The Impact of Urbanization on Soil Bacterial Diversity and Community Composition in Long Island, New York

by

KAUNG MYAT SAN

Thesis submitted to the Graduate Faculty in Biology in fulfillment of the requirements for the degree of Master of Art, The City University of New York

March 2019

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Sincerely yours,

_____
Chair of Examining Committee
Dr. John. J. Dennehy
Queens College
Biology Department

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ABSTRACT

Soil accommodates many different types of microorganisms such as bacteria, archaea and viruses. They collectively play an essential role in important terrestrial ecosystem services such as provision of nutrients to plants and other macro-organisms through driving carbon and nitrogen cycles, amending the soil structure, maintaining the soil fertility by recycling the organic wastes. However, the environmental drivers that cause variations in soil microbial communities have not been fully explored, especially on how human activities impact soil microbial communities via urbanization. In this study, I collected soil samples from 30 locations along urbanization gradient that spanned from Manhattan, NY to Montauk, NY during 2014-2015. I used a 16srRNA meta-genomic approach to measure the bacterial diversity. Soils were characterized for their pH. There is significantly higher human population density and bacterial species diversity in urban area than in rural areas. pH of rural soil was found to be more acidic in natural soil than those of the urban soil. The positive correlation between pH and bacterial diversity was observed which made me conclude that pH was one major driver that controls the bacterial diversity in soil. Therefore, I've concluded that highly-populated urban centers significantly raise pH level in soil, which, in turn, increases the bacterial diversity. Not only urbanization increases bacterial diversity, but it also shifts the community composition of bacteria inhabiting in soil. The dominant bacteria phyla found across all soil are *Acidobacteria, Proteobacteria, Actinobacteria, Verrucomicrobia, Plantomycets* and *Chloroflexi*. Among them, *Verrucomicrobia* have the preference for acidic pH. They are most likely to be found and abundant in acidic soils. However, the preference for distinct pH was not observed for *Proteobacteria*, *Actinobacteria* and *Plantomycetes*.

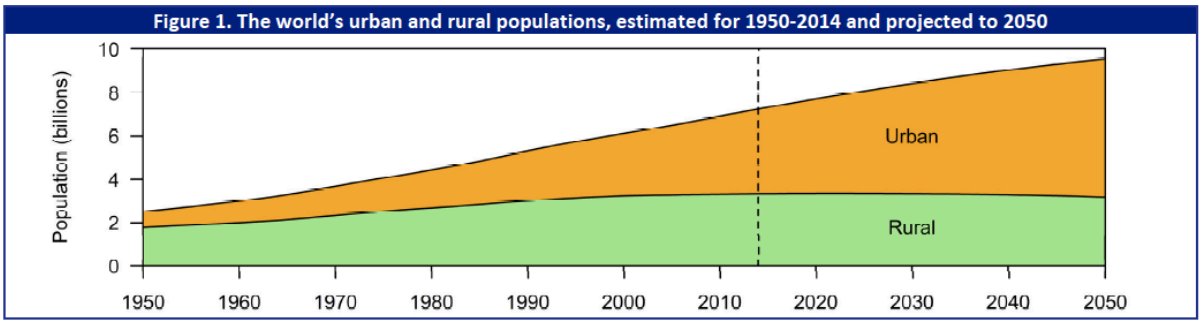
ACKNOWLEDGEMENT

This interdisciplinary project is supported by many of our collaborators. I would like to thank my mentor/PI Dr. John Dennehy for allowing me to be part of his lab to work on this project and his supports. I would also like to thank Dr. David Lahti for his supports and being a committee member for the defense of this project. I would also like to thank Dr. Jeffrey Bird from Earth and Environmental Science for soil analysis, Dr. Jose Anadon and Irene Hoxie from Biology Department for R statistical data analysis and QIIME (Quantitative Insight Into Microbial Ecology) analysis, Dr. Theodore Muth and Jessica Joyner from Brooklyn College for providing 96 barcoded 16SrRNA reverse primers. We also would like to thank Dr. Mitchell Baker, Fraida Straiter, Hisham Alrubaye, Elsa Rosario. Jessenia Soriano, Nanami Kubota, Sasha Balkaran, Paola Lozada, Boryana Baric for all their help with the samples collection.

Introduction

1.1 Urbanization

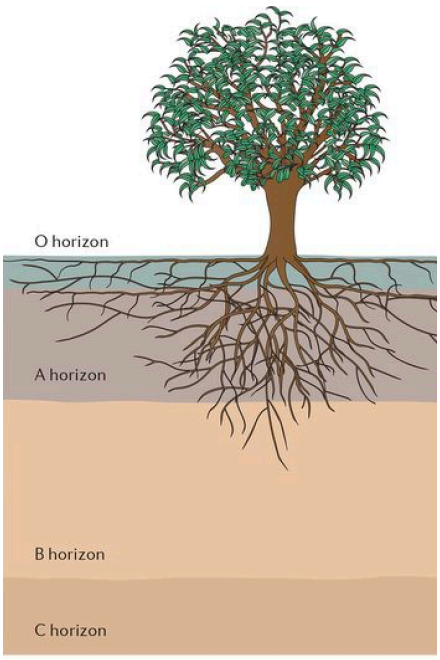
Urbanization results when a large number of people condense in a relatively small area. Urbanization occurs because of the concentration of resources, facilities, jobs and businesses in a small area. More than 80% of US population resides in urban area and similar pattern is also found around the world. (Brown et al., 2005).



Data from United Nation indicates that the world has urbanized rapidly since 1950. (United Nations, 2014, Figure.1). By 2030, the urban land cover will increase by 1.2 million km^2, tripling the global urban land area present in 2000 (Seto et al, 2012). As the urbanization continues to grow all over the globe, it is important that we study and improve our understanding of the effects of urbanization on biological communities in soil. In addition, the constant stresses added by human activities in the urban environment can make the microorganisms to become adapted to the condition and causes variations in microbial community composition, structured in urban center to be different from rural area. In this study, I assessed the effect of urbanization on bacterial diversity and community composition in the soils, as well as the edaphic factors such as soil's physical and chemical properties that can be altered by human activities and ultimately influence the microbial community.

1.2 Soil profile

Soil is composed of different layers called horizons. The top layer of soil called O horizon (organic layer) is about 2 inches thick and composed of dead materials from animals and plants. Right below O layer, there is A horizon (topsoil). A horizon is about 5-10 inches, where the microorganisms primarily live to break down materials into nutrients for plants to use. B horizon (subsoil layer) is right below A horizon and contains clay, minerals and organic matter that are washed down by the rain. This is the horizon where the roots of plants get in to find the nutrients for them to grow. Plants need nutrients in small amount (micronutrients) such as iron, zinc, copper, as well as nutrient in large amount (macronutrients) such as nitrogen, phosphorus, potassium, calcium, magnesium, sulfur. Below B horizon, there is a C horizon. This layer contains rocks and no organic materials and is made of weathered parent materials. Just below that, there is R horizon which is made of bedrock.



Nature Review Microbiology (Noah Fierer.,2017) **Figure 2.** Soil profile displaying different horizon

1.3 Role of Microbes in Soil

Soil is a component of earth where many ecologically relevant living organisms thrive. Microorganisms such as bacteria, viruses and fungi are especially abundant in the soil and they work together to provide important terrestrial ecosystem services. We cannot underestimate the importance of microorganism in soil. Soil microbes are paramount because they impact Carbon, Nitrogen and Phosphorous in soil. They impact carbon cycle by decomposing organic matter from dead organisms, which releases carbon dioxide to atmosphere and used by trees, plants for the photosynthesis. In addition, soil microbes drive soil nitrogen cycles in which nitrogen-fixing bacteria convert nitrogen (N_2) from the atmosphere into ammonium, a form of nitrogen that can be assimilated by other organisms.

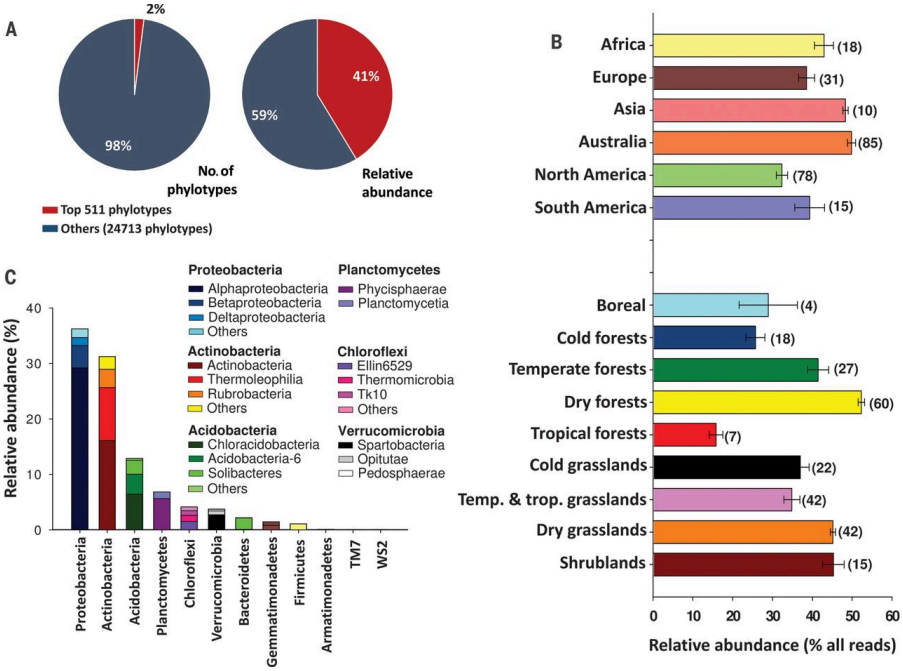
1.4 Soil Bacterial Diversity and pH is linked to Human Population Density in Urban Centers

As a result of urban expansion, there is an increase of human population, land covers, managements and anthropogenic activities. The soil microbiome studied at latitudinal urbanization gradient, across the City of Chicago had shown that human population density as a proxy of anthropogenic activity altered soil characteristics in urban centers, and ultimately increased the bacterial diversity (Wang et al., 2018). There are several environmental factors that impact the bacterial diversity. In this study, I will be assessing how the urbanization gradient in New York affect soil pH, and how pH influences the bacterial diversity and their community composition. The urbanization study done in arable soil of Kumasi, Ghana (West Africa) has shown that pH of urban soils was

significantly higher than those of rural and forest soils (Asabere et al., 2018). And this increase in urban soil pH is the result of the anthropogenic activities, that have a similar effect as liming, even though liming is not used as part of field management (Asabere et al., 2018). Similarly, the higher pH was reported for urban soil of Hong Kong, in comparison to rural soils. The urbanization study done in arable soil of Kumasi, Ghana (West Africa) has shown that pH of urban soils was significantly higher than those of rural and forest soils (Asabere et al., 2018 (Jim et al, 1998). According to these previous studies, there are many possible reasons why urban soils are more alkaline than rural soils. One possible reason for higher pH in urban soils is due to the release of alkaline leachate from construction materials, calcareous materials such as limestones (calcium carbonate) in the cements (Jim et al, 1998), as well as from decomposing organic wastes (Boatang et al., 2006; Cofie et al., 2009). Most urban area are covered with concretes that are made with cements. The carbonate compounds from cements can be leached off by the rain and raise the pH in soil (Brady and Weil et al., 2017). Other sources of carbonates come from household wastes such as eggshells, animal bones, batteries, charcoal, ashes and they can also raise pH in soil. (Asabere et al., 2018). All these factors attribute to the increased pH in urban soils. Based on these studies, I hypothesize that the pH level of soils increases as the soils get closer to urban center.

1.5 The Effects of Soil pH on Bacterial Diversity and the community composition

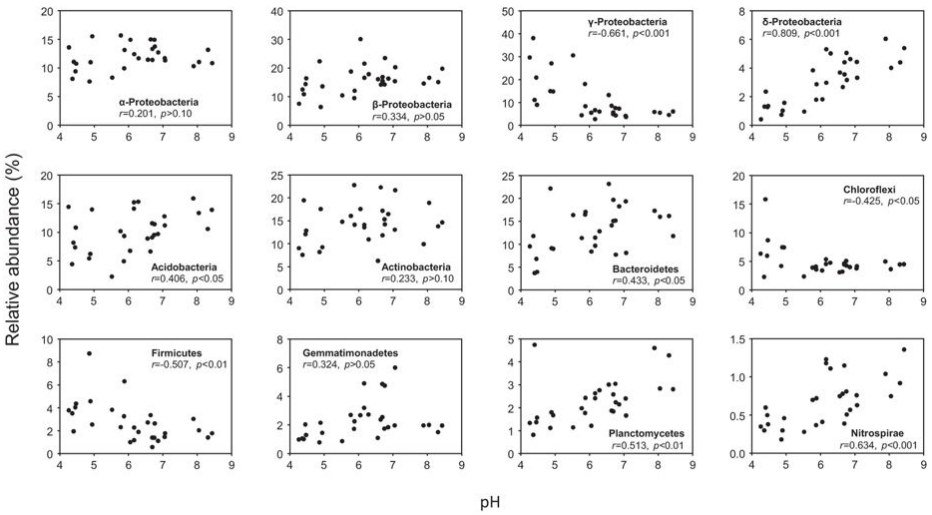
Soil pH is known to be one significant factor that governs the bacterial diversity and their community composition (Jones et al., 2009; Chu et al., 2010; Shen et al., 2013; Liu et al., 2014). There is a direct correlation between soil pH and bacterial diversity, in which the bacterial diversity decreases due to the soil acidification. (Yuan et al., 2016, Fierer et al., 2013). The pH also plays a pivotal role in shaping the bacterial community composition by affecting the relative abundance of certain group of bacteria in soils (Shen et al., 2013, Wu et al., 2017). Although the bacteria communities are very diverse, only a few dominant phyla of bacteria such as *Proteobacteria, Actinobacteria, Acidobacteria, Plantomycetes, Chloroflexi, Verrucomicrobia* are observed in soil across the globe (Delgado et al., 2018).



(Delgado et al., 2018).

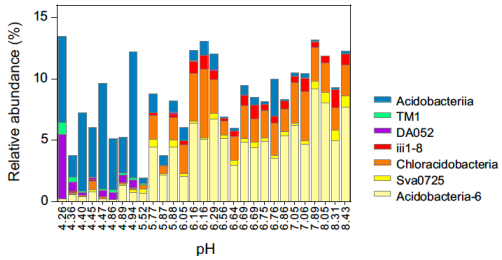
Figure 3a. Dominant Bacterial Phyla and Classes in Soil Across Continent.

Among them, the bacterial communities in the soils that have a relationship with pH are found to be *Acidobacteria, Chloroflexi, Plantomycetes*, *Nitrospirae* and *Bacteroidetes* phyla. (Wu et al., 2017, Figure 3b). *Acidobacteria* is found to be one of the bacterial phyla that contains many groups of bacteria, who respond well to the change in pH and their relative abundance increases as the pH increases to neutral level (Wu et al., 2018). Within *Acidobacteria* phylum, *Acidobacteria-6, iii1-8* and *Chloracidobacteria* are found to be the dominant classes of bacteria at the slightly acidic and near neutral pH, whereas *Acidobacteriia, TM1 and Da052* are dominant in low pH environment (Wu et al., 2018, Figure 4).



(Wu et al., 2017)

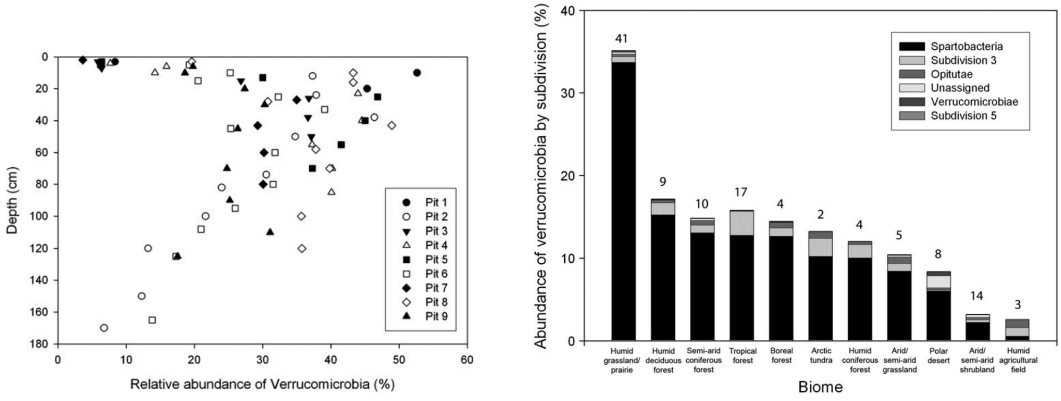
Figure 3b. The correlation between soil pH and relative abundance of some dominant bacterial phyla.



(Wu et al., 2017)

Figure 4. The class level composition of *Acidobacteria*.

Verrucomicrobia is another dominant bacterial phylum in the soil that is under-recognized. A study had shown that the relative abundance of *Verrucomicrobia* was highest in sub-surface soil horizon of *grassland* comparing to other biomes and *Spartobacteria* was found to be the dominant verrucomicrobial class in soil (Bergmann et al., 2011 (Figure 5), Sangwan et al., 2004, Janssen et al., 2006). However, no study had been done specifically on verrucomicrobial diversity, abundance, composition and their relationship to pH. Tracking the abundance and presence of particular group of bacteria is crucial since they not only have the impact on the soil health and ecosystem, but also human health (Parajuli A, Grönroos M, Kauppi S, Płociniczak T, Roslund MI, et al; 2017)



(Bergmann et al., 2011)

Figure 5. Abundance of *Verrucomicrobia* at different depths and biomes.

Objectives and hypotheses:

The goal of this project is to study the impact of urbanization on soil pH and how pH changes affects the "bacteria diversity and community composition" in soil. In order to study soil microbiome on the urbanization gradient, I will collect soil samples from 30 locations along urbanization gradient that spanned from Manhattan, NY to Montauk, NY . Then, I will use a 16srRNA meta-genomic approach to measure the bacterial diversity. The 16srRNA meta-genomic approach involves extracting DNA from the environmental samples, picking out the DNA of the bacteria by amplifying the bacterial 16srRNA ribosomal gene with primer and sequencing the amplified DNA.

Once the samples are sequenced, I will use the DNA sequences and analyze using **QIIME (Quantitative Insights into Microbial Ecology)** to taxonomically arrange these sequences into OTU (operational taxonomic unit) based on 97% sequence similarity. I will perform the taxonomic composition analysis by creating a taxa summary plot in QIIME. This will allow me to determine the dominant bacterial phyla that reside in the soil.

Then, I will determine the diversity and OTU richness of samples by calculating the alpha diversity. The alpha diversity, in ecology, is the mean species diversity or the species richness within the same habitat/ site or sample. This alpha diversity value will be used to represent the bacterial diversity in the soil. The beta diversity will also be calculated in order to see the variation of the species composition between two different habitats or sites. Also, Soil pH will be measured to examine the acidity of soil along urbanization gradient.

In order to find the correlation between each variable, I will then determine the population density of the areas on longitude that spanned from Manhattan to Montauk in

Long island, New York using data from United State Census Bureau (*https://www.census.gov/geo/*) and then find the relationship between longitude and population density to see if longitude is correlated with population density and can be used as a proxy for the urbanization gradient. Since Manhattan is well known to be highly-populated and Long Island to be less populated, I hypothesized that there will be relationship between longitude and the population density **(Hypothesis#1).**

The previous study done in arable soil of Kumasi, Ghana (West Africa) and Hong Kong both had shown that pH of soils in urban centers was significantly higher than those of rural and forest due to the release of alkaline leachate from construction materials, calcareous materials such as limestones (calcium carbonate) in the cements as well as from decomposing organic wastes (Asabere et al., 2018, Jim et al, 1998). Therefore, I hypothesize that urbanization will increase the soil pH **(Hypothesis#2).** I will test this hypothesis by determining the significance of the correlation between the longitude and soil pH, as well as the population density and soil pH.

Then, I will assess if there is a relationship between pH and bacterial diversity in soil. Based on the previous study done, there is a direct positive correlation between soil pH and bacterial diversity, in which the bacterial diversity increases as the pH becomes neutral (Yuan et al., 2016, Fierer et al., 2013). Therefore, I hypothesize that high bacterial species richness will be observed as the pH increases to the cell's physiologically possible condition (pH 7) and bacterial species richness will decrease as pH decreases to acidic condition **(Hypothesis#3).** I will test this hypothesis by calculating the alpha diversity. And I will plot this alpha diversity value against soil pH to find their correlation.

In addition, the previous study done at latitudinal urbanization gradient, across the City of Chicago had shown that high human population density altered soil characteristics in urban centers and increased the bacterial diversity in soil (Wang et al., 2018). Therefore, I hypothesize that urbanization will increase the soil bacterial diversity in NY longitudinal urbanization gradient as well **(Hypothesis#4).** To test this hypothesis, the significance of correlation between 'soil pH and alpha diversity' will be determined.

Many previous studies had also shown that pH also affects the relative abundance of certain group of bacteria in soils (Shen et al., 2013, Wu et al., 2017). *Proteobacteria, Actinobacteria, Acidobacteria, Plantomycetes, Chloroflexi, Verrucomicrobia* are observed in soil across the globe (Delgado et al., 2018). Among them, *Acidobacteria and Plantomycetes* are the dominant bacterial phyla in soil, that their relative abundance of Acidobacteria increases as pH becomes more neutral, but the relative abundance of *Chloroflexi* decreases as pH becomes neutral based on the previous study done (Wu et al., 2018). Based on that, I hypothesize that the same relationship will be found in this study **(Hypothesis#5, Hypothesis#6, Hypothesis#7).** To test this hypothesis, the significance of correlation between 'soil pH and the relative abundance of *Acidobacteria, Plantomycetes and Chloroflexi* ' will be determined.

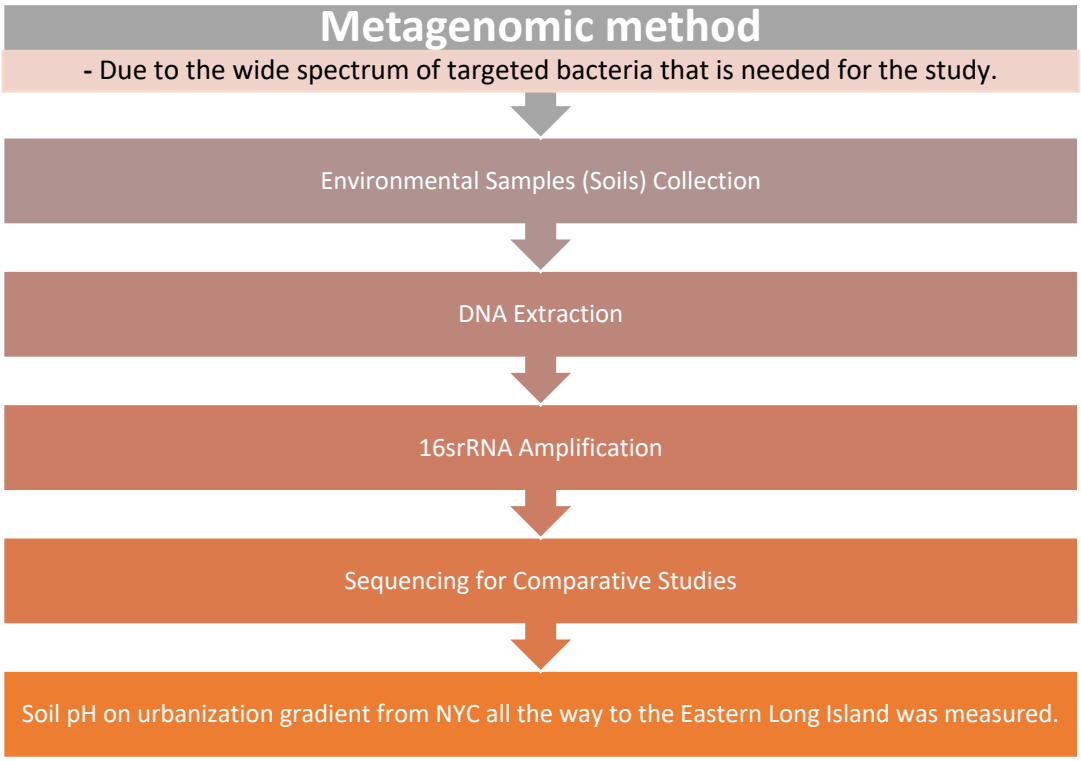
However, *Verrucomicrobia* was under-recognized due to the primers used in the previous studies and the type of soil being studied. Until recent year, *Verrucomicrobia* phylum become more recognized in the soils. Studies had found that the relative abundance of *Verrucomicrobia* was found to be highest in grassland soils comparing other biomes (Bergman et al., 2011). However, the relationship between soil pH and *Verrucomicrobia* was not explored in the previous studies. Therefore, I will be assessing whether there is a

relationship with the pH and *Verrucomicrobia* phylum in this study. Therefore, I will test and see if there is a relationship between the soil pH and the relative abundance of *Verrucomicrobia* phylum in my study **(Hypothesis#8).** I will test this hypothesis by finding the correlation between soil pH and the relative abundance of *Verrucomicrobia*.

Hypothesis	Prediction and Approach
$H1_0$: There is no relationship between longitude and population density. **$H1_A$:** There is a relationship between longitude and population density.	Longitude will be negatively correlated with population density. The population density of the areas will be determined on longitude that spanned from Manhattan to Montauk in Long island, New York using data from United State Census Bureau (*https://www.census.gov/geo/*).
$H2_0$: Urbanization decreases soil pH. **$H2_A$:** Urbanization increases soil pH.	Urbanization will increase soil pH. The significance of correlation between 'longitude and soil pH' will be determined, as well as between 'population density as a proxy of urbanization and soil pH'.
$H3_0$: Bacterial diversity increases as soil pH becomes neutral. **$H3_A$:** Bacterial diversity increases as soil pH becomes neutral.	Bacterial diversity will be increased as soil pH become neutral. Bacterial diversity will be high in the pH neutral soil, whereas it will be low in acidic soil. Alpha diversity of soil samples will be measured. The significance of correlation (R-value and P-value) between 'soil pH and alpha diversity' will be determined.
$H4_0$: Urbanization decreases the bacterial diversity in soil. **$H4_A$:**Urbanization increases the bacterial diversity in soil.	Urbanization will increase the bacterial diversity in soil. The significance of correlation between 'longitude and alpha diversity will be determined, as well as between 'population density and alpha diversity'.
$H5_0$: The relative abundance of *Acidobacteria* decreases as soil pH becomes neutral. **$H5_A$:** The relative abundance of *Acidobacteria* increases as pH becomes neutral.	There will be a positive correlation between *Acidobacteria* and pH. The relative abundance of *Acidobacteria* will increase as pH becomes neutral. The significance of correlation between pH and the relative abundance (in proportion) of Acidobacteria will be determined.
$H6_0$: The relative abundance of *Plantomycetes* decreases as pH becomes neutral. **$H6_A$:**The relative abundance of *Plantomycetes* increases as pH becomes neutral.	There will be a negative correlation between *Plantomycetes* and pH. The relative abundance of Plantomycetes will increases as pH become neutral. The significance of correlation between 'Plantomycetes and pH' will be determined.
$H7_0$: The relative abundance of *Chloroflexi* increases as soil pH becomes neutral. **$H7_A$:** The relative abundance of *Chloroflexi* decreases as pH becomes neutral.	There will be a positive correlation between *Chloroflexi* and pH. The relative abundance of *Chloroflexi* will increases as pH become neutral. The significance of correlation between '*Chloroflexi* and pH' will be determined.
$H8_0$: There is no relationship between pH and Verrucomicrobia. **$H8_A$:** There is a relationship between pH and Verrucomicrobia.	There will be a relationship between *Verrucomicrobia* and pH. The significance of correlation between '*Verrucomicrobia* and pH' will be determined.

Materials and Methods

Due to the wide spectrum of targeted bacteria that is need for the study, the metagenomic method was used. Metagenomics, is a branch of genomics in which the genomes of entire communities of microbes are studied, without having the need to isolate them. This is a great advantage, since it is believed that with traditional methods of the isolation and cultivation of microorganisms, most of the microbes in samples are "lost", which basically limited microbial diversity within a study. Metagenomic projects start from taking a sample of a particular environment, soil in this case. The DNA is then extracted from the samples, amplified 16srRNA gene and sequenced for comparative studies.



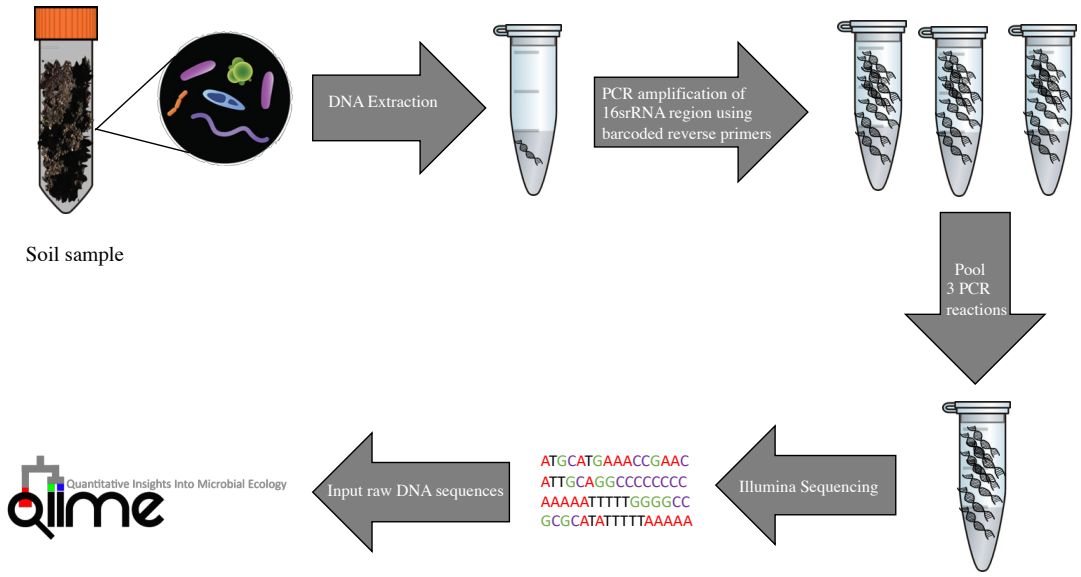


Figure 6. Metagenomic method is used to quantify the soil bacterial diversity and abundance by extracting environmental DNA, amplifying 16srRNA region and illumina sequencing. Raw DNA sequences are analyzed using QIIME (Quantitative Insights into Microbial Ecology).

2.1 Environmental Soil Sample Collection

In order to assess if the urbanization affects bacterial diversity and community composition in this study, I chose to collect the topsoil at 15 cm depth, along urbanization gradient. Sample collection was carried out through four boroughs of NYC (Queens, the Bronx, Brooklyn, Manhattan) and Long Island (Suffolk and Nassau). Soil samples were collected from the grassy lawn of 30 public parks. Within each park, the soils were sampled from 4 randomly chosen plots. Within each plot, soil samples were obtained from five points that are 5-meters apart along the transect, total of 25 meter transect from each plot. Using AMS 401.02 7/8" x 21" soil sampler probe, 15 cm of topsoil was obtained from each point within transect (Figure.8). The soils were pooled and mixed in a Ziploc bag. The mixed soil was then subsampled in the 50 mL falcon tube and stored in the -80◦C freezer for genomic analysis. 15 g of mixed soils (5 g x 3 replicates) were used to determine the average moisture content of the sample at the time of sampling using thermogravimetric method using convective oven drying. The remaining soil samples were air-dried and sieved through 0.5-1.0 cm to remove stones and roots, followed by 2mm-seive. The sieved soils were then used for the characterization of soil pH.

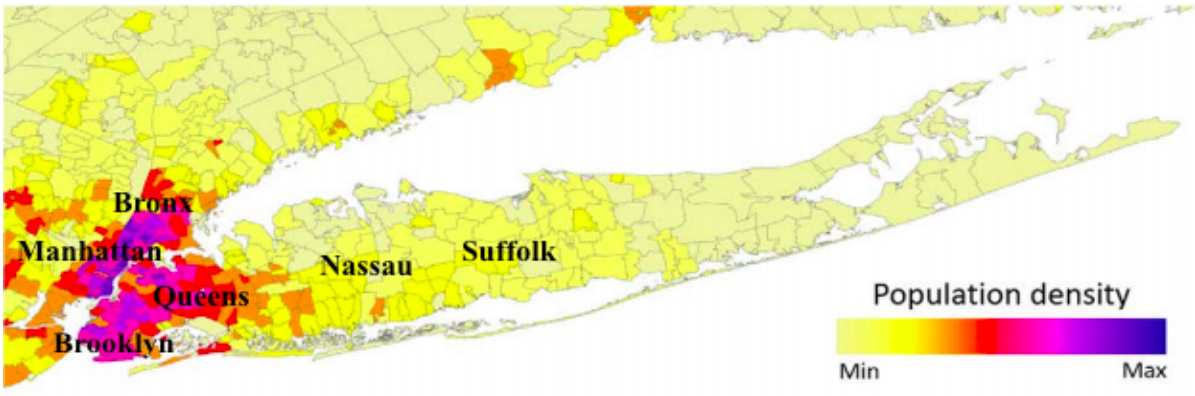
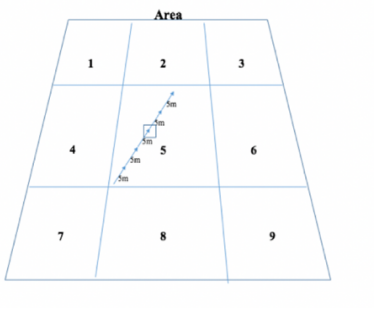


Figure 7. Gradient of human population density from NYC to eastern Long Island, NY. (*https://www.census.gov/geo/*)



Location	City	Date	Coordinates	Lat	Lon
Bethpage Park	Farmingdale (NYS)	10/17/14	N 40° 45' 12.3, W 73° 28' 03.2"	40.7534167	-73.467556
Caumsett Park	Huntington (NYS)	10/31/14	N 40° 55' 4", W 73° 28' 12"	40.9177778	-73.47
Robert Cushman County Park	Calverton (NYS)	12/2/14	N 40° 52' 32" , W 72° 48' 34"	40.8755556	-72.809444
Broad Channel American Park	Queens (NYC)	11/24/14	40°35'50.7"N, 73°49'17.4"W	40.5974167	-73.8215
Cantiague Park	Hicksville (NYS)	10/17/14	N 40° 46' 20.4, W 73° 33' 00.7 "	40.7723333	-73.550194
Brentwood State Park	Brentwood (NYS)	11/21/14	N 40° 48' 4.9176", W 73° 16' 37.3656"	40.801366	-73.277046
Grant Park	Hewlett (NYS)	11/7/14	N 40° 38' 43.2384", W 73° 40' 25.2984"	40.645344	-73.673694
Rider Ave Park	Patchogue (NYS)	10/27/14	N 40°45'04.5" W 73°00'24.4"	40.7475278	-73.742861
Alley Pond Park	Flushing (NYC)	10/17/14	N 40°44'51.1" W 73°44'34.3"	40.75125	-73.006778
Cunningham Park	Fresh Meadows (NYC)	10/17/14	N 40° 44' 7.0836, W 73° 46' 1.3944"	40.735301	-73.767054
Eisenhower Park	East Meadow (NYS)	11/12/14	N 40° 43' 29.2, W 73° 34' 04.1"	40.7247778	-73.567806
Hempstead Lake State Park	West Hempstead (NYS)	11/12/14	N 40° 40' 18.7, W 73° 38' 56.5"	40.6718611	-73.649028
Brookville Park	Rosedale (NYC)	11/7/14	N 40°39'27.6" W 73°44'51.1"	40.6576667	-73.747528
Queens College	Queens (NYC)	10/12/14	40°44'08.1"N 73°49'07.2"W	40.7355833	-73.818667
Soundview Park	Bronx (NYC)	11/21/14	N 40° 49' 12" , W 73° 52' 14"	40.82	-73.870556
Randall's Island Park	Manhattan (NYC)	11/21/14	N 40° 47' 28" , W 73° 55' 31"	40.7911111	-73.925278
Juniper Valley Park	Middle Village (NYC)	11/14/14	N 40° 43' 14.3" , W 73° 52' 45.1"	40.7206389	-73.879194
Prospect Park West	Brooklyn (NYC)	10/24/14	N 40°39'49.3" W 73°58'33.1"	40.663694	-73.975861
Friends Field	Brooklyn (NYC)	11/24/14	N 40° 37' 8" , W 73° 58' 23"	40.6188889	-73.973056
Thomas Paine Park	Manhattan (NYC)	12/5/14	N 40° 42' 52", 74° 0' 11"	40.7144444	-74.003056
Owl's Head Park	Brooklyn (NYC)	10/24/14	40°38'22.5"N 74°01'55.5"W	40.639583	-74.032083
Elmhurst Park	Queens (NYC)	11/14/14	N 40° 43' 50", W 73° 53' 8"	40.7305556	-73.885556
Herbert Von King Park	Brooklyn (NYC)	11/24/14	N 40° 41' 22" , W 73° 56' 48"	40.6894444	-73.946667
Claremont Park	Bronx (NYC)	12/1/14	N 40° 50' 29.1, W 73° 54' 28.8"	40.8414167	-73.908
East River Park	Manhattan (NYC)	12/5/14	N 40° 42' 50.8", W 73° 58' 33.6"	40.7141111	-73.976
Central Park Gr Hill	Manhattan (NYC)	12/1/14	N 40° 47' 49", W 73° 57' 30"	40.7969444	-73.958333
Hither Hill Park	Manhattan (NYC)	6/24/16	N 41 2' 7", W 71 58' 18"	41.035278	-71.971667
Pine Meadow County Park	Manhattan (NYC)	4/11/17	N 40 51' 32", W 72 43' 23"	40.858889	-72.723076
Southhaven County Park	Manhattan (NYC)	4/11/17	N 40 48' 26", W 72 53' 34"	40.807222	-72.892778
Caleb Smith State Park	Manhattan (NYC)	9/4/15	N 40 51' 18" ,W 73 13' 28"	40.855	-73.224444

Figure 8. Soils were collected at 30 sites across the population gradient, 4 soil samples taken per location (25-m transects) to 15 cm depth. All sites were sampled from turfgrass sites within public parks. (map: Google Earth)

2.2 Soil Characterization of pH

pH is measured in the lab using a pH meter (Mettler Toledo SevenEasy). pH is a measure of the acidity and is the concentration of protons [H^+] in solution. It is expressed as –log [H^+]. Soil pH influences many facets of plant productivity and soil chemistry, including availabilities of nutrients and toxic substances, activities and nature of microbial populations. I used a combination pH/reference electrode probe constructed around a H^+-sensing glass membrane. The protons create an electrical potential once the salt bridge completes the circuit. A mixture of soil and water (in beaker) is stirred and the electrode is immersed in the suspension. The H^+ ions create an electrical potential once the salt bridge completes the circuit. Soil pH was determined using 0.01M $CaCl_2$ buffer solution. This was prepared by dissolving 1.47 g $CaCl_2.2H_2O$ in 1 L of distilled water. Prior to measuring for pH of soil samples, the pH meter was calibrated using standard buffer solution, ranging from 4.0 to pH 10.0. To determine the pH of each sample, a 1:2.5 mixture of soil and 0.01M $CaCl_2$ buffer solution was made. This was accomplished by adding 10 g of soil to 25 mL of 0.01M $CaCl_2$ buffer solution. The resultant soil slurry was then stirred vigorously for 15 seconds and let stand for an additional 60 minutes. At the end of this process, the pH electrode was placed into the slurry to record its pH. The pH values of all the samples were measured at 25°C.

2.3 Metagenomic Methods

Total DNA Extraction

Total DNA was extracted from 0.25 g of soil using PowerSoil DNA Isolation Kit according to the manufacturer's protocol (MoBio, Carlsbad, CA, USA). Due to the fact that DNA is inside the bacteria cell membrane, the cells are needed to be lysed. To accomplish this, a lysis buffer and lysis enhancer with beads were used. The beads physically break open the cells while the lysis buffer and lysis enhancer degrade it chemically. This lysis buffer is a detergent solution in which together with the lysis enhancer help to break the main structure of the cell; Lysis buffers might also contain salts, and proteases that deal with proteins. the resulting material or debris was centrifuged. The fact that these proteins and lipids are denser than DNA, it will form the pellet at the bottom of the tube after centrifugation. This unwanted material was pelleted while the DNA remained floating in the supernatant. A cleanup buffer is added to this supernatant and centrifuged again to further remove any inorganic and non-DNA organic material. A high concentration salt solution together with the supernatant that contains DNA was then pass through a silica membrane in the spin column. At higher level of salt concentration, DNA binds to the silica membrane and any debris pass through the filter. A wash buffer (Ethanol) was used to wash off DNA. Lastly, an elution buffer (ddH_2O) was used to bring down the DNA from the silica filter into a collection tube. The eluted DNA was then stored in the -20°C for the subsequent PCR amplification of DNA. The DNA product from extraction was checked for purity and concentration using a **Implen** nano-photometer (P-Class).

PCR Amplification of 16SrRNA gene in bacterial DNA

In this project, I specifically screened for the prokaryotes (bacteria and archaea). In order to solely select the DNA of prokaryotes out all other microorganisms (viruses and fungi) in the soil sample, I used the *original* universal primer pair **515F/806R** (Capraso et al.,2011), which is designed to amplify V4 region of 16s SSU rRNA gene in bacterial DNA that gives the amplicon size of approximately ~390bp. The current 515F/806R primers have been modified in the earth microbiome project's 16S illumina amplicon protocol. In the original protocol, the reverse primers(806R) are barcoded whereas the forward primers (515F) are barcoded in the modified protocol.

Primer name	**Primer sequence**	**Reference**
515f (Original)	5'-GTGYCAGCMGCCGCGGTAA-3'	Caporaso et al.
806r (Original)	5'-GGACTACNVGGGTWTCTAAT-3'	Caporaso et al.

Table.1 The primers used in this study

The DNA samples were amplified using Applied Biosystems (Veriti 96 well Thermocycler) under the following condition: initial denaturation at 95oC for 3 minutes, followed by 30 cycles of denaturation at 98°C for 20 seconds, annealing at 65°C for 10 seconds, extension at 72°C for 15 seconds. And the final extension at 72°C for 1 minute. The 50 ul-PCR reactions were performed using KAPA HiFi HotStart PCR Kit.

PCR Purification

The triplicate of PCR products was pooled together for PCR purification prior to sequencing. This procedure was performed to remove or degrade any unused primer and unincorporated nucleotides. Exonuclease I help to degrade any residual Primer. Whereas, Anatartic Phosphatase is an alkaline Phosphatase, which dephosphorylates the unused dNTPs. Antartic Phosphatase, Antartic phosphatase buffer and Exonuclease I purchased from New England BioLabs were the reagents used for the purification of the PCR products. 4.0ul of unpurified PCR product was mixed with, 0.5ul Antartic Phosphatase buffer, 0.6ul of Antartic phosphatase, 0.6ul of Exonulease I and 3.3ul of nuclease free water. The mixture was then placed in Applied Biosystems-Veriti 96 well Thermal cycler under the following conditions: (1) 37°C for the first 20 minutes (2) 80°C for an additional 20 minutes.

2.4 Sequences Processing

The sequences are analyzed using **QIIME (Quantitative Insights into Microbial Ecology)**. This software focuses on analysis of raw DNA sequences and allows us to taxonomically arrange the data, as well as analyze and visualize the diversity and OTU richness of the sequences.

2.5 Statistical Analysis

I first tested our edaphic variable data for normality by plotting normal Q-Q plots and residuals. The data for population density, alpha diversity and pH were not normally distributed. So, I transformed these data by converting to log scales to approximate normality. (Supplementary materials) First, I wanted to test if environmental variables were

changing across a longitudinal gradient, as I suspected longitude could correlate as an urbanization gradient. I performed spearman rank correlations on all data set (soil pH, population density, and alpha diversity) since they are not normally distributed. I used our output tree and OTU table files from the QIIME pipeline to generate phylogenetic distance based alpha diversity measures for the sample. Chao1 diversity measurement was also calculated but does not use branch lengths so PD is a better measurement for a microbial dataset because taxonomic differences alone at the bacterial level are especially arbitrary, though they correlated strongly with each other. To see how alpha diversity was changing across environmental gradients, I did spearman's rank correlations between alpha diversity and soil pH, population density and longitude. Next, I wanted to see which pH affects the bacterial diversity and their community composition. To do this I made a CCA plot in R using the beta diversity distance matrix, mapped with all environmental variables. To test for significance between environmental factors and the beta diversity, I performed distance matrix correlations on the beta diversity dissimilarity matrix and Euclidean distance matrices of soil pH, population density, alpha diversity, and longitude. Then I wanted to see what bacteria tended to be seen together and what environmental drivers might be driving this. To do this I used a data set containing the proportions of top classes of bacteria in the soil samples. I made a CCA plot using a beta diversity dissimilarity matrix fitting the strongest environmental drivers with the classes of bacteria.

Results:

In this study, I performed the taxonomic composition analysis by creating a taxa summary plot in QIIME. According to the taxa summary plot, the dominant phyla of bacteria found across all soil samples are *Acidobacteria, Proteobacteria, Actinobacteria, Verrucomicrobia, Plantomycetes and Chloroflexi. (in the order by their relative abundance*. (Supplementary material: Figure 13).

In order to study soil microbiome on the urbanization gradient, I first determined population density of the areas on longitude that spanned from Manhattan to Montauk in Long island, New York using data from United State Census Bureau (*https://www.census.gov/geo/*), as an characteristics of urban centers. Population density significantly changes across the urban gradient and is negatively correlated with distance from urban center ($R=-0.91$, $p<0.001$), which indicated that these areas were a good fit for this study. I observed a significantly high population density in Manhattan, Bronx and Brooklyn and Queens. And the population gradually decreased as the areas got closer to Montauk (Figure 9, Supplementary materials: Figure.16).

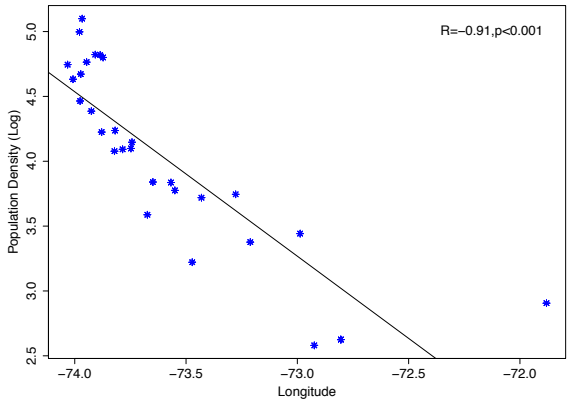


Figure 9. The population density along urbanization gradient (most urban=-74.0, least urban = -72.0)

The Effect of Urbanization on Soil pH

Using the obtained data, I tested my hypothesis#1 that urban centers where population density is high/urbanization increases soil pH. The result showed that soil pH is significantly correlated with and urbanization gradient (R= -0.26, p-value <0.005) as well as the population density (R= 0.31, p<0.001). In other words, soil pH levels are high in urban center where the population density is highest, which indicates that anthropogenic activity is one of the contributing factors to the soil pH. This confirmed my hypothesis#1. (Figure 10, Supplementary materials: Figure.16).

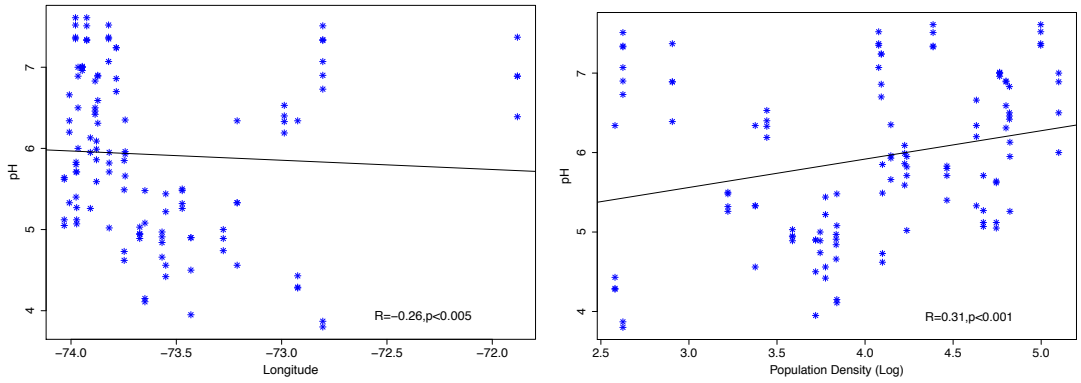


Figure 10. The distribution of soil pH on urbanization gradient (most urban=-74.0, least urban = -72.0) (left), the correlation between population density and pH (right).

The Effect of pH on Bacterial Diversity

Then, I tested my hypotheses#2 that bacterial diversity decreases as pH level decreases. The result showed that pH was significantly and directly correlated to bacterial diversity in soil, which confirmed my hypothesis#2 (R= 0.17, p-value <0.07) (Figure 11, Supplementary materials: Figure.16) .

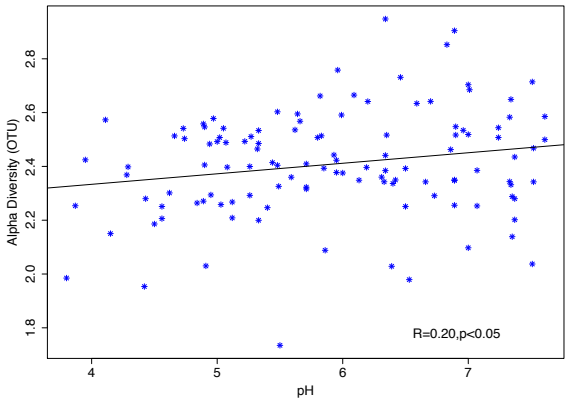


Figure 11. The bacterial diversity at pH gradient (R=0.20, p<0.05).

The Effect of Urbanization on Bacterial Diversity

Then, I tested my hypothesis #3 that highly-populated urban centers/urbanization increases bacterial diversity. The result showed the positive correlation between urbanization gradient and diversity, in which bacterial diversity was greatest closest to urban areas and declined as it becomes distant from urban center (R=-0.21, p<0.005) (Figure 12). Bacterial diversity was also positively correlated with human population density (R=0.28, p<0.005) which confirmed my hypothesis#3. And the high bacterial diversity observed in urban soil samples were driven by pH (Figure 11).

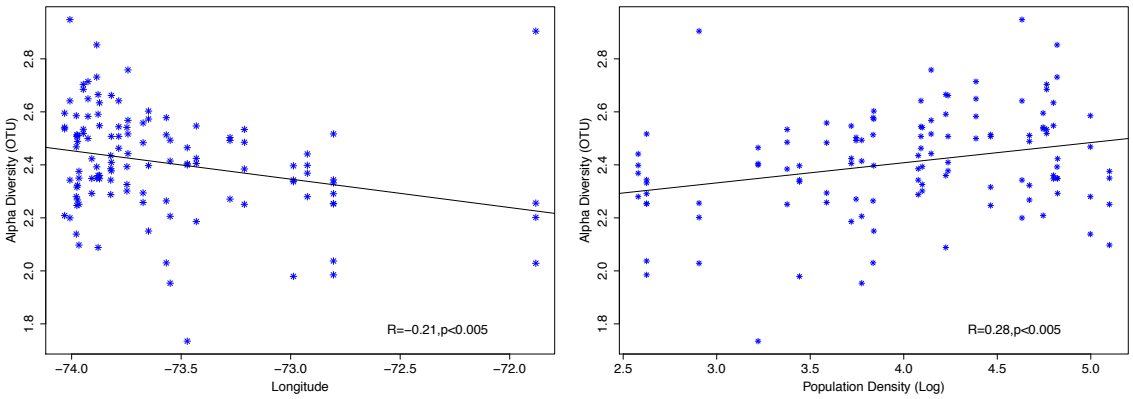


Figure 12. The bacterial diversity on urbanization gradient (most urban=-74.0, least urban =-72.0) (left), the correlation between bacterial diversity and population density (right).

Based on all these results, I've concluded that highly-populated urban centers significantly raise pH level in soil, which, in turn, increases the bacterial diversity.

The Effect of pH on Relative Abundance of Dominant Bacterial Phyla in Soil

pH affects not only the bacterial diversity but also can shift the bacterial community composition in soil by affecting particular groups of bacteria in soil. Therefore, I also assessed if pH shifts particular groups of bacteria in the soil by finding the correlation between pH and the relative abundance of dominant bacterial phyla. Based on the previous study by Wu et al., 2017, *Acidobacteria*, *Chloroflexi, Proteobacteria and Plantomycetes* are the dominant groups in soil that respond to change in pH level among six dominant bacterial phyla. Therefore, I hypothesized that I will find the correlations between these three bacterial phyla and pH. I then tested the hypotheses#4,5,6 that there is a correlation between pH and the relative abundance of *Acidobacteria*, *Chloroflexi*, *Plantomycetes*. The results showed the significant positive correlation for *Acidobacteria* ($R= 0.33$, $p<0.01$) and *Chloroflexi* ($R=0.20$, $p<0.05$), thereby confirming my hypothesis#4 where there is a

positive correlation between pH and *Acidobacteria*, but it contradicted with my result for *Chloroflexi* having negative correlation with pH according to Wu el al., 2018 (Table.2). My results showed the positive correlation between *Chloroflexi* and pH (Table.2, Supplementary materials: Figure 14). However, my results showed no significant correlation between pH and *Plantomycetes* (R=0.037, p<0.07), this also contradict with the results from Wu et al., 2018, thus I rejected the hypothesis#6 (Table.2, Supplementary materials: Figure 14). The relative abundance of *Proteobacteria, Actinobacteria, Plantomycetes* are not affected by soil pH. Then, I tested the hypothesis#7 that there is a relationship between pH and *Verrucomicrobia*. The results showed that the relative abundance of *Verrucomicrobia* is significantly and negatively correlated with pH, which confirms my hypothesis#7 (R=-0.30, p<0.01) (Table.2, Supplementary materials: Figure 14).

pH					
Positive			**Negative**		
OTU (phylum)	**R**	**P**	**OTU (phylum)**	**R**	**P**
Chloroflexi	0.20	<0.05*	*Verrucomicrobia*	-0.30	<0.01*
Acidobacteria	0.33	<0.01*			
Proteobacteria	0.03	<0.07			
Plantomycetes	0.037	<0.07			
Actinobacteria	0.03	<0.07			

Table.2 The relative abundance of six dominant phyla recording statistically significant correlation (P<0.05*) with pH.

The Effects of pH on Relative Abundances of Dominant Classes of Bacteria in Soil

I based on Delgado et al., 2018 and looked into the top bacterial group in soil at the class level and their response to the pH. This increased the resolution for me to see if there is a correlation between pH and the classes of bacteria within some dominant bacterial group in soil that doesn't show correlation at the level of phylum such as *Proteobacteria*, *Plantomycetes* and *Actinobacteria*. Even at the class level, the bacterial groups within *Proteobacteria*, *Actinobacteria* and *Plantomycetes* phyla showed no significant relationship with pH. (Table.3, Supplementary materials: Figure.15) Based on my data, I observed that top bacterial groups that belong in *Proteobacteria*, *Actinobacteria* and *Plantomycetes* do not have distinct pH preference and are not affected by pH (Table.3, Supplementary materials: Figure.15). However, the top bacterial classes in *Acidobacteria* phylum: *Chloracidobacteria*, *Acidobacteria-6* and *Solibacteres* all have the distinct pH preference. My result showed there is a positive correlation between pH and *Chloracidobacteria* ($R=0.38$, $P<0.01$) and *Acidobacteria-6* ($R=0.40$, $P<0.01$). And there is a negative correlation with pH and Solibacteres. In *Chloroflexi* phylum, pH is significantly and positively correlated with two top classes: *Anaerolineae* ($R=0.40$, $P<0.001$) and *Ellin6529* ($R=0.35$, $P<0.005$). There is a slight correlation with *Thermomicrobia*, but not significant enough. Within *Verrucomicrobia* phylum, the top two bacterial classes: *Spartobacteria* and *Pedospharae* are both significantly and negatively correlated with pH [($R=-0.25$, $P<0.005$), ($R=-0.27$,$p<0.005$), respectively], but no significant correlation was observed for class *Optitutate* ($R=-0.0041$, $P=0.97$) (Table.3, Supplementary materials: Figure.15).

I observed that bacterial groups that belong in *Verrucomicrobia* have the preference for acidic pH and they are most likely to be found and abundant in acidic soils. However, the distinct pH preference was not observed for bacterial groups that belong to *Proteobacteria*, *Actinobacteria* and *Plantomycetes*. Those three phyla are not significantly affected by change in pH level.

pH							
Positive				**Negative**			
OTU (Phylum)	**OTU (Class)**	**R**	**P**	**OTU(Phylum)**	**OTU(Class)**	**R**	**P**
*Chloroflexi**	*Thermomicrobia*	0.17	<0.7				
	Anaerolinae	0.40	<0.001*				
	Ellin6529	0.35	<0.005*				
*Acidobacteria**	*Chloracidobacteria*	0.38	<0.01*	*Acidobacteria**	*Solibacteres*	-0.40	<0.01*
	Acidobacteria-6	0.40	<0.01*				
Proteobacteria	*Beta-proteobacteria*	0.13	<0.15	*Proteobacteria*	*Alpha-proteobacteria*	-0.15	<0.15
	Gamma-proteobacteria	0.035	0.70		*Delta-proteobacteria*	-0.005	0.95
				Plantomycetes	*Phycispharae*	-0.12	<0.5
					Plantomycetia	-0.15	<0.5
Actinobacteria	*Thermoleophilia*	0.095	0.3	*Actinobacteria*	*Actinobacteria*	-0.13	0.17
					Rubrobacteria	-0.045	0.62
				*Verrucomicrobia**	*Spartobacteria*	-0.25	<0.005*
					Pedospharae	-0.27	<0.0005*
					Optitutae	-0.004	0.97

Table. 3 The relative abundance of dominant classes within 6 phyla recording statistically significant correlation (p<0.05*) with soil pH.

Discussion:

One of my most significant results I found is the bacterial diversity in soil, which was directly proportional to the human population density. Also, high population density in urban centers altered the soil pH, which in turn influence the soil bacterial diversity and the community composition. Based on my data, I concluded that high population density in urban centers increased bacteria diversity in soil, which corresponded with the result from the soil microbiome study done at latitudinal urbanization gradient, across the City of Chicago. My data also indicated that urbanization increased the soil pH, which corresponded with the results from the previous study done in arable soil of Kumasi, Ghana (West Africa) and Hong Kong. Furthermore, my data showed that pH was highest in soils that were closer to dense urban centers and became more acidic as distance increased from the urban center. The soils furthest from dense urban centers had the greatest range of pH. Based on all these results, I reasoned that the low pH decreases bacterial diversity in soil.

According to Delgado, the bacterial groups such as *Acidobacteria, Proteobacteria, Actinobacteria, Verrucomicrobia, Plantomycetes* and *Chloroflexi* were the dominant bacterial phyla, found in the soil across the globe (Delgado et al., 2018). The same dominant phyla were observed across all soil samples in in this study. Although low soil pH usually affected majority of bacterial groups, I found a bacterial phylum that their relative abundance increased as the pH decreased. That dominant bacterial phylum that seemed to favor low pH was *Verrucomicrobia* (Table.2, Supplementary materials: Figure.14). No other study had mentioned the relationship between pH and *Verrucomicrobia* phylum. On the other hand, *Acidobacteria* and *Chloroflexi* seemed to be dominant in slightly acidic and neutral condition of pH . However, other dominant bacterial

phyla such as *Proteobacteria, Actinobacteria* and *Plantomycetes* thrived in wide range of pH and there is no distinct pH where they are most abundant (Table.2, Supplementary materials: Figure.14). According to Wu et al., 2018, *Acidobacteria* and *Plantomycetes* phyla reside and are most abundant in pH neutral soils. However, *Chloroflexi* phyla reside and are most abundant in acidic soils. This results from Wu el al., 2018 contradicted with my results, where I found *Acidobacteria* and *Chloroflexi* to be most abundant in pH neutral soils and *Plantomycetes* to have no distinct pH preference (Table.2, Supplementary materials: Figure.14)

In summary, *Proteobacteria*, *Actinobacteria* and *Plantomycetes* don't have the distinct pH preference among the top six phyla. In other word, their relative abundance is not affected by change in pH and there is no correlation between them and pH. I observed the bacterial groups that belong in *Verrucomicrobia* have the preference for acidic pH. They are most likely to be found and abundant in acidic soils. However, the preference for distinct pH was not observed for bacterial groups that belong to *Proteobacteria*, *Actinobacteria* and *Plantomycetes* (Figure. 13). Those three phyla are not significantly affected by change in pH level. Therefore, pH cannot be used to determine and relative abundance for those bacterial groups belong to *Proteobacteria*, *Actinobacteria* and *Plantomycetes* phyla. Even at the class level, I observed no correlation with pH and the relative abundance of the top bacterial groups that belong to *Proteobacteria*, *Actinobacteria* and *Plantomycetes* (Figure. 14). The bacterial phyla in soil that their relative is most affected by pH are *Acidobacteria*, *Chloroflexi* and *Verrucomicrobia*. Even the bacterial classes within these phyla are significantly affected by pH (Figure.14).

My data indicated that Acidobacteria tend to dominate in the urban soils where pH is mostly neutral. The abundance of Acidobacteria has many benefits to the soil. Their ecological function is very diverse that includes their ability to: use of nitrite as N source, respond to soil macro-, micro nutrients and soil acidity (Kielak, Anna M et al, 2016). My data also indicated that there is a positive correlation between *Chloroflexi* and pH. This means *Chloroflexi* tend to dominate in urban soil, where pH is mostly neutral soil. They participate in the oxidation of ammonia or ammonium into nitrites in the soil, making nitrogen available to plants. However, I found *Verrucomicrobia* negatively correlated with pH and they were dominant in the natural soil, where pH is acidic. The bacteria that belong in *Verrucomicrobia* phylum are methanotrophs and these bacteria possess copper contained methane monooxygenase enzyme (MMO) to break the methane bond and metabolize methane as their carbon energy source, and they participate in the soil ecosystem by reducing the methane gas in the atmosphere. The reduction in abundance of methanotrophs in urban soil means there will be less methanotrophs in soil to reduce the methane gas in urban environments.

I created CCA plot to see which environmental variables seemed to be driving the bacterial diversity and community composition. My CCA plot indicates that pH is one major driver of bacterial diversity, based on the observation that there is a positive correlation between pH and bacterial diversity (Supplementary materials). This result agreed with the finding from the previous study done in arable soil of Kumasi, Ghana (West Africa) and Hong Kong. I also observed that the population density is also driving the bacterial diversity on my CCA plot, which agreed with the result from the soil microbiome study done at latitudinal urbanization gradient, across the City of Chicago. My CCA plot

shows that *Gammaproteobacteria* is positively correlated with human population density. This indicates that gram-negative Gammaproteobacteria such as Enterobacter, E. coli, Vibrio, Salmonella, Pseudomonas, Legionella, Shigella and other potentially pathogenic bacteria humans and other animals carry are more abundant in the soils from the highly-populated urban centers.

Supplementary materials:

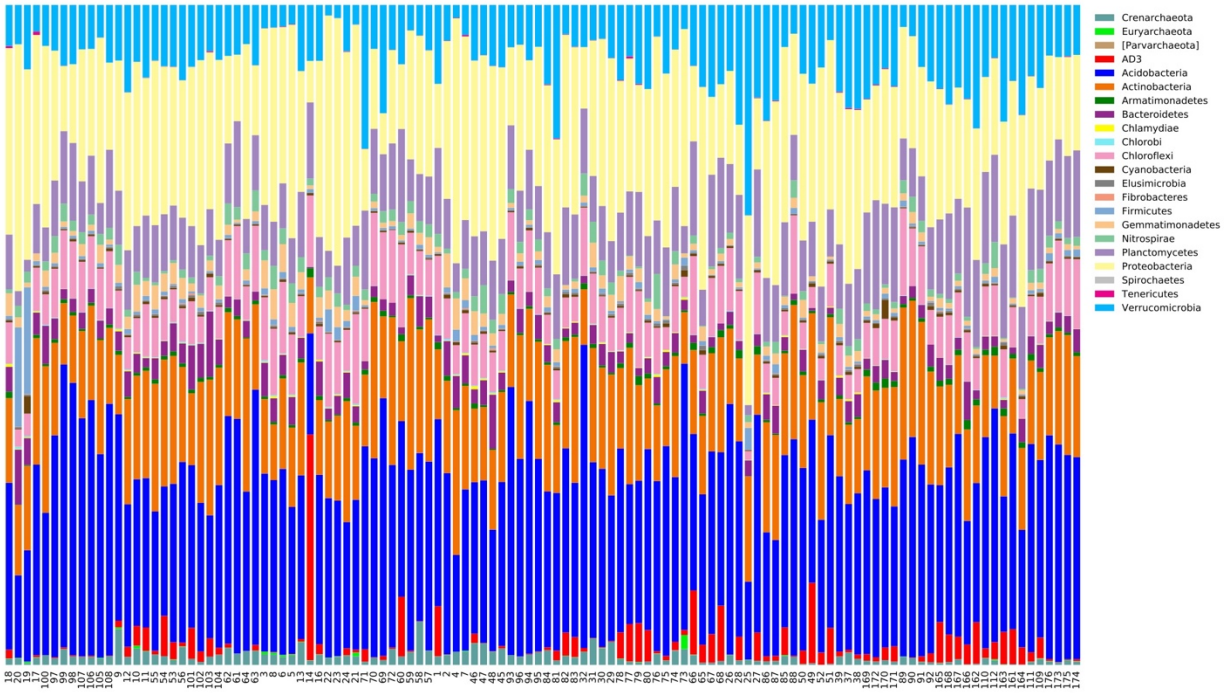


Figure 13. Taxa summary plot: Acidobacteria, Proteobacteria, Actinobacteria, Verrucomicrobia, Plantomycetes and Chloroflexi are the dominant bacterial phyla found across all soil samples.

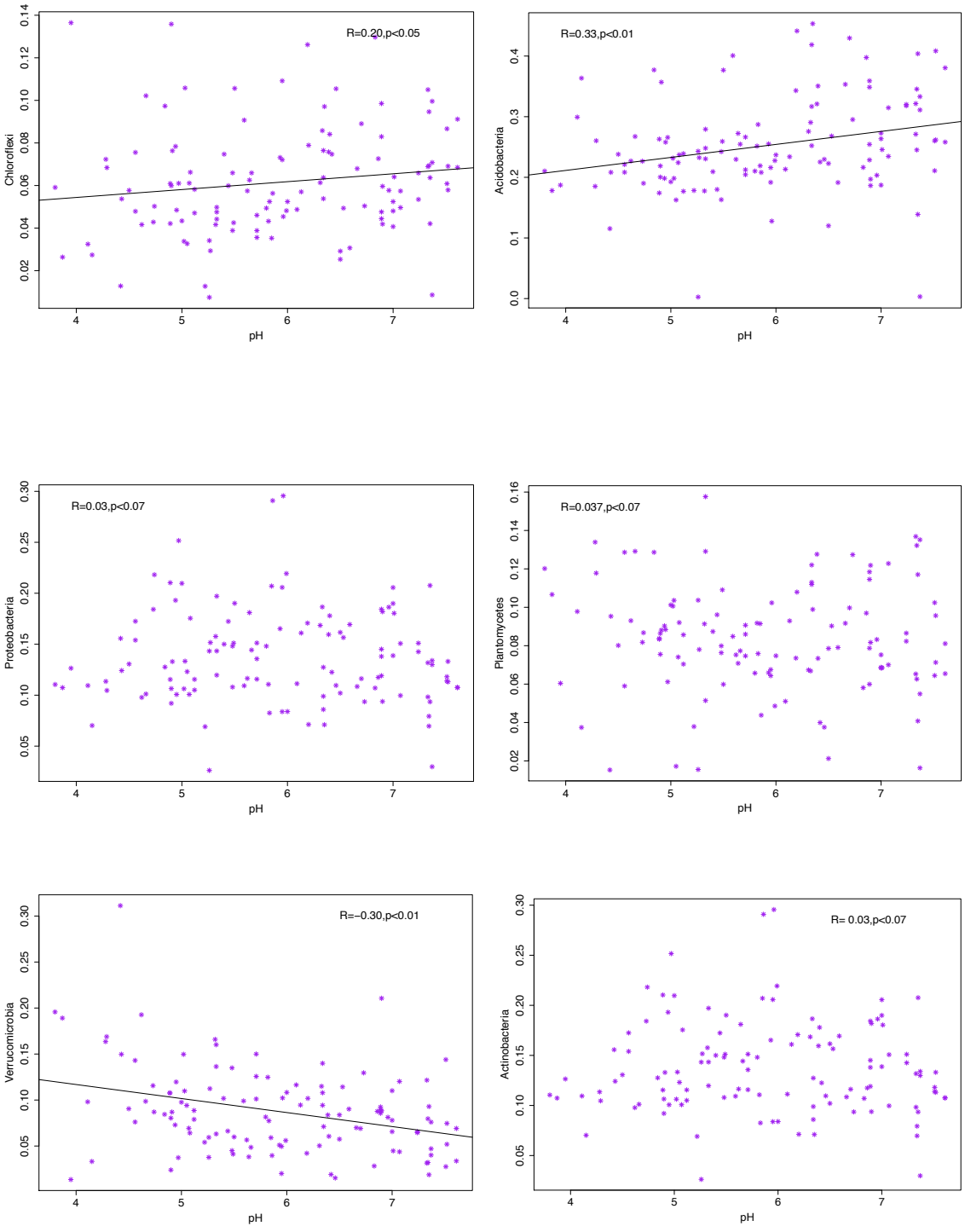
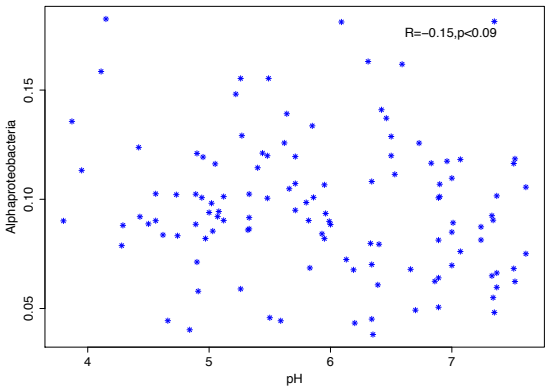
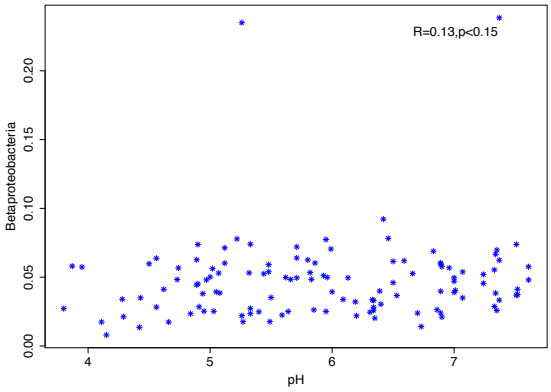
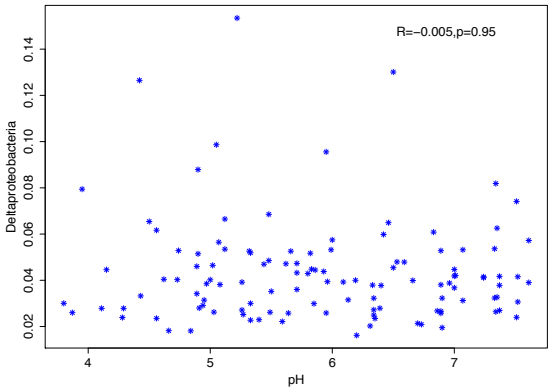
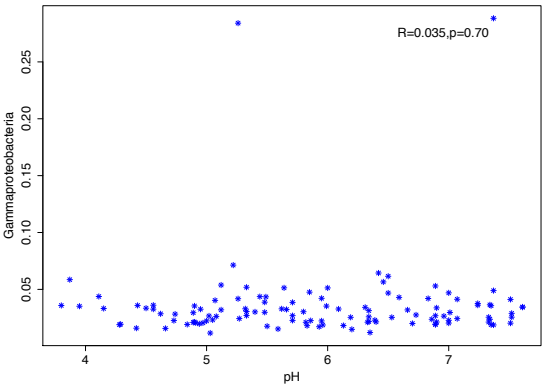


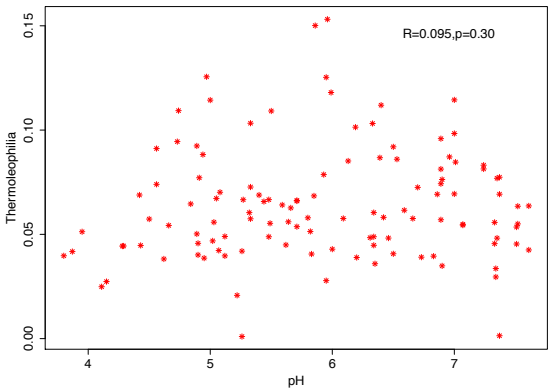
Figure 14. The Correlation between pH and Dominant Bacterial Phyla

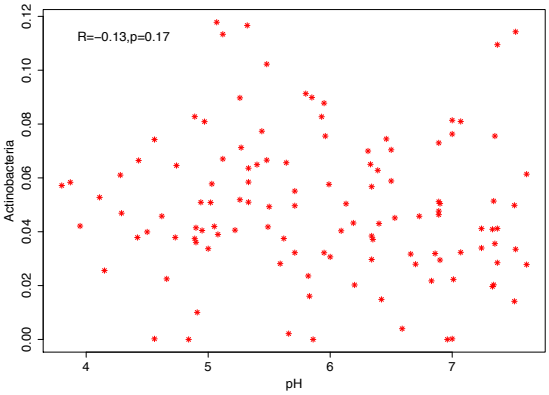

R=−0.15,p<0.09
Alphaproteobacteria
pH

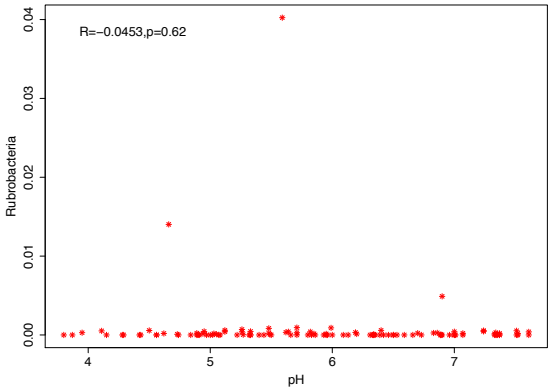

R=0.13,p<0.15
Betaproteobacteria
pH

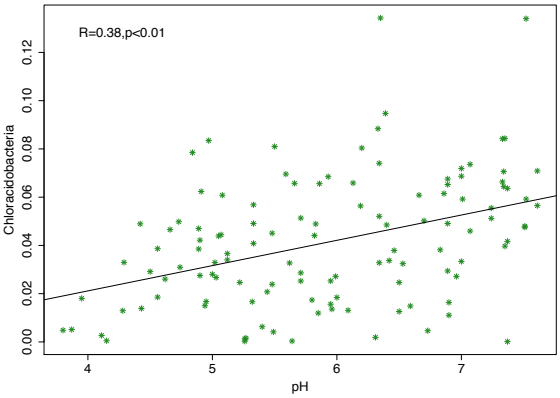

R=−0.005,p=0.95
Deltaproteobacteria
pH

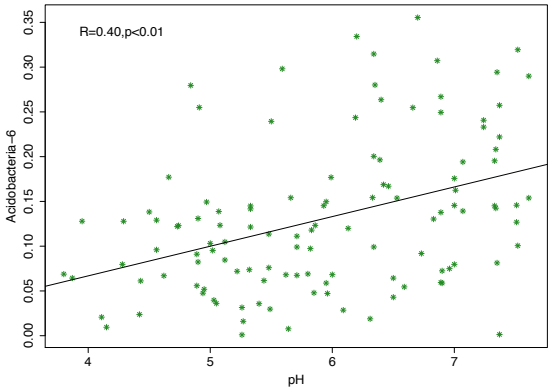

R=0.035,p=0.70
Gammaproteobacteria
pH

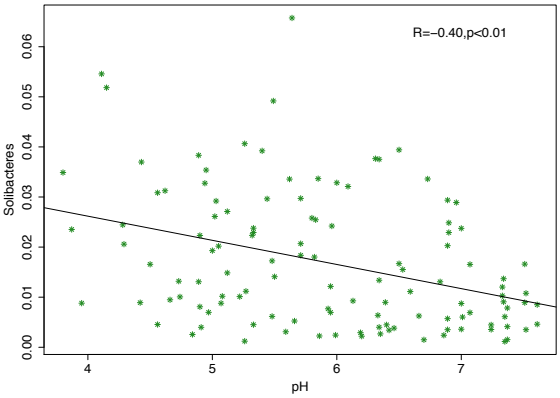

R=0.095,p=0.30
Thermoleophilia
pH

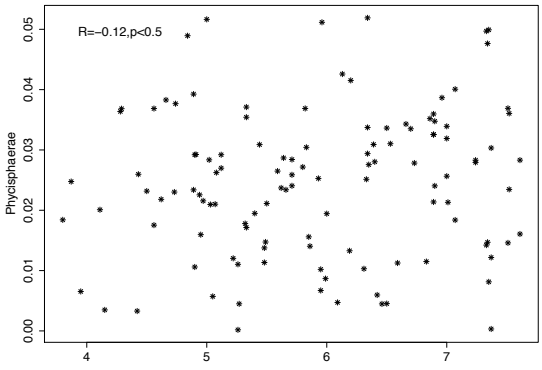

R=−0.13,p=0.17
Actinobacteria
pH

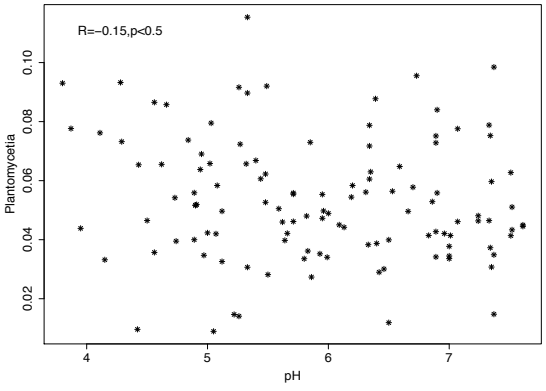

R=−0.0453,p=0.62
Rubrobacteria
pH


R=0.38,p<0.01
Chloracidobacteria
pH


R=0.40,p<0.01
Acidobacteria−6
pH


R=−0.40,p<0.01
Solibacteres
pH


R=−0.12,p<0.5
Physisphaerae


R=−0.15,p<0.5
Plantomycetia
pH

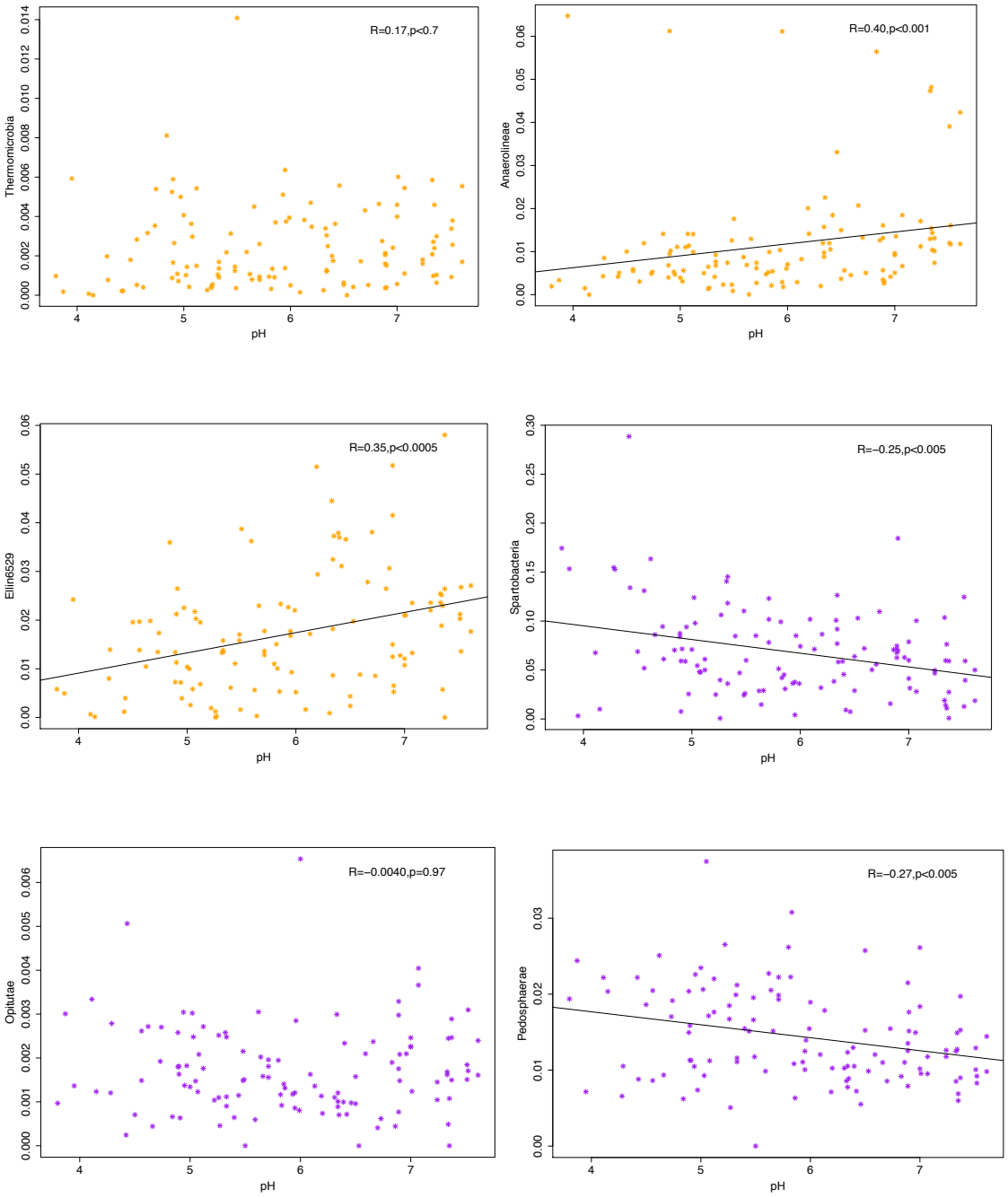


Figure 15. The Correlation between pH and Dominant Bacterial Classes: proteobacterial classes (Blue), actinobacterial classes (Red), plantomycetal classes (Black), acidobacterial classes (Green), chloroflexal classes (Orange) and verrucomicrobial classes (Purple).

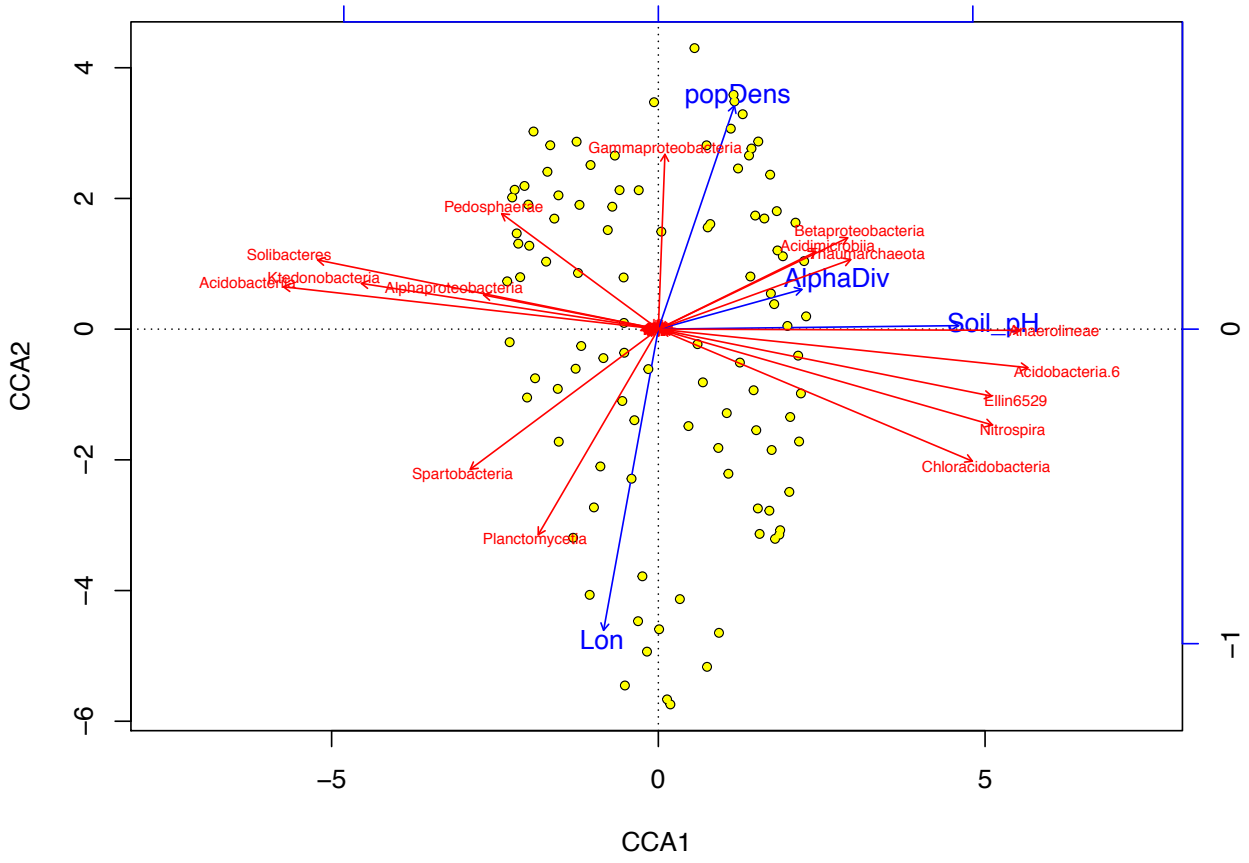
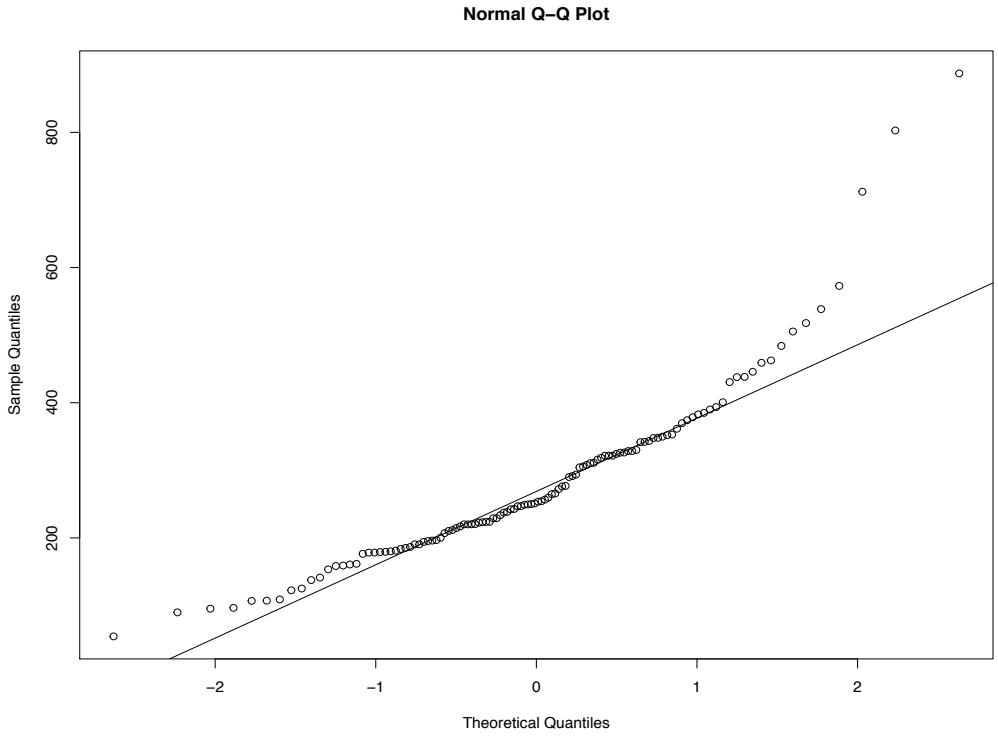


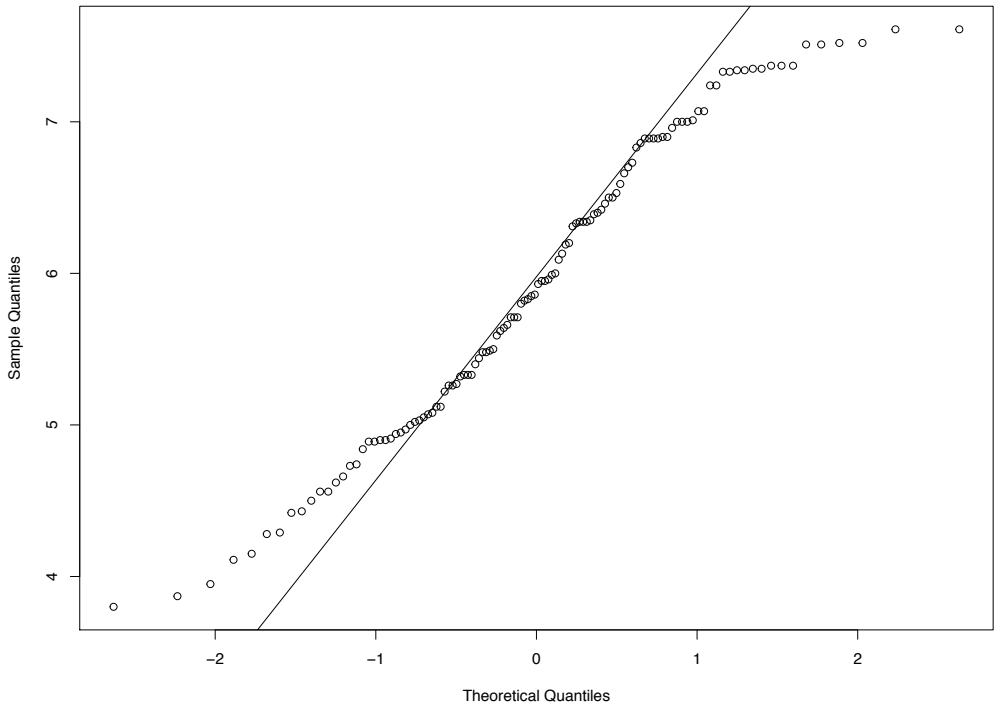
Figure 16. The Canonical Correspondence Analysis (CCA plot) depicting the bacterial communities driven by soil pH. The pH was found to be one of the significant drivers of bacterial diversity, based on the observation that both vectors are parallel to each other. The vectors that are parallel indicates the positive correlation and anti-parallel indicates the negative correlation. The angle between each vector for the environmental variable indicates how strongly each variable correlate to each other. The length of the vector indicates the significance of correlation.

QQ plot (Normality Test)

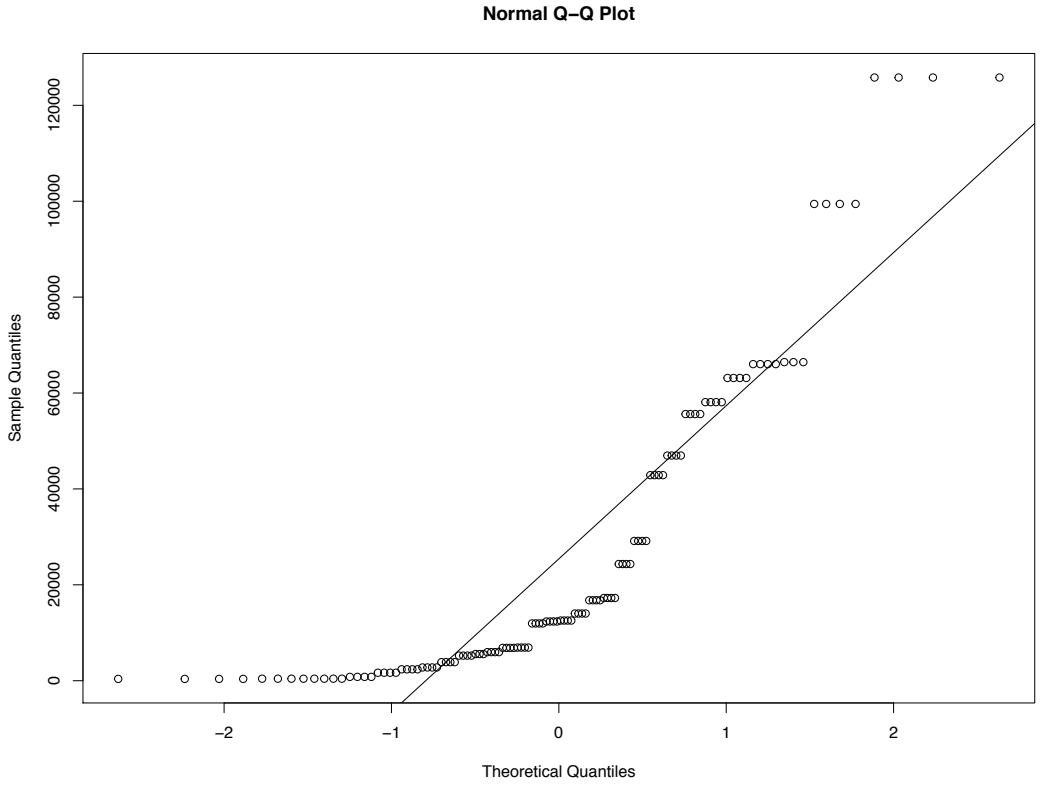
Alpha Diversity



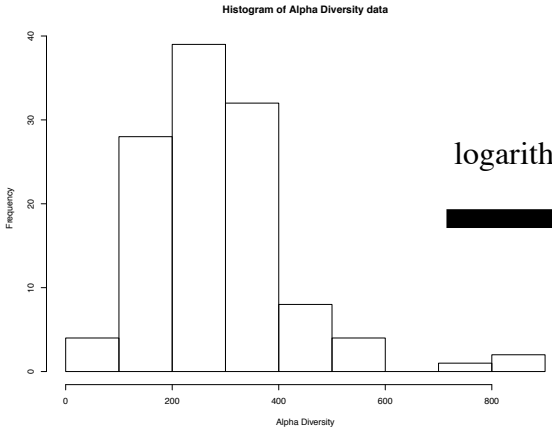
pH



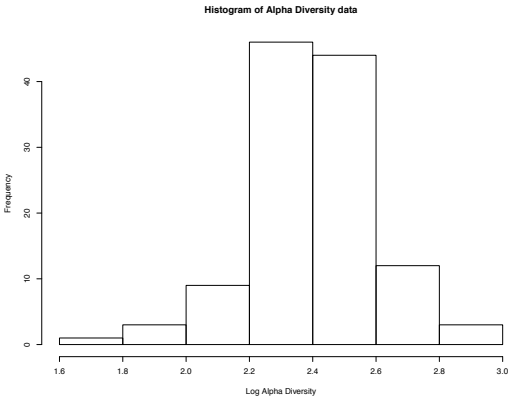
Population Density



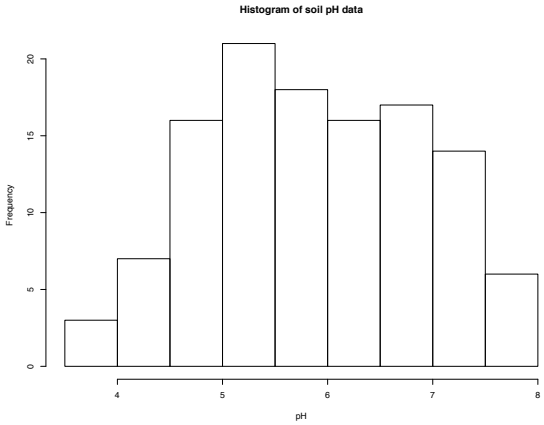
Data transformation



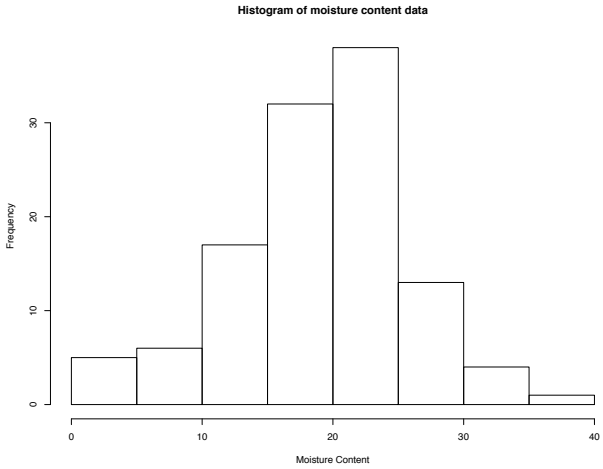
logarithm



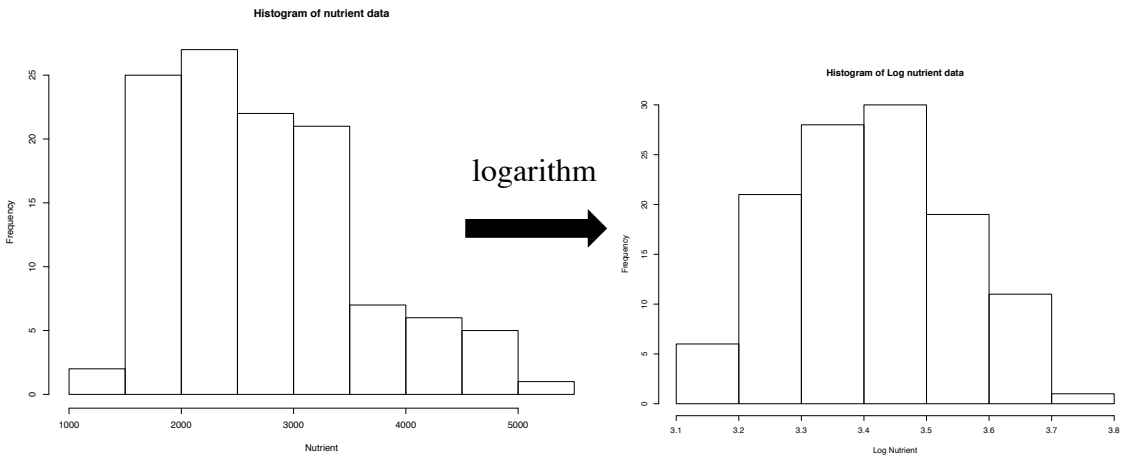
Data is transformed to approximate normality.



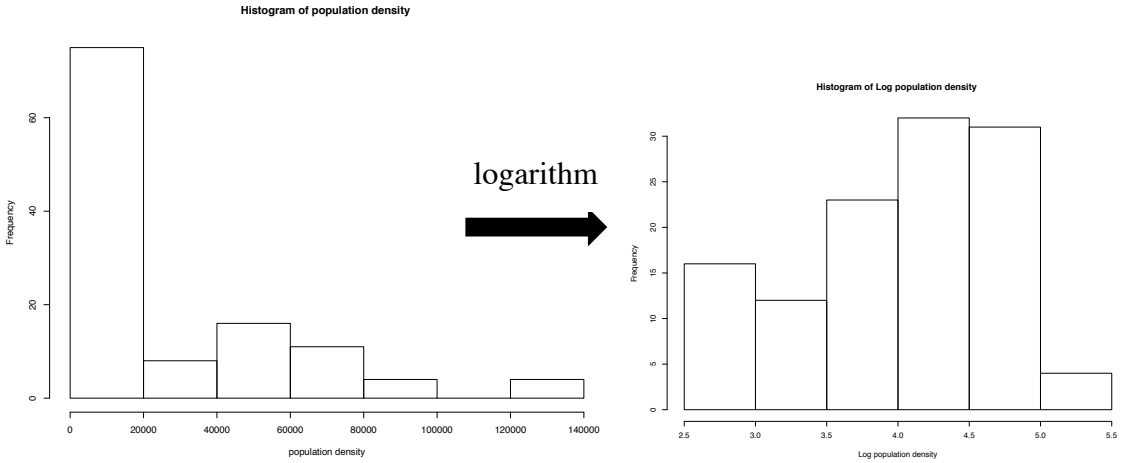
Data cannot be transformed.



Data is normal.



Data is transformed to approximate normality.



Data is transformed to approximate normality.

Figure 17. Data checked for normality by Q-Q Plot Test. Data that were not normal were transformed into approximate normality by converting into log scale.

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