercial Agriculture, Regenerative Agriculture, Urban Soils, DNA Genomic Sequencing, Soil Health, Kansas City

1. Introduction

Urban areas play an increasingly important role in human society as 56.61% of people globally lived in urban areas in 2021, with this number expected to increase to 68% by 2050 [1]. Healthy biodiverse soils are critical for supporting food production, managing carbon, and providing healthy environments for both urban and rural agricultural communities [2]. However, testing of urban soil reveals the soil severely lacks biomass and will not be effective for urban farming, or water filtration and retention. Urban soils are a growing focus for population health and the need for healthy urban agriculture [3].

Remediated urban soils are an important resource for food production, mitigating urban heat island impacts, and human health. Urban soils will need to be integrated into food production to keep up with growing populations and inflation of food costs, address urban food deserts, and provide healthy produce. Mostly ignored by modern descriptive soils science, urban soils are considered too mixed, too contaminated, or too removed from natural soil processes to be of interest or value [4] [5]. Urban soils are a complex matrix of degraded soil, contaminants, and fill. They have traditionally been ignored world-wide because of their anthropocentric origin, and environmental and engineering sciences have not effectively accounted for them in current soil classifications [5] or management [6]. When addressed, the contamination aspect of urban soils has been the focus of the EPA Urban Brownfields Program since 1995 with the goal of contamination clean-up and enhancing community health and safety [7]. As such, soil health is an environmental health issue at the nexus of climate change, environmental degradation, and food access [8].

Agricultural soils are the foundation of global food production, but only since the end of World War II has western agriculture become commercial in scale. Commercial agriculture is the industrialization of food production for markets based largely on technological advancements, mechanization, and chemical fertilizers. The development of the Haber-Bosch process of producing nitrogen fertilizer transformed generations of farming. This industrial process of nitrogen production has fed half the world with short-term increases in biological production; however, the tradeoffs are increasing soil acidification, salinization, eutrophication, nutrient run-off, reduced biological diversity, and substantial greenhouse gas (GHG) emissions. Degradation of soils is one of the greatest consequences of human land-use and climate change, impacting not only food production, but also greatly reducing soil as a healthy source of carbon sequestration [9].

The foundation of terrestrial ecosystems is soils and soil microbes are central to

their formation and functioning. Soils contain some of the most diverse microbiomes on Earth. Yet, the role of soil microbes remains little understood [10]. The physical and chemical indicators traditionally used to determine soil health are not exclusively a product of physical and chemical characteristics [10]. They do not reflect the health of soil microbial communities, which in turn hampers our understanding of their ecosystem functions and long-term health of soils. In comparison, a whole ecosystem approach considers the extensive ecology of the soil microbiome, providing a clearer picture of the soil's health and multiple functions. This is because fungi and bacteria within the soil are increasingly recognized as critical for increasing crop yield, providing and cycling essential nutrients, preventing erosion, encouraging carbon sequestration, and maintaining the soil's overall ecosystem [11]. Understanding the complexity of soil microbial communities produced by a range of agricultural settings informs best practices for restoring soil health for food production and community health in the urban environment.

To sustainably remediate soils more precise indicators of soil ecosystem health and function are needed. This project developed a baseline of soil microbial communities from a range of agricultural and environmental settings. The soil sampling sites include: a commercial farm growing soybean and corn crops, a high intensity, small acreage regenerative organic farm, three fungal dominant composters, and soil from two vacant urban lots. Our whole ecosystem approach to assessing soil health included environmental parameters and microbial identification [12]. The significance of this research is the potential for transformative perspectives on urban soil health and potential impacts on public health in impacted urban settings, especially for children, and increasing the capacity for healthy urban soils and agriculture. This study compares soil environmental characteristics and soil fungal and bacterial composition from two different farming approaches (commercial and regenerative), organic compost, and urban soil to characterize microbial diversity and abundance as a measure of soil health.

2. Materials and Methods

2.1. Project Background

Management of soil impacts microbial biomass, which is a very sensitive indicator of soil biological health. Yet it is important to recognize soil as a dynamic living system and move beyond individual parameters for characterizing soil health [13]. Defining commercial farming is very broad but generally includes large-scale agriculture, use of technology to increase efficiency and production, and crop specialization. Many of the commercial agricultural techniques used today have increased crop yields due to new genetic crop varieties, mechanical farm equipment, and the expanded use of agri-chemical inputs. Monocultures, the planting of a single type of crop through two crop cycles, is another prominent feature of commercial farming operations. In the United States, the typical monoculture operations produce crops such as corn and soybeans. These commercial practices de-

grade soil health, require large amounts of chemical nutrient inputs, don't consider soil ecosystem function, and in turn severely alter the microbial community.

Regenerative agriculture refers to the need to not only lessen impacts of commercial farming approaches but rebuild the health and diversity of the natural soil base. There is significant difference between the methods used by commercial agriculture and regenerative agriculture, which results in different effects on microbial biodiversity, invertebrate species diversity, soil health, nutrient density/cycling, and water usage [14]. Regenerative practices can be broken down into two principles: 1) reducing uniform disturbances, and 2) increasing diversity, specifically the diversity of plants, microbes, invertebrates, and revenue streams. Regenerative practices are also broad, being adaptable and operation specific, but include practices such as reduced tilling, cover cropping, diverse crop rotations, intercropping, livestock integration, compost, and reduction of synthetic agrichemicals common in commercial agriculture. Regenerative techniques prioritize organic matter, soil biology, ecological processes requiring fewer inputs, use resources more effectively, and they have been found to be more profitable [15]. Few studies exam soil microbiomes much less compare commercial agriculture, regenerative agriculture, urban soil and compost microbiomes. Regenerative farming methods have high potential to be used in urban locations due to their small scale and abundant potential sources of organic material [16]. Characterizing and comparing soil microbial diversity under different approaches could provide strategies for revitalizing degraded urban and commercial agricultural soils and supporting urban agriculture.

Microbial biomass in soil is crucial to many pathways and processes. They transform elements within the soil such as nitrogen, calcium, sulfur, zinc, phosphorus, and magnesium [17]. This nutrient cycling deconstructs deceased animals and plant matter to allow living plants access to these nutrients via root uptake [18]. Bacteria produce polysaccharide gels which bond soil aggregates reducing erosion [19]. Filamentous hyphae from fungi also bind soil particles together, which aids in stability and carbon sequestration [20]. Diversity of the microbial biomass aids in the prevention of plant [21]. There is a strong correlation between fungal diversity and the function of the soil ecosystem, with fungal dominant soils flourishing.

During composting microbial diversity varies with the variety of composting materials [22]. Composting of green waste (GW) organic matter as a source of natural nutrients and regenerating degraded soils is little studied. Green waste is leaf litter, grass debris, and branches which is a common source of organic matter in urban areas. Little is known of microbial communities and dynamics involved in the processes of compost [23].

The commercial farm (know from here on as commercial), located in Grain valley, MO (**Figure 1**), is mostly flat with no prominent hills and is only slightly sloped near the shoulder of the roads bounding the field. This field floods regu-

larly and was unable to support corn from 2016-2022 so soybeans were the only crop grown on the field from 2016-2022. Before 2016 the field produced optimal yields each year corn was planted and tested well regarding the chemical and nutrient content compared to other monoculture plots. The farm planted corn on April 24th, 2022. For fertilization the farm uses municipal waste (sewage) from Blue Springs, which is neutralized and applied 7 - 8 inches down into the soil during the fall. Nitrogen fertilizer combined with humic acid was applied using a self-propelled sprayer on May 22nd, 2022. The soil was tilled in spring of 2023, but not 2022. No cover crops have been applied on this plot. No fungicides had been applied during the growing season prior to soil sampling.

The Kansas City Farm School in Kansas City, KS was the source of the regenerative farm and compost soil samples. The field sampled has been under organic practices such as no-till, organic compost amendments, and crop rotation, for 30 years. On-site Biologically Enhanced Agriculture Management (BEAM) composters [24] constructed in the fall of 2019, 2020, and 2021 are housed within a large greenhouse were labeled 1—newest, 2—middle, and 3—oldest and sampled in August of 2021.

The urban soil samples were collected from two empty urban lots; one in a neighborhood in Kansas City, KS, and one on a corner in Kansas City, MO. Both lots consisted of a mix of compact dirt and weed patches.

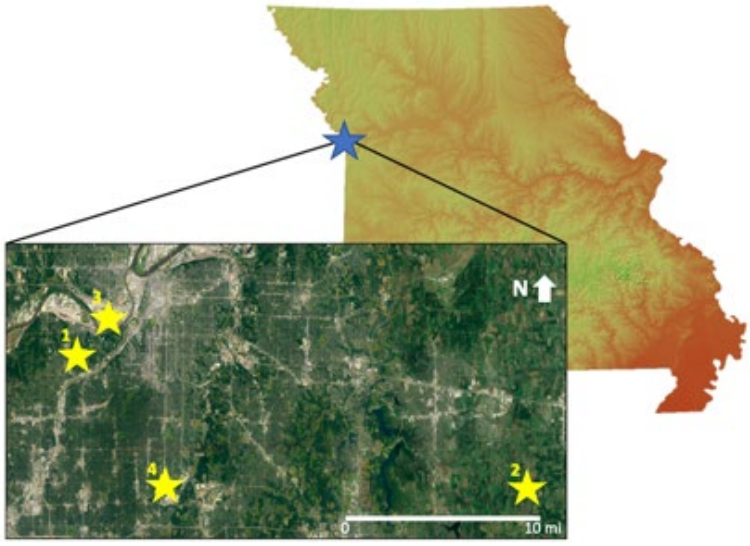


Figure 1. Site map showing the soil sample locations from the Kansas City metropolitan area and surrounding rural area. 1. KC Farm School regenerative farm and compost, 2. soybean and corn commercial farm, 3. urban lot in Kansas City, KS, and 4. urban lot in Kansas City.

2.2. Methods

2.2.1. Soil Characterization

The microbial composition of soil samples from differing agricultural settings (commercial and regenerative) and BEAM composters are compared to urban soil

from empty lots as a means of baseline characterization of soil health from a variety of settings. Three soil samples each were collected in fall of 2021 and spring 2022 from a crop row within a corn field at a commercial farm, a crop row from a 30-year-old section of a no-till, regenerative organic urban farm, three BEAM composters, and two vacant urban lots. The soil samples were collected six inches down from the surface or six inches horizontally into the composters, in all locations and weighed approximately five ounces each. Soil samples were collected in multiples of three from the commercial farm and the regenerative farm in August of 2021 and June of 2022. The regenerative farm soil samples were collected specifically from a row used to grow beets in the oldest area of the farm. The composter samples were collected in August of 2021 from the Top, Middle and Bottom of each of the three composters. The urban soil samples were collected in December of 2022.

Soil samples from both farms were deep brown in color and fine grained in texture. No rocks were included in any samples, and minimal to no roots were included from each of sampled plots. Environmental data and samples collected from three BEAM composters, each of a different age. Samples in each were collected from the top, middle, and bottom to explore microbial distribution with depth. Soil organic matter (SOM) is critical to soil health for a range of functions including moisture holding capacity, and yet it typically comprises only 2% to 6% of a soil matrix [25]. Increased soil moisture is linked to higher SOM concentrations [26]. Soil microbes bind moisture within the soil and therefore are critical for retaining moisture [27]. All soils were assessed for soil moisture and organic matter content by Loss-on-Ignition (LOI) following the methods of [28].

2.2.2. DNA Extraction

All soil and compost samples were processed for metagenomic DNA analysis following the bead beating protocol for soil microbial genomic DNA extraction [29]. This method of DNA extraction allows large volume soil samples, up to 100 mg, rather than the small (1mg), more standard DNA extraction protocols. This method allowed the use of large scale soil preparations providing greater probability of detecting species present in low abundance in the soil environment. Briefly, 100 g soil samples were soaked overnight in 100 mL extraction buffer followed by bead beating and SDS membrane disruption to release DNA. Soil and cell debris was pelleted by centrifugation and supernatants containing DNA were transferred to new tubes and DNA was further isolated and purified by phenol/chloroform and chloroform/isoamyl alcohol extraction. DNA was precipitated by isopropanol and resuspended in TE for storage and polymerase chain reaction (PCR).

2.2.3. PCR

For bacterial identification, genomic analysis of prokaryotic 16S ribosomal RNA gene (16S rRNA) was used. This gene region is approximately 1500 bp long and contains nine variable regions interspersed between conserved regions [30]. Variable regions of 16S rRNA are frequently used in phylogenetic classifications such

as genus or species mixed microbial populations [31]. Amplification was across the region of V4, V5, and V6. While 16S hypervariable regions can vary dramatically between bacteria, the 16S gene as a whole maintains greater length homogeneity than its eukaryotic counterpart (16S Forward primer 5' AGA GTG CCA GCM GCC GCG GTA A 3' 16S Reverse primer 5' GCC CCC GTC AAT TCM TTT GA 3').

For fungal identification, the region of ribosomal RNA genes (rRNA) including the genes ITS-1 (Internal Transcribed Spacer), the 5.8S gene, and the ITS-2 region were amplified and sequenced. ITS-1 and 2 are rapidly evolving, highly multicopy regions which harbor enough variability to allow taxonomic discrimination between most fungal species (ITS-1 S Forward primer 5' TCC GTA GGT GAA CCT GCG G 3' ITS-4 Reverse primer 5' TCC TCC GCT TAT TGA TAT GC 3') [32].

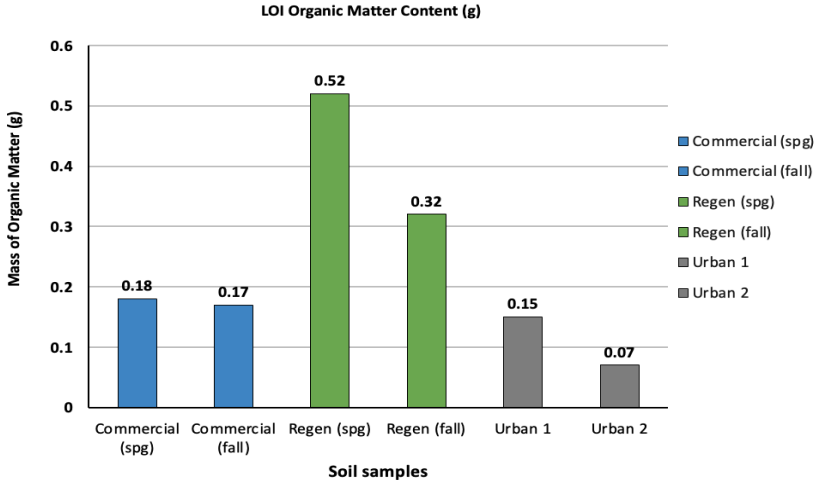
For Illumina sequencing, adapter sequences must be added to both the forward and reverse primers for binding to the flow cell. The forward adapter sequence is 5' ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT 3' and the reverse adapter sequences is 5' GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC T 3'. Successful PCR amplification was checked using agarose gel electrophoresis and quantitated using 260/280 values by Biotek Take3 (Agilent, Santa Clara California, USA) spectroscopy. The bacterial 16S-region and fungal ITS PCR products were purified, prepared for sequencing, and sent to the DNA Core Facility at the University of Missouri-Columbia for MiSeq Illumina sequencing. The MiSeq run output is approximately 20 million reads per plate and can be sent as a group of 96 indexed samples. This process can generate ~200,000 reads per sample. Illumina On-Board Filtering (%PF) consisted of the Chastity Threshold in which clusters pass filter (%PF) if no more than one base call has a chastity value below 0.6 in the first 25 cycles. Quality score benchmarks (Q-Scores) are Q30 Standard: Standard MiSeq runs are expected to deliver at least 80% of bases with a Phred quality score of Q30 or higher (meaning 99.9% base call accuracy), particularly when run with standard v2/v3 chemistry and balanced nucleotide diversity. Samples were run and analyzed by the MU's Bioinformatics Core. Bioinformatic analysis of the sequence results can identify bacterial and fungal taxonomy at the species level. The workflow clustered highly similar sequences into Operational Taxonomic Units (OTUs) at a standard 97% identity threshold. Upon receiving the OTUs they were run through the Basic Local Alignment Search Tool (BLAST) to assign taxonomic identities [33].

Visual examination of microbial composition of the soil samples consisted of streaking on standard microbial nutrient agar plates with solution obtained after soaking the soil in a buffer. One set of plates were made from fungal-specific CHROMagar™ Candida Plus medium [34] and another set of plates were a rich growth medium which supports nonspecific growth. The plates were incubated at 30° C for multiple days until growth occurred. These plates provided a qualitative visual representation of the fungal and bacterial components of each of the soil

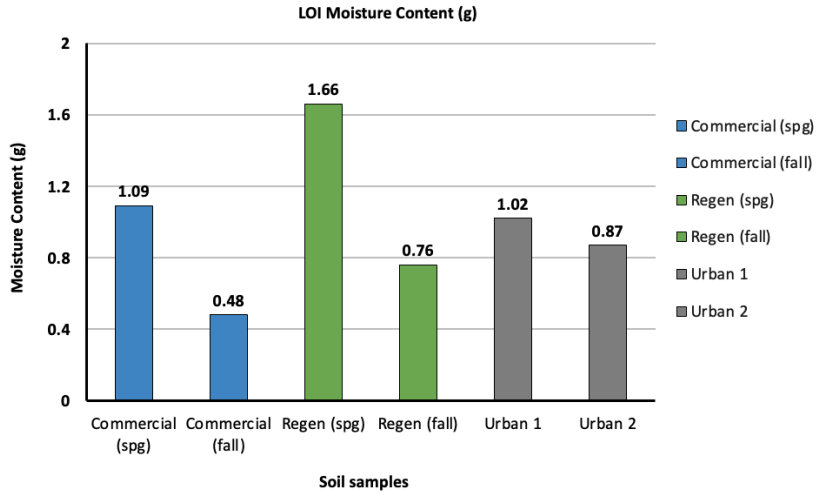
sample locations.

3. Results

The sediment composition of both the commercial and regenerative farm soil samples is silty loam. LOI characterized all soil samples for organic matter and soil moisture. The results indicate organic matter content, as a percentage of the total sample, was highest in the regenerative farm samples in both spring and fall with a decrease in the fall relative to its spring value (Figure 2(a)). The regenerative soil organic matter is 65% higher than the next highest commercial farm samples in both spring and fall and in fall 71% higher than the urban soil. The seasonal difference in organic matter for the commercial farm samples from spring to fall is only a 0.01 percent change, whereas the regenerative farm had a 38% decreased in organic matter. The two urban lot soils had the lowest organic matter.



(a)

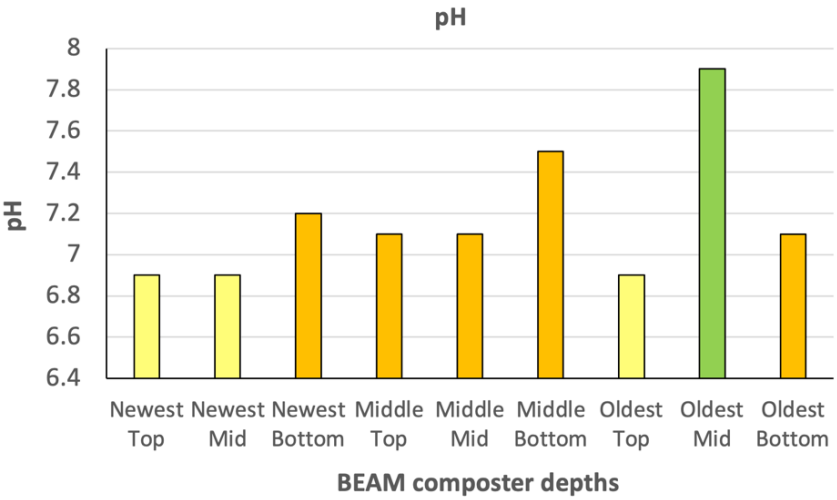


(b)

Figure 2. Results of the Loss-On-Ignition (LOI) analysis of farm and urban lot soil samples for (a) soil organic matter and (b) soil moisture content.

Soil moisture content, determined by LOI in grams, reveals regenerative farm soils had the highest moisture in spring, 34% higher than the commercial farm soil, and 39% higher than the urban lot soil (Figure 2(b)). Both the commercial and regenerative farm soils had a fall reduction in moisture content by approximately half. The two urban lot soils similarly had the lowest moisture content except for the commercial farm in the fall after the growing season. Soil moisture in the urban lots was below the highest commercial moisture in spring and above the regenerative farm's lowest moisture in fall.

(a)



(b)

Figure 3. (a) Photo of BEAM composters (oldest, middle, and newest in age) and the compost sampling depths, top (T), middle (M), and bottom (B). (b) Charts of the range of pH in the three BEAM composters from newest to oldest. Yellow is pH in the 6.8 range, orange in the low to mid 7 pH range, and green is a pH score of nearly 8.

Analysis of compost from the three BEAM composters of different ages characterize the environmental conditions at different depths of each (Figure 3(a)). Environmental metrics collected by soil probe include temperature, moisture, and

pH. The environmental variables are very similar across all units and depths except for pH values (Figure 3(b)). In general, pH ranges from slightly acidic (pH 6.0) to neutral (pH 7.0) in the top samples of all three composters. The newest composter (#1) was slightly acidic until the bottom sample which was neutral. The middle composter (#2) recorded neutral from top to bottom, and the oldest composter (#3) was acidic at the top increasing to slightly alkaline (almost 8.0) in the middle and declining to neutral at the bottom.

CHROMagar and non-specific fungal and bacterial agar plates provide visual comparison of both farms and urban soils which illustrate microbial correlations and similarity between soil settings (Figure 4). Very strong visual similarities in microbial populations occur between the vacant urban lot soil and commercial farm in that both settings lack fungal colony growth and lack bacterial diversity. The commercial and urban soils had virtually no fungal growth, while bacterial growth was similar in type, but limited in diversity. Conversely, the regenerative farm soil plates had very similar visible microbial growth with high fungal and bacterial presence (Figure 4).

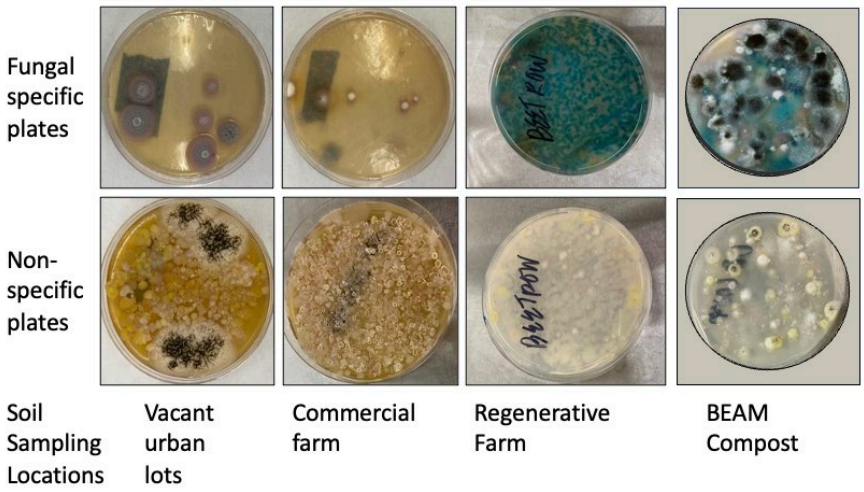


Figure 4. Soil microbial colonies for urban lot, commercial farm, regenerative farm, and BEAM compost for fungal specific and non-specific agar plates.

Additionally, both the regenerative farm fungal and bacterial colonies were very different in type from commercial farm and urban soil colonies. The regenerative fungi are dominated by *Aspergillus* sp., a blue-green, filamentous fungus whose role in soil is recycling carbon and nitrogen. The regenerative and bacterial colonies are potentially Actinomycetes and other bacteria.

The compost fungal specific CHROMagar plates from composter #1 exhibited high fungal and bacterial growth most similar to the regenerative farm soil samples (Figure 4). The plates with by far the most fungal and bacterial growth and diversity are the regenerative farm soil and compost samples. The other soil samples most closely related visually are the commercial farm and urban lot samples, notable for their sparce growth and lack of diversity.

The PCR amplification of fungal ITS (Internal Transcribed Spacer) and bacterial 16S rRNA targets for DNA sequencing demonstrated the presence of multiple species of fungi and bacteria. The fungal ITS only returned very minimal presence. There was a single large spike of *Clavispora lusitaniae* in the Urban lot #2 soil. *Clavispora lusitaniae* is an environmental saprophytic yeast commonly found in soil. The commercial farm soil recoded a presence of *Aspergillus niger* and *Fusarium equiseti*. *Aspergillus niger* is both a naturally occurring fungus and also a common agricultural amendment, although the farm stated they had not added amendments to the soil. *Fusarium equiseti* is a cosmopolitan organism classified as a secondary invader potentially causing root rot. Fungal ITS were not included further. 16S rDNA revealed a range of diversity and abundance of distinct bacterial communities present at each location. The regenerative farm soil has the highest bacterial diversity followed by commercial farm soil and lastly the urban lots (Figures 5(a)-(d)).

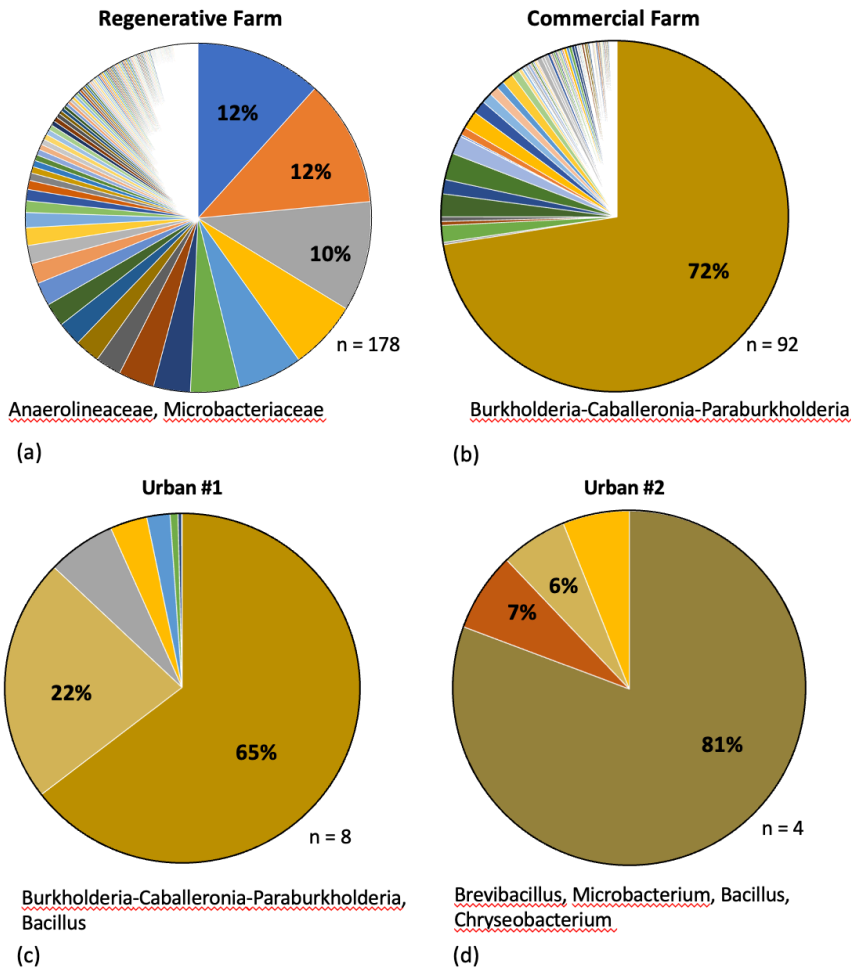


Figure 5. Bacterial 16S sequencing diversity by percentage for (a) regenerative farm soil, (b) commercial farm soil, (c) urban vacant lot soil #1, and (d) urban vacant lot soil #2.

The regenerative farm soil returned 178 bacteria sequences, the highest variety

of the soil sites (Figure 5(a)). A dominant family at 12% is Anerolineaceae and 175 families are each under one percent, revealing broad diversity. Conversely, sequences from the commercial farm (Figure 5(b)) and urban lot (Figure 5(c) and Figure 5(d)) soils contain different bacterial communities from the regenerative farm, and they share a pattern of low to very low diversity with a single species being overwhelmingly dominant. The commercial farm sample had the highest abundance of bacteria (72%) from the Burkholderia-Caballeronia-Paraburkholderia (BCP) group and the urban soil from lot #1 also contained (65%) of bacteria from the genus Burkholderia-Caballeronia-Paraburkholderia (BCP) group (Figure 5(b) and Figure 5(c)).

The commercial farm soil is in stark contrast to the regenerative farm soil with nearly half the amount of diversity and dominance by a single species (Figure 5(b)). The urban soils are very similar in pattern and composition to the commercial farm soil, but at significantly lower bacterial diversity. Urban soil #1 with only eight sequences identified is dominated by Burkholderia-Caballeronia-Paraburkholderia (BCP) group at 65% and urban soil #2 is dominated by Brevibacillus at 81%.

The bacterial sequencing results for three levels (Top, Middle, and Bottom) of BEAM composter #1 had the highest diversity (Figure 6). The major components were the same at each level but only represented seven percent of the total species. The increase in species with depth occur in very small percentages. Three levels of compost samples have high bacterial community diversity with a slight increase at the bottom. They share Anerolineaceae as the dominant community at only seven percent. The remaining bacterial community abundances are all below four percent demonstrating broad diversity throughout the composter. The BEAM compost bacterial diversity is double that of the regenerative farm soil and its diversity increases slightly with depth which potentially represents the downward processing of the compost. This may be the consequence of vertical aeration tunnels through the interior of the compost bin.

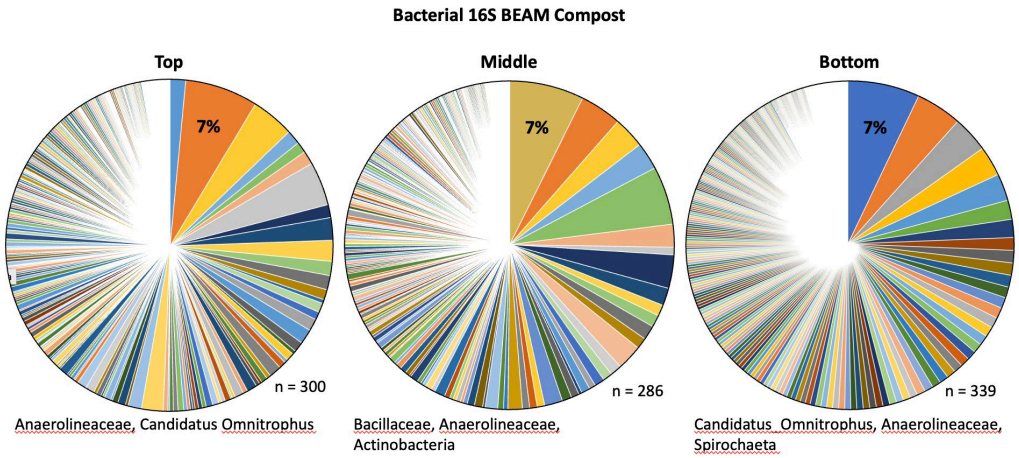
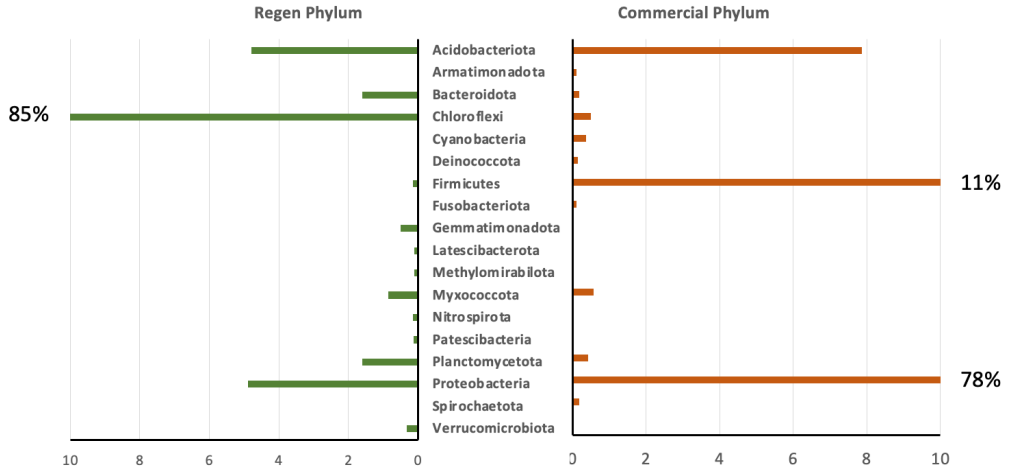
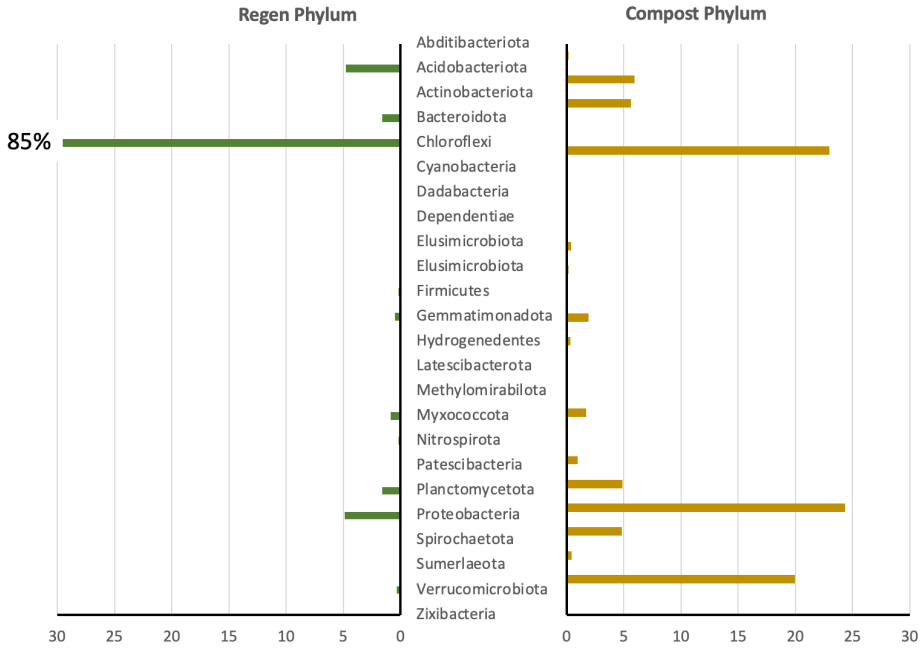


Figure 6. Bacterial 16S sequencing of BEAM composter #1 showing community diversity by percentage at the family level for three depths (Top, Middle, and Bottom).

Additional comparison of the percent abundance at the phylum level between regenerative and commercial soils show differences in dominance by site (Figure 7(a)). Regenerative soil is dominated by Chloroflexi (85%) and the commercial soil is dominated by Protobacteria (78%). Regenerative soil also contains Protobacteria and Acidobacteriota, but at low levels (<6%). Firmicutes appears in moderate amounts (11%) in the commercial soil, but virtually no presence in the regenerative soil. Comparing the percent abundance at the phylum level of regenerative soil and compost exhibits close similarity of the phyla present and abundances (Figure 7(b)).



(a)



(b)

Figure 7. (a) Comparison of abundances of dominant regenerative and commercial farm soil bacteria at the Phylum level. (b) Comparison of abundances of dominant regenerative soil and compost bacteria at the Phylum level.

A Shannon diversity index analyzed the taxonomic diversity of the microbial communities by accounting for relative abundance [35].

$$H' = -\sum^{S} p_i \ln(p_i)$$

where: H' is the Shannon diversity index, p_i is the relative abundance of each genus within each sample group, S is the number of observed taxa, and ln is natural logarithm, J', Pielou's measure of evenness, is $H'/\ln(S)$, and Simpson's diversity ($1 - D$) which is less sensitive to rare taxa and serves as a cross-check.

The results reveal compost has by far the highest microbial diversity (**Table 1**). The Shannon Index of compost (4.01) has substantially greater diversity than the regenerative farm (1.92) and the commercial farm (1.38) (**Figure 8**). Conversely, the commercial farm has the lowest diversity (1.38) and is strongly dominated by a small number of taxa.

Table 1. Calculated values for richness, Shannon diversity, evenness, and community composition metrics for the observed sample groups.

Sample Group	Total Reads	Richness (S)	Shannon (H')	Evenness (J')	Simpson ($1 - D$)
Commercial	2802	65	1.38	0.33	0.43
Regen.	3731	93	1.92	0.42	0.69
Compost	3162	182	4.01	0.77	0.94

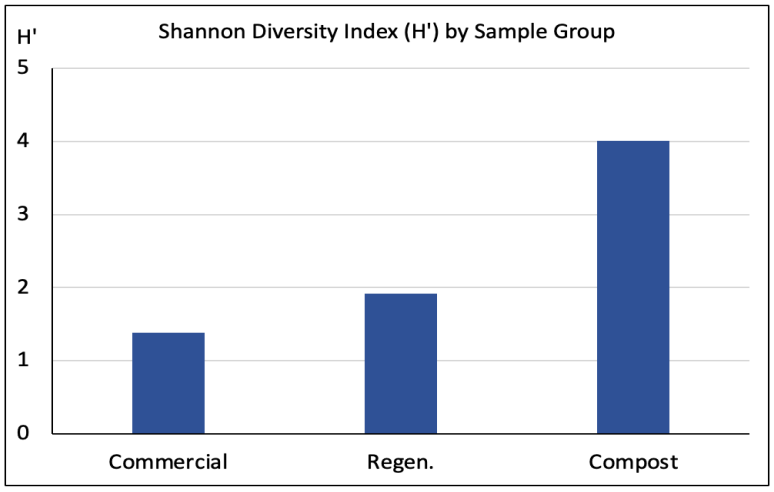


Figure 8. Plot of Shannon Diversity Index by sample group illustrating the range of diversity between sites.

The Shannon diversity analysis reveals pronounced differences in microbial community structure between commercial, regenerative, and compost samples. Compost exhibits consistently greater diversity across all taxonomic levels, with Shannon diversity increasing from Phylum to Genus indicating dramatically higher taxonomic richness. At the genus level compost contains 2.5 times as many observed categories than the commercial samples, demonstrating compost has both

greater richness and evenness. Therefore, compost isn't simply more genera, its highest evenness (0.77) indicates the reads are evenly distributed across taxa, whereas microbial evenness of the commercial farm is low and dominated by relatively few taxa (0.33). Shannon diversity by taxonomic level reveals compost is consistently the most diverse community at each taxonomic level (Figure 9). The commercial samples are strongly dominated by a few taxa such as Burkholderia-Caballeronia-Paraburkholderia.

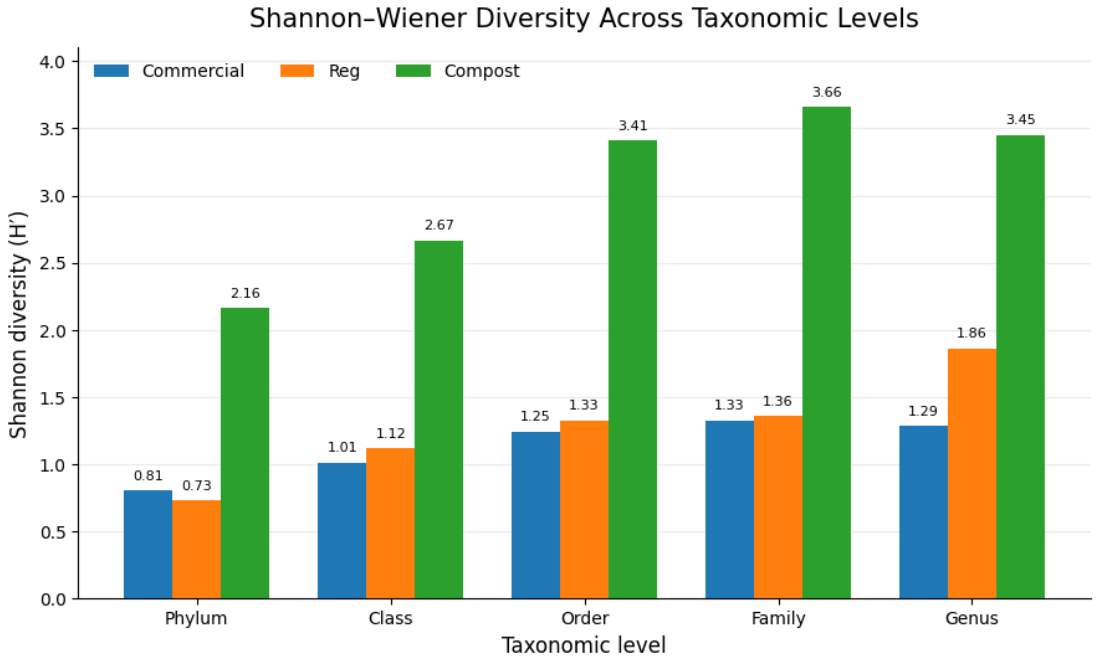


Figure 9. Shannon diversity (*H*) across taxonomic levels illustrates compost substantially higher than the regenerative farm and the commercial farm at each taxonomic level.

The diversity analysis was followed by a hierarchical cluster analysis (HCA) using Ward clustering with Bray-Curtis measure of dissimilarity. Combining the Shannon diversity analysis with the HCA and Bray-Curtis dissimilarity demonstrated considerable differences in composition at the genus level between the groups (Table 2). Regenerative and compost cluster first with more similar genus-level composition (0.654) than either has with commercial. Commercial is identified as the clear outlier.

Table 2. Calculated Bray-Curtis dissimilarity values for the observed sample groups. Compost and regenerative have much more in common than either have with commercial.

Bray-Curtis Dissimilarity			
	Commercial	**Regenerative**	**Compost**
Commercial	0.000	0.924	0.907
Regenerative	0.924	0.000	**0.654**
Compost	0.907	**0.654**	0.000

4. Discussion

Studies focused on soil microbial communities enhancing SOM increasingly support the critical need for maintaining and improving agricultural soils [36]. The LOI analyses of the three soil sites reveal regenerative farm soil has double the organic matter in both spring and fall than either the commercial farm or urban soil (**Figure 2(a)**). The commercial farm soil has half the organic matter content of the regenerative farm soil and very little change in amount of organic matter in both seasons. This may be due to high soil compaction at the commercial farm. The urban soil has the lowest organic matter content in both seasons. The SOM results suggest the regenerative farm soils are functioning at a higher level than the commercial or urban soils.

Microorganisms alter soil physical properties and can through a variety of pathways promote more effective water retention [37]. LOI results also show regenerative farm soil has the highest moisture content in spring and the largest moisture loss by fall (**Figure 2(b)**). Commercial farm and urban lot #1 soils have similar spring moisture content, but the commercial farm soil has the next largest seasonal decrease and urban soils remain much the same. The lack of variation in seasonal moisture flux may indicate less water retention across the growing season for commercial and urban soils.

Soil biodiversity plays a strong role multiple ecosystem functions such as plant diversity, decomposition, nutrient retention, and nutrient cycling [38]. The fungal-specific agar plates clearly illustrate commercial farm, and urban soils share extremely low percentages of overall fungal abundance, fungal diversity, and exhibit similar fungal patterns (**Figure 4**). While one would expect an empty urban lot with a bare sediment surface to have low fungal presence and low microbial diversity. It is remarkable to see their to similarly to the low fungal abundance and diversity of the commercial farm soil. The other visual similarity in fungal-specific agar plates is between the regenerative farm soil and compost which share similar patterns of high abundance and diversity of fungal growth (**Figure 4**). The same pattern in similarities is also visible in bacterial growth on the non-specific agar plates. The agar plates provide a visual demonstration of the similarity and differences in abundance and diversity of the soil settings. There is a strong correlation between fungal diversity and the function of the soil ecosystem with fungal dominant soils flourishing. Diverse microbial populations increase soil productivity and yield over time [38] and are linked to increased soil nutrient cycling [39]. The visual display of the soil fungal and bacterial communities from different agricultural and urban settings could indicate differences in productivity and ecosystem functioning.

When examining the 16S rRNA sequencing of these same samples the similarities in pattern hold up (**Figure 10**). The regenerative farm soil has broad diversity with most families under one percent. *Anaerolineaceae* dominate the regenerative farm soil and compost are recognized for contributing to inorganic CO_2 fixation and as degraders of large amounts of organic contaminants [40]. Sequencing of

the commercial farm and urban lot soils reveal different bacterial communities dominated by the Burkholderia-Caballeronia-Paraburkholderia (BCP) group. These soils share a pattern of low to very low diversity with a single species being overwhelmingly dominant. Associated with rhizosphere soil, Burkholderia-Caballeronia-Paraburkholderia (BCP) group contribute to plant growth and degrading pollutants. They are often associated with contaminated sites [41]. Associated with plant growth, inhibition of pathogens, and drought stress, BCP are considered keystone species [42].

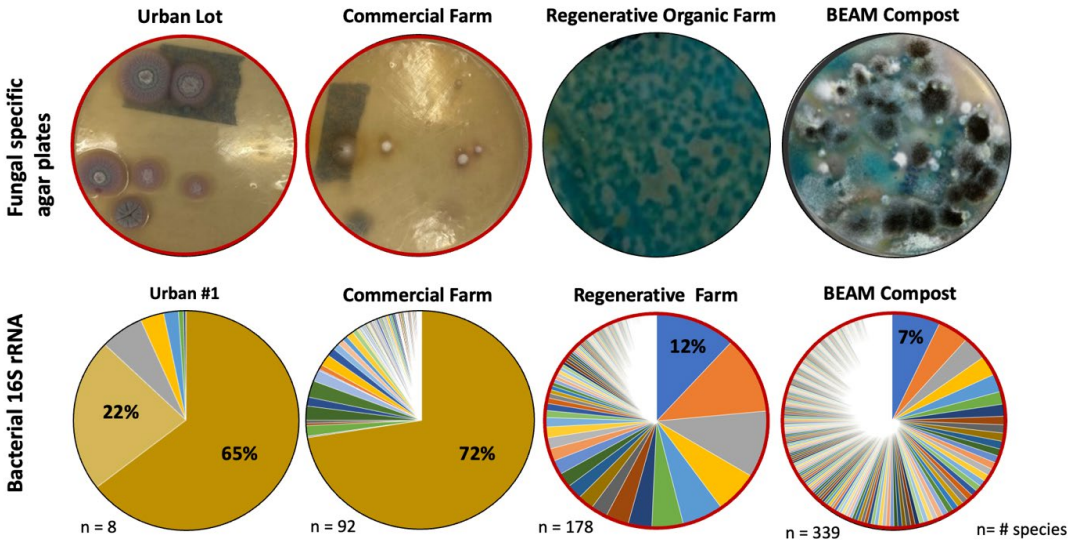


Figure 10. Comparison of fungal specific agar plates (top) to bacterial 16S rRNA sequencing percentages (bottom) by sample location and type. Urban and commercial farm soils are more similar in both agar growth and 16S rRNA sequencing. They are more similar than the two farm soils are to each other. The regenerative farm soil and BEAM compost are also similar to each other in both fungal colonies and 16S rRNA sequencing than they are to either the commercial farm or urban soils.

The regenerative soil and compost have the highest percent of bacterial abundance and diversity (**Figure 11**). The bacterial abundance and diversity reveal a larger pattern across the agricultural and urban soils. Biodiversity consistently decreases from regenerative farm to commercial, then again from commercial farm to urban soils (**Figure 9**). These microbial communities are critical for nutrient cycling, maintaining soil health, preventing diseases in plants, and stimulating plant growth [43].

While the diversity analyses do not establish statistical significance, they do characterize community structure. Taken together they reveal pronounced differences in microbial community structures between commercial, regenerative, and compost. Compost exhibited greater diversity across all taxonomic levels. This elevated diversity is associated with both greater taxonomic richness and greater community evenness making compost highly diverse, taxonomically rich, and evenly distributed microbial community. In contrast, commercial soil exhibited the lowest Shannon diversity, with strongly uneven community, and characterized

by dominance of a limited number of taxa. Additional taxa contribute little to overall community structure. The regenerative farm soil has substantially more taxonomic richness than the commercial but remains less diverse than compost. Compost has greater genus-level evenness, suggesting a more distributed community.

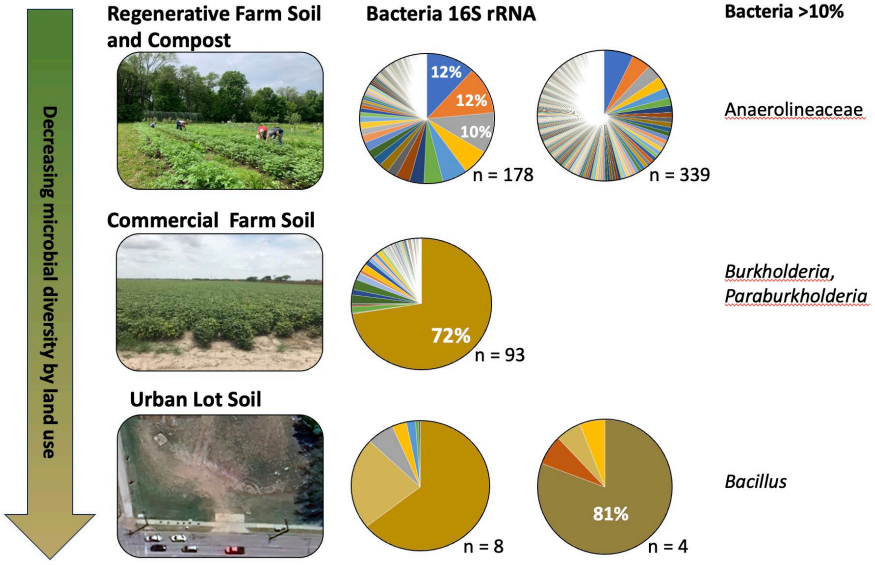


Figure 11. Comparison of bacterial 16S rRNA species percentages for regenerative farm, commercial farm, and urban soils. Bacterial percent diversity decreases from regenerative farm to commercial farm to urban soils. Far right, bacteria species present at greater than 10 percent. Conversely, biodiversity increases from urban to commercial farm to regenerative farm.

Regenerative agriculture requires fewer nutrient inputs and pesticide applications. Regenerative farm techniques can be utilized to restore degraded soils, constructing healthier soil ecosystems [44]. It could also contribute to regenerating poor urban soils and allow them to host healthy, self-dependent areas providing crucial greenspace and a fertile substrate for urban agriculture. A highly functioning soil ecosystem can also bioremediate contaminated and polluted soils due to fungi and bacteria's ability to absorb and transform hazardous pollutants. Fungi are especially effective at remediating soils as fungal groups hyperaccumulate toxicants in their mycelia [45]. This type of remediation would be a practical approach in removing bio contaminants such as heavy metals and petroleum on smaller scales like individual lots and helping reduce polluted wastewater which also percolates through poor urban soils [46].

5. Conclusions

The LOI, agar plate observations, 16S rRNA sequencing, and statistical analyses of soils samples from different land-use types reveal distinct patterns of soil properties and microbial communities. The regenerative farm soil and BEAM compost have high organic matter content and high water retention. They are also similar

in fungal and bacterial diversity and abundance. The similarity between organic compost and regenerative farm soil is not surprising. The regenerative farm soil and compost have very diverse and abundant bacterial presence. The robust similarity in the phyla present and relative abundance indicate a strong correlation between land use, and the farming practices used. Results strongly support the potential for organic compost to improve soil microbial diversity.

The commercial farm and urban plot soil have reduced SOM and lower water retentive capacity. The similarity of the commercial farm and urban lot soils in their starkness is clear, if not surprising. The commercial farm and urban soils have strikingly similar profiles of suboptimal health lacking diverse microbial communities. This lack of microbial diversity and overall microbial mass results in a deficit of nutrients available for plants and a cascade of other ecosystem issues such as a lack of pore space for oxygen and water. Soil compaction and or cropping may contribute to their condition.

Our data reveal commercial and urban soils have poor biodiversity and health, while the regenerative farm soil and compost microbiome are shown to have significantly higher diversity, functionality, and soil health. This study demonstrates both a common assumption that organic soil and compost have extraordinarily rich biodiversity, and an unexpected result revealing commercial farm soils devoid of microbial diversity are more akin to abandoned urban lots in their microbial profile. The data reveal the microbial characteristics of a healthy soil are lacking in commercial farm and urban soils. These soils severely lack biomass, water retention capacity, and fungal and bacterial diversity. This has important implications for urban agriculture, environmental health, and the critical need for remediation of urban soils. The study also reveals a surprising lack of microbial diversity and abundance in the commercial farm soils, at levels similar to urban soils. The implication of an unhealthy microbiome speaks to the extractive and non-regenerative nature of our modern agriculture practices which are significant challenges for sustaining commercial agriculture. This study supports using regenerative techniques to help remediate both poor urban and exhausted commercial agricultural soils. Although the scalability of remediating commercial agricultural soil remains unknown, and more work is necessary to understand the complexity of microbial functions and interactions.

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Author Contributions

Conceptualization, Caroline Davies; methodology, Brooke Esquivel and Caroline Davies; investigation, Caroline Davies, Brooke Esquivel, and Gavin Hysten; reporting, Gavin Hysten, data processing, Caroline Davies and Brooke Esquivel; validation, Brooke Esquivel, Caroline Davies, and Theodore C. White, formal analysis, Caroline Davies, and Brooke Esquivel; resources, Caroline Davies, Theodore C. White, and Brooke Esquivel; data curation, Caroline Davies; writing—original draft preparation, Caroline Davies; writing—review and editing, Caroline Davies, Brooke Esquivel, and Theodore C. White; visualization, Caroline Davies; supervision, Caroline Davies, Brooke Esquivel, and Theodore C. White; project administration, Caroline Davies; funding acquisition, Caroline Davies, Theodore C. White, and Brooke Esquivel; All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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