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The Influence of Conservation Tillage and Conventional Tillage on Soil Bacterial Diversity in Southern Illinois

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THE INFLUENCE OF CONSERVATION TILLAGE AND CONVENTIONAL TILLAGE ON SOIL BACTERIAL DIVERSITY IN SOUTHERN ILLINOIS

by

Nasser Syed

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ABSTRACT

THE INFLUENCE OF CONSERVATION TILLAGE AND CONVENTIONAL TILLAGE ON SOIL BACTERIAL DIVERSITY IN SOUTHERN ILLINOIS

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Agriculture in the Midwest United States (Illinois, Indiana, Iowa, Michigan, Minnesota, Ohio, and Wisconsin) is a critically important component of the United States economy and also for world exports of food grain. This is well reflected in the 2012 Census of Agriculture which showed that these states had a market value of crop and livestock products sold in excess of $80,000,000,000 (USDA, 2012). Within the U.S. the three Midwest states, Illinois, Iowa, and Minnesota are ranked 2nd, 3rd, and 4th for the economic value of crops sold. This economic value of agriculture in the Midwest encompasses not only corn, soybeans, livestock, vegetables, fruits, tree nuts, and berries but also nursery and many greenhouse plants. Soil is the one common underlying platform for agriculture and if agriculture has to remain profitable and sustainable, a scientific understanding of soils and their relationship to plant productivity is critical.

Soils harbor probably the most diverse microbial ecosystems on Earth (Delmont et al., 2011) and we are just beginning to understand the full extent of this diversity and how it influences agricultural productivity and how in turn agricultural practices influence the microbial diversity. Estimations indicate that approximately 1,000 Giga base pairs (Gbp) of microbial genomic sequences exist per gram of soil (Vogel et al., 2009). Microorganisms occupy almost every available niche on Earth and directly affect the environment and agricultural systems by a range of mechanisms that include

biological nitrogen fixation (Hungria, Franchini, Campo, & Graham, 2005), suppression of diseases (Mendes et al., 2011), decomposition of organic components (Schmidt et al., 2011), plant growth promotion (Bhattacharya & Jha, 2012), soil nutrient cycling (Brussard, 2012) and bioremediation (Ali et al., 2012). Soil microbial community structure and its associated and interdependent biological processes can be affected by the way land is used and managed. Since a vast majority of soil microorganisms do not respond to "traditional" culturing techniques (Delmont et al., 2011), it has been difficult to study and characterize the functional and phylogenetic diversity of these important ecosystems until recent advances in next-generation DNA sequencing which have begun to unravel what is beneath our feet (Caporaso et al., 2010). According to Food and Agricultural Organization (FAO), the amount of land used for agriculture is about 11% (http://www.fao.org/docrep/005/y4252e/y4252e06.htm) and the emissions which can have serious environmental and health effects from agricultural food production far outweigh the total emissions from all the other industries combined (Bauer, Tsigardis, & Miller, 2016). Thus, any steps to fine-tune the management practices and the way the agricultural land is utilized can go a long way in sustaining our way of life while maintaining a healthy environment.

The purpose of this study is to examine the shifts in the taxonomic diversity of bacteria in soils at phylum, class and order level between two distinct agricultural practices – Conventional Tillage (CT) and Conservation Tillage (NT) in Southern Illinois along with changes in soil compaction and soil phosphatase activity. The larger idea, based on results reported here and elsewhere, is to encourage conservative tillage practices using a combination of diverse cover crop systems and continuous soil cover

which seem to enhance functional microbial diversity in the soil (Ajay & Ngouajio, 2012; Verzeaux et al., 2016). Research also indicates the presence of higher numbers of bacteria of varied trophic groups, as well as increased species richness in bacteria in well-managed soils with minimal tilling and this, may correspond to more resilience to drying and rewetting disturbances in the soil (Anne et al., 2006).

This research may be the first to reconstruct the entire soil bacterial community in agricultural fields of Southern Illinois and will also hopefully be a precursor for more studies aimed at not only understanding soil from a biological bacterial perspective but also in deciphering interesting patterns that can help correlate changes in land management practices and how they impact bacterial communities. It may help us in developing a methodology to use bacterial taxa as indicators of soil management practices. The study will also detect previously unreported rare bacterial taxa-specific for this region and regional geochemistry.

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DEDICATION

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CHAPTER I – INTRODUCTION

Background of the Study

For the past several decades, many studies have measured the impact of agriculture on soil microorganism's diversity and function, but using methodologies that could only identify a small fraction of microorganisms and characterize microbial activities at a very limited depth in relation to the microbial biomass (Kaschuk, Alberton, & Hungria, 2010, 2011). New technologies such as Next-generation DNA sequencing (NGS) combined with metagenomic platforms such as MEGAN & QIIME are revealing how anthropogenic activity may be impacting soil biology and the ecosystem (Souza, Cantão, Vasconcelos, Nogueira, & Hungria, 2013). With almost 40% of the Earth's available surface used for agriculture (including both crops and pasture) and agriculture-related industries (Foley et al., 2005; The World Bank Data - http://data.worldbank.org/indicator/AG.LND.AGRI.ZS) there is a need to find innovative methods to reduce land usage while conserving soil and the soil ecosystem. This study is one of the first to examine how tillage practices influence soil bacterial diversity in southern Illinois. By utilizing an NGS approach, limitations of using specific methodologies or genes which rely on bias of specific primers towards detecting uncultivable bacteria can be avoided or minimized, and thus, more in-depth information revealing genetic and metabolic diversity as well as new metabolic pathways, genes, and their products can be generated (Bengtsson-Palme, Bowland, Fick, Kristiansand & Larsson, 2014; Singh et al., 2014). Therefore, to better understand the impact of anthropogenic agricultural practices on southern Illinois soils, a shotgun metagenomic approach was used with taxonomic analyses comparing conventional tillage (CT) vs

conservation tillage (NT) soils. This research study will be the first for southern Illinois and also novel in several ways. It will be the first to analyze and reconstruct the entire soil bacterial community. These techniques should also increase our ability to detect rare taxa. We will be comparing no-till soils to conventional tillage treatments.

A word of caution must be placed here to emphasize the fact that these techniques are all new and are subject to biases both in the experimental aspect and the analytical part. Many of the software packages that are used here are updated continuously as and when the genomic databases are updated based on inputs from groups and individuals. In addition to this, new information on newly discovered species and pathways are constantly being added. A big part of the study is the use of universal primers for bacteria, and the choice of the primers and the annealing temperatures used can influence the study (Deagle, Thomas, Shaffer, Trites, & Jarman, 2013). Finally, due to the novelty of this study and of this field and the geographical area used for sampling, these results may require future investigation.

Agriculture is facing major challenges and unless production is doubled in the next 50 years we may face shortages in both our growing food demand and bioenergy needs (Foley, Ramankutty, Brauman, Cassidy, & Gerber, 2011). Ideally, this should be achieved without risking environmental damage, biodiversity loss, and degradation of natural resources. The biggest impediment for such an approach is the trade-off among economic and environmental goals combined with insufficient understanding about the biological, biogeochemical, and ecological processes that underlie functioning of a healthy soil ecosystem (Balmford, Green, & Scharlemann, 2005). A majority of our soil fertility requirements are met by our dependence on a range of external inputs which can

have many negative impacts (Matson, Parton, Power, & Swift, 1997). The key to understanding and conserving soil lies in how various geochemical events are modulated by the underlying biology (Scholes & Scholes, 2013). These approaches should involve not only studying the physical and chemical parameters but also studying and integrating the diversity and functional analysis of the biology in that ecosystem (Fierer, Ladau, Clemente, Leff, & Owens, 2013).

Agriculture based on the amount of land used is one of the major influencers of both local and global ecological changes and can affect many important ecosystem properties (Vitousek, Mooney, Lubchenco, & Melillo, 1997). In today's world conservation programs aimed at soil conservation are necessary to preserve the biodiversity of these ecosystems because, as our food needs increase, conversion of more and more lands to agricultural use can lead to loss of ecological functions and thus cause major ecological imbalances (Griffiths & Philippot, 2013).

Ever since the dawn of human agricultural activity tillage in some form has been a part of the practice that impacts the soil quality. Physical manipulation or disturbance of the soil leads to changes in many aspects of soil including soil water content, the mechanical properties, and composition of soil particles, and the degree to which crop residues get incorporated within the soil matrix thus causing perturbations in physical, chemical and biological aspects of the soil (Kladivko, 2001). If done continuously, it can disrupt existing plant communities and their associated biological dependencies while loosening the soil and redistributing organic matter. In the early 1920s and 1930s, extensive ploughing with subsequent removal of any plant residue was a standard practice to prepare soils for the next season (Coughenour & Chamala, 2000). Such

practices can ultimately damage the soil environment to a point where it may no longer be able to support cropping systems (Lal, 1993). Lal (1993) also reported that many important characteristics such as soil structure, resistance to wind and water induced erosion, cycles of water, nutrients and organic matter could be disturbed in a manner that is very difficult to repair or rectify. The effect of conventional tillage appears to correlate with the changes in soil structure that can disturb microbial diversity including impact on the community dynamics, diversity, and relative abundance of microbes compared to conservative tillage practices (Brussaard, de Ruiter, & Brown, 2007). New age tillage practices including reduced or no-till practices have resulted in soils that are more resilient to degradation, resource efficient and more productive (Holland, 2004). The exact mechanisms through which the new reduced or conservation tillage or no-till practices impact soil biodiversity and ultimately soil resilience is open for research and studies.

Research Questions

1. Do agricultural soils under conservation tillage practice have lesser soil compaction than those under conventional tillage practice?
2. Do agricultural soils under conservation tillage practice have higher phosphatase (a commonly used indicator of soil fertility) activity than those under conventional tillage practice?
3. Do agricultural soils under conservation tillage practice have a more diverse community of bacteria than those under conventional tillage practice?

Purpose of the Study

Without functioning and dynamic soil ecosystems, our planet may not support the living systems as we know them. With all its multitude of forms and functions, soil ecosystems affect our climate and many aspects associated like carbon sequestration, the quantity and quality of water, water storage, the productivity and nutritional value of plants growing in soils, the successful establishment of invasive organisms, the health of bays and estuaries downstream and the prospects of discovering new medicines or molecules for human health. Soil ecosystems are the key drivers of global cycles of carbon, water, nitrogen, phosphorus and sulfur and many more yet to be discovered dependencies and geochemical transformations.

Soils are probably the most diverse environments and may contain more species than any other terrestrial ecosystem (Thiele-Bruhn, Bloem, de Vries, Kalbitz, & Wagg, 2012). The diversity of the living systems especially the diverse soil organisms have a fundamental role in nutrient cycles as they decompose organic matter, fix nitrogen and sequester carbon and hence soil is one of the key drivers of global carbon, water, nitrogen and many more cycles. The extent and the speed with which these processes occur is largely dictated by the diversity of microbial taxa (Heemsbergen, Berg, Loreau, van Hal, Faber, & Verhoef, 2004). Soil biologist David Wardle (2002) a distinguished author of over 160 articles on soil/plant issues, made it very clear in his book, "*Communities and Ecosystems: Linking the Aboveground and Belowground Components*" that less than 3 percent of papers published in major ecological journals involved studying organisms below the ground. In one of the earliest and my favorite books, author Rachel Carson devoted an entire chapter to the soil microorganisms which by their very presence and by

their activities make this planet capable of supporting life as we see it and understand it today (Carson, 1962). Wardle (Wardle, Bardgett, Klironomos, Setälä, van der Putten, & Wall, 2004) acknowledged in the 2004 *Science* special report that there is an increasing recognition of the fundamental role played by above and below ground feedbacks in controlling the dynamic properties and processes underlying the ecosystem. What is more important is that the complexity of soil as an ecosystem can only be studied and understood better with an interdisciplinary approach because no one discipline on its own will be enough to understand the most complex material or matrix on the planet (Young & Crawford, 2004).

The premise of this thesis is that "in order to push for fundamental changes in the way we approach agriculture and sustainable living we have to provide evidence on how we are affecting and impacting one of the largest and most important diverse ecosystem to both land users and policymakers to push for changes in policies and practices. The conceptual models on which most of our present agricultural practices function must be corrected. This study in predominantly farming based Southern Illinois hopes to add to that momentum by generating data and evidence both for understanding and educating ourselves on how we are damaging what is right beneath our feet. The work done as part of this thesis will study the impact of conventional tillage practices and conservative tillage practices on the bacterial diversity in the soils in southern Illinois.

Hypotheses

H1. Agricultural soils with conservative tillage practice will have lesser compaction than soils with conventional tillage practice.

H2. Agricultural soils with conservative tillage practice will have higher phosphatase activity.

H3. Agricultural soils with conservative tillage practice will have a more diverse community of bacteria.

Assumptions

The researcher classifies the soils as conservation tillage (NT) or conventional tillage (CT) based on what kind of tillage practice was established prior to sampling the soils. A set of practices broadly classified as NT and CT were used for this purpose and explained in the methodology. However, the conservation tillage has many variations from region to region based on the soil conditions, crop to be grown, available resources and organic content.

Delimitations

The study will be restricted to agricultural land in Saline County of southern Illinois and may not represent the entire range of soils types for this geographical area. The study takes into consideration only three years of history of the soil before being divided into plots for CT and NT. The plots were managed under NT and CT practices for two years before collecting samples in June of 2016.

Definitions and Acronyms

The following are acronyms and definitions of terms in the context of how they are used in this study:

DNA: Deoxyribonucleic acid. It is a molecule that carries the genetic framework of instructions used by all known living organisms in their growth, development, functioning, and reproduction.

RNA: Ribonucleic acid. It is an essential molecule for many biological roles (including reproduction in many viruses) such as coding-decoding, regulation, and expression of genes.

OTU: Operational taxonomic unit. An OTU is an operational definition mainly used to classify groups of closely related individuals based on similarity in genomic data.

PCR: Polymerase chain reaction. PCR is a technique used for amplifying and making multiple copies of a unique segments of DNA strand across several orders of magnitude to generate a few thousand to millions of copies.

SOM: Soil organic matter. It is the organic component of soil, made up of primarily plant and animal residues in various states of mineralization, decomposition, cells, and tissues of soil organisms and their metabolic products.

NGS: Next-generation DNA sequencing. It is also referred to as high-throughput sequencing which forms a broad umbrella under which large-scale parallel sequencing and resequencing of genomes, RNA-sequencing (also known as transcriptome profiling) is carried out and software-based algorithms are used to align, bine and parse the data and finally compare to existing constantly updated databases to achieve specific characterization of samples based on their genetic material.

16S: 16S rRNA gene. 16S ribosomal RNA is the component of the 30S or smaller subunit of a prokaryotic ribosome. The genes coding for it are referred to as 16S rRNA genes and are used in reconstructing prokaryotic phylogenies and identification of prokaryotes.

Sustainable Development: Sustainable development is a development model or strategy or approach that meets the needs of our present without compromising on the ability of

future generations to meet their own needs while taking into account the interest of all stakeholders.

NT: Conservation, or no-till, tillage is any tillage and planting system that leaves or tends to leave a good percentage (usually 30%) of the soil surface covered by residue after planting.

CT: Conventional, or traditional, tillage leaves the soil surface bare and loosens soil particles, making them more susceptible to various elements of nature like erosion by wind and water.

P: Phosphorus.

Justification

Although the influence of tillage practices on soil biology is an extensively studied area of research, there is still a gap in our understanding of this influence. A major part of that gap is in our understanding of the soil bacteria in terms of its community composition and diversity. An even larger gap is in how the diversity of bacteria, fungi, nematodes and any other prokaryotic and eukaryotic systems in the soil community are influenced by various land management practices and, in turn, how they influence soil characteristics which ultimately impact agricultural productivity. My research aims to understand one aspect of the complex soil ecosystem by studying bacterial diversity in relation to tillage practices. This research aims to reconstruct the entire soil bacterial community of these ecosystems. Soil microbes drive and regulate many of the processes underlying the ecosystem services provided by the soils and understanding how tillage practices affect soil microbial communities is important for productive and sustainable management of agriculture. Several studies in the Midwest

region of the United States have been conducted in relation to many variables including cover crop type and environmental variables (De Bruin, Paul, Porter & Jordan, 2005; Jarecki, Parkin, Chan, Kaspar, Moorman, Singer, Kerr, Hatfield & Jones, 2009), but this would be the first one from the region of southern Illinois. Although this study is more narrowly focused on looking at only the influence of tillage practices on soil bacterial biodiversity, it may help pave the way for more complex and larger studies to be conducted. This study will increase our ability to detect not only the taxa that may be commonly found in these soils, but also help identify - for the first time - any rare taxa for soils from this geographical area. The data in relation to the number of sequences generated will be an order of magnitude more for agricultural soils from this region than any available now. 11% of world's arable land is used for agricultural food crop production (http://www.fao.org/docrep/005/y4252e/y4252e06.htm) but the agricultural industry contributes to only 3.79% of world's GDP (https://data.worldbank.org/indicator/NV.AGR.TOTL.ZS) and accounts for about 29% of world's employment (https://data.worldbank.org/indicator/SL.AGR.EMPL.ZS), yet the emissions which can have serious environmental and health effects from agricultural food production far outweigh the total emissions from all the other industries combined (Bauer, Tsigardis, & Miller, 2016). Thus, any steps to fine-tune the management practices and the way the agricultural land is utilized can go a long way in sustaining our food sources while maintaining a healthy environment.

CHAPTER II – REVIEW OF RELATED LITERATURE

Metagenomic analyses can provide extensive information not only on the composition but also the predicted functions of diverse microbial communities in a particular environment. Added to that each environmental ecosystem presents a unique set of challenges. The complexity of the soil ecology (Schmidt et al., 2011), means that the metagenomic investigation requires a carefully planned and designed approach to accommodate both biotic and abiotic factors unique to each specific environment that can pose technical hurdles and/or bring bias to the metagenomic analyses and the final interpretation. Therefore, selection of the agricultural ecosystem samples should even out any major differences in the way the ecosystem is managed, what is growing above ground, what amendments have been applied and many more variables. For any real-time field study in relation to one particular treatment, the rest of the variables have to be relatively similar and they have to be within the same geographic region so that even the climate and weather conditions are more or less similar if not exact. Any successful application of a soil metagenomic study depends on selecting the appropriate sites and appropriate and similar if not exact sampling techniques, same exact DNA extraction, purification methods, and any other downstream methods for analyses. This literature review highlights many such aspects of modern metagenomic approaches for soil biodiversity studies and some of the issues that may arise.

The classic or rather standard illustration of soil in classroom textbooks usually shows a cross-section of a grassland or an agricultural field with plant shoots on top followed by lower layers of different colors or patterns. These layers are labeled and described according to qualities like texture, pore size, particle size, plant root infiltration

and sometimes mineral composition. Most of these illustrations (soils.usda.gov/education/facts/soil.html) and definitions fail to address soil as a living dynamic system. Hence, for the most part, we fail to see the connectedness of the soil processes through both biotic and abiotic systems and how they both modulate and impact each other. These processes occur within the larger framework of the changes in environment in which the soil is and are performed by that part of biology which call soil its home. In most soil ecosystems life below the ground exceeds by an order of magnitude (10 to 1) both in the population and diversity of life above the ground. A single gram of soil extracted from rhizosphere may contain over 9000 species (Paul & Clark, 1996) and most of them are yet to be characterized. Richter and Markewitz (2001) define soil and I interpret in my words as "a biologically active, organized mixture of organic matter and mineral matter which makes life possible on this planet". Many studies have indicated that the phylogenetic as well as functional diversity of microorganisms in soil ecosystems, far exceeds that of what we know from microbial cultivation/culture (Breitbart et al., 2003; Dinsdale et al., 2008).

Soil

Soil is the outermost layer of the earth's crust and depending on the location could be anywhere between a few centimeters to a meter thick. It is a complex matrix that supports life and can take up to a thousand years for formation of just about 2.5 cm (1 inch) of soil (Ahrens & Arnold, 2000). Throughout history, there are examples of how anthropogenic use and management of soil and water resources have shaped and directed the development, persistence, downfall and subsequent regeneration of human civilizations, sustained by agriculture (Harlan & Crops, 1992). Hence it is critical to

manage the soil in a sustainable manner if we are to preserve our food production. Soil is made up of biotic and abiotic components. It is the complex interaction between these components and the influence of anthropogenic activity on top which defines today's soil environment (Lehmann & Kleber, 2015). Soil is made of mineral components, organic components, water, and air which can be considered abiotic factors. The solid mineral components and organic components combined make up about 50 percent of the soil, and the average or typical soil is composed of about 45 percent mineral matter and about 5 percent organic matter (Brady & Weil, 2008). These fractions are not tightly packed but arranged, and this arrangement is dictated by many variables including but not limited to weather, gravity, wind, and rain and is modulated by the complex interactions between the biotic and abiotic factors. The living soil as a matrix is composed of five components — minerals, soil organic matter, living organisms, gas, and water. Soil texture is divided into three classes based on the size of the mineral particles— clay, silt, and sand (Brady et al., 2008). These classes help us define soil texture based on their relative proportions and distribution (Thien, 1979). The soil mineral content is diverse and the most common mineral in soils is quartz (Thien, 1979). The mineral particles form variable size aggregates consisting of silicate clays, ash minerals of volcanic origin, organic matter, and oxides (Edwards & Bremmer, 1967). It is these mineral particles, their aggregates, and the pore space between the aggregates which together form one of the most complex ecosystems which can only be viewed as a multi-scale assemblage of ecosystems (Ponge, 2015). Soil organic matter is composed of residues of plants, animals, and microbes in various states of decomposition/mineralization and is a critical ingredient for agricultural soil quality (http://soils.usda.gov/sqi/). The visible soil colors and structure are due to the

complex and numerous interactions between the various components of the soil both biotic and abiotic. Hence the study of soils has become more interdisciplinary in nature as no single component on its own can be used to define soils and the soil ecology (Brevik et al., 2015). Soil studies are moving from narrowly focused studies to broader view in order to truly understand soil and to address some of the global challenges related to environmental preservation as well as sustainable agricultural (McBratney, Field, & Koch, 2014).

Soil Compaction – Indicator of Soil Physical Health

Soil compaction is one of the major problems facing modern agriculture today. Sustained continuous use of machinery, intensive cropping, shorter crop rotations, intensive grazing and inappropriate soil management can lead to compaction. Soil compaction can occur in a wide range of soils and climates and can increase soil strength or bulk density and decrease fertility through a reduction in the storage and supply of water and nutrients (Hamza & Anderson, 2005). The reduction in both pore spaces and the variability in the soil texture brought about by compaction can have a negative impact on soil biology, aeration, water retention and root penetration (Hoorman, Sa, & Reeder, 2011; Unger & Kaspar, 1993). Soil compaction is a measure of soil bulk density, a soil physical property. Soil physical properties are a set of parameters (USDA) and are divided into six categories (www.nrcs.usda.gov/wps/portal/nrcs/detail/nj/home/?cid=nrcs141p2_018993#Physical_Properties).

1. Horizons: These are the discrete identifiable layers that make up a soil profile and are usually parallel with the ground surface.

2. Soil Color: Soil color is described using color reference chart and is a result of sum of all the chemical, physical properties of the soil and their interactions put together. A commonly used chart is the Munsell Color Chart (Pendelton, Robert, Nickerson, & Dorothy, 1951).
3. Soil Texture: Soil texture refers to the proportion of the soil "separates" that form the mineral component of soil. The separates are further classified based on particulate size and are called sand (0.05 mm to 2 mm), silt (0.002 mm to 0.05 mm) and clay (smaller than 0.002 mm).
4. Soil Structure: The soil aggregates can come together to form discrete structural units called "peds" and these peds are organized into a repetitive pattern called as soil structure. Spaces between the peds are called "pores" which conduct or move air and water. Shape of the individual peds is used to describe soil structure within each horizon.
5. Soil Consistence: It is ranked based on the ease with which a particular type of ped can be pressed or crushed between the fingers. Soil consistence usually depends on soil moisture content. The terms commonly used to describe consistence are 1. Moist (Loose, Friable, Firm), 2. Wet (Plastic, Sticky) and 3. Dry (Soft, Hard).
6. Bulk Density: It is the ratio of the weight of a given type of soil relative to the volume it occupies. It is expressed as weight per volume and is commonly measured in units of grams per cubic centimeters (g/cc) or kilograms per cubic meter (kg/m^3). Bulk density tells us about the amount of available pore space within the soil and is inversely proportional to pore space.

Being a dynamic and heterogeneous environment, there will always be competition within the species (biotic component) and with other species for nutrients and space in soil. Soil microbes can be subjected to abiotic stress like temperature, pressure, moisture, etc. Hence, the soil ecosystem is a dynamic system where any abiotic stress can alter the physical attributes of the soil (Ivo & Mielniczuk, 1999). Tillage disturbs the physical attributes which in turn affects the chemistry and the biology (Filho & Tessier, 2009: Ivo et al., 1999). Tilling influences the biology by impacting the soil physical structure by causing changes in soil porosity, density, aggregate stability, and water retention. Additionally, tillage has the tendency to create two distinct layers which over time can become discontinuous as the top few inches are constantly tilled (Carof, De, Coquet, Hallaire, & Roger-Estrade, 2007). Soil compaction is one of the early indicators of soil degradation and affects soil biology primarily by negatively affecting soil erosion, soil water, and nutrient availability. The two major factors which cause soil compaction are (Badalikova, 2010)

1. Natural factors such as innate predisposition of soil, rain, waterlogging, and poor penetration of soil by roots.
2. Artificial factors like mechanical disturbance, improper management practices, improper use of fertilizers-amendments, monoculture leading to lack of plant diversity.

Compacted soils are characterized by low porosity, low water and air permeability (Anikwe & Agbin, 2003). Bulk density and penetration resistance are the most commonly used variables used to evaluate soil compaction (Mapfumo, Chanasyk, Naeth, & Baron, 1998), and one of the well-used tools for measuring compaction through penetration

resistance is the Cone Penetrometer (Bouwman & Arts, 2000; Horn & Rostek, 2000). The penetration resistance also serves as an indicator of the how deep roots can penetrate and how much the root grows radially (Materechera & Mloza-Banda, 1997). Compaction leads to increases in soil strength and decreases in air permeability and hydraulic conductivity and thus can affect soil biological activity (Beylich, Oberholzer, Schrader, Höper, & Wilke, 2010; Whalley, Dumitru, & Dexter, 1995). Tillage followed by conventional agricultural practices lead to soil compaction and hence impact the soil biology (Beylich et al., 2010). Although tillage is used to increase soil porosity for better root penetration, this increase in porosity is very short lived due to less structural stability imparted by tilling of the soil (Foldesi, Gyuricza, Miko & Nagy, 2006; Liiri, Häsä, Haimi, & Setälä, 2012) and impacts soil biology (Gregory et al., 2007). It also has the compounding effect of reduction in invertebrate as well as vertebrate diversity due to the inherent impact on the soil pore structures (Pagliai, Vignozzi, & Pellegrini, 2004).

Soil Organic Matter (SOM)

Soil organic matter (SOM) is that fraction of the soil that consists of plant or animal or any biological tissue in various stages of decay/decomposition or mineralization (http://www.fao.org/docrep/009/a0100e/a0100e.pdf). Soil organic matter contributes to soil productivity and resilience in many ways and is a major source of ecosystem stability in agricultural ecosystems (Burke et al., 1988). SOM plays an essential role in maintaining soil fertility and hence conserving it is essential for halting soil degradation and making cropping sustainable. SOM composition is extremely diverse, with both labile fractions which decompose in a short time frame of days to a few months and the nonlabile fractions which tend to persist for years (Ross, 1993). The

main source of the organic matter in soils is the CO_2 fixed by the plants (Trumbore, 1997) and its turnover rate is influenced by factors such as climate, weather, vegetation, parent material, topography and time (Jenny, 1980). As stated earlier SOM is a major component of soil productivity and overall agricultural ecosystem performance and hence any practice which can reduce its levels can impact soil health (Morrow, Huggins, & Reganold, 2017). Studies indicate SOM accumulation is higher in cool and humid regions while relatively lesser accumulation occurs in warm and dry climates (Lal, 2002). SOM influences several physical attributes including reduction in bulk density, increasing aggregate stability, mitigating compaction issues and resisting compaction, reducing erosion and nutrient leaching and sequestering carbon (Bot & Benites, 2005; Krull, Skjemstad, & Baldock 2004).

SOM losses resulting from conversion of native vegetation to agriculture (Paul, 1997) and also reduction in SOM quality due to agricultural activity have been well documented in soil ecosystems (Lal, 2002). Research also indicates up to 50% reduction in SOM in the Midwest due to modern practices (Lal, 2002). Because of the cultivation practices, agricultural soils under traditional management practices have generally lower levels of SOM than grazing soils (Lal, 2002).

Although there are no currently accepted standards for classifying soils based on their SOM, there is a consensus that depending on the geographic regions and the climate soils have different minimal thresholds of SOM levels (Doran et al., 1997; Krull et al., 2004) above which they are most agriculturally productive. Tillage results in the loss of SOM through (Lal, 2002),

1. Mineralization of carbon due to breakdown of soil aggregates and greater exposure of the organic matter to the environmental vagaries such as temperature and loss of moisture.
2. Leaching of organic carbon and subsequent loss of the elements sequestered by the organic matter.
3. Increased erosion brought about by more exposure to factors such as wind, water, and temperature.

There seems to be a direct relationship between SOM levels and their ability to stabilize soil structure within the limitations imposed by the inherent physiochemical characteristics of the soil (Six, Conant, Paul, & Paustian, 2002). There are studies that have shown that the increase in SOM decomposition rate after addition of fresh organic matter to soil is a result of increased microbial activity. This, in turn, can be attributed to higher availability of energy substrates from which energy is released through decomposition (Fontaine, André Mariotti, & Luc Abbadie, 2003).

Agricultural Soil Ecosystems and Bacterial Diversity

Soil ecosystem provides a range of habitats which include not only micro niches but also entire landscapes. The soil biodiversity includes all varieties of life living and dwelling below and above ground. Soils are a result of millions of years of weathering processes involving physical and chemical processes modulated by the biology which occupies the various niches within that system. The soil is a complex, multifaceted environment and thus provides by far the most wide-ranging niches for a diverse group of biology (http://www.fao.org/3/a-Y4810.pdf). The large diverse group of living organisms in the soil contributes to many critical ecosystem services including formation of soil,

organic matter decomposition and incorporation, nutrient availability, carbon sequestration, nitrogen fixation, bioremediation of degraded and contaminated soils and many more (Lavelle et al., 2006; Dominati, Patterson, & Mackay, 2010). The soil environment likely houses the most complex biological community and contributes to the ecosystem services at many levels directly and indirectly and hence impacts land productivity (Barrios, 2007). Despite the overwhelming evidence for the soil being fundamental to overall earth's productivity and the very basis of human existence, soil biology has fallen somewhat behind both in the level of our understanding (Giller, 1996) and implementation of our understanding (Lehman et al., 2015) despite soil being one of the most biologically diverse ecosystems (Pelletier & Newton, 1999).

Studies of soil ecosystems have shown that the number of prokaryotic species in a single soil sample is far higher than that of known cultured and enumerated prokaryotes (Curtis & Sloan, 2005) and thus there is a large gap in understanding to what extent the soil biology contributes to the soil ecosystem and the potential for many more bioactive compounds and interactions to be discovered (Monciardini, Iorio, Maffioli, Sosio, & Donadio 2014). Microbial activity and their population dynamics in soils are influenced by its physical, chemical, and biological properties and in return the microbial processes transform the soil environment (Sessitsch, Weilharter, Gerzabek, Kirchmann, & Kandeler, 2001). There has been a rapid succession of technologies after World War II which defines the present agricultural industry (Karlen, Andrews, Weinhold, & Zobeck, 2008) which seems to rely more on physical and chemical manipulation while undermining the role of soil biology and the soil ecosystem (Karlen, 2012). This has led to rapid decline in soil bio-diversity at almost every taxonomical level directly and

indirectly (Dong et al., 2012; Luo et al., 2015). To define in simple language soil ecosystem is the habitat of plant roots and a diverse range of organisms including but not limited to bacteria, fungi, protozoa, many invertebrates and vertebrates, which together contribute to the maintenance and productivity of agroecosystems (Giller, Bare, Lavelle, Izac, & Swift, 1997; Kibblewhite, Ritz, & Swift, 2008). Modern agricultural practices have reduced the regulation of functions brought about by soil biodiversity and relies more upon chemical and physical regulation. There is an overwhelming bias towards production of food as a basis for assessing soil health and seems to pay little attention to the other ecosystem services soil can provide through the biodiversity (Kibblewhite et al., 2008). Only a small minority of policymakers and researchers outside soil-related fields probably understand the extent of the ecosystem services provided by interactions of soil biology with the soil.

Just the top 5 to 15 cm (two to six inches) of soil may contain trillions of microflorae (bacteria, fungi, and algae), billions of protozoa grazing on bacteria, millions of nematodes feeding on the microflora and the protozoans interlinked to many other organisms – predatory, saprophytic, autotrophic and this is again linked not only to the plants below and above ground but also to the processes above ground in a multitude of ways we barely understand (Westcoat & White, 2003). Within this complex ecosystem, soil biology is not evenly distributed and follows a complex hierarchy and organization which we are just trying to understand (Brimecombe, De Leij, & Lynch, 2001).

Soil Bacterial Diversity Studies

Soil bacterial diversity studies so far have depended upon cultivation-based studies (Staley & Konopka, 1985) in which soil bacteria are inoculated on culture plates

and then characterized using a set of biochemical techniques and microscopy (Galvez, Maqueda, Martinez-Bueno, & Valdivia, 1998; Hugenholtz, 2002). This approach can help characterize only those bacterial groups that can grow on the culture plates (Hugenholtz, 2002). It is time-consuming and required wide range biochemical tests and a trained eye for the microscopy. As far back as in the 1940s, it has been recognized that soils can be a great resource for the discovery of novel potentially useful microbial products (Schatz and Waksman, 1944) but there is a discrepancy between the numbers of microorganisms visible under a microscope and the colonies obtained from laboratory cultivation. This difference is several orders of magnitude for most of the soils and this phenomenon is frequently referred to as "the great plate count anomaly" (Staley & Konopka, 1985). It is estimated that about only 1% of wild bacterial species can grow in an environment provided in agar plates (Stanley & Konopka 1985) and this 1% only represents about 8 bacterial genera which are easy to grow in situ and have also been referred to as "weeds" of the microbial world (Hugenholtz 2002). Studies from all over the world have shown that the phylogenetic and functional diversity of bacteria in various environments, including soil, far exceeds the diversity of bacterial phyla we know from cultivation alone (Breitbart et al., 2003; Dinsdale et al., 2008; Tringe et al., 2005).

Soils, as stated earlier, are dynamic and heterogeneous environments. Bacteria, fungi, protozoa, and other eukaryotes compete for nutrients and space at multiple levels in this environment. In such an environment, bacteria like any other organisms are subjected to both biotic stress and abiotic stress (Buckley & Schmidt, 2002) leading to a very dynamic ecosystem that encourages a variety of microbial interactions and functions on top of the microbial diversity (Fierer & Jackson, 2005). Our understanding of soil

microbial biogeography is still in its early phase, despite the consensus that the diversity and composition of soil bacterial communities has a direct influence on a very wide range of ecosystem processes (Fierer et al., 2005). Hence the soil environment is an abundant but under-characterized source of seemingly endless genetic diversity which has the potential to greatly enhance and enrich our understanding of soil ecosystem (Rondon et al., 2017) and how soil bacteria can be used not only as indicators for the state of the soil ecosystem but also a platform to gauge soil's true value which is more than just agriculture, yield, and profits. Molecular techniques and advances in technology are providing us the tools for analyzing the entire bacterial communities including those which we are not able to cultivate in the laboratory. Studies as far back as in 1996 have indicated that soils may contain bacterial species in the order of 10,000 and the diversity of the total soil bacterial community may be almost 200 times higher than the diversity of the entire collection of bacterial isolates cultured from the same soil (Torsvik, Sørheim, & Goksøyr, 1996). They carry out processes critical for the whole ecosystem and in the larger picture the environment itself. These processes range from degradation of organic matter to nutrients cycling to carbon sequestration and are essential for the soil biogeochemical cycles (Falkowski, Fenchel, & Delong, 2008). They not only influence the fertility of the soil (Falkowski et al., 2008) but also contribute to net carbon exchange through metabolic activity, heterotrophic respiration, interactions with plants and modulating the nutrient availability in the soil (Heijden, Bardgett, & Straalen, 2008).

Although much research has been carried out on plant and animal communities and their relationships with the ecosystem (Hooper et al., 2012) it is not the same scenario when it comes to the bacterial world (Prosser et al., 2007). Moreover many of

the studies so far on relationships between soil bacteria and ecosystem functions have been conducted in controlled closed experiments (Hallin, Jones, Schloter, & Philippot, 2009), but there is strong evidence from field studies that soil microbial communities have a critical role to play in ecosystem functioning (Allison et al., 2013). Bacterial community composition and numbers are influenced and prone to changes brought about by disturbances and these changes are relevant to the stability of the ecosystem (Allison & Martiny, 2008). Thus, in an environment where a microbial community provides eco-services to that ecosystem, any disturbance that can perturb the microbial community composition and numbers will have an impact on those eco-services and ultimately on that ecosystem.

Soil Bacterial Diversity and Metagenomics

Our current understanding of bacterial interactions and metabolic pathways is limited to our inability to grow the vast majority of environmental bacteria in a pure culture (Epstein, 2013), and even if we were able to grow a good majority of the lab, it is difficult to understand the interdependent metabolic pathways which rely upon the community composition and which cannot be replicated in pure cultures (Pham & Kim, 2012). This is where, metagenomics, the study of DNA isolated directly from the environment, has become crucial for our understanding of microbial communities in their natural environment (Daniel, 2005; Handelsman, 2004). The term metagenome was first used by Handelsman et al. (Handelsman, Rondon, Brady, Clardy, & Goodman, 1998), with reference to the genomes of all the microbes living in the soil. The initial metagenomic approaches were aimed at identifying novel functions and novel enzyme systems using cloned libraries of small random DNA fragments (Henne, Daniel, Schmitz,

& Gottschalk, 1999). The next evolution was using high throughput sequencing technologies to sequence all the extractable DNA fragments from the environmental samples. Some of the common approaches used to analyze metagenomic samples are summarized below. Some approaches may require a combination of the methods described while some may not.

Functional Analysis

Functional analysis is a method that can provide direct access to genes that perform a particular function or confer a specific phenotype within the ecosystem from which the sample is obtained. Briefly, metagenomic DNA is cloned randomly into vectors such as plasmids if it is small inserts (<15 kb), or cosmids/fosmids for larger inserts (~ 40 kb) and bacterial artificial chromosomes (BAC) for even larger inserts (up to 350 kb) (Hårdeman & Sjöling, 2007). Libraries of clones are then screened for a particular phenotype. This is a widely used method (Hårdeman & Sjöling, 2007; Kim, Kweon, Jones, Edmondson, & Cerniglia, 2008; Lämmle et al., 2007).

Sable Isotope Probing (SIP)

Stable Isotope Probing involves incorporation of a heavy isotope, usually ^{15}N and ^{13}C into biomolecules using a specially made growth substrate containing the isotope (Chen & Murrell, 2010). It allows for the separation of DNA or the proteins that belong to bacteria capable of utilizing that substrate. This procedure can help researchers to link the identity of a microbe from a complex community structure to the degradation and consumption of individual compounds but needs to be followed up with other downstream methods to identify the microbial species or their genes of interest using

other sequencing methods (Chen et al., 2008). SIP can be used to narrow down the range of metagenomic sequences being analyzed in a sample (Chen & Murrell, 2010).

Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE)

These techniques are based on PCR amplification of 16S rDNA genes (Winsley, Dorst, Brown, & Ferrari, 2012). The rRNA gene is one of the most conserved DNA in all cells. Portions of the rDNA sequence from distantly related organisms show remarkable similarity and can be used to precisely align sequences from distantly related organisms and study the differences in the sequences beyond the 16s rDNA gene (Boye, Høgdall, & Borre, 1999). Using the 16S gene, it is possible to classify variants as operational taxonomic units (OTUs) among the amplified sequences. This technique is relatively cheaper and involves gel-based techniques to separate the amplified regions. Visualization of the differences is done using denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE). The amplified regions after separation based on their sizes on the gel can be visualized using either standard DNA binding dyes or under UV light using fluorescent DNA binding dyes.

Restriction Fragment Length Polymorphism (RFLP)

This technique is an extension of DGGE and TGGE and is used to study differences in homologous DNA sequences that can be detected across the samples by the presence of fragments of different lengths after digestion of the DNA samples with specific restriction endonucleases (Uddin & Cheng, 2012). After a segment of DNA is digested into pieces with restriction enzymes, the fragments are examined using DGGE or TGGE (Kirk et al., 2004).

Next Generation Sequencing (NGS)

The Next Generation Sequencing method can also be called as high throughput sequence-based screening. It involves direct sequencing of metagenomic DNA without additional processes to enhance the quantity of the living organisms in that sample. The raw sequence data is then subjected to bioinformatic analyses (Sleator, Shortall, & Hill, 2008). Recent developments and advances in next-generation sequencing (NGS) technologies have made available, different methods that can be used for sequencing. The capabilities and costs vary. GS20 which is now under Roche acquisition was one of the first instruments based on the 454-pyrosequencing technology which could sequence up to 25 million bases of a bacterial genome in a four to five hour run time and had an average read length of 120 bp (Abbai, Govender, Shaik, & Pillay, 2011). The current model from Roche, the 454 GS-FLX sequencer using Titanium chemistry can achieve read lengths of up to 500 bp. The Illumina/Solexa technology which is leader in NGS uses a technique which immobilizes random DNA fragments on a surface and then performs solid-surface PCR amplification resulting in multiple copies or clusters of identical DNA fragments or segments. These are then sequenced with reversible terminators in a process called sequencing-by-synthesis (Bentley et al., 2008). Another well-known platform is the Ion Torrent system which is based on measuring the protons (H^+ ions) released during DNA polymerization and can give up to 400 bp read lengths (Salipante et al., 2014). Both the Illumina and Ion Torrent platforms sequence DNA by monitoring the addition of new nucleotides during DNA synthesis but they differ in the principles of operation or how they monitor the new nucleotide addition. DNA fragments are processed and prepared for Illumina sequencing by isothermic “bridge PCR,” which

involves simultaneously amplifying single DNA molecules and covalently linking the amplicons to a solid substrate (hence the term bridge) in order to form randomly arrayed "clusters." These clusters are then sequenced through repeated cycles of single-base extension using a mixture of four fluorescently labeled (one for each nucleotide) reversible chain terminators in the Illumina platform. Sequence data is built on imaging and identifying the chain termination reactions. (Shendure & Ji, 2008). Ion Torrent sequencing involves preparation of templates by using a technique called emulsion PCR (Shendure et al., 2008). PCR reagents/chemicals, primer-coated particles, and template fragments are combined with oil and emulsified to prepare picoliter-scale micro reactions. These reactions are then subjected to PCR to prepare clonal amplicons of single DNA molecules on the surfaces of individual particles. Particles are then deposited into individual, nano well chambers on a semiconductor-based sequencing chip (Rothberg et al., 2011). Individual nucleotides (one for each nucleotide) are introduced in the presence of DNA polymerase. Each successful incorporation of a specific nucleotide is registered by the release of hydrogen ions which is read as an electrical signal corresponding to the nucleotide base added. Unlike in Illumina chemistry, multiple nucleotides can get incorporated during a single sequencing reaction cycle (Loman et al., 2012). Metagenomics adds on a range of ever-evolving genomic technologies and bioinformatics tools to analyze the information generated through NGS. The field of metagenomics has been responsible for substantial and critical advances in understanding microbial ecology, evolution, and diversity over the past 10 years (Miller, Koren, & Sutton, 2010) and will advance our understanding in the future too. Many research

laboratories are actively engaged in it now. The various steps involved next-generation sequencing can be summarized in the figure 1 (Thomas, Gilbert, & Meyer, 2012).

Soil Phosphatases

Soil phosphatases, particularly acid and alkaline phosphomonoesterases, have been well studied (Martinez, 1968; Oshima, Ogawa, & Harashima, 1996; Sakurai, Wasaki, Tomizawa, Shinano, & Osaki, 2008; Rejsek, 1991). Data supporting naturally occurring rhizospheric phosphorus solubilizing microorganism has been reported as early as 1903 (Khan, Zaidi, & Wani, 2007) and bacteria seem to be more effective in phosphorus solubilization than fungi (Afzal & Bano, 2008). Soil microbes given their intimate interaction with soil particles and their associated processes are probably the most sensitive and the earliest biological indicators of soil health and the direction in which the soil is headed as enzyme activities play key roles in the functioning of soils, including soil organic matter formation and degradation and most importantly in nutrient cycling (Acosta-Martinez, Zobeck, Gill & Kennedy, 2003). Studying and quantifying enzyme activities can be used to correlate changes in soil ecosystem to agricultural activity. The microbial biomass which usually only constitutes 1% -4% of total soil organic carbon (Anderson & Domsch, 1989) plays a critical role in not only the decomposition of organic matter in soils but also is the major source of soil enzymes involved in nutrient cycling. Hence soil enzymes activities and their levels can indicate the status of the soil environment and specific biochemical reactions of the entire microbial community in soils as they are involved in the transformation of the soil nutrients such as C, N, S, and P (Nannipieri, Grego, & Ceccanti, 1990). Field or soil enzyme activity is well correlated with soil biomass which includes soil bacteria and can

be a good indicator of soil use (Jordan, Kremer, Bergfield, Kim, & Cacnio, 1995). Especially phosphatase enzyme activity has been shown to be significantly correlated with microbial biomass and can be a good indicator of cropping and management practices (Dick, 1984; Frankenberger & Dick, 1983). This study may further help characterize and quantify which specific bacterial genera and species may be responsible for the increased phosphatase activity in no-till systems if an increase is observed. Phosphatase is a term used to describe a particular group of enzymes that catalyze the hydrolysis of esters and anhydrides of H_3PO_4 (Tabatabai, 1994) and play an important and major role in the mineralization of soil organic P (Speir & Ross, 1978). The commission on enzymes of the International Union of Biochemistry has described five groups of phosphatases; phosphoric monoester hydrolases (EC 3.1.3), phosphoric diester hydrolases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5), enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1). and enzymes acting on P-N bonds (EC 3.9), such as the phosphoamidase (EC 3.9.1.1). The most extensively studied group among the phosphatases in soils is the phosphomonoesterases, acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1), and phosphodiesterase (EC 3.1.4) (Browman & Tabatabai, 1978; Tabatabai & Bremner, 1969).

Further, the enzymes are classified as acid and alkaline phosphatases based on whether their optimal activity is in acidic or alkaline environments respectively (Nannipieri, Giagnoni, Landi, & Renella, 2010). Studies have also indicated that alkaline phosphatase activity in soils is majorly derived from microbial systems (Dick, Juma, & Tabatabai, 1983). Further Eivazi and Tabatabai (1977) showed that the acid phosphatase activity was predominant in acidic soils while alkaline phosphatase activity was

predominant in calcareous soils. Research has also indicated that for the modern cropping systems which are dependent on natural biological processes to sustain productivity, measuring the alkaline phosphatase activity and acid phosphatase activity and using that ratio might be preferable compared to chemical approaches to evaluate and measure effective soil pH and calculations for pH adjustment requirements using lime (Dick, Cheng, & Wang, 2000).

As stated earlier soil microbes and their associated processes are potentially the most sensitive and earliest biological indicators of soil health and the direction in which the soil is headed. The microbial biomass which usually constitutes 1 - 4% of total soil organic carbon (Anderson & Domsch, 1989) plays a critical role in the decomposition of organic matter in the soil and is also the major source of enzymes in the soil. Maintaining soil organic matter is beneficial for agricultural purposes (Reeves, 1997) and can be used to measure soil quality but optimal concentrations of organic matter vary among soils due to factors that hardly change especially physical and chemical properties. Hence soil quality indicators should be chosen based on rapid and more dynamic changes (Tscherko & Kandeler, 1997). Usually, the effects of tillage on the total organic matter are experimentally detectable in soils of temperate areas only over long time frames, but the changes in the microbial biomass and microbial processes may be measurable over a far shorter time scale (Christensen, 1996).

Increased inputs of phosphorus (P) and their subsequent transport in agricultural runoffs can rapidly accelerate freshwater eutrophication (Sharpley, Daniel, Sims, & Pote, 1996). This is of great concern especially in areas of intensive crop and livestock farming, where soil P has reached to levels that are not only of agricultural concern but

rather a serious environmental concern. Microorganisms play an important role in the acquisition and cycling of nutrients in soil. Especially when it comes to phosphorus (P), soil microorganisms are involved in multiple processes that affect transformation of P and therefore influence the downstream availability of P (as phosphate) to plant roots (Richardson, 2001). Microorganisms can solubilize and mineralize P from inorganic and organic pools of total soil P (Richardson & Simpson, 2011) and hence utilize microorganisms to increase the availability of P in soil, is a very attractive and sustainable proposition for agriculture and environment as such (Sánchez, 2010). Uptake of P from soil solution/soil environment occurs in orthophosphate form through transporters in plant root epidermis. These transporters are expressed in response to P deficiency or P requirement (Bucher, 2007). Total soil P occurs in both organic and inorganic form in soils. The organic form of P usually organic phytic salts are not readily available to plants as a source of phosphorus because it has a tendency to form complexes with cations or adsorbs to various components of soil (Singh & Satyanarayana, 2011). It can also get incorporated within the biomass or be in association with soil organic matter (Richardson et al., 2011). The inorganic form of P which is predominant is present either adsorbed to soil mineral surfaces or as sparingly available precipitates (Richardson et al., 2011).

Phosphate solubilizing microorganisms which are ubiquitous in soils can influence and play an important role in P supply to plants (Abd-Alla, 1994). P mobilization and availability in orthophosphate form and its diffusion represent the major limitations to an optimal supply of P required for plant growth (Richardson et al., 2011). Addition of more phosphates as amendments adds onto this unavailable pool of

phosphates rapidly and makes it necessary to add P amendments year after year while a huge unavailable P reserve builds up and leaches out or runs off. This study is also aimed at looking at if there is any significant increase in microbial phosphatase activity because of adopting conservative tillage practices which may or may not impact microbial diversity and quantity.

Soil Compaction and Soil Biology

Soil compaction is a term used to describe soil bulk density (Whalley, Dumitru, & Dexter, 1995) and increased compaction leads to increases in soil strength, increased resistance to penetration and finally decreases in air & water permeability and hydraulic conductivity (Horn, Domżżał, Słowińska-Jurkiewicz, & Ouwerkerk, 1995). This, in turn, can impede root development while reduced water permeability may result in soil erosion, with serious negative effects on the environment. On top of that compaction may also contribute to global warming as it leads to increased emission of CO_2, CH_4, and N_2O from compacted soils (Horn et al., 1995). Studies indicate soil management strategies impacting soil compaction can affect microbial activity and the associated biological interactions (Horn et al., 1995). Because the effects of tillage and compaction on biological processes in the soil are only partly understood more effort needs to be put into studying these interactions so that appropriate management methodologies and technologies can be developed. Investigations on soil compaction so far have focused mainly on effects on variables such as soil physical parameters and on plant growth (Beylich, Oberholzer, Schrader, Höperd, & Wilke, 2010) but its adverse effects on soil structure and soil environment especially on the microbial parameters has been indicated by many studies (Weisskopf, Reiser, Rek, & Oberholzer, 2010). Studies have also

revealed that parameters such as denitrification were increased in compacted and irrigated soils (Abbasi & Adams, 1998; Barken, Bosressen, & Njoss, 1987) where the occurrence of anoxic conditions in the field was more frequent. Soil compaction can retard mineralization of organic matter and N and increase gaseous losses of N along with other gases as the pore space decreases (Breland & Hansen, 1996). Thus, changes in soil physical characteristics induced by soil compaction may alter soil habitat diversity, soil microhabitats and therefore, play a significant role in influencing microbial populations and their functions (Pengthamkeeratia, Motavalli, & Kremer, 2011) which in turn can affect the field plant productivity. The ability of a system to withstand stress, both abiotic and biotic depends on its level of biodiversity which in turn defines the functioning of ecosystems. The standard methods used or employed in agriculture are more or less common over a wide a range of soil types and essentially are rooted in the idea that yield is more important. Hence most of the practices essentially give rise almost similar soil types at least in its physical characteristics. These, in turn, affect the biological diversity and also the chemical characteristics which are compounded by many chemical amendments which are added. Studies have also indicated that in compacted soils many of the microbial parameters like number of species, microbial enzyme activities and microbial catabolic activity are significantly reduced (Pignataro, Moscatelli, Mocali, Grego, & Benedetti, 2012). Increased soil compaction effects these microbial parameters by probably reducing the pore space in soils, macroaggregate formation which in turn impact the water infiltration, air diffusion and reduction in root penetration (Jung, Kitchen, Sudduth, Lee, & Chung, 2009)

Tillage in Agriculture

Tillage can be defined as the mechanical manipulation of the soil for crop production and has been employed in some form or the other from dawn of agriculture. It is preparation of soil by mechanical agitation of various types and can impact various aspects of soil which are sometimes noticeable immediately as soil runoff/erosion (Bhatt & Khera, 2006). Tillage methods and soil surface management affect how soil resources can be used sustainably because of their influence on soil structure, soil stability, soil resilience and soil quality. Conventional tillage practices can negatively affect soil productivity due to erosion and organic matter losses in soils (Alvarez, 2005). Different tillage practices can cause changes in soil physical properties like bulk density (Wander, Bidart, & Aref, 1998), water holding capacity (Trojan & Linden, 1998), and aggregation (Chan & Mead, 1988). Such, altered soil conditions can alter the habitats for microorganisms and hence impact soil microbial community structure (Helgason, Walley, & Germida, 2009; Staley, 1999). The other aspect is the balance between aerobic and anaerobic systems which together constitute the soil ecosystem. Conventional tillage can lead to predominantly aerobic systems, while the no-till system can lead to predominantly anaerobic systems (Balota, Colozzi-Filho, Andrade, & Dick, 2003). There is data indicating conservation tillage practices can increase microbial populations and their activity (Staley, 1999) along with an increase in microbial biomass (Balota et al., 2003). In the last hundred years of agriculture, tillage has decreased soil organic levels by more than 50% (Lal, 2004) which has decreased the amount of substrates that provide energy for soil microbes (Ghimire, Norton, Stahl, & Norton, 2014).

Tillage practices on soil microbial communities seem to have the largest impact in the early growing season and the later fallow period (Feng et al., 2003) and within that, management practices seem to have a larger role to play than climatic conditions like rainfall (Ibekwe et al., 2002). This forms an important basis for the framework of our study which has excluded using climate variables such as rainfall and temperature. Added to this, large-scale cultivation of monoculture crops will not only lead to a loss of soil organic matter (Holland, 2004), but also lead to poor water and nutrient holding capacity (Gregory, Shea, & Bakko, 2007). Relative to plants, microbes have much shorter turnover rate and can, therefore, respond more quickly to changes in land management than plants. It is this exact characteristic feature which makes them the most sensitive indicators to anthropogenic activities (Entry et al., 2008).

Based on how the Conservation Technology Information Center (CTIC) from Purdue University and Pesticide Action Network - Germany Online Information Services for Non-Chemical Pest Management (OISAT) define tillage practices, I present broad definitions of the tillage practices below.

1. Conventional Tillage refers to tillage practices usually defined based on location and the crop and leaves less than 15% of crop residue in the soil. The ploughing can be intensive, repetitive, and uses mechanical means in a big way.
2. Conservation tillage practices tend to be less invasive and are grouped into three types: no-till, ridge- till, and mulch-till. This approach tends to leave more than 30% of the plant residue from the previous crops.

Advantages of conventional tilling (CT):

- Disruptive to pests.
- Exposes pests to predators and unfavorable conditions
- Redistributes soil nutrients throughout the soil and creates even conditions.
- Easier to follow up with other standard farm practices.
- Provides additional access to O_2 for plant roots.

Disadvantages of conventional tilling (CT):

- Disruptive and damages soil cover and its structure.
- Increases soil erosion and moisture loss.
- Along with the pests it also destroys beneficial biology.

Advantages of conservation tilling (CS):

- Conserves moisture and reduces evaporative water losses.
- Reduces soil erosion.
- Reduces damaging impact of environmental/climatic conditions.
- Increases organic matter content.
- Stabilizes the ecosystem which houses a multitude of species at different trophic levels.

Disadvantages of conservation tilling (CS):

- Needs in-depth understanding of the concept and the ecosystem functions.
- Must be followed up with specific management practices to be successful.
- Soil pest populations and weeds may increase.
- Uneven distribution of minerals and organic matter may occur.
- The benefits may not be immediate.

Types of Conservation Tillage Practices

(Boller et al., 2004; Uri, 1999; & www.extension.purdue.edu/extmedia/ct/ct-1.html).

Zero Tillage (no-till/minimum tillage): A set of practices where the soil is not disturbed or physically manipulated between cycles of harvesting and planting the next crop. It is a crop production system where the soil is not conventionally tilled although simple or complex planting equipment is used for seeding. Planting is done in narrow less than 6-inch-deep slots or furrows created by in row chisels, coulters, row cleaners, disk openers, or roto-tillers.

Ridge Tillage: Slightly different compared to no-till where a new crop is planted on pre-formed ridges or hills or bunds which are a result of the previous cropping. Post-harvest crop residue is left until the next seeding or planting. This kind of tillage involves planting into a seedbed prepared on ridges with sweeps, disk openers, coulters and row cleaners. The ridges are rebuilt during every cultivation season and are not disturbed from harvest to the next planting. This method works best on level and makes drainage more efficient. Ridge-till systems leave most of the residues on the surface between ridges. Soil conservation depends on the amount of residue and the row contour and row direction. Contour-based seeding or planting is a part of this tillage method.

Mulch Tillage: Sometimes also referred to as stubble mulch tillage is another conservative tillage practice where the soil is prepared in such a way that plant residue or other mulching materials is left on the surface. This practice uses chisel plows, field cultivators, disks, sweeps, or blades to till the soil before planting. This tillage method, however, does not invert or turn the soil upside down but leaves it rough with clods so as to incorporate the residue well.

Deoxyribose Nucleic Acid (DNA)

DNA is a molecule that carries the genetic information which acts as a framework or a foundation in the development, growth, functioning, and reproduction of all known living organisms including many viruses. It essentially is that information based on which most of the organisms can be identified and this information is stored as a code made up of four nitrogen bases: adenine (A), guanine (G), cytosine (C), and thymine (T) (Alberts et al., 2014). The monomer or fundamental unit of the DNA molecule is a nucleotide and each nucleotide is composed of one of four nitrogen bases, a sugar called deoxyribose, and a phosphate group. Monomers form a polynucleotide string through covalent bonding between the sugar moiety of one nucleotide and the phosphate moiety of the next nucleotide (Brown, 1999). The nitrogenous bases of the two separate polynucleotide strands are linked and bound together through hydrogen bonds according to the base pairing rules (A with T, and G with C) to form the double-stranded DNA (Brown, 1999). Most important property of DNA is that it can replicate, or make copies of itself based on the existing sequence of the bases. Each strand of DNA in the double helix can serve as a foundation for replicating the sequence of bases in the exact order (Pray, 2008). The two strands of DNA run in opposite directions to each other, and thus the complementarity in the base pairs makes the two strands antiparallel. The order or sequence of these bases determines the guiding framework available for building and maintaining an organism by encoding biological information (Dahm, 2008).

DNA usually occurs as linear structures packed in chromosomes in eukaryotes, and as circular chromosomes in prokaryotes including bacteria (Brown, 1999). Initial investigations into the structure of the bacterial chromosomes came in the formwork done

using light microscopy of stained specimens which was furthered by electron microscopy (Robinow & Kellenberger, 1994). The circular chromosome in the bacteria usually exists as a compact structure with distinct supercoiled or superhelical domains (Postow, Hardy, Arsuaga, & Cozzarelli, 2004).

CHAPTER III - METHODOLOGY

Collection of Soil Samples

This study was conducted on two 4-acre plots (Latitude & Longitude: 37.789394 & -88.416613) belonging to Quorum Laboratories LLC in the town of Eldorado in southern Illinois (IL 62930). The experimental agricultural plots are operated and maintained by Quorum Laboratories LLC. For the past 4 years, the plots have been maintained on strict regiments with either no-tillage or some common conventional tillage practices. The treatments that will be examined in this study are no-till (NT) and conventional tillage spring moldboard plow practice (CT). The direction of tillage is reversed between even and odd years. The two plots measured 4 acres each and were divided into 4 equal quadrants. Both the plots were not in use for any commercial agriculture since 2008. Cover crop combination of Rye (*Secale cereal*), Kale (*Brassica oleracea*), Fava beans (*Vicia faba*) and Clovers (*Trifolium pretense* & *Trifolium incarnatum*) was raised for hay. The two plots were separated by a stream and tree line running in between and a separation of 200 feet was maintained all along the entire length of the plots which were adjoining each other. The soils are classified as Loess over Illinoisan Drift soils with some alluvium (NRCS data). Plot on the east was maintained without tilling from 2013 December while the plot on the west which was marked for conventional tillage was tilled twice a year, once in spring and once in fall using Mold Board ploughing system dragged by a tractor. A mix of cover crops including Rye, Clover, Kale and Fava beans were planted every year. Rhizosphere soil (usually defined as soil rich in roots and/or soil adhering to the roots and affected by root activity) was collected from 5 to 15 cm depth from the experimental fields used by Quorum

Laboratories. A minimum of 10 and maximum of 30 soil samples were cored from each plot randomly in May of 2016 and 2017. Care was taken to avoid collecting the samples from the edges of the plots. The rhizosphere soil was cored at a distance of 5 to 10 mm from the plant roots. The samples were transferred into pre-labeled sterile 50 mL centrifuge tubes (VWR brand) and transported to the laboratory, where they were stored at 4 °C until appropriate processing could be done for DNA extraction and bioassay of phosphatase enzyme activity. All the samples were processed for metagenomics profiling and phosphatase assay within 24 hours and within 48 hours for all other assays including elemental analysis. Soil compaction analysis data was recorded in the field where the compaction was measured using the cone penetrometer.

Before all the samples were studied and analyzed for the soil compaction, soil phosphatase activity and metagenomics profiles, soil samples from both treatments were analyzed for pH, and the elemental composition. pH analysis using soil paste method (Thomas, 1996; Soil Survey Staff, 2004 https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_052226.pdf) showed that the average pH ranged between 6.54 to 6.63. A minimum of 20 samples per treatment was used for pH analysis. The pH and elemental analysis were recorded so as to take into account any major differences that may skew the rest of the studies.

Elemental Analysis of Soil Samples

Elemental analysis of the soil samples was conducted using a Thermo Inductively Coupled Plasma Mass Spectrometer (ICP-MS). All soil samples were analyzed for 30 different elements using a Thermo Scientific Quadrupole RQ ICP-MS and analyzed using Qtegra ISDS software. The instrument is able to measure more than 40 elements at a

detection limit of parts per billion (PPB) and can also be ramped up to measure at parts per trillion (PPT). ICP-MS was used to analyze for both the macro trace elements as well as micro trace elements which are found at very low concentrations. The instruments were calibrated using the US EPA method 6020A (http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6020a.pdf). The data acquisition and processing was controlled by ICP-AES and ICP-MS software. The results were expressed as PPM or mg per kg. Two approaches were used to measure the elemental concentrations. Method one which will be referred to as Haney's protocol (Haney) is based upon use of biologically relevant nondestructive extraction where soil is treated with a solution made up of organic acids mimicking nature to extract the elements (Haney, Haney, Hossner, & Arnold, 2006). Briefly, the soil sample is dried at 50^{o} C for 24 hours and 4 grams of the dried soil sample is digested with 40 mL of H3A extract (Haney, Haney, Hossner, & Arnold, 2010) for 10 minutes on a tabletop shaker set at 250 RPM. H3A extract is composed of 0.55 grams Malic acid, 0.35 grams Citric acid and 0.225 grams Oxalic acid in one liter of deionized distilled water. After one hour of shaking, the samples are centrifuged at 1200g for 15 minutes. About 20 mL of the supernatant is carefully poured through a Whatman #2 filter. The filtered solution then filtered further through a 2 µm cellulose filter using a syringe filter system (VWR). The final clear solution is used for elemental analysis using the RQ ICP-MS. This is usually a small fraction of the total elemental pool in the soil.

A second method referred to as Total Nutrient Extraction (TNE) which involved destructive method using a microwave digestion step was also used to look at the total pool of the elements (Chen & Ma, 2001; Uddin et al., 2016). Briefly, 4g of dried soil

sample is suspended in 40mL of distilled water for 10 minutes on a tabletop shaker set at 250 RPM. 1mL of suspended soil extract is added to a 3:1 (v/v) nitric acid: hydrochloric acid solution in a pressurized quartz digestion vessel (Perkin Elmer) and digested in a Anton-Paar Physica Multiwave (microwave sample preparation system) digester set at 350W power, 30 bar pressure for 60 minutes (Uddin et al., 2016). Post-digestion, samples were centrifuged at 1200g for 5 minutes to remove particulate matter. Supernatant is poured off into a clean 5mL tube and capped. A small volume of this (microliter range) is injected into the RQ ICP-Plasma Mass Spectrometer for elemental analysis using an auto sampler.

Soil Compaction Analysis

Soil hardness is the resistance of the soil to deformation. Hard soils can be a major problem for plant growth and seedling emergence (Jones, 1983) as well as for the soil microbiology (Whalley, Dumitru, & Dexter, 1995). Penetrometers are commonly used to measure soil hardness (Sandusky, 2003). Soil's resistance to penetration was measured by a hand-held recording cone penetrometer (Anderson, Pidgeon, Spencer, & Parks, 1980) to a depth of 50 cm in 5-cm increments. The number of replications per plot was between 10 to 15 per treatment. The cone used for our studies had an included semi-angle of 15^{o} and a diameter of 12.8 mm. The penetration rate was maintained between 1 cm to 1.5 cm per second. One-way MANOVA was performed on all the data.

Acid Phosphatase Activity in Soils

Phosphatase activity assay was performed as described by Tabatabai (Tabatabai et al., 1994). 1 gram of moist soil sample (stored at 4 °C) was weighed in duplicates into polypropylene vials for each of the 10 to 15 replicate soil samples. Acid or alkaline

phosphatase activity was determined by adding 4 mL of a pH buffer (pH 6.5 for acid and pH 11 for alkaline phosphatases respectively), and 1 mL of 0.1 M disodium phenylphosphate as a substrate. The mixture in polypropylene vials was incubated at 37 °C for 1 hour after which, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to arrest the reaction. The mixture was then filtered through Whatman #2 filter paper and the absorbance of the supernatant was measured using a GE UV–VIS spectrophotometer at 420 nm wavelength. P-nitrophenol standards were used to prepare a standard curve for comparison. Additionally, for each assay, a control was included to account for non-enzymatic substrate hydrolysis to account for the background. All the assays were performed in triplicates (n = 3) per sample. Statistical *t-test* was performed.

Metagenomic Analysis

DNA for metagenomic analysis was extracted from 200 mg of each soil sample using the ZR Soil Microbe DNA Kit (Zymo Research, USA) following the manufacturer's recommendations with slight modification using Ferric Chloride as an additional flocculating agent to remove humate inhibitors (Braid, Daniels, & Kitts, 2003). The quality and the quantity of the DNA extracts was verified by using Agilent Bioanalyzer 2100 and the Agilent DNA 1000 kit according to manufacturer's protocols. DNA concentrations were also determined using a Qubit dsDNA HS Assay Kit (Invitrogen, USA) on a Qubit fluorometer (Life Technologies, USA).

The library for Ion Torrent sequencing was prepared by amplifying seven different 16S rDNA hypervariable regions (V2, V3, V4, V6 + V7, V8, and V9) (Baker, Smith, & Cowan, 2003; Bates et al., 2011; Aloisio et al., 2016) using Life technologies 16s Metagenomics kit according to the manufacturer's instructions

(https://tools.thermofisher.com/content/sfs/manuals/MAN0010799_Ion_16S_Metagenomics_UG.pdf). The amplified and enriched products for the two primer sets used for each soil sample were pooled together for the final sequence run. These primer sets are also predicted to amplify a wide range of microbial taxa and have been confirmed through submission to blast of public sequence databases (Fonseca, Nichols, Lallias, Quince, & Carvalho, 2012). Each forward primer was tagged with a unique 10-12 bp barcode for multiplexing during sequencing run using the Ion Xpress barcode adapters procured from Life technologies (Thermo Scientific). This allows for multiple samples to be run on a single next-generation sequencing run. PCR was performed in 25 μL of reaction volume using Ion Plus Fragment Library kit (Thermo- Life Technologies) and the products after each amplification and enrichment step were purified using Agencourt AMPure XP Beads with 1.5X concentration (Beckman Coulter, Inc., Brea, CA), the concentrations of the purified PCR products were measured using Bioanalyzer 2100 (Agilent Technologies Inc.) and DNA 1000 kit (Agilent Technologies Inc.). The library mix was prepared by adding an equal amount of DNA from each barcoded sample. The amplicon size and concentration of the library mix were confirmed using Agilent BioAnalyzer 2100 and Agilent High Sensitivity DNA kit. The library mix was diluted with ultra-pure water to a final concentration of 26 pM. Finally, equal volumes of each were processed and sequenced in Ion S5 sequencer.

All the manipulations including dilutions were performed in a dedicated DNA extraction and PCR-mixing hood using sterile DNA/RNA free water (Ambion, USA) and DNA/RNA and DNAse/RNAse free plasticware (VWR-USA, Axygen-USA, Eppendorf-Germany). All the procedures and steps of the extraction and amplification were

conducted with the necessary no-template controls, including extraction blank controls. Library preparation from DNA from the corresponding soil sample was performed using Ion Torrent technology (Ion S5 Sequencer; Life Technologies, USA). Each sample was enriched and processed along with the addition of a unique barcode sequence added to each soil sample according to the manufacturer's instruction. The processed samples, 30 at a time were loaded onto an Ion 520 chip using Ion Chef System (Life Technologies).

Statistical comparison of metagenomes was conducted using QIIME pipeline (Caporaso et al., 2010). The Ion Torrent sequencing data was analyzed using the QIIME pipeline (Group Jumpstart Consortium Human Microbiome Project Data Generation Working Group, 2012). Both preset and scripted filters were applied to sequences prior to phylogenetic analysis to avoid using the barcode data. Depending upon appropriate fragment size for PCR (150 to 250 bp), bases after position 250 were trimmed and reads shorter than 150bp were removed. The reads with more than 30% of bases showing <Q20 were removed with Ion Torrent QC algorithms built into the Ion Reporter Metagenomics workflow (Di Gioia et al., 2016). After filtering and trimming, on average about 27,348 reads were obtained per sample. The rarefaction curve indicated that a reasonable number of individual samples had been taken. The sequence of each OTU was assigned to the lowest possible taxonomic rank with QIIME. Two-dimensional maps of the analyzed data identifying the bacterial phyla and their relative abundance were created using QIIME and KRONA. NCBS database and EMBL database were used for the analysis. Clustering was visualized using principal coordinates analyses and Bray Curtis similarity plots were created using the statistical software built into the QIIME pipeline. QIIME is an open

source platform running based on UNIX command platform. Figure 1 outlines the steps in this study.

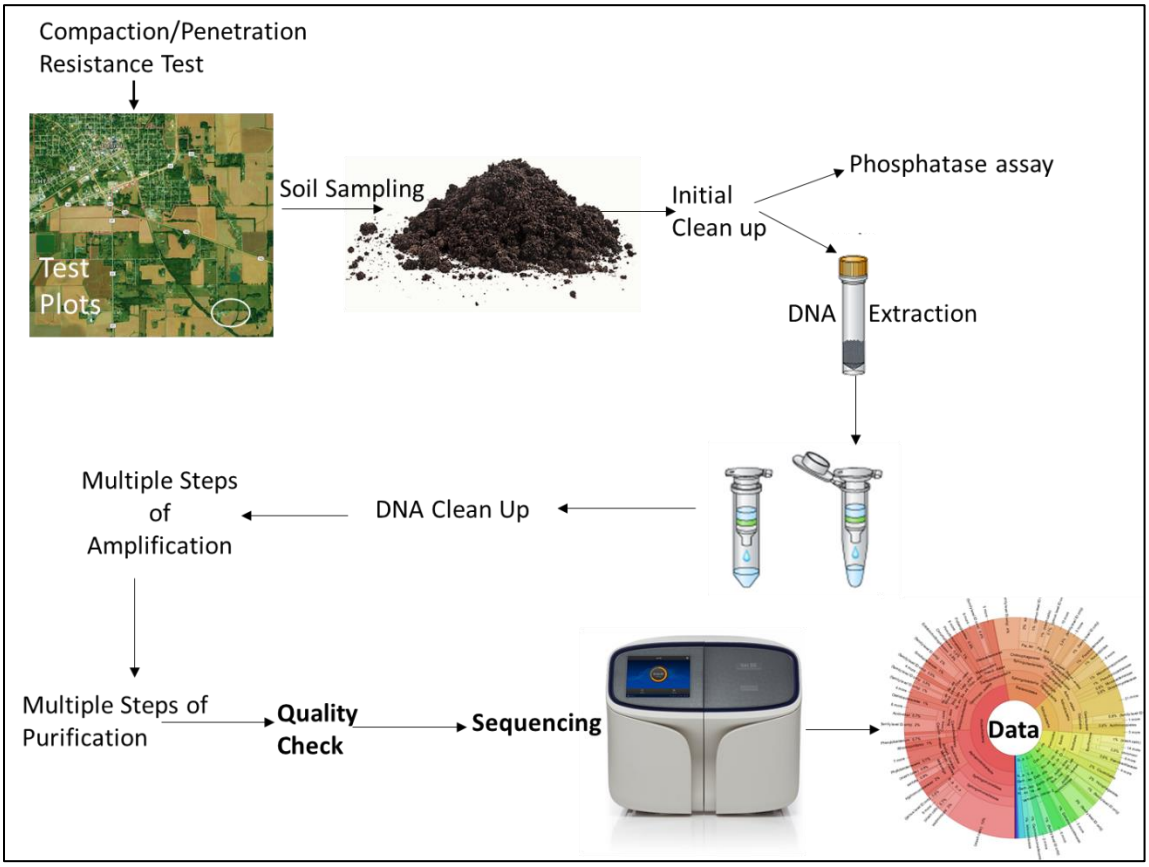

Figure 1. This figure outlines the steps in this study.

Figure 2. Mulch tiller used for the study.

Figure 3. John Deere moldboard plough used in the study.

Figure 4. John Deere Model 4025 tractor used in the study.

Figure 5. Soil grinder used for processing soils for elemental analysis.

Figure 6. Thermo ICAP mass spectrometer at the facility.

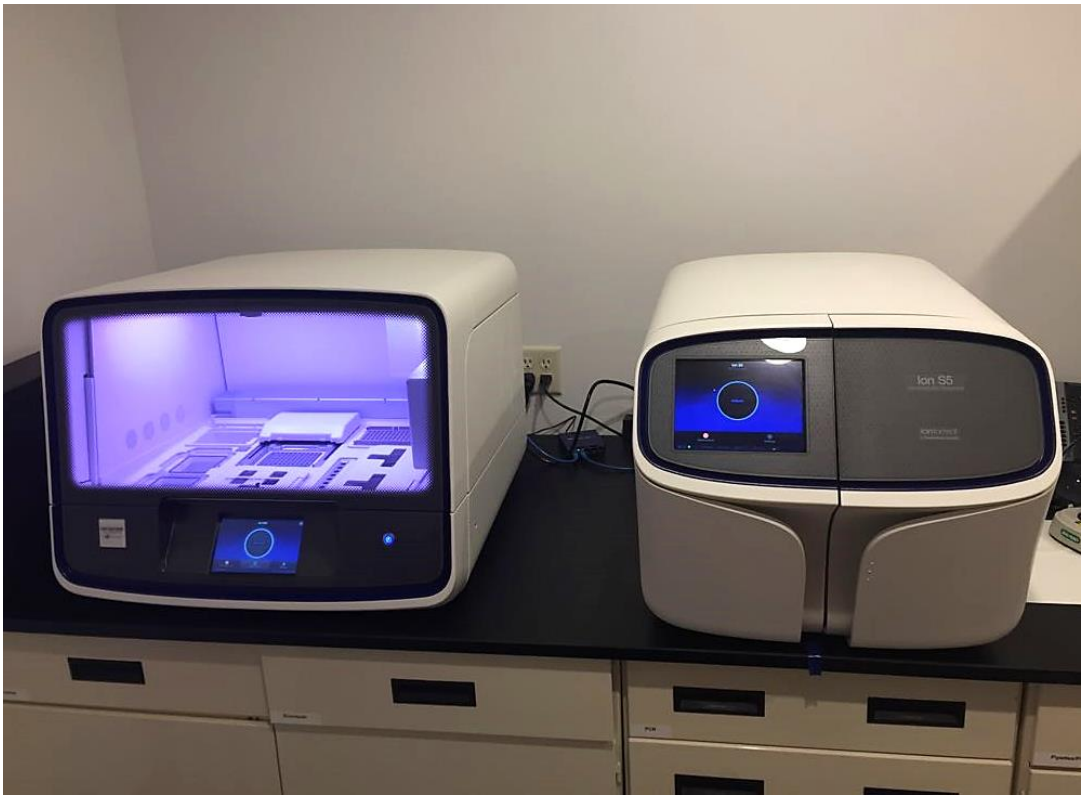

Figure 7. Ion S5 Next Generation Sequencer at the facility.

CHAPTER IV – ANALYSIS OF DATA

The purpose of this study was to examine the shifts in the taxonomic diversity at phylum, class and order level between two distinct agricultural practices – Conventional Tillage (CT) and Conservation Tillage (NT) in Southern Illinois (see Figures 8, 9, & 10).

Figure 8. Image of U.S. Map showing the location of the facility and field site – Eldorado, IL 62946 (Map Source: Bing Maps).

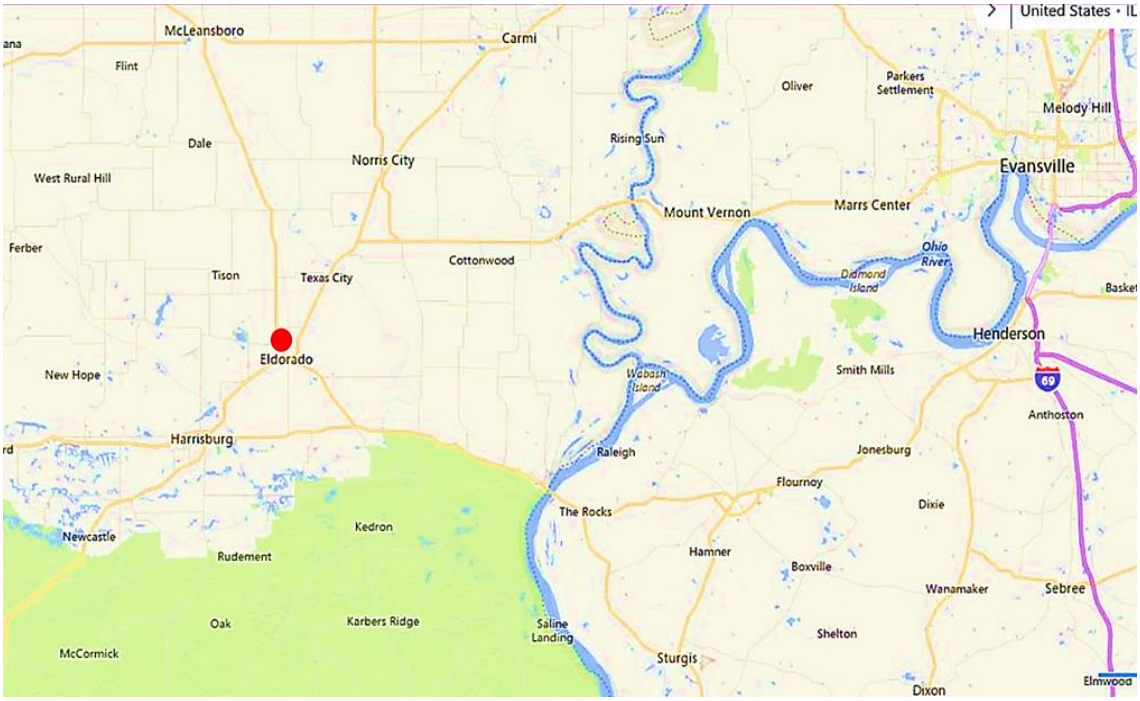

Figure 9. Zoomed in image of U.S. map showing the location of the facility and field site – Eldorado, IL 62946 (Map Source: Bing Maps).

Figure 10. Aerial image of the field site (Latitude/Longitude) and facility – Eldorado, IL 62946 (Map Source: Bing Maps).

Each plot measured 4 acres (420 ft. x 420 ft.) in size and was planted with a mix of kale, winter rye, crimson clover and field peas. A minimum of 15 soil samples from the CT and the NT were taken with 7 samples along an imaginary line between the diagonally opposite corners and was repeated from the other two diagonally opposite corners. The distance between the two diagonally opposite corners was approximately 590 feet. Samples were collected at every 70 to 90 feet and the 15th sample was collected at the approximate point where the two lines intersected (see Figure 11).

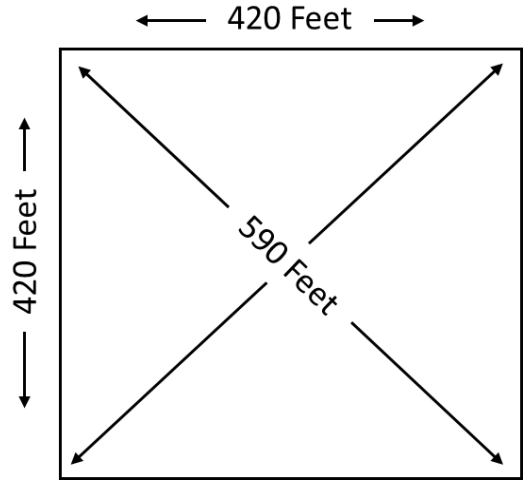

Figure 11. Soil sample collection pattern.

The 15 samples were analyzed in order to test the following hypotheses:

H1. Agricultural soils with conservation tillage (CT) practice will have lesser compaction than soils with conventional tillage (NT) practice.

H2. Agricultural soils with conservation tillage practice will have higher phosphatase activity.

H3. Agricultural soils with conservation tillage practice will have a more diverse community of bacteria.

We now present the results of the following analyses: pre and post soil pH; pre macro elemental components; pre micro elemental components, post soil penetration resistance (as measured by the soil cone index), and post-acid phosphatase activity (measured as µmol pNP/gram). Post bacterial metagenomic analyses measuring number of operational taxonomic units (OTUs), number of phyla, and taxonomic distribution were conducted.

Soil pH

The pH of the soils was determined pre and post CT and pre and post NT. A standard *t*-test of the soil pH data was performed. The *t-test* (df=18, p=0.33) showed that there is no significant difference at alpha = 0.05 for the phosphatase activity between the soils samples before CT and NT were implemented. However, the *t*-test (df=18, p=0.002) indicated that there was significant difference at alpha = 0.05 between both the plots two years after implementing CT and NT practices. (see Figure 12).

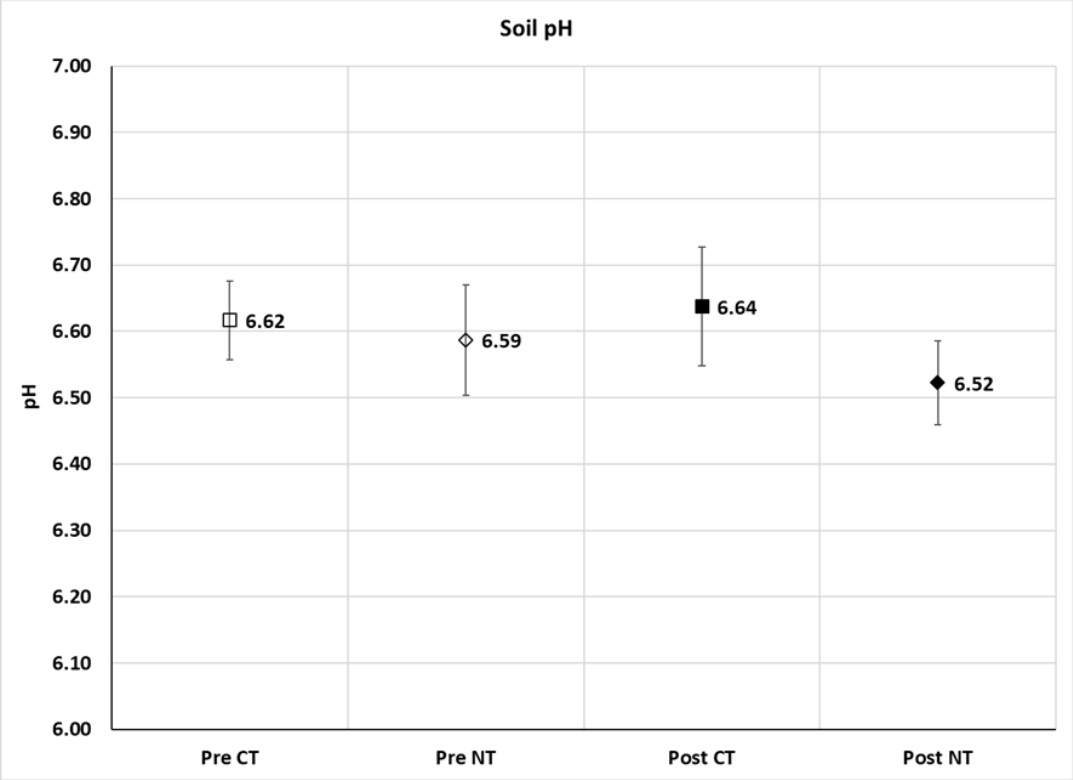

Figure 12. Soil pH from the test plots.

Elemental Analysis of the Soil Samples

Elemental analysis of the samples from CT and NT plots before the treatment was done to make sure that if any differences did arise in the measured dependent variables, it was not because of underlying differences in the soil elemental composition. Figure 13 shows the elemental analysis report for CT and NT plots for the macro elements while Figure 14 shows the elemental analysis report for CT and NT plots for microelements using the Haney protocol. The results are for n = 3 replicates per plot.

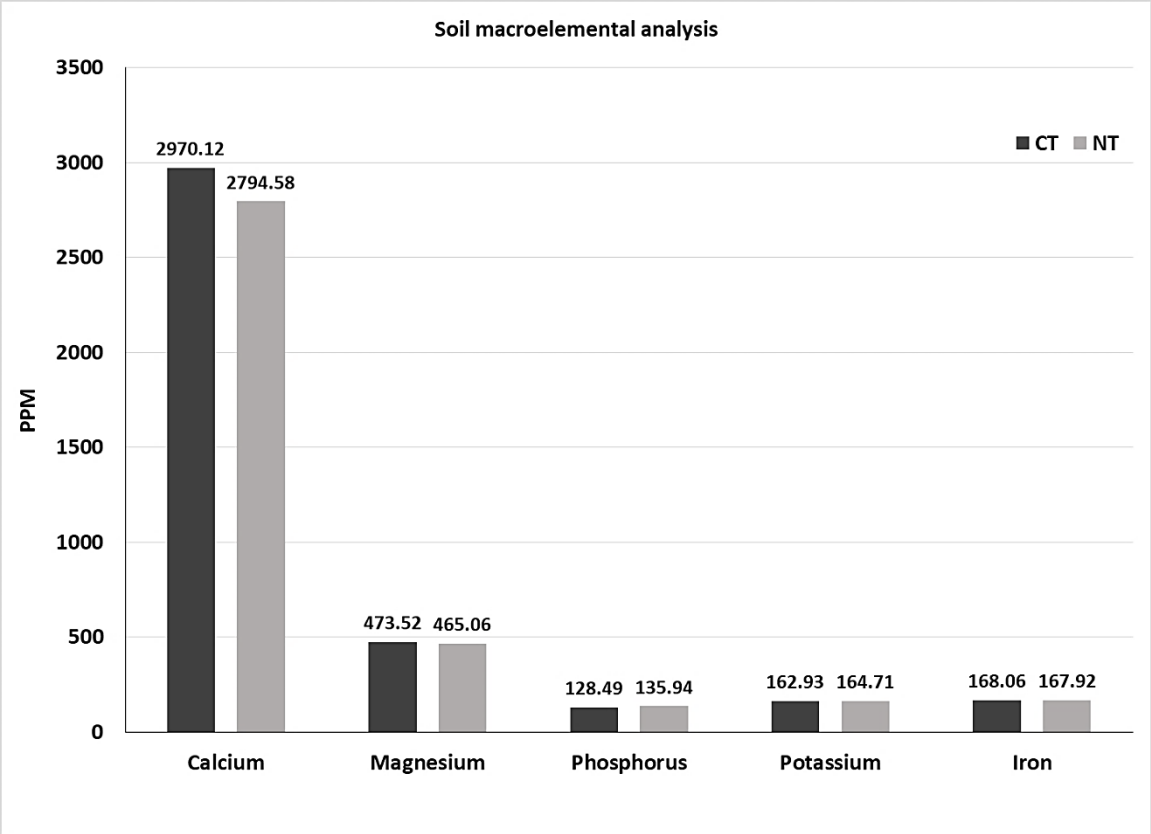

Figure 13. Macro elemental analysis for soils from the test plots.

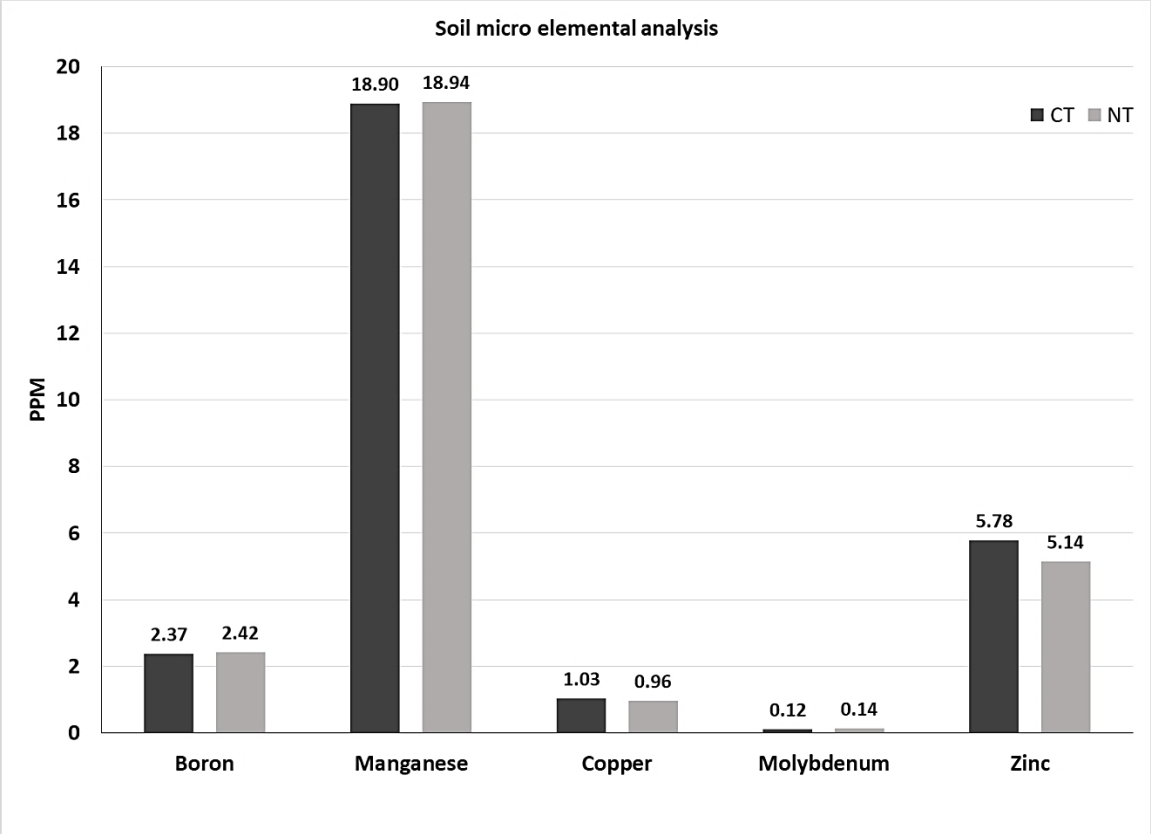

Figure 14. Micro elemental analysis for soils from the test plots.

The pre-treatment analysis of soil pH, macro, and micro elements indicated there were no major differences in the soils from both the plots. These pre-tests were performed to make sure that there are no significant underlying factors which may influence the post-treatment test variables – soil compaction, phosphatase activity and bacterial diversity.

Soil Cone Index – Penetration Resistance

The soil cone index (measure of penetration resistance) ranged from a low of 422 Kpa at 5 mm depth to a high of 2298 Kpa at 50 mm depth (see Figure 15). A Wilks Lambda multivariate analysis statistically significant difference in soil compaction as measured with a cone penetrometer, in relation to the type of tillage treatment. F (10, 13)

= 130.853, $p < .005$; Wilk's $\Lambda = 0.010$. The hypothesis H1 that there is a significantly lower compaction in the soils with NT compared to CT treatments was supported by the data.

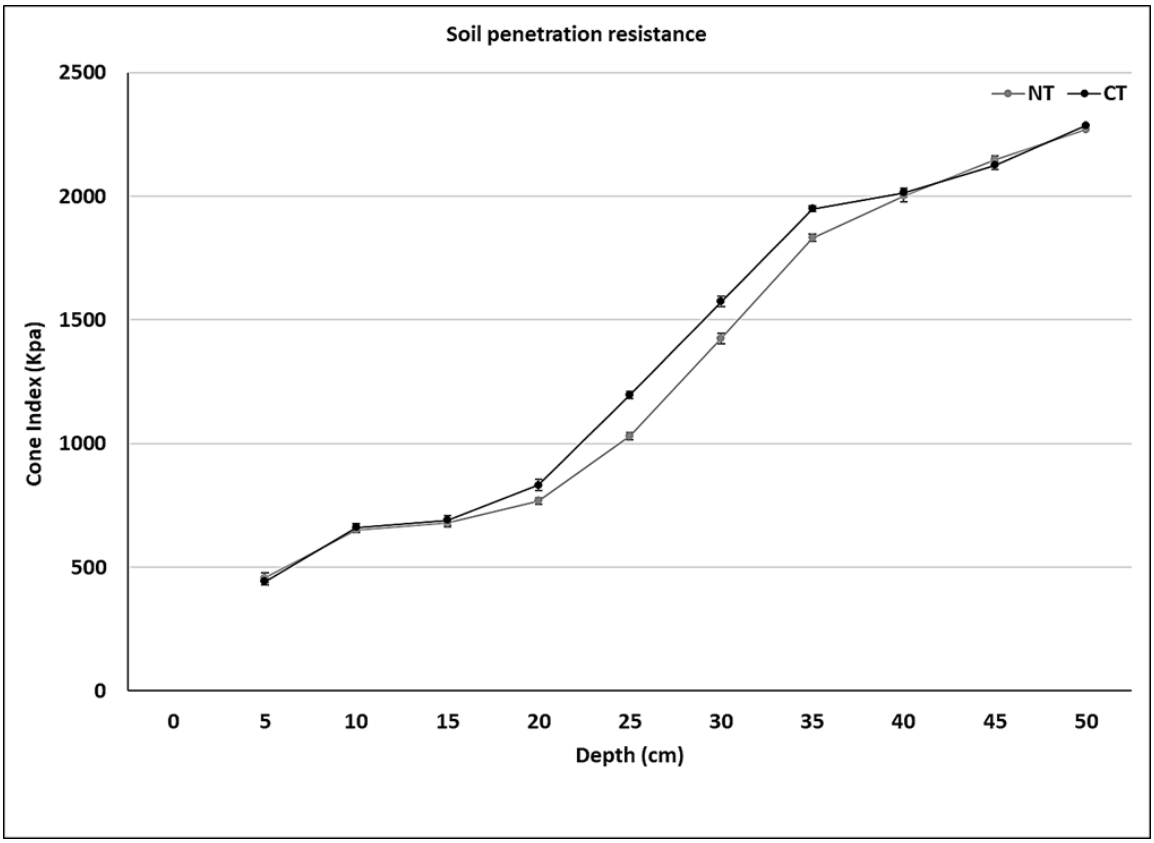

Figure 15. Average soil compactions at various depths in the soil grouped b CT and NT.

Acid Phosphatase Activity

The soil phosphatase activity ranged from 2.82 to 3.56 μmol pNP/gram of soil (see Figure 16). The *t-test* (df=28, p=0.054) showed that there is no significant difference at alpha = 0.05 for the phosphatase activity between the soils samples from CT and NT. The hypothesis H2 that soils under NT will have higher phosphatase activity was not supported by the phosphatase assay data. There were no statistically significant differences in the phosphatase activity between the soils from CT and NT practices.

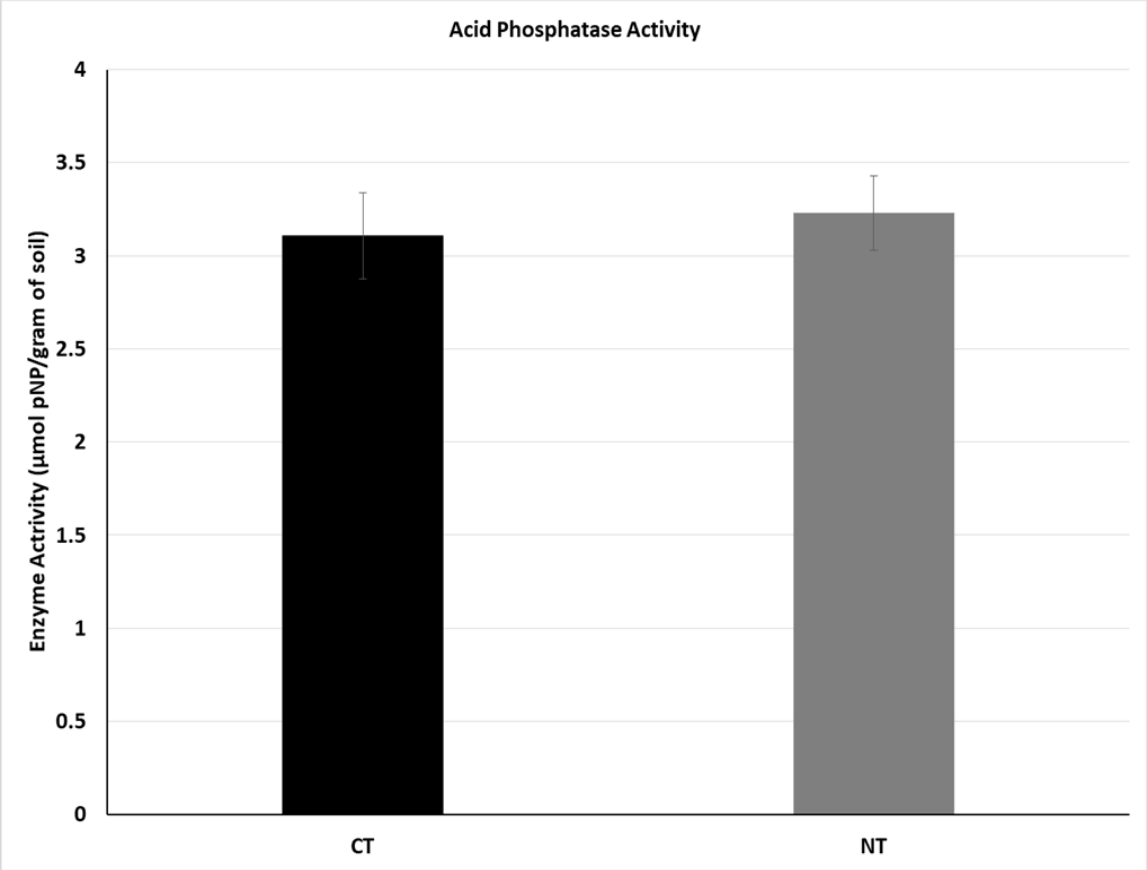

Figure 16. Average soil phosphatase enzyme in the soil grouped by CT and NT.

Metagenomic Analysis: Number of Operational Taxonomic Units (OTU).

The average number of OTU reads obtained for soil samples from CT was 27,142 and for soil samples from NT was 28904 (see Figure 17) . The *t-test* (df=28, p=0.009) showed that there is a significant difference at alpha = 0.05 for the number of OTUs detected between the soils from CT and NT.

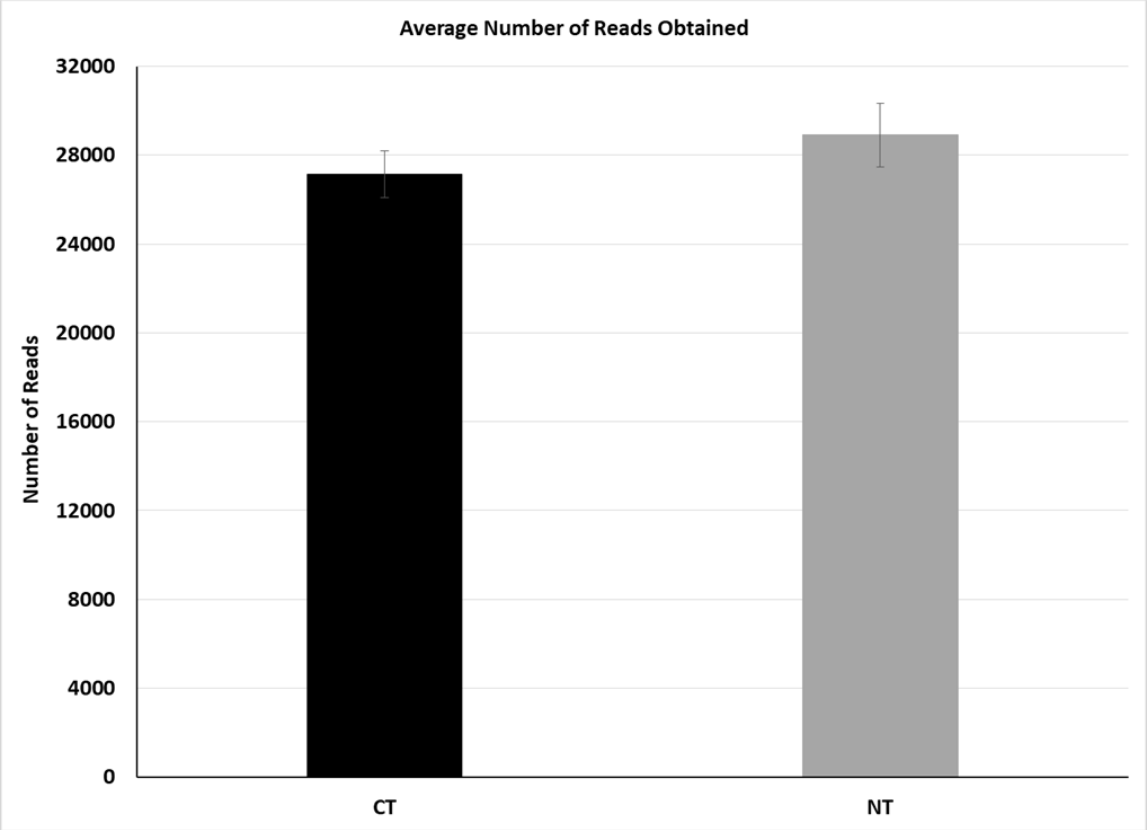

Figure 17. Average number of reads obtained from soils samples from CT and NT.

Metagenomic Analysis: Number of Phyla.

The average number of phyla detected for soil samples with CT was 16.2 and the average number of phyla detected in soil samples with NT was 17.8 (see Figure 18). The *t-test* (df=28, p=0.004) showed that there is a significant difference at alpha = 0.05 for the number of phyla detected.

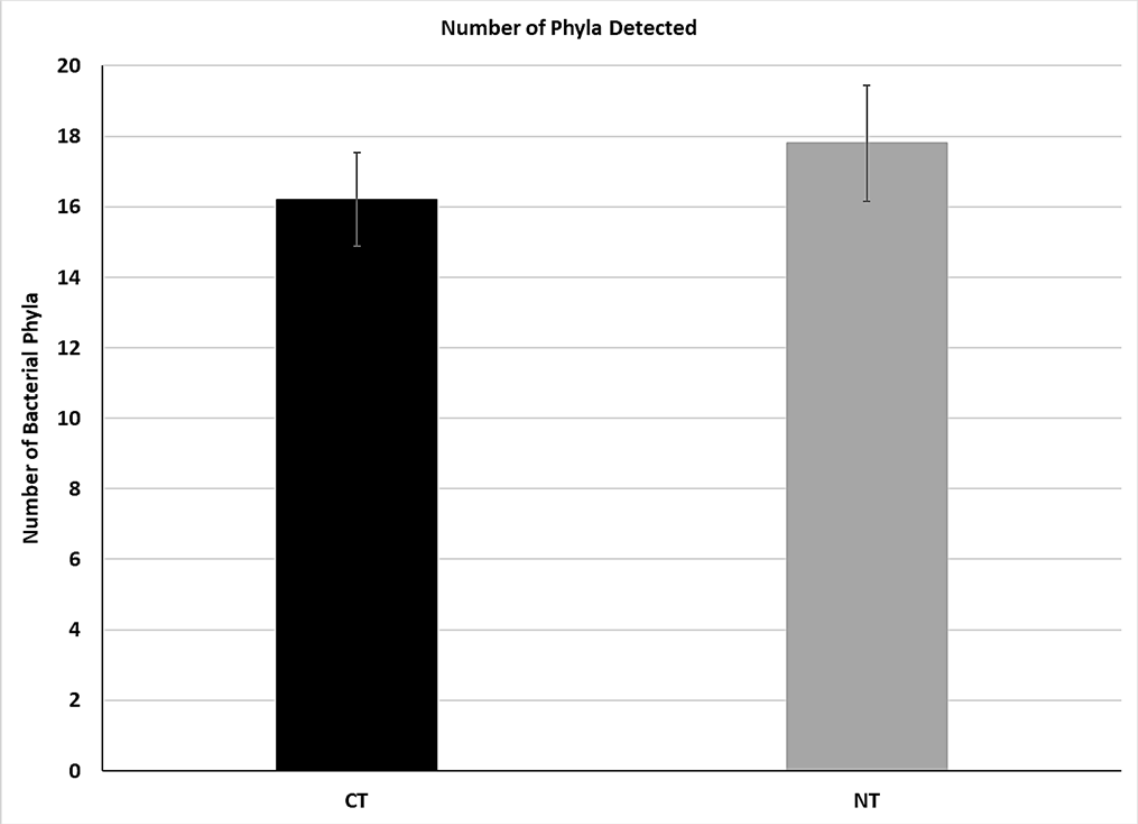

Figure 18. Average number of phyla detected from soil samples from CT and NT.

Metagenomic Analysis: Taxonomic Distribution

The taxonomic results for the different phyla detected were plotted as a bar graph with relative percentages (see Figure 19). A Wilks Lambda multivariate analysis showed that there was a statistically significant difference in composition of the phyla in relation to the type of tillage treatment. $F(19, 10) = 133.73$, $p < .005$; Wilk's $\Lambda = 0.004$. The hypothesis H3 that there is a higher diversity in the microbial communities of the soils with NT is supported by the metagenomic analysis data.

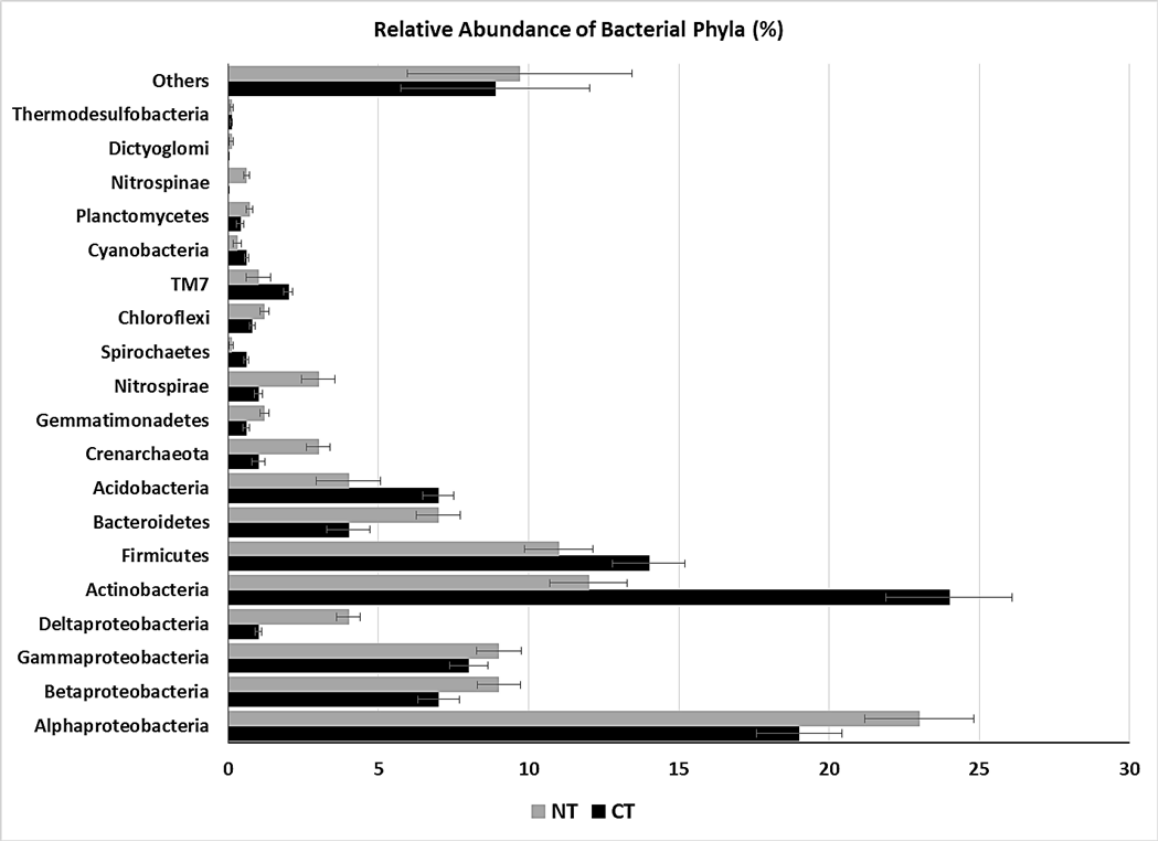

Figure 19. Taxonomic results for the relative abundance of various phyla detected in the soil samples from CT and NT.

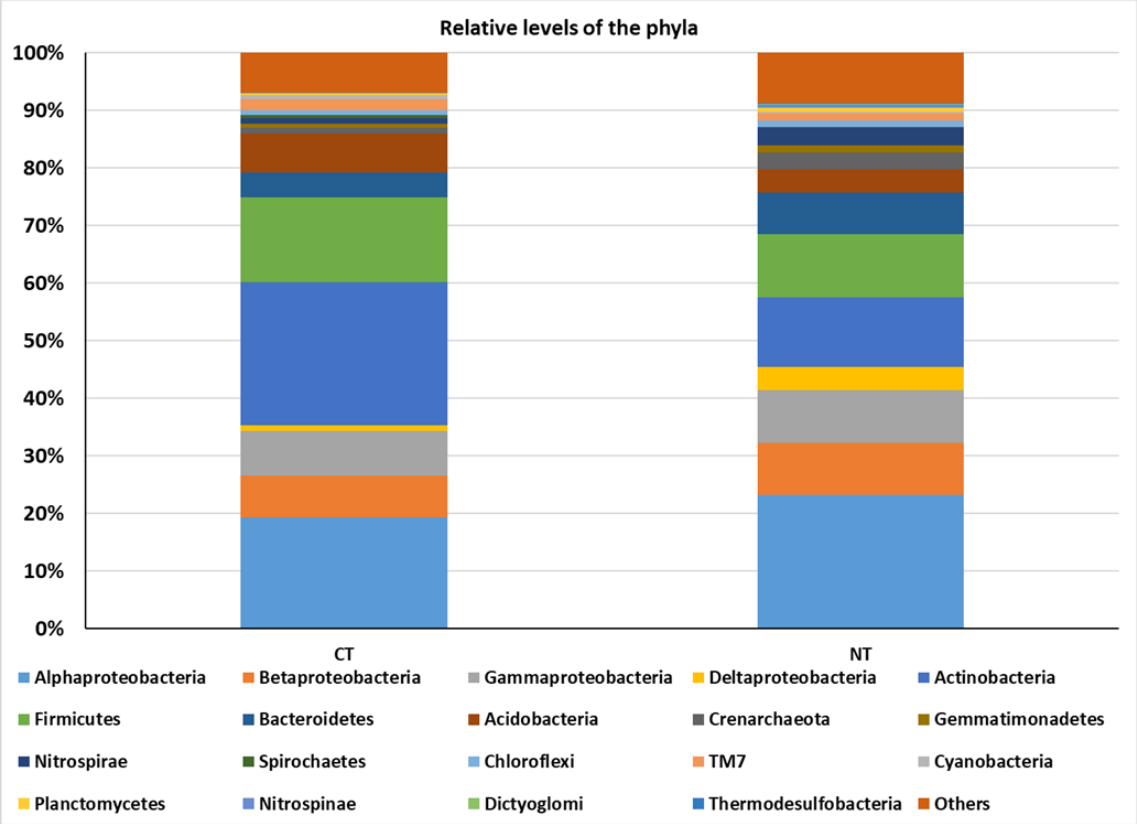

Figure 20. Relative levels of the detected phyla in soils samples from CT vs. NT.

All the dependent variables measured were significantly different for CT and NT and supported the respective hypothesis except for one variable – soil phosphatase activity. Although the phosphatase activity was higher in the soils from NT, it was not statistically significant at alpha = 0.05. Taken together there is strong evidence to support the hypotheses H1 and H3.

CHAPTER V – SUMMARY

Impact of Soil Tillage on Soil

In the present work, we tested three hypotheses:

H1. Agricultural soils with conservative tillage practice will have lesser compaction than soils with conventional tillage practice.

H2. Agricultural soils with conservative tillage practice will have higher phosphatase activity.

H3. Agricultural soils with conservative tillage practice will have a more diverse community of bacteria.

The study was conducted on two 4-acre plots (Latitude & Longitude: 37.789394 & -88.416613) that had been maintained on strict regiments with either the conventional tillage spring moldboard plow practice (CT) or no-tillage (NT) for four years. A cover crop combination of rye, kale, fava beans and clover was raised for hay each year on both plots. Between 10 and 30 rhizosphere soil samples (defined as soil rich in roots, and/or soil adhering to the roots and influenced by root activity) were randomly cored at a 5 to 15 cm depth from each plot in May of 2017 in the middle of the growing season. The variables measured were pre and post soil pH; pre macro elemental components; pre micro elemental components, post soil penetration resistance (as measured by the soil cone index), post-acid phosphatase activity (measured as μmol pNP/gram) and post bacterial DNA metagenomic analyses, including number of operational taxonomic units (OTUs), number of phyla, and taxonomic distribution. All of the post samples were processed for DNA metagenomics profiling (bacterial diversity studies) and soil phosphatase assays within 24 hours of collection. For pre and post-treatment soil pH and

pre-soil elemental analysis, the samples were processed within 48 hours after collection. Post soil compaction analysis data was recorded in the field where the compaction was measured using the cone penetrometer.

Effect of Tillage on Soil Compaction

The results from this study show that tillage indeed effects soil compaction. The soil's inherent properties may influence this but management practices like tillage can modulate and impact the soil compaction. In this study as the two plots were in close vicinity, the only variable which was different was tillage practiced. Therefore, the hypothesis that conservative tillage can reduce soil compaction is supported by the data. Soil tillage-based manipulations have a strong influence on the soil and can impact the soils for a considerable depth depending upon the frequency and type of tillage system used (Alvarez & Steinbach, 2009). Surface sealing a phenomenon brought about by tillage involves the soil particles falling back on itself and creating an almost impervious layer (Foley, Loch, Glanville, & Connolly, 1991) has been shown to reduce infiltration over a time frame (Azooz & Arshad, 1996). This effect is due to the rearrangement of soil particles and aggregates into new arrangement with changes in void space distribution as well as the overall particle orientation making it more uniform and more tightly packed (Tafangenya, Mthembu, Chikoore, Ndimande, Xulu, & Gcwensa, 2011). This reduced infiltration may affect the availability of water, aeration and hence result in reduced habitat diversity and ultimately impact the microbial diversity. Soil compaction due to tillage practices is a global concern and has serious implications on soil conservation, ultimately affecting soil productivity. In modern commercial agriculture, inappropriate soil management along with the use of heavy machinery followed with intensive

cropping and grazing are common causes of soil compaction (Jung, Kitchen, Sudduth, Lee, & Chung, 2010). As compaction increases so does the cost of tilling and managing because of increased use of machinery and the additional costs of irrigation and fertilization due to reduced infiltration. Another major impact of tillage is on soil erosion. Tillage-induced erosion seems to be also correlated with decreased soil organic carbon (SOC) content (Heckrath, Djurhuus, Quine, Van Oost, Govers, & Zhang, 2005). There is good evidence to indicate that decrease in SOC levels can reduce microbial activity and microbial count (Araújo, Luiz, Leite, Valdinar, Santos, & Carneiro, 2009). It is possible that in the study conducted increased compaction in the CT plot could be a combination of erosion, surface sealing, changes in aggregate shape and arrangement and reduced microbial diversity.

Effect of Tillage on Soil Phosphatase Activity

Soil biology, especially microbes play a crucial role in soil nutrient cycling, maintenance of soil structure and bioremediation (Stockdale & Brookes, 2006). Therefore, any practices aimed at using soil for anthropogenic activity should take into consideration the biological component of the soil. Nutrient cycling and organic matter decomposition, two very important dynamic components of soil are brought about by the enzymes (Pavel et al., 2004). The present study provides information on soil phosphatase activity as influenced by tillage. Although several studies have indicated a significant increase in soil phosphatase activity (Gupta & Germida 1988; Mohammadi, Heidari, Nezhad, Ghamari, & Sohrabi, 2012), the present study was inconclusive and did not find any statistically significant increase in soil phosphatase activity. Therefore, the hypothesis "**H2 -** Agricultural soils with conservation tillage practice will have higher phosphatase

activity" was not supported by the results. One reason could be that most of the studies involved comparison between treatments which also included the use of fertilizers. There are studies indicating a reduction in soil phosphatase activity under conventional fertilizer regimen (Chang, Chung, & Tsai, 2007). As any kind of fertilizer was not applied in this study, the soil phosphatase activity may not have been influenced by any strong chemical gradients and hence a big difference was not noticed in the soil phosphatase activity between CT and NT. Phosphatases are involved in the transformation of organic phosphorus compounds in soil and the objective of the present study was to determine the effects of two different tillage practices. The hypothesis was that no-tillage will lead to increased habitat diversity and hence increased bacterial diversity. Although the study does support increased bacterial diversity, the microbial biomass based on the number of OTUs may not have been significantly big enough to impact the inherent soil phosphatase activity. The trends found in the impact of tillage on phosphatase activity are in line with previous studies (Acosta-Martınez & Tabatabai, 2001; Balota, Kanashiro, Filho, Andrade, & Dick, 2004) showing lower values in tilled soils but did not show a significantly lower value. The increased diversity may not transform to increased phosphatase activity probably because of the redundancy in the services provided by various bacterial phyla (Allison & Martiny, 2008). Although literature review indicates that microbial communities are sensitive to disturbance, shifts in their community composition may not necessarily shift their functional output due to presence of taxa that are functionally redundant. As long as there is a need to scavenge for or perform certain nutrient cycling reactions without the added stress of chemical gradients like fertilizer applications, soils with different microbial diversities may perform enzyme based activity

at similar levels (Tilman, Knops, Wedin, Reich, Ritchie, & Siemann, 1997). Different or diverse communities can function differently but as complete supra organism can result in a similar outcome. Within the limitations of present techniques available, this may be difficult to prove but research indicates a high probability of such dynamics (McGill, Enquist, Weiher, & Westoby, 2006). Another reason could be that the bioavailability of P was similar in both the plots.

Effect of Tillage on Soil Bacterial Diversity

The microbial communities which are a part of the soils whether agricultural or forest lands or simply our home gardens significantly contribute to nutrient uptake and cycling and the overall ecosystem architecture (Buckley & Schmidt, 2001; Young & Crawford, 2004). The contributions can be both positive and negative on the plants growing on those soils (Daims, Lebedeva, Pjevac, Han, Herbold, & Albertsen 2015; Fierer, Leff, Adams, Nielsen, Bates, & Lauber, 2012). It has also been reported that increased tillage will cause increased turnover of species and hence reduced ability to adapt and stabilize (Mueller & Sachs, 2015). This may, in the long run, have detrimental effects on overall soil ecosystem and the services the soil ecosystem can provide. Taken together, the present study which was in a real field setting supports the hypothesis that reduced or no-tillage (NT) can increase the soil microbial diversity and stabilize the microbial communities. The diversity can add to an increased number of ecological services provided by the soil. There are a few studies examining the microbial communities under different tillage practices from a metagenomic microbial diversity perspective, a few have reported increased diversity in tilled lands (Souza et al., 2013). However, closer inspection reveals that the significant differences were limited to only a

small proportion of taxonomic groups. A possible explanation could be that tilled soils tend to lose SOC and nutrients with time (Hungria et al., 2009). This may lead more diverse taxonomic profiles in tilled soils as a result of a depleted environment which may be conducive to more commensal microbial communities than mutualistic microbial communities. However, the number of studies indicating an increase in microbial diversity in tilled soils is a small fraction of the total number of studies so far. A good example to support the hypothesis is a recent study conducted in Argentina (Carbonetto, Rascovan, Álvarez, Mentaberry, & Vázquez, 2014). No-till soils were found to have a significantly higher taxonomic diversity. The amount of land used to raise corn and soybean crops is large and the agricultural practices employed for farming these crops have major effects on environmental health on a global scale.

Soils with no-tillage have also been shown to have higher number of gene sequences associated with important nitrogen cycle steps, suggesting that a larger potential for microbial nitrogen fixation that could be low or lost in conventional tillage (Smith et al., 2016). Hence, any decrease in microbial diversity combined with soil erosion and increased compaction can actually magnify the nutrient losses as well as losses in SOM. The results from this study indicate that tillage practices, especially in the long term, can impact soil microbial population diversity and community profile. As this study only analyzed samples from same single time point over two years, it will be more useful to run experiments analyzing samples over an extended period of time in order to gain a better understanding of microbial dynamics in response to the different weather patterns. This should also be followed with studies correlating crops and

amendments to gain a better understanding. This study demonstrates that the no-tillage system can significantly enhance the soil microbial diversity.

Conclusions

In an agro-ecosystem biological diversity is probably highest in the soil and their functions may impact many aspects of agriculture including but not limited to plant health, yield, geochemical cycling and transformations and overall soil productivity (Roger-Estrade, Anger, Bertrand, & Richard, 2010). Although there are potentially a large number of factors that can influence the soil bacterial community composition, diversity and overall function, anthropogenic activity in agricultural lands could be the single largest driver of changes that impact soil properties on many levels as mentioned earlier. In addition, the interaction between soil microbial communities, plant species, other biotic and abiotic factors is very complex and we have only started scratching the surface. Some ways in which tillage on soils can decrease the bacterial populations and diversity is by increasing desiccation, mechanical disturbance, reduction in pore volume, soil compaction and changing the habitat diversity. It may also negatively impact access to food substrate resources (Giller, 1996). This study touches on just one aspect of many factors which can influence the soil bacterial diversity, bacterial quantity and the ecological services provided by the soil. Hence, this field of investigation is highly heterogeneous, multi-disciplinary and wide open for intensive research which is necessary to understand the ecological role of soil bacterial communities. Another aspect which comes into light is development of unique microbial pattern fingerprints for different soil types under different management practices, plants grown and weather. Agroecosystems are inherently prone to more stress when compared with other soil

ecosystems because of relatively higher anthropogenic activity. Using markers such as soil compaction, soil phosphatase activity and microbial diversity in correlation with management practices and crops to be grown, yield and inputs can help not only offset some of the negative impacts agricultural industry has on the environment and but also help improve human health while preserving food security. This research provides results which can help in agroecosystem optimization with respect to the inputs, practices, yield and environmental quality. One aspect I would like to add in any future experiments is measure and characterize the soil organic matter in such approaches to gauge the changes in not only the organic matter but also the changes in the type of functional groups found in the soil organic matter. The soil organic matter changes could potentially throw more light on the metabolic and functional aspects of soil bacteria. The biggest limitation to this approach for studying soils is the relatively nascent reference databases, disconnect in metagenomic analysis platforms from various parts of the world, lack of common globalized protocols to access and use the databases that enable even more comprehensive analyses of diverse metagenomic datasets. There is also lag in populating these databases with new data on difficult to culture individual bacterial species as cultivation of a very large proportion of such bacteria is very time-consuming and not necessarily successful. However continued research and work in this area can help us understand our environment better and also help us profile such environmental samples to characterize the true functional output of the soils and their environmental services which is a result of the chemistry, physics, and biology in the soil. Although metagenomics, theoretically enables us to study any sample for their biological content, especially microorganisms and get a taxonomic profile, even more, important is connecting the dots

linking their interactions with each other and the environment they live in. By understanding such interactions, a true picture of the dynamic soil environment can be drawn and used to fine tune the way anthropogenic activity influences the soil environment towards true sustainable agriculture. Combining such metagenomic taxonomic studies with functional gene profiling for functions such as nitrogen fixation, denitrification, phosphatase activity, pathogen resistance can help understand the true potential of soil and ways to fine tune or enhance it.

APPENDIX A – Data Tables

Table A1.

Summary of Soil pH Studies for pre-CT and NT, and post CT and NT

Treatment	Mean	n	Std. Dev.	Highest	Lowest
Pre CT	**6.62**	**10**	**0.06**	**6.71**	**6.52**
Pre NT	**6.59**	**10**	**0.08**	**6.74**	**6.48**
Post CT	**6.64**	**10**	**0.09**	**6.79**	**6.51**
Post NT	**6.52**	**10**	**0.06**	**6.61**	**6.43**

Table A2.

Summary of Soil Penetration Resistance (kPa) for CT.

Depth (mm)	n	Mean	Std. Dev.	Highest	Lowest
5	**12**	**441.75**	**17.68**	**470**	**438**
10	**12**	**660.50**	**12.62**	**689**	**646**
15	**12**	**689.67**	**13.85**	**717**	**684**
20	**12**	**832.17**	**19.31**	**868**	**811**
25	**12**	**1196.25**	**21.50**	**1249**	**1172**
30	**12**	**1574.17**	**15.87**	**1592**	**1547**
35	**12**	**1948.33**	**20.66**	**1990**	**1916**
40	**12**	**2013.42**	**10.97**	**2034**	**1998**
45	**12**	**2124.17**	**17.30**	**2159**	**2106**
50	**12**	**2285.42**	**15.28**	**2313**	**2271**

Table A3.

Summary of Soil Penetration Resistance (kPa) for NT

Depth (mm)	n	Mean	Std. Dev.	Highest	Lowest
5	12	442.58	13.86	482	441
10	12	650.08	18.95	699	631
15	12	678.42	10.18	701	668
20	12	768.08	15.96	801	754
25	12	1030.83	12.73	1056	1014
30	12	1424.92	14.47	1455	1411
35	12	1831.50	21.74	1883	1806
40	12	2000.58	15.14	2031	1975
45	12	2147.42	23.74	2192	2304
50	12	2269.17	15.60	2304	2255

Table A4.

Summary of Phosphatase Activity (µmol pnp/gram of soil) Assay Studies

Treatment	Mean Activity	n	Std. Dev.	Lowest	Highest
CT	3.106	15	0.212	2.82	3.487
NT	3.229	15	0.187	2.89	3.56

Table A5.

Summary of Number of OTU Reads from Soil Samples

Treatment	Mean	n	Std. Dev.	Lowest	Highest
CT	27142.8	15	1054.425	25652	29314
NT	28904.46	15	1432.504	26707	31216

Table A6.

Summary of Number of Phyla Detected in Soil Samples

Treatment	Mean	n	Std. Dev.	Lowest	Highest
CT	16	15	1.549	13	19
NT	17.86	15	1.627	16	22

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