# ANTHROPOGENIC INFLUENCES ON SOIL MICROBIAL PROPERTIES

by

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B.S, Kansas State University, 2013

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

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# Abstract

Human activities have the potential to alter soil biochemical properties in a number of different ways. This thesis will focus on how agricultural practices (tillage and cropping system), climate change, and urban soil pollution (primarily lead and arsenic) affect soil biochemical properties. Two incubation studies were conducted to determine how human activities influence soil biochemical properties. The first study focused on how altered temperature and moisture regimes affected soil properties from four different agroecosystems. Four different soils were incubated under two different soil preparation methods (sieved <4mm and <0.25 mm), three different temperature treatments (12, 24, and 36°C), and two different moisture treatments (field capacity and 80% of field capacity) for 180 days. Destructive samples were taken at 7, 30, 60, 120, and 180 days and the soil microbial community was analyzed using phospholipid fatty acid analysis (PLFA). The second study investigated how soil amendment treatments (Mushroom Compost and Composted Biosolids) of an industrially contaminated site affected the biochemical properties of that soil. Surface soil samples collected 435 days after compost addition from urban garden test plots located adjacent to a former rail yard in Monon, Indiana. Soils were incubated for 30 days to stimulate microbial activity. Following incubation, the soil was analyzed for PLFA, soil enzymes, and available metal fractions. In the first study the greatest differences were found between the <4mm and the <0.25 mm size fractions – which highlights the effect of soil aggregation and structure on microbial populations. After aggregation effects, temperature treatment had the next largest effect on microbial populations, with the greatest biomass in the middle (24°C) treatment. The second study assessed different soil amendments on soil microbial properties and metal availability. Composted biosolids reduced metal availability and increased microbial enzyme activity and biomass.

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# List of Abbreviations

SOC: Soil Organic Carbon

NTM: KS soil managed under no-till with manure fertilizer

CTM: KS soil managed under conventional till with manure fertilizer

NTR2: BR soil managed under no-till with intensive rotation

CTR2: BR soil managed under no-till with intensive rotation

S1: Sampling time 1; 7 days after start of incubation

S2: Sampling time 2; 30 days after start of incubation

S3: Sampling time 3; 60 days after start of incubation

S4: Sampling time 4; 120 days after start of incubation

S5: Sampling time 5; 180 days after start of incubation

M1: Field Capacity

M2: 80% of Field Capacity

T1: 12°C

T2: 24°C

T3: 36°C

## Acknowledgements

I could not do this thesis alone and I would like to greatly thank those that helped me along the way. First, I would like to acknowledge my advisor, Dr. Hettiarachchi, and my committee members, Dr. Rice and Dr. Kirkham as well as the rest of the KSU faculty that assisted me, in particular, Dr. Ransom, and Dr. Kluitenberg. I would like to thank Pavithra Pitumpe Arachchige for her help with running with first study and for developing the SAS codes that were used in both studies. I would also like to thank Oshadhi Athukorala Arachchige and Caleb Gravesen for helping to keep the lab running smoothly and for doing a lot of sample and glassware prep work. I appreciate Andrew McGowan for his help in running the GC-MS and with PLFA results calculations and interpretations. I would like to thank Dr. Nora Bello of the statistics department for help with developing the statistical approach used for my first study. I would like to thank Chammi Attanayake for allowing me to use her soils for my second study. I would like to thank Madhubhashini (Buddhika) Galkaduwa for helping with the GF-AAS.

# Chapter 1 - The Effects of Human Influence on Soil Microbial Properties – an Introduction

## Climate Change and Soil Carbon

Climate change is one of the most pressing problems of this time and there are many suggested solutions and mechanisms for mitigation and adaptation, many of which involve agricultural practices. One of the major agriculture related mitigation/adaptation options to change soil management on croplands. Since the industrial revolution, burning of fossil fuels and ever more extensive changes in land management have caused global carbon dioxide concentrations to rise from around 280 parts per million (ppm) to the present value of 401 ppm (Tans and Keeling, 2015).This rise of over 100 ppm has already correlated to an approximately 0.75 °C average change in global surface temperature (Shaftel et al., 2015). Given current emission rates, atmospheric $CO_2$ is likely to continue to increase to over 600 ppm by 2050. This will correlate to a 2.6 °C change in average surface temperature; which will have severe consequences for human habitation and development (Intergovernmental Panel on Climate Change, 2014). The increased amount of heat in the atmosphere is already altering climate patterns and is at least partly responsible for the recent increase in droughts and tropical storms (Shaftel et al., 2015).

Climate change and agricultural production are deeply intertwined. About 11 % of global greenhouse gas emissions are associated with land-use change (deforestation, etc.), which is often done to provide new land for agricultural production (as well as for harvesting timber) (Intergovernmental Panel on Climate Change, 2014). Additionally, about 14 % of global

greenhouse gas emissions are estimated to come from ongoing agricultural production (Intergovernmental Panel on Climate Change, 2014) . These emissions are partly due to decreased soil carbon stocks. A loss of soil carbon typically follows land-use change – especially when soils that are high in carbon, like native grasslands and wetlands, are tilled or drained. Soil disturbance brought on by landscape alteration and tillage practices accelerates soil carbon decomposition – releasing stored carbon back into the atmosphere as $CO_2$. Decreasing soil carbon (along with livestock production and rice production) is one of the major sources of agricultural greenhouse gas emissions (Intergovernmental Panel on Climate Change, 2007). Soil and crop management practices that stop and reverse the trend of declining soil carbon have the potential to play a role in the mitigation of climate change.

The reason that soil carbon stocks are important for climate change is that soil carbon constitutes a large pool in the global carbon cycle that has the potential to be a net source or sink of atmospheric $CO_2$. Worldwide, soils store approximately 1550 Gt of carbon as organic carbon; this is over twice that of what is currently in the atmosphere (approximately 750 Gt) (Lal, 2004a). This carbon pool is controlled by the amount of carbon inputs via plant growth and the amount of carbon mineralization to $CO_2$ via microbial respiration. It is well understood that increasing temperatures (i.e. from climate change) leads to an increase in metabolic rate for microorganisms. Thus, increasing temperatures have the potential to decrease soil organic carbon stocks as microbial demand for carbon substrate increases; which in turn could further exacerbate climate change by releasing even more $CO_2$ into atmosphere. Climate change also has the potential to change local, regional, and global moisture regimes; which affects soil carbon stocks and soil greenhouse gas balance– although less directly. Waterlogged soils tend to have

more carbon stored due to slow decomposition from low oxygen; however they are also sources of methane and nitrous oxide, both of which are greenhouse gases and by products of anaerobic metabolism. Altered moisture regimes could mean previously dry soils become wetter, and previously wet soils dry. Climate change has potential to alter soil carbon balances in a number of different ways and understanding how the multiple factors add up is an important endeavor for mitigating and adapting to climate change.

While there are natural feedback loops that could cause a decline in soil organic carbon due to climate change; how we manage agricultural land still has a large impact on global soil carbon stocks. Agriculture takes up about 34% of all land on earth (Ramankutty et al., 2008). Since agricultural land makes up a significant fraction of the earth's land surface, how that land is managed and how that management affects soil organic carbon is an important part of the global carbon cycle and thus important for addressing climate change. As a whole, agricultural soils tend to have less carbon than non-agricultural soils; due to removal of crops and residue, disturbance from cultivation/tillage, and the tendency for domestic plants to have a less vigorous root system than native plants (Lal, 2004a). However, the actual carbon stocks in agricultural soils can vary widely based on soil properties, climate, and management practices.

Soil properties, climate, and management practices all control soil carbon storage capacity in two basic ways. Soil carbon content ultimately depends on the rate of addition of plant residues and the rate of decomposition. When decomposition rate exceeds the rate of residue input, the soil loses carbon. When the rate of residue input is greater than the decomposition, soil carbon increases. Better soil fertility and management practices that increase

residue input (No-till, composts, manures, cover crops, etc.) increase the amount of organic carbon being put into the soil system. Climate and management practices that affect aeration and microbial access to residue (tillage, tile drains, etc.) affect the rate of decomposition. The purpose of this study was to observe the microbial response to changes in environment in different soil types.

## Soil Contamination and Agriculture

Contamination with potentially toxic metal(loid)s and organic compounds is another major anthropogenic impact on soils. In the U.S., the problem of soil contamination is most pronounced in urban and industrial areas. Soils in these areas are often contaminated with potentially hazardous substances like lead, arsenic, and industrial compounds that stem from historical use of leaded gasoline and paints, As containing household pesticides, as well as metal processing industries (i.e. smelting).These contamination problems can have serious impacts on human and environmental health. A review of child lead poisoning cases as reported to the Center for Disease Control (indicating that blood lead concentrations were high enough to cause serious symptoms) found that between 40 and 45% of cases had no lead paint in the home – indicating that the lead likely came from ingestion or inhalation of contaminated soil or dust (Mielke et al., 1983). Humans can be exposed to lead in soils in a number of different ways. Some exposure happens through direct consumption of soil – through unwashed produce or through eating the soil directly (particularly with young children through hand-to-mouth behavior and putting soil covered toys/objects in their mouths) (World Health Organization, 2010a). There is also the risk of indirect soil exposure through produce grown in contaminated soil and through dust inhalation as soil particles become air borne (Taylor and Lovell, 2014; Tu

et al., 2013). Children exposed to lead are at greater risk for learning disabilities, such as ADD and ADHD, as lead damages brain tissue – which is particularly problematic for children with actively growing and maturing brains (McClintock, 2015). Arsenic is another soil contaminant that can be common in urban areas, often as a by-product of the burning of coal and petroleum as well as metal smelting and processing (Han et al., 2003). Chronic environmental exposure to arsenic has a number of potential health hazards. Chronic arsenic exposure can cause skin problems – including irritation, rashes, lesions, changes in pigmentation, and gangrene. Arsenic can cause gastrointestinal upset and peripheral neuropathy – which is nerve damage of the hands and feet that leads to numbness and weakness. In pregnant women arsenic exposure is linked to a higher risk of miscarriage and birth defects (Environmental Protection Agency, 2012; National Institute of Neurological Disorders and Stroke, 2016). Arsenic tends to accumulate in the liver and kidneys– where it causes organ damage. Lastly, arsenic is a known carcinogen and is linked cancer, particularly cancers in the lungs, liver, skin, and bladder (Environmental Protection Agency, 2012; Mandal and Suzuki, 2002). Polycyclic Aromatic Hydrocarbons (PAH's) are a class of organic compounds that are widespread in the soils of urban areas as a result of fossil fuel burning (Li et al., 2014a). Sixteen different PAH compounds are classified as priority contaminants by the US Environmental Protection Agency due to their carcinogenic potential (Lorenzi et al., 2011).

The problems of soil contamination and pollution are worldwide problems and cause problems in human health in many regions around the world. In some areas of China, long term use of lead based pesticides have caused many of the agricultural soils to have elevated lead (Tu et al., 2013). In urban China industrial processes such as smelting and coal burning have resulted in problems with soil and road dust contamination in 65% of cities (Yang, 2010). In Canada,

metal smelting has caused nearby soils to have As concentrations 200 times the natural background levels (Mandal and Suzuki, 2002). In Bangladesh soil-plant-human transfer via rice grown in As contaminated soil accounts for more than half of human As uptake (Meharg and Rahman, 2003). Soil contamination from Cd containing P fertilizers has created problems with human Cd exposure in, Australia, Sweden, Taiwan, the Netherlands, and likely many more countries around the world (Bandara et al., 2011; Franz et al., 2008; Lu et al., 2007; Satarug et al., 2003).

Urban populations are increasing rapidly and are expected to double by 2030 (Seto et al., 2012). With rising urban populations and increasing pressure on surrounding agricultural land – there is a growing trend towards urban agriculture to provide a portion of the food consumed in urban areas. Urban agriculture improves food security and provides a source of jobs – in some global cities, such as Dar Es Salaam, Tanzania – the majority of the leafy green produce (up to 90%) that is consumed in the city is also grown in the city (Halloran and Magid, 2013; Taylor and Lovell, 2014). Urban agriculture improves distribution of quality nutrition by providing people in inner-city areas with access to fresh produce that is not always available. Urban agriculture has been practice for millennia – particularly in poorer regions. In the United States, urban agriculture is still fairly new but growing. Currently it is estimated by the U.S. Census that about 6% of U.S. farmland and 14% of US farms are in cities (Lee-Smith, 2010; Rogus and Dimitri, 2015). However in many urban areas, soil contamination is a prevalent issue – 88% of gardens in Boston, USA, were contaminated with lead (Taylor and Lovell, 2014). It is important to work with the soils in urban areas as well as agricultural areas to ensure healthy ecosystem function and the production of healthy food.

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# Chapter 2 - Literature Review

## The Significance of Soil Carbon Storage

Soil carbon storage is significant because of large amount of carbon stored in the soils and the connection of that carbon to the carbon in the atmosphere, which is a major greenhouse gas. Soils store approximately 1550 Gt of organic carbon (compared to the 750 Gt in the atmosphere) (Lal, 2004b). Practices that reduce soil carbon storage (thus converting soil organic carbon into atmospheric $CO_2$) have been a major driver of anthropogenic climate change. Improving practices to improve soil carbon storage in agricultural lands can be an effective mitigation strategy for climate change (Lal, 2004a).

In addition to its significance with regards to anthropogenic climate change, soil carbon influences a whole host of soil biochemical and physical properties. Increased soil carbon is known to improve soil aggregate stability and thus increase water infiltration rate and soil water retention. Soil organic carbon, like soil colloids, has active charge sites where soil cations and anions can be adsorbed. While soil clays tend to have just negatively charged sites, organic carbon also has positively charged sites. Organic carbon also has functional groups that interact with soil minerals and ions in solution. Despite being a small fraction of the soil, organic carbon is significant in soil chemical processes Soil organic carbon, through decomposition, is also a source of nutrients and substrate to plants and soil microorganisms (Brady and Weil, 2010). Since soil organic carbon has a net beneficial impact on soil properties and the capacity of soils to support plant life, practices that increase soil carbon – or at least minimize its loss are favorable from a strictly agronomic perspective. Therefore, improving soil organic carbon

storage to mitigate climate change is a benefit for environmental protection and for increased crop production.

## Drivers of Soil Carbon Storage

### *Temperature*

One of the major driving controls of soil carbon storage is the ambient temperature. Rising temperatures increase the reaction rate of chemical processes; which includes those in and controlled by organisms (Davidson and Janssens, 2006a). Microorganisms, to a general extent, increase their metabolic respiration rate in response to increasing temperatures. This increase in respiration rate leads to a greater demand for carbon compounds as a food source from decomposers; which over time could deplete soil carbon stocks. Theoretically, this would lead to a universal decrease in soil carbon content which would cause further emissions of $CO_2$ from the soils – creating a positive feedback loop to climate change. The positive feedback loop is especially alarming in boreal and tundra regions where permafrost and water-logged conditions lock up large amounts of carbon. Because of this, most climate change models include the effect of rising temperatures on SOC stocks (Schlesinger and Andrews, 2000). While it is generally understood that there will likely be a decrease in SOC following an increase in global temperatures, there are situations where this trend might not occur as predicted. Modeled predictions might not be completely accurate. For example, in some localized areas an increase in primary productivity due to elevated temperatures (or another aspect of climate change) could offset any increases in decomposition. This has been observed in situ in low-SOC mineral soils in very cold climates (i.e. montane soils) (Davidson and Janssens, 2006b; Lavigne et al., 2003). In order to better understand how climate change will affect soil ecosystems – including potential feedback loops and effects on agricultural production – it is important to have a solid

understanding of how the various aspects of soil carbon is affected by climate change and increasing temperatures.

Multiple studies have found that not all soil carbon compounds respond the same way to increasing temperatures (Davidson and Janssens, 2006a; Erhagen et al., 2013a; Schindlbacher et al., 2010). Simpler and more easily consumed compounds are removed from soil rapidly upon increasing temperatures; while more recalcitrant ones often remain (Davidson and Janssens, 2006a). Some compounds are more sensitive to increasing temperatures than others due to the intrinsic nature of their chemical structure; while others are more sensitive to increasing temperature because they are the main substrate for microorganisms. Recalcitrant soil C (i.e. humic substances) tends to be more chemically sensitive to changes in temperature. While recalcitrant C is typically less chemically stable under elevated temperatures, it is not the preferred substrate of most soil microbes. Simpler carbon compounds like carbohydrates and acetate have low intrinsic sensitivity to elevated temperatures but are the preferred food source of soil microbes. As microbial metabolism increases with temperature – there is greater demand for these compounds. Microbes generally prefer to attack simpler and easier to digest compounds (Bosatta and Ågren, 1999; Davidson and Janssens, 2006b; Erhagen et al., 2013b; Leifeld and von Lützow, 2014). The temperature sensitivity of a compound is expressed quantitatively as a Q10 value; which is the amount a reaction rate (in this case, the rate of decomposition) changes with a 10°C increase in ambient temperature. Most estimates assume the average Q10 of SOC to be between 2 and 3 based on general knowledge of organic decomposition reactions; but there is not much work determining Q10 values for SOC experimentally (Davidson and Janssens, 2006c).

Determining a precise Q10 value for each soil in the world would be nearly impossible, but estimating based on local SOC chemistry might be more simplistic but might also be a bit more feasible. It is not possible to measure every aspect of every soil – so some assumptions will always have to be made. One of the goals of advancing research into topics like this is to make the estimates that have to be made more accurate. Q10 values for reactions generally depend on the rate limiting step. Activation energy for rate-liming steps is a major factor in the Q10 of any chemical compound. Compounds with higher activation energies (i.e. aromatic compounds) tend to have higher Q10 values. As ambient temperature increases, so does the energy of the system and as the energy of the system increases, overcoming the activation energy barrier becomes more likely (Bosatta and Ågren, 1999). Wagai (2013) suggested that aromatic and R-O containing compounds have higher activation energies, and thus Q10 values than alkyl carbon compounds (Wagai et al., 2013). In contrast, Erhagen (2013) found that the amount of aromatic compounds, relative to other compounds in a soil tended to have no effect on overall Q10 for SOC (Erhagen et al., 2013a). Interestingly, Leifeld (2014) found that SOC thermal stability was largely unaffected by SOC chemistry and more strongly affected by soil microbial properties and SOC protection (i.e. aggregation). While chemical characteristics, such as organo-mineral complexation affect inherent thermal stability of the compound (in lab conditions), they have much less influence on in-situ stability. Instead the thermal stability when catalyzed by soil enzymes was far more important in determining in-situ decomposition potential. Soil enzymes are dependent on microbial activity and speciation. Microbial enzyme catalysis potential depends on soil pH, microbial speciation, and nutrient content of substrate. Changes in soil pH alter the charges of functional groups on large organic molecules, this is often called pH-dependent charge (Pierzynski et al., 2005). Active sites on enzymes, where reactions take place, are often

charged functional groups – making pH important for enzyme function. Microbial speciation and substrate quality determine the types of enzymes being produced, for example, in a soil with abundant phosphate mineral and minimal organically bound phosphate, microbes will produce very little phosphatase enzymes, which release organically bound phosphates. (Leifeld and von Lützow, 2014).

Since not all soil organic C compounds are as sensitive to increasing temperatures; some studies have found that there is an initial burst of increased $CO_2$ emissions (and thus a decrease in SOC) following warming that then dies down (Giardina and Ryan, 2000; Pold et al., 2015). However, Pold et. al (2015) found that following a warming period the initial increase and then decrease in soil $CO_2$ emissions was followed by another increase in emissions. This is likely tied to changes in microbial populations. In a 20 year in-situ study, soil respiration rate initially increased sharply under elevated temperatures and then decreased to lower equilibrium rate. It is likely that the warmer conditions favored a burst of microbial activity that then reached equilibrium, as the preferred substrate was consumed. However, after 20 years, the soil respiration rate began to increase once more, as soil microbial communities changed and became more capable of consuming the remaining less-preferred substrate. DNA analysis of soil microbes showed that the microbes were adapting and acquiring traits that allowed them to consume a wider variety of SOC compounds (Pold et al., 2015). In an incubation study, Waldrop (2003) traced C-isotopes to determine that indeed the first wave of $CO_2$ release from increased temperatures was from young, labile carbon; and that later releases of $CO_2$ were from the decomposition of older and more recalcitrant C. The overall $CO_2$ emission was found to increase

with increasing temperatures in accordance with standard kinetic models (Waldrop and Firestone, 2004a).

Decomposer microbes are often classified by their substrate preferences. Oligotrophic microorganisms are microorganisms that can survive in low-nutrient environments. They are capable of degrading more recalcitrant C and can survive in a low nutrient environment. They generally grow slower and could be considered as K-selected (slow growing with stable populations). In contrast, copiotrophic microorganisms are microorganisms that can only survive in high nutrient environments. These microbes require a high nutrient environment with plenty of labile C, as they are often not capable of degrading recalcitrant C. Copiotrophs tend to grow much faster and are thought of as being more r-selected (fast growing with unstable, frequently fluctuating populations) (Colwell and Grimes, 2000; Koch, 2001) . At least from these studies, it appears that over time, a warmer environment can lead to a higher prevalence of oligotrophic microorganisms as the labile C is quickly consumed by copiotrophic microorganisms.

Another in-situ warming experiment found that warming soils only increased $CO_2$ emissions to a certain extent. Temperatures up to +5°C warmer than ambient were found to greatly increase soil $CO_2$ emissions; however, past +5°C above ambient the $CO_2$ emission rate did not continue to increase and in fact, began to decrease. This could be due to heat sensitivity of microbes or of their substrate (McHale et al., 1998). Microbes increase their respiration rate as temperature increases, however given too much of an increase their cellular proteins denature and they die. The minimum, maximum, and optimal temperatures for growth vary by species, but for most temperate soil microbes, the maximum is around 40 ° C (Eddleman, 1998).

Soil microorganisms are not the only part of the soil ecosystem to be affected by rising temperatures. Plants are affected as well, and if they increase production at a rate high enough to offset the decomposition rate, then the net change would be zero. This is particularly the case in colder climates (i.e.; Polar Regions) where low temperatures are a major limiting factor for plant growth. Even in already temperate climates, some warming can stimulate increased plant biomass production (Garten et al., 2009). However, it is unknown if the increase in plant growth would be enough to offset emissions, especially in areas with very large reserves of carbon stored under permafrost or as peat (Lavigne et al., 2003).

There is a global overall trend for higher SOC values to be found in areas with lower temperatures. In North America, it is found that SOC and soil carbon turnover time increases with increasing latitude (Garten, 2011). This can be explained both by cooler climates and greater tendency for water-logged conditions. Cooler climates have less ET and thus more soil water. Waterlogging can cause SOC to build up by inhibiting decomposition via low oxygenation.

Increasing soil temperatures is likely to increase soil SOC loss and thus soil $CO_2$ emission; however it is still possible to protect soil carbon from degradation. Even in warm climates, soils can store significant amounts of carbon given the right conditions that keep it protected from microbial decomposition. Knowing what affects the temperature sensitivity of soil organic carbon could lead to practices that protect soil carbon from elevated temperatures. A study of in-situ field data and climate change modeling on barley fields in Spain found that

management practices could reduce the impact of elevated temperatures on SOC. While SOC declined under all climate change scenarios – this decline was less significant in systems with No-till, residue cover, and no fallow (Alvaro-Fuentes and Paustian, 2011). A similar study in Italy found the lowest SOC under anticipated climate change with conventional tillage and the most SOC under ambient climate with no-till and a wheat-corn rotation. Under a warmer climate the wheat-sunflower rotation under no-till was the only treatment to have increased SOC compared to ambient climate. Conventional tillage and a warmer climate amplified each other resulting in large losses of soil carbon. No-till reduced the effects of a warmer climate on SOC and selecting a crop that performs well under altered climate (like sunflower) increases residue C inputs to the soil (Farina et al., 2011).

### *Moisture*

The other major climatic variable that affects soil carbon storage and soil greenhouse gas balance is soil water content. Microbes tend to be most active at water contents around 50 – 60 % of pore volume filled with water (Uhlírová et al., 2005). Microbes, like all organisms, need water to survive, and thus at very low water contents, they will not be active. Additionally, at lower water contents, plant production will also be limited, resulting in low SOC (Tissue et al., 2004). Conversely, at high water contents, the soil will have little to no available oxygen. Without available oxygen, organic matter is broken down anaerobically, which is a much slower process than aerobic decomposition (Soil Survey Staff, 2015). While anaerobic soil microorganisms breakdown SOC more slowly, they also release more potent greenhouse gases,

$N_2O$ and $CH_4$ (298 and 28 - 36 times more powerful than $CO_2$, respectively) (United States Environmental Protection Agency, 2015).

An in-situ study of altered temperature and moisture regimes found that moisture conditions were actually more important than ambient carbon in determining C flux rates in a Tennessee soil. Drought conditions decreased C flux as lack of water availability decreased microbial decomposition (Garten et al., 2009). Another in-situ study on pine plantations in Georgia found an interaction between temperature and moisture on soil C flux and Q10. Drier conditions resulted in decreased Q10 and decreased C flux only at higher temperatures. Elevated temperatures increase microbial demand for C substrates but they also increase microbial demand for other resources such as water and nutrients. Soil microbes can only take advantage of warmer conditions if their other needs (i.e. water) are met (ArchMiller and Samuelson, 2016). When water (or other resources required for growth) is limited then growth will be determined by the resource that is the most limiting. This is the ecological concept of a limiting factor; where one condition or required resource limits organism growth (Edwards and Edwards, 2011).

Soil water content depends primarily on the precipitation but also on soil texture / mineralogy, landscape position, and evapotranspiration. Since soil texture, mineralogy, and landscape position are not changeable through management and are not going to be altered via climate change, they will be discussed very briefly. With precipitation, the amount of rain is not the only important variable, timing and intensity are as well. Generally more major rain events (>5mm) correlates with more rain overall (Tissue et al., 2004). Obviously, areas with more rain will tend to have wetter soils, but what time during the year the rain arrives does seriously affect

the potential for plant and microbial growth. The intensity of the rainfall affects how much of a given rain event will actually be absorbed by a soil.

Climate change is predicted to alter precipitation patterns in a number of ways. Due to increased temperatures, the intensity and magnitude of rain events is expected to increase while the average amount of time between rain events is expected to increase as well (Cole and Hansen, 2013). The net result would likely be the same amount of water but in less frequent rain events (Cole and Hansen, 2013). This change would likely result in drier soils; especially in soils with a low water holding capacity. Larger and more intense rain events often have lower percentages of the rainfall actually absorbed by the soil; as the rain falls faster than the soil can absorb it; which when coupled with longer time intervals between rainfall; would likely result in drier soils.

Soil texture, mineralogy and landscape position affect the fate of water added to a soil system. Generally less sloped positions that are lower in the landscape will be wetter as gravity carries rainwater down. Finer textured soils will hold more water as clay minerals adsorb water much more readily than silts or sands. While these effects are significant on soil processes, they are not going to be altered due to climate change and (usually) cannot be altered via management practices.

Evapotranspiration is the main mechanism that removes water from the soil system. Evapotranspiration depends greatly on weather conditions. Warm, dry, and windy weather favors higher ET, while cool, humid, and calm conditions favor low ET. These are likely to be affected

by climate change. There is some suggestion that climate change will result in higher ET in many areas of the world. Elevated temperatures associated with climate change for sure increase ET but what is uncertain is the availability of water and other factors like cloud cover and wind. Changes in ET from climate change are non-uniform and have considerable (31%) uncertainty to them (Lofgren et al., 2013; Nam et al., 2015).

From the literature there is a general trend on how soil decomposition and C-flux change with soil moisture. Soil decomposition rate will rise with increasing moisture content until the soil becomes anoxic; upon which decomposition will decrease. However, the gases released from anaerobic decomposition are more powerful greenhouse gases than $CO_2$. Ultimately is important to think about how climate change will alter soil moisture regimes when thinking about soil carbon sequestration.

### *Microbial Speciation*

Breakdown of soil organic carbon is largely driven by microbial processes. While many species and taxa utilize soil organic carbon, they do not utilize it in the same manner. Boyero et, al (2014) found that in a tropical forest site, the decomposer species distribution and residue composition were just as important in ambient temperatures in determining decomposition rate. Of particular importance were larger macro-organisms that shred residue into smaller pieces; allowing for greater breakdown (Boyero et al., 2014). Different soils will have different decomposer assemblages due to location, climate, and soil type.

Changing climate is expected to change microbe speciation; which could have consequences for soil carbon storage. A number of studies have found that altered soil temperature regimes alters the composition and functional diversity of microbial communities.

In an in-situ pasture study, Gray et al. (2011) found that a number of microbe groups respond to climate change with changes in relative abundance. When temperatures were increased, the relative abundance of gram positive bacteria compared to gram negative bacteria was found to increase as well. Like gram positive bacteria, Actinobacteria were found to increase in population under warmer conditions. Firmicutes biomarkers were more abundant in warmer and drier soils. In contrast to these, AM Fungi were found to be detrimentally affected by increased temperature (Gray et al., 2011). In a similar in-situ study in a deciduous forest, Pold, et al. (2015) found that some species were more sensitive to changing temperatures than others. Acidobacteria, Rhizobiales, Xanthomonadales, and some Actinobacteria were found in greater abundance in plots subjected to warmer conditions, while Alpha-proteobacteria, some Actinobacteria, and fungi overall were negatively impacted by warmer conditions. Additionally, an assessment of functional genes found that a warmer climate favored oligotrophic (capable of surviving in low-nutrient environments and on poor quality substrate) species over copiotrophic (requiring higher amounts of nutrients and higher quality substrate) ones (Pold et al., 2015). In an incubation of alpine tundra soil study by Wu et al (2015), it was found that increasing temperatures increased the prevalence of Actinobacteria and Firmicutes while decreasing the prevalence of Bacteriodetes and Delta-Proteobacteria. This increase in temperature was also accompanied by an increase in biodiversity up until 40 °C; at which point there was a sharp decline. The system shifted from using more labile C to more recalcitrant C with increasing

temperatures. The shift in bacterial community was smooth up to 30°C - 40°C, above which, changes became more abrupt (Wu et al., 2015). There are very clear changes in soil microbial communities under altered climate and how these changes affect soil function depends on the affected microbial group. To understand that, it is important to first go over how these microbial groups function in a soil environment.

These changes in functional microbial community structure could alter soil carbon storage/decomposition patterns. A field study in southern China found that the ratio of gram positive to gram negative bacteria was positively correlated with soil C respiration rate; while the inverse was true for the ratio of fungi to bacteria (Wang et al., 2013). Traditionally gram positive bacteria and fungi are thought of as being oligotrophic and K-selected and thus consuming more recalcitrant carbon compounds. Gram positive bacteria are also thought of as being more resistant to environmental stress such as altered temperature regime and altered moisture status. Meanwhile, gram negative bacteria are often thought of as copiotrophs and as being more r-selected and thus preferring more labile C (Fierer et al., 2007). Despite this, the study by Wang, et al. (2013) found inverse trends with gram positive bacteria and the fungi – so it's important to also consider that both of these are very broad groups where assumptions might not always hold true (Wang et al., 2013).

There are many different groups of gram positive bacteria in the soil environment, all with differing roles. Actinobacteria are a group of colony forming gram positive bacteria that tend to resemble fungi. Being gram positive, Actinobacteria are resistant to warmer, drier conditions. Actinobacteria are important in forming soil humic substances – which is an

important mechanism of soil carbon stabilization (Encyclopedia of Life, 2007). The study by Wang et al (2013) also found that actinomycete biomass was negatively correlated with soil C respiration. However, another study by Fierer et al. (2007) found no correlation between actinomycetes and soil respiration. Firmicutes are another group of gram positive bacteria, however current work suggests there is little correlation between Firmicutes abundance and soil C mineralization rates (Fierer et al., 2007).

Alpha-proteobacteria are a large and diverse group of mostly gram negative bacteria. The decline of Alpha-proteobacteria found by Pold, et al (2015) fits with the trend of gram negative bacteria being more sensitive to environmental stress such as elevated temperatures. However, like with the Firmicutes group little correlation was found between Alpha-proteobacteria and C mineralization rate (Fierer et al., 2007). Pold et al. (2015) found that both Rhizobiales and Xanthomonadales increased in abundance under elevated temperatures, however neither of these groups are decomposers so they have no direct effect on C cycling. However, there could be indirect effects as Rhizobiales are legume associated N-fixers (which elevate plant growth and decrease plant C:N ratio), and Xanthomonadales are parasitic and detrimental to plant growth. Acidobacteria are another group of gram negative bacteria that are common in soil environments – particularly in acidic soils. Acidobacteria have been shown to be inversely correlated with C respiration rate (Fierer et al., 2007). Bacteriodetes are another common group of gram negative bacteria that Pold noted a change in the abundance of. Unlike Acidobacteria, Bacteriodetes are positively correlated with C mineralization rates (Fierer et al., 2007; Pold et al., 2015).

Changes in microbial community structure can have major impacts on soil carbon storage capacity. A study on the Konza Prairie, Manhattan KS, found that the application of a fungicide, with no-other alterations of the soil, significantly reduced soil carbon storage and soil structural stability – indicating that changes in the microbial community can have a profound impact on soil functioning (Wilson et al., 2009a). Another survey of soil organic carbon found that soils with low OM tended to have low fungal biomarkers (Yevdokimov et al., 2013).

### *Substrate Quality and Availability*

Substrate quality is a way of describing the chemistry of C compounds based on how easily they can be broken down by organisms. The chemistry and placement of organic carbon (i.e. incorporated versus on the surface) substrate/residue due to plant species can be just as important as climate in determining soil C stocks and storage patterns (Boyero et al., 2014). Bosatta and Ågren (1999) defined substrate quality as the inverse of the number of steps required to break down a compound and then added a modifier based on the activation energy of the rate limiting step. "High quality" substrate is broken down with few steps and minimal activation energy and is a preferred food source for soil organisms. Simple sugars, cellulose, proteins and compounds like lactate and acetate would fall under the "High Quality" category. "Low Quality" refers to substrates with many breakdown steps and high activation energies. These can be older more recalcitrant humic substances or they can be more recent residue compounds such as lignin or chitin (Bosatta and Ågren, 1999). Low quality residues tend to be much more resistant to microbial degradation, however modeling work has shown that under increased temperatures they become more vulnerable (Bosatta and Ågren, 1999). Other authors have used the C:N ratio

as a means of defining SOC quality (Bonanomi et al., 2014; Bossuyt et al., 2001; Turner and Jones, 2002; Vesterdal et al., 2012). Generally low C:N ratios tend to break down quicker than high C:N ratios because adequate nitrogen content allows microbial populations to greatly multiply. However, some very recalcitrant humic compounds have low C:N ratios and many high C:N ratio compounds can degrade quickly if there is enough free nitrogen in the soil – i.e. from fertilizers. Clearly indicating that the C:N ratio is not always an accurate assessment of C quality.

Substrate availability refers to how easily soil microorganisms can access and degrade SOC. Substrates can be unavailable to microbes due to their composition/chemistry – they are difficult for microbes to break down. They can also be unavailable due to physical protection within soil aggregates or due to organo-metal complexation. All mechanisms combined, the reduction of availability of SOC will reduce $CO_2$ emissions and increase SOC storage. It is the decline in substrate availability that is thought to be the main mechanism behind the often observed decline in increasing decomposition rate over time with soils subjected to increased temperatures. In the classical decomposition sequence, when a plant or cell dies the carbon compounds that are metabolized by microorganisms first are largely simple and often considered labile C (i.e. sugars). With time compounds consumed are more recalcitrant and the amount of available energy declines. The majority of the C loss as $CO_2$ that occurs will happen in the first year or so after decomposition begins. When a soil environment is warmed – the decomposition rate increases but the general sequence of C attack remains the same – just occurring faster. When microbial growth is faster due to higher temperatures, the microbial population can easily consume all of the labile C before more is added to the soil – resulting in a die-off and a decrease

in $CO_2$ flux. (Giardina and Ryan, 2000; Hartley et al., 2007; Koelbl et al., 2006; Leifeld and von Lützow, 2014; Pold et al., 2015; Swift, 2001; Xu et al., 2012).

### *Land Management Practices*

On land used by humans for food production or building construction or other purposes, the fate of the SOC stocks often depend on how the humans use that land. It is estimated the up to 80% of cropland and about 75 – 80% of grazing land globally has some degree of degradation – mostly due to tillage and overgrazing (Burgess and Pimentel, 2013). This degradation is strongly associated with a loss of soil organic carbon. A survey of SOC stocks in the UK since 1978 has found that soils in the UK have been losing 0.6% of their initial C content per year – with a significant chunk of this likely due to changes in land use and management (and the remainder due to climate change (Bellamy et al., 2005). Another study in New Zealand of pasture soils found that over the last 20 years, there has been an average loss of 2.1 kg C / $m^2$ area attributed to soil respiration. While the average was a net loss, some pastures gained C but this was not correlated with soil order or grazing species (sheep vs. cattle). Which suggests that other management decisions such as stocking density, forage species, fertilization and irrigation practices has a greater impact on soil C (Baisden et al., 2007). After long periods of cultivation or intensive grazing, SOC carbon stocks can drop dramatically. Taking native ecosystems and converting them to agriculture or grazing lands is generally associated with a loss of soil carbon from the disturbance. In a meta-analysis of 385 studies, Don et al (2011) found that the average loss of soil carbon after 5 years for tropical forests converted to cropland was between 25 and 30% depending on the type of crop (da Rocha et al., 2014; Don et al., 2011). Another meta-analysis of 74 studies across both tropical and temperate climates found an average soil carbon

loss of 50% after 30 to 50 years of conversion of undisturbed systems to cropland. In this study the majority of the carbon loss had occurred by 30 years of cultivation (Guo and Gifford, 2002). The carbon loss reported by Guo and Gifford (2002) is much higher than that reported by Don et al. (2011), however, it is important to keep in mind that the latter study looked at sites where crop production had been in place for 5 or more years and the former focused on studies of sites 30 – 50 years old. In a meta-analysis of 95 studies (with 322 sampling sites) in IPCC defined temperate climates, Poleplau et al (2011) examined the effect of various land use-conversions on soil carbon stocks. For the conversion of native grassland to cropland there was an average loss of 36%. For forests the loss was 32%. Most of the site to site variation in both cases was explained by annual temperature (with warmer sites have greater soil carbon losses) and soil texture (with higher clay content sites having less soil carbon loss) (Poeplau et al., 2011). The losses reported by Poeplau et al. (2011), while from older sites, were less than reported by Gao and Gifford (2002), likely because they were restricted to only temperate climates and tropical systems typically have greater losses of soil carbon upon conversion to cropland (Lal, 2004b).

There are a number of agricultural and other land management practices that are known to have negative impacts on soil SOC stocks. One of these practices is conventional soil tillage. Tillage decreases residue cover and increases aeration in the surface horizon – both of which allow for water from the soil to evaporate more easily, while the compaction associated with tillage typically decreases infiltration. As a result no-till soils tend to have greater water infiltration and storage than their conventional counterparts (Colozzi et al., 2004; Presley et al., 2013; Rusu et al., 2013). The changes in residue cover and water storage properties affects how the soil temperature changes with the air temperature. The cover and higher water content cause

no-till soils to heat and cool more slowly than tilled soils; effectively insulating soil from very cold weather and shading it from extreme heat. In humid tropical climates the shading can be important in protecting seedlings and reducing loss of SOC, conversely in very cold climates the slow heating can be a bit of a problem (Colozzi et al., 2004; He et al., 2010; Iamaguti et al., 2015). The incorporation of the residue allows for more contact between microbes and residue; causing a more complete and more rapid decomposition. This creates an environment where stable SOC is exposed to decomposition – and over time this can reduce soil SOC stocks (Colozzi et al., 2004; Farina et al., 2011; Lal, 2004b; Schlesinger and Andrews, 2000). Tilling the soil promotes the destruction of soil aggregates which create a less diverse soil habitat and allows for a soil microbial community that is less efficient at C sequestration (Berns et al., 2015; Colozzi et al., 2004).

The reduction of SOC loss caused by tillage practices can be reversed by adapting no-till technologies and practices. No-till increases SOC storage and soil microbial population (Alvaro-Fuentes and Paustian, 2011; Colozzi et al., 2004; Farina et al., 2011; Lal, 2004b; Presley et al., 2013; Rice et al., 2009; Rusu et al., 2013; Swedrzynska, 2013; West and Post, 2002). No-till and reduced tillage practices keep the soil in a condition that is more similar to the one found in native ecosystems – which allows for greater biodiversity of soil microbes. No-till promotes better aggregate structure, which protects SOC and provides a more suitable microbial habitat (Berns et al., 2015). In particular, fungi and oligotrophic bacteria are more abundant in less disturbed systems – and it is precisely these organisms that are important for the maintenance of soil aggregate structure and SOC storage (Bossuyt et al., 2001; Rice et al., 2009; Swedrzynska, 2013). Converting to no-till can have significant impacts on SOC stocks. A meta-analysis across

67 global studies in 2002 found that, on average, a conversion from conventional tillage to no-till sequestered 48 g $C/m^2/yr$ (Soil Science Society, 2002).

No-till and reduced tillage systems, while ideal on average are not ideal in every corner of the globe. Certain environments have unique features that require different management practices for maintaining soil SOC stocks and having sustainable yields. For example, no-till did not improve SOC stocks in an Aridisol prone to crusting. In contrast, conventional tillage with regular applications of organic matter resulted in better SOC sequestration and better agronomic functioning (Gutiérrez-Castorena et al., 2015). Another long term study, conducted in southern Finland, compared conventional tillage to a reduced tillage system and found greater SOC loss under the reduced tillage compared to the conventional tillage (Singh et al., 2015). Similar trends have been found in other boreal peat soils (Elder and Lal, 2008; Regina and Alakukku, 2010).

Another practice that can have significant impacts on soil carbon storage capacity is the cropping system. Monocrops of soybean (and other legumes with low residue production) generally lead to decreased soil carbon because their residues breakdown quickly due to the low lignin content and high nitrogen content. This effect can be reduced by planting these crops as part of a rotation cycle (Colozzi et al., 2004). Use of cover crops and crop rotations can enhance the benefits of no-till practices, and both combined is better than either practice alone (Soil Science Society, 2002).

Practices that improve SOC such as no-till, cover crops, composting, and agroforestry management are not only important for combating climate change, but they also can improve soil productivity and quality in ways that improve yields sustainably (Lal, 2004b). There is much to be gained in terms of carbon sequestration and productivity by working to restore degraded soils.

Restoring previously degraded soils allows for increased yields without cultivating native ecosystems (da Rocha et al., 2014).

## Soil Contamination Issues

Soil contamination with industrial by-products, toxic heavy elements, and a number of other anthropogenic pollutants is a widespread problem. Major concerns are potentially toxic heavy elements (lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd)), and organic contaminants such as polycyclic aromatic hydrocarbons (PAH's), antibiotics, and industrial solvents (Brown et al., 2016; Chaney and Ryan, 1994; Chen et al., 2014; Chen et al., 2007; Kachenko and Singh, 2006; Lourenco et al., 2010; Taylor and Lovell, 2014; Wortman and Lovell, 2013; Yang, 2010). This type of contamination is common in urban areas and is in conflict with a growing trend of urban agriculture. Urban agriculture increases food security and provides greenspace, jobs, and fresh produce to urban communities, however without knowledge of urban soil contamination there can be some serious risks (Halloran and Magid, 2013; Lee-Smith, 2010; Smith, 2009; Wortman and Lovell, 2013).

### *Scope and Risks*

In order to understand soil contamination issues, it is important to first know the scope of the problem and the risks associated with soil contamination. Each soil contaminant has a different source, scope, and risks associated with it.

#### *Lead*

Soil contamination with Pb is a widespread problem; and is one of the most common soil issues in urban and industrial areas with associated risks to human health. Almost half of the

U.S. EPA's Superfund sites have Pb contamination as one of the causes for concern (Brown et al., 2016; Hettiarachchi and Pierzynski, 2004; McBride et al., 2014). As an example of how pervasive Pb can be, a study in Boston, USA, found that 88% of gardens in Boston had Pb contamination – with soil Pb content above EPA residential yard soil limit of 400 ppm (Clark et al., 2006). This high level of soil lead in the city is not unique to Boston. Lead and other toxic metals tend to be much more common in the soil of urban areas than of their surrounding countryside, particularly in older and more industrial urban regions, which are often poorer parts of cities (Henry et al., 2015; McClintock, 2015). A survey of cities across the United States found that 52% of lots with buildings constructed before 1978 had elevated Pb in the nearby soil (McClintock, 2015). Much of this contamination is due to historical use of leaded paint and leaded gasoline that released Pb containing dust. That dust was then deposited on the nearby ground and incorporated into the soil (Chaney and Ryan, 1994; McClintock, 2015; Yang, 2010). Another common source of Pb contamination is from mining activities and spills, as well as from industrial processing of mined material, such as smelting (Kachenko and Singh, 2006; Perez-de-Mora et al., 2006). While Pb contamination is more prevalent in urban areas, rural soils can have Pb issues as well. Use of organic fertilizers that contain Pb (i.e. manures, composted biosolids, sludge, municipal composts) as well as use of Pb based pesticides can also introduce lead to agricultural (Smith, 2009; Tu et al., 2013). Even when the heavy element concentration of the soil is below the legal "safe" limit, repeated applications of the materials with high concentrations of potentially toxic elements or compounds to the same field can elevate the level of that element beyond safety limits. Lead is persistent and does not degrade over time and can thus easily build up in the soil (Smith, 2009; Tu et al., 2013).

Lead contamination is not restricted to the U.S., it is a global problem. A survey of soils in various cities across China found an average Pb content of about 65% (Yang, 2010). Use of Pb based pesticides has resulted in elevated Pb in rice fields, with 2.5 to 3 times the Pb of surrounding uncultivated soils (Tu et al., 2013). A survey of Pb in garden soils in Australia found 100% of gardens in the smelting region, Boolaroo, to exceed national safety limits. While this was an extreme case, about 30% of garden sites in Sydney and Port Kembla had elevated Pb (Kachenko and Singh, 2006). Soil Pb contamination in urban areas has been identified as a problem in Brazil and Europe (Cruz et al., 2016; Levonmäki et al., 2006a; Lourenco et al., 2010; Perez-de-Mora et al., 2006).

Lead exposure poses human health risks which are particularly significant risk for children. Young children (particularly kids under 3 years of age) absorb 4 – 5 times as much Pb from their environment as adults (Chaney and Ryan, 1994; Henry et al., 2015). Lead has serious impacts brain development in young children – which can cause lifelong cognitive problems – even at low bloodstream concentrations (Hettiarachchi and Pierzynski, 2004; McClintock, 2015). Lead exposure is responsible for approximately 600,000 cases of intellectual disability annually (World Health Organization, 2016). Blood concentrations as low as 5.0 ppm have been linked to learning difficulties and decreased IQ (Henry et al., 2015; Hettiarachchi and Pierzynski, 2004; World Health Organization, 2016). Children exposed to Pb are also likely to develop behavioral problems such as shortened attention span and antisocial behaviors. Due to the decreased IQ and behavioral problems, Pb exposed children are less likely to graduate high school or college (McClintock, 2015; World Health Organization, 2016). Much higher levels of blood Pb are associated with anemia, kidney damage, and high blood pressure, in addition to the cognitive issues (Hettiarachchi and Pierzynski, 2004; World Health Organization, 2016). Regulations on

use of Pb in products like gasoline and paint has significantly decreased the incidence of blood Pb levels high enough to cause systemic problems – however cognitive Pb associated problems are still a public concern. The current CDC "safe limit" for blood Pb in children is 5.0 ppm, above which there is a significantly decreased IQ (Henry et al., 2015; McClintock, 2015). A significant amount of child Pb poisoning cases (as reported to the Center for Disease Control), 40 – 45%, is thought to come from contaminated soil or dust in industrial and urban areas (Mielke et al., 1983).

Lead contamination poses environmental risks as well. Elevated soil Pb is correlated with a decrease in microbial biomass and microbial activity (Gai et al., 2011; Liao et al., 2007; Wang et al., 2010; Wang et al., 2007). Lead has also been shown to inhibit or decrease the activity of soil microbial enzymes (Hinojosa, 2004; Makoi and Ndakidemi, 2008). Besides a decline in microbes over all, soil Pb alters microbial community composition. Multiple studies have reported that bacteria, particularly actinomycetes and gram positive bacteria are harmed by available soil Pb more than other microbial groups (Abaye et al., 2005; Liao et al., 2007; Wang et al., 2010; Wang et al., 2007; Yang, 2010). Meanwhile, there is usually a minimal effect of Pb on soil fungi and gram negative bacteria – sometimes even an increase in population following Pb exposure (Abaye et al., 2005; Liao et al., 2007; Wang et al., 2010; Wang et al., 2007; Yang, 2010). Given a particular Pb concentration and bioavailability, some microbial groups are more affected than others. This difference in response to Pb from different microbial groups is likely due to differences in cell walls. Gram positive bacteria (including actinomycetes) have very simple cell walls that bind easily to metals, like Pb, while gram negative bacteria and fungi have more complex cell walls that likely have better mechanisms for protecting the cell from harmful metals (Abaye et al., 2005; Wase, 1997). This change of the soil microbial community can affect

nutrient cycling pathways and the ability of the soil to support plant and animal life. A study of contaminated sediments in Brazil found that areas with elevated lead had lower populations and higher mortality rates of invertebrates (Cruz et al., 2016). Other studies have found that soil lead is detrimental to a variety of soil invertebrates such as mites, nematodes, and springtails (Bongers et al., 2004; Kolesnikov et al., 2015). Like with soil microbes, soil invertebrates are important for the soil ecosystem and for the breakdown of residues. Removal or reduction of soil invertebrates can slow or impair soil processes. Ecosystem damage from lead contamination is another important thing to consider when working in lead contaminated soils.

### *Arsenic*

Arsenic (As), like Pb, is a naturally occurring element that is elevated in some regions due to a number of human activities. Arsenic is has been used in a number of products; pesticides, herbicides, wood treatment, wood preservatives, and animal feed additives all of which can contaminate soil (Mandal and Suzuki, 2002; Wang and Mulligan, 2006a). Additionally, as with Pb, As can be introduced into soils via mining and industrial processing of mined material, like smelting and foundries (Han et al., 2003; Mandal and Suzuki, 2002; Perez-de-Mora et al., 2006). Soils can also become elevated in As when high As groundwater is used for irrigation (Chaney and Ryan, 1994; Gillispie et al., 2015; Zhao et al., 2010a). Most groundwater As is from As containing rock formations. When a well is drilled into an As containing rock formation, a number of biochemical changes can occur, which leads to the dissolution of the As in the rock. Most of the As minerals in groundwater formations are Fe or S containing (ex. Arsenopyrite and Scorodite). Anaerobic bacteria attack on the exposed surfaces of the rock for use as an electron acceptor, this process dissolves the rock and releases As ions (Garcia-Sanchez et al., 2010a; Zhao et al., 2010a). Further, most As in these groundwater

formations is the in the $As^{+3}$ form, which is more bioavailable and more toxic than $As^{+5}$ (Gillispie et al., 2015; Mandal and Suzuki, 2002; Wang and Mulligan, 2006a; Zhao et al., 2010a). When water from these As rock formations is used to irrigate crops and flood rice paddies, As will build up in the soil. Over time this can lead to highly contaminated soils (Gillispie et al., 2015; Meharg and Rahman, 2003; Zhao et al., 2010a).

Arsenic in urban soils is a potential hazard to human health. Arsenic is a known human carcinogen, chronic exposure to arsenic increases risks of developing liver, bladder, skin, and lung cancers (caused by the inhalation of As containing dust), and is classified by the US EPA as a "Group A – human carcinogen (Environmental Protection Agency, 2012; Mandal and Suzuki, 2002; United States Environmental Protection Agency, 2004). Other health problems caused by As exposure are skin irritation and damage, gastrointestinal problems, kidney and liver disease, nerve damage, and increased risk of miscarriage and birth defects (Environmental Protection Agency, 2012; Mandal and Suzuki, 2002; National Institute of Neurological Disorders and Stroke, 2016). Since, As is linked to many serious human health problems, soil As contamination is a problem worldwide. Contamination of rice field soils from irrigation water has led to elevated arsenic in the harvested rice. Due to the nature of rice production, rice is more susceptible to As uptake than other major crops – something that will be discussed in more detail the following sections (Chaney and Ryan, 1994; Gillispie et al., 2015; Meharg and Rahman, 2003; Zhao et al., 2010a).

Arsenic contamination can damage ecological functioning as well. A prime example of this is the Aznalcollar Disaster, an acid mine waste spill in Spain, contaminated thousands of acres with primarily with As, as well as lead, copper, and zinc. The affected soils have been shown to have decreased soil microbial population, biodiversity, and functional diversity

(Hinojosa, 2004; Perez-de-Mora et al., 2006). A diverse and stable microbial population is vital for nutrient cycling, which is something that growing plants are dependent on – when soil pollution harms the soil microbial community – the ability of that soil to support plant growth is seriously impaired. Multiple studies have found that soil contaminated with arsenic, as with many other heavy elements, often have a decrease in the biodiversity and activity of soil microorganisms (Hinojosa, 2004; Wang et al., 2010). Arsenic does not affect all microbial groups equally. Fungi have greater resistance to arsenic than bacteria (Kaloyanova, 2007; Oliveira and Pampulha, 2006; Wang et al., 2010). Bacteria involved with N cycling, nitrifying bacteria and nitrogen fixing bacteria, are particularly sensitive to As when compared to other bacterial groups (Kaloyanova, 2007; Oliveira and Pampulha, 2006; Yeates et al., 1994). The decline in bacteria that are important for N-cycling can have detrimental effects on plants that depend on these bacteria. Plants are also harmfully affected by As contamination – often having slower growth rates and lower biomass (Claassens, 2006; Szakova et al., 2007; Yeates et al., 1994). Arsenic soil contamination was found to have detrimental effects on earthworm populations through increased mortality and lower average body weight in contaminated soils (Sizmur et al., 2011) A similar study also found As to be detrimental to earthworms as well as nematodes and roundworms (Yeates et al., 1994).

***Cadmium***

Cadmium (Cd) is a widespread urban and agricultural soil pollutant. In urban areas much of the Cd contamination is from a variety of industrial activities: plastic manufacturing and incineration, battery manufacture and disposal, dye/pigment production, and processing (mining and smelting) of zinc and copper metal ores (Chaney and Ryan, 1994; Franz et al., 2008; Kaji, 2012; Lu et al., 2007). In rural areas, most of the Cd contamination was from the use of low-

grade rock phosphate based fertilizers that were mined from rock formations that contained Cd minerals (Bandara et al., 2011; Chaney and Ryan, 1994; Chaney, 2015; Chen et al., 2007; Satarug et al., 2003). Cadmium is a particular concern because it is often more soluble and thus more bioavailable than other potentially toxic metals (Sauve and Hendershot, 1997a; Weber and Hrynczuk, 2000). Currently, Cd soil contamination is a common urban soil problem in regions where metal ore smelting was a major economic activity. Surveys of lakeside and riverbed sediments across the U.S. have found a trend of increasing Cd concentrations in urban areas (Mahler et al., 2006; Rice, 1999). Changes in regulations have resulted in significant reductions on the deposition of new Cd into urban soils (Mahler et al., 2006). Cadmium contamination from mining and smelting activities has become very common problem in urban China (Chen et al., 2014; Qiu et al., 2011; Yang, 2010; Zhao et al., 2016). In Europe, metal ore smelting (particularly Zn) and use of high-Cd P fertilizers has led to fairly widespread Cd contamination. One study found that up to 74% of Cd in rural European soils was thought to come from use of Cd containing P fertilizers (Chen et al., 2007; Franz et al., 2008). In rural areas of Sri Lanka, New Zealand and Australia, use of Cd-containing rock phosphate in P-fertilizers, has led to widespread areas of Cd contamination (Loganathan et al., 2003; Oliver et al., 1998; Satarug et al., 2003). A study of urban areas in Australia found Cd contamination to be quite common. One city, Boolaroo, in New South Wales, had 50% of sampled sites with Cd contents above national limits for soil (Kachenko and Singh, 2006).

Cadmium causes harm to human health. Ingested Cd replaces Zn (an essential micronutrient) in biological pathways and when consumed leads to chronic kidney disease, osteoporosis and gastrointestinal issues. The first well documented case of Cd toxicity in humans was the outbreak of a disease called Itai-Itai in Japan. Zinc ore smelting and disposal of waste

minerals in rivers used for irrigation led to soils and waterways around the mine to become contaminated with Cd. In the 1930's a number of people in those areas began experiencing the symptoms of Cd toxicity, which was then named Itai-Itai. It was not until 1948 that it was established that the illness was caused by Cd toxicity from the contaminated soils and water (Kaji, 2012). On a related note, there is an ongoing epidemic of kidney disease of unknown origin (CKDu) in northern and central Sri Lanka as well as parts of India and Central America. . One of the suspected causative factors is the of contaminated P fertilizer on rice fields. However there is still not a clear causative agent for this disease. While Cd soil contamination (drinking water has been shown to be low in Cd) is common throughout the affected region – there are unaffected regions that are also using high Cd fertilizers. Other suspected causative agents are pesticides, fluoride, or a genetic factor (Bandara et al., 2011; Johnson et al., 2012; Noble et al., 2014).

Due to the widespread nature of Cd contamination and the high bioavailability of Cd, consumption of food grown on Cd rich soils is an important pathway for human Cd exposure. According to the World Health Organization food is the most common route of Cd exposure in non-smokers with rice, organ meats (i.e. liver, kidney), and seafood being responsible for most of the food related Cd exposure (World Health Organization, 2010b). In a study of Cd contaminated soil on grazing land in the Netherlands, the organ meats (i.e. liver) had Cd content above EU regulation for meat for human consumption (Franz et al., 2008). Cadmium accumulates in the liver and kidneys of animals that consume it and when cattle or sheep graze on high-Cd soil – the Cd is transferred from the soil to the grass to the animal and ultimately to the human that eats that animal (World Health Organization, 2010b). A study of Cd intake in

Sweden found that around half of all human Cd exposure was from food with the remaining half from cigarette smoke (Satarug et al., 2003).

In addition to damage to human health, Cd can impact ecosystems and wildlife. Erosion of Cd-contaminated soil has caused harm to fish and aquatic crustaceans (Bandara et al., 2011; Satarug et al., 2003). A study on tubifid sludge worms found that Cd contamination decreased their population and reproductive success (Delmotte et al., 2007). A study on invertebrate populations in Cd contaminated soils found that annelid worms (i.e. earthworms) and terrestrial crustaceans (i.e. pill bugs and wood lice) experienced significant declines with soil Cd contamination (Hunter et al., 1987). Cadmium can have harmful effects on soil microbes as well (Khan et al., 2010; Wang et al., 2010; Yang, 2010). Like Pb, the groups of soil microbes most sensitive to Cd are gram positive bacteria and actinomycetes while gram negative bacteria and fungi are more resistant (Wang, 2010, Yang, 2010). It seems likely that once again the differences in cell walls are driving the different responses (Abaye et al., 2005; Wase, 1997).

### *Polycyclic Aromatic Hydrocarbons (PAH's)*

Polycyclic Aromatic Hydrocarbons (PAH's) are a class of persistent organic pollutants that are comprised of two or more fused aromatic carbon rings. Some of these compounds are known to be carcinogens and/or mutagens (Li et al., 2014a; Rengarajan et al., 2015). While there are hundreds of different PAH compounds, sixteen of them have been classified as priority pollutants by the U.S. EPA for their cancer-causing potential (Lorenzi et al., 2011). These compounds typically are formed during the combustion of fossil fuels and during incineration processes and are released as airborne particulate matter (PM). The airborne PAH's then occur as dry deposition where they are incorporated into the soil. Naturally occurring PAH's are much less prevalent and are mainly due to fires and volcanic activity (Li et al., 2014a; Lorenzi et al.,

2011; Rengarajan et al., 2015). Polycyclic aromatic hydrocarbons can also be added to soils via contaminated amendments such as sewage sludge (Evans et al., 2014; Wilcke, 2000). Polycyclic aromatic hydrocarbons concentration is typically highest in urban areas and along roadsides – where combustion of fossil fuels is highest (Chen et al., 2016; Khan and Cao, 2012a; Li et al., 2014a; Wilcke, 2000; Woodhead et al., 1999). Since PAH's are a class of many different compounds, they vary greatly in molecular weight and properties - larger PAH compounds fall out of the air quicker and thus tend to be more concentrated closer to the source while smaller compounds are more widely distributed over a broader area (Li et al., 2014a; Plachá et al., 2009; Yunker et al., 2002). Larger compounds also tend to be more persistent in the environment and have greater potential to be carcinogenic (Canadian Council of Ministers of the Environment, 1999; Cerniglia, 1992; Plachá et al., 2009). Smaller molecular weight compounds, on the other hand, tend to be more mobile and acutely toxic, especially to aquatic organisms (Boonchan et al., 2000a; Canadian Council of Ministers of the Environment, 1999). In addition to causing cancer, PAH exposure has been linked to skin/eye irritation, endocrine disruption, male infertility, increased risk of birth defects (Evans et al., 2014; Han et al., 2011; Rengarajan et al., 2015).

Polycyclic aromatic hydrocarbon contamination is widespread in many developed countries with economies that are largely based on fossil fuel consumption (Canadian Council of Ministers of the Environment, 1999; Han et al., 2011; Khan and Cao, 2012a; Li et al., 2014a; Lorenzi et al., 2011; Plachá et al., 2009; Rengarajan et al., 2015; Wilcke, 2000; Woodhead et al., 1999). A study of soils in Delaware found that nearly all sites had PAH levels elevated above background levels. While the U.S.A. has no national legal safety limit on PAH's in soils, there are limits in Canada and so do some U.S. States (limits vary based on individual PAH) (Canadian Council of Ministers of the Environment, 2010). Nearly all of the sites in Delaware

with elevated PAH's were above the legal PAH limit for soil in Canada (Zhang et al., 2013). Multiple studies of PAH contaminated soils in urban China have also found that the PAH concentration exceeds the legal limit set by the Canadian government (like the US, China has no legal PAH limits) (Chen et al., 2016; Khan and Cao, 2012a; Wang et al., 2012). A survey of PAH exposure in the UK found that up to 90% of human PAH exposure came from contaminated soils (Wilcke, 2000).

### *Other Contaminants*

There are many possible soil contaminants and it would be nearly impossible to cover every single one, however it is possible to cover some of the larger groups of contaminant types. Other organic compounds, such as chlorinated hydrocarbons, industrial solvents, and pharmaceuticals, can also become soil contaminants (Pierzynski et al., 2005; U.S. Environmental Protection Agency, 2011). These compounds are almost everywhere and can sometimes be hard to characterize and predict the distribution of. Everyday over 70,000 different synthetic organic compounds are used in the USA for everything from industrial solvents to nail polish. While many of these compounds are indeed useful, they can also have undesirable effects on ecosystem function and human health – especially when disposed of improperly (Pierzynski et al., 2005).

In addition to organic contaminants, there are many other potentially toxic inorganic compounds and elements that can end up in soils, such as Zn, Cu (while Zn and Cu are micronutrients, they can be hazardous in sufficiently large concentrations), Cr, and Hg (U.S. Environmental Protection Agency, 2011). These types of contaminants are likely to become more of a concern as new types of waste (i.e. e-waste) are being disposed of. For example, antimony is a potentially hazardous metalloid that can leach into soils from the heating of plastics during manufacturing, recycling, and solar degradation (Cheng et al., 2010). Antimony

also makes its way into soils during mining, smelting, coal burning, and incineration activities. Antimony is genotoxic, meaning it damages DNA – which can cause birth defects and cancers (Hockmann et al., 2014; Wu et al., 2011).

## *Fate and Transport*

Fate and transport of soil contaminants depends largely on the nature of the contaminant but also on the nature of the soil itself (Tu et al., 2013). This section aims to explore the prominent fate and transport pathways for important soil contaminants. Some of the major process of transformation and transportation of mineral soil contaminants are: leaching, sorption, formation of secondary minerals, erosion, uptake, and dissolution.

### *Lead*

Once introduced to a soil environment, Pb is particularly prone to sorption processes and the formation of secondary minerals (Hettiarachchi and Pierzynski, 2004; Rooney et al., 2007; Sauve and Hendershot, 1997a; Scheckel et al., 2013; Tu et al., 2013). Lead binds readily to cation exchange sites on clay minerals and organic matter (Brown et al., 2012; Chaney and Ryan, 1994; Cruz et al., 2016; Levonmäki et al., 2006a; Smith, 2009). As such the mineralogy, texture, and organic matter content of the soil greatly determines the fate and transport of Pb, (Cruz et al., 2016; Hettiarachchi and Pierzynski, 2004; Rooney et al., 2007; Tu et al., 2013). The formation of Pb phosphates and Pb carbonate minerals is fairly common in Pb containing soils and can reduce Pb mobility (Hettiarachchi and Pierzynski, 2004; Rooney et al., 2007; Sauve and Hendershot, 1997a). Pb phosphate minerals typically form when solution P is high, and Pb carbonate minerals tend to form under higher pH conditions. Ultimately, since Pb is highly sorbed to clays, Fe-Mn minerals, and SOC, the mobility of soil Pb is usually very low. However, in very acidic

conditions (soil pH 4 or less), many of the Pb minerals are more prone to dissolution and soil Pb becomes more mobile (Chaney and Ryan, 1994; Levonmäki et al., 2006a; Rooney et al., 2007; Sauve and Hendershot, 1997a). A study on lead leaching on an acidic (pH 4.1) peat soil found leaching to be a transport pathway via both Pb cations and Pb bound to soluble SOM (Levonmäki et al., 2006a). Another study in Brazil found that Pb leaching was significant only in areas with very sandy soils. (Cruz et al., 2016). Since soil properties are important in determining the fate and transport of Pb, management practices that alter these properties can reduce Pb mobility (Hettiarachchi and Pierzynski, 2004).

Lead has limited capacity to transfer to plant tissues – plants typically only uptake Pb when it is in the soil solution and even then in typically small quantities (Chaney and Ryan, 1994; Hettiarachchi and Pierzynski, 2004; U.S. Environmental Protection Agency, 2014). A study on the uptake of metals by wheat crops found that Pb had a much lower plant uptake than Zn or Cd (Weber and Hrynczuk, 2000). Even with the limited capacity for Pb uptake, plant Pb uptake level does vary by type of crop grown. Crops harvested for fruits and seeds (i.e. tomatoes) will tend to have the lowest Pb tissue concentration while those harvested for the roots (i.e. carrot) will have the most (Attanayake et al., 2015; Brown et al., 2016; McBride, 2013; McBride et al., 2014; Wortman and Lovell, 2013). As a result, human risk from lead contaminated soil is mostly linked to direct contact with and consumption of soil (Attanayake et al., 2015; Brown et al., 2016; Henry et al., 2015; McBride et al., 2014). In adults most direct contact and consumption of soil is through dust on unwashed produce and in the home. With children hand-to-mouth play, toy-to-mouth play, and directly consuming soil (pica) are common exposure routes for Pb contaminated soil (Attanayake et al., 2015; Brown et al., 2016; Chaney and Ryan, 1994; McBride et al., 2014; Mielke et al., 1983; U.S. Environmental Protection Agency, 2014).

### *Arsenic*

Arsenic exists in multiple oxidation states – which have different fate and transport pathways; As(-III), As(+0), As(+III) and As(+V). The first two are uncommon in nature and are not important for this discussion (Zhao et al., 2010a). Arsenate, As (+V), is the most oxidized form of As and readily sorbs to Fe-Mn minerals and forms As sulfur minerals. Arsenate is more typically strongly sorbed to soil or in insoluble minerals and is thus less mobile. Arsenite (As +III) is the more reduced of the As forms common in nature and is less readily sorbed and thus more readily mobile (Garcia-Sanchez et al., 2010a; Mandal and Suzuki, 2002; Wang and Mulligan, 2006a; Zhao et al., 2010a). Due to the affinity of As(+V) for Fe-oxide minerals and the effect of oxidation state on mobility – the redox potential of the soil is very important for determining the fate and transport of As (Mandal and Suzuki, 2002; Wang and Mulligan, 2006a; Zhao et al., 2010a). Under oxidized conditions, As is generally in the oxidized form, As(+V) and is sorbed to clays, Fe-Mn minerals and SOC (Attanayake et al., 2015; Han et al., 2004; Sizmur et al., 2011; Zhao et al., 2010a). Arsenate is also commonly found in As-Fe secondary minerals such as Arsenopyrite and Scorodite (Brown et al., 2012; Garcia-Sanchez et al., 2010a; Han et al., 2004; Zhao et al., 2010a). Arsenic bound to Fe minerals and clays are typically less soluble and thus less mobile (Garcia-Sanchez et al., 2010a; Zhao et al., 2010a). Arsenic bound to organic compounds is typically more mobile and can be leached and is available for plant uptake (Han et al., 2004; Wang and Mulligan, 2006a; Zhao et al., 2010a). However, when conditions in the soil become reduced and oxygen is limited, many anaerobic soil microbes will attack the Fe minerals (i.e., biologically mediated reductive dissolution of Fe minerals) that As is sorbed to or bound in, thereby releasing reduced As into the soil solution (Garcia-Sanchez et al., 2010a; Zhao et al., 2010a). The combination of organic-bound As and saturated conditions makes peat soils very prone to As leaching (Miller et al., 2010; Wang and Mulligan, 2006a).

A study in 2007 found that leaching was the primary mechanism for arsenic translocation in soils (Chen et al., 2007). Similarly, a study of land application of wastewater found that the saturated conditions increased As mobility and leaching (Aryal and Reinhold, 2015). It is this increase in mobility of As under reduced conditions that make crops that require saturated conditions, like rice, much more susceptible to As than dryland crops (Meharg and Rahman, 2003; Zhao et al., 2010a).

***Cadmium***

Cadmium exists in the soil solution as a divalent cation, $Cd^{2+}$, and in the soil solid phase as bound to or part of carbonate, phosphate, Fe-Mn oxides, organo-mineral complexes, and soil clay minerals (Barrow, 2000; Fan MeiRong et al., 2012; U.S. Department of Health and Human Services, 2012). Cadmium in the soil solution is readily mobile and thus prone to leaching and capable of plant uptake (Barrow, 2000; Satarug et al., 2003). The soil factors that most influence Cd mobility are thus those that influence the ability of Cd to either adsorb or precipitate out of solution (Barrow, 2000; U.S. Department of Health and Human Services, 2012).

Predicting Cd fate and transport can be more difficult than other soil metals, a modeling study in 2008 found that attempts to model Cd transport in soils had very little success – especially when compared to the modeling attempt for Pb, which was largely accurate (Hutzell and Luecken, 2008). The mobility of Cd largely depends on the availability of potential adsorption sites – which largely depends on soil pH and concentrations of minerals and organic compounds that Cd adsorbs to (Barrow, 2000; de Livera et al., 2011; Simmler et al., 2013; U.S. Department of Health and Human Services, 2012). Cadmium is more adsorbed and less mobile in soils that are less acidic; Cd availability increases considerably at pH 5.0 (Barrow, 2000; U.S.

Department of Health and Human Services, 2012). While Cd has a leaching potential, it still easily accumulates in soils due to its affinity for cation exchange sites. Cadmium does adsorb to cation exchange sites, although it is less tightly bound to them than lead and other metals (Chen et al., 2013; Zhao et al., 2009).

### *Polycyclic Aromatic Hydrocarbons*

Once in the soil, polycyclic aromatic hydrocarbons (PAH) have many of the same transformation and translocation pathways of other contaminants, however as they are organic contaminants they can also be decomposed by microbes (Cerniglia, 1992; Chen et al., 2016; Kawasaki et al., 2012). The major transport and transformation pathways for PAH's: sorption, leaching, erosion, plant uptake, volatilization, and microbial decomposition (Wilcke, 2000). What processes dominate varies greatly both by soil type and by the molecular weight of the PAH compound (Khan and Cao, 2012a; Wilcke, 2000). Sorption processes involve the sorption of PAH's to clays and soil organic matter, which increases the capacity of a soil to retain PAH's while decreasing bioavailability (Wilcke, 2000; Zhang et al., 2010). PAH compounds are generally more readily adsorbed to SOC which much more limited ability to absorb to clays (Khan and Cao, 2012a; Wilcke, 2000; Zhang et al., 2010). Leaching potential of PAH compounds depends greatly on their molecular weight. Small compounds that are readily soluble are prone to leaching, while leaching is rare for heavier compounds (Li et al., 2014a; Plachá et al., 2009; Reilley et al., 1996; Yunker et al., 2002). Plant uptake of PAH's varies considerably with both PAH compound and plant type. Typically plant uptake of PAH's is very low; lower weight PAH's are often more plant available than heavier PAH's (Attanayake et al., 2015). Brassicas and leafy vegetables like spinach tend to have higher uptake of PAH's – especially of

lower molecular weight compounds. The uptake level of these vegetables occasionally crosses safety thresholds for small children (Khan and Cao, 2012a; Wang et al., 2012). When compared to leafy vegetables, root vegetables uptake less PAH's and much of what they do uptake is typically stored in the peel, which is easily removed and discarded (Attanayake et al., 2015; Wang et al., 2012; Zohair et al., 2006). A significant portion of human-plant PAH exposure comes not from the plant tissues, but from PAH laden dust or sediment on the surfaces of the plant (Attanayake et al., 2015; Wilcke, 2000).

The most important pathway for PAH removal from soils is decomposition by microorganisms (Boonchan et al., 2000a; Cerniglia, 1992; Chen et al., 2015; De Nicola et al., 2015). The degradation processes of PAH's is thus correlated with soil properties that affect microbial growth; such as SOC content and climate (Cerniglia, 1992). PAH accumulation in soils tend to be more of a problem in regions and soils where microbial activity is low, typically due to cold temperatures or anaerobic conditions (Cerniglia, 1992; Li et al., 2014a). The chemistry each individual PAH compound affects its decomposition rate; with larger PAH's being more resistant to microbial attack (Boonchan et al., 2000a; Cerniglia, 1992; Plachá et al., 2009). An incubation study on microbial degradation of high molecular weight PAH's found that differing compounds of the same weight may degrade at different rates (ex; Pyrene degrades much faster than chrysene). Much of this is due to differences in molecular structure that makes some compounds easier to break down (Boonchan et al., 2000a).

### *Other Contaminants*

Other organic contaminants follow similar trends to PAH compounds in their main fate and transport pathways. Many organic contaminants are preferentially adsorbed by SOC rather than clay (Fabietti et al., 2010; Liu et al., 2012; Schlebaum, 1998). The main pathway that most

organic contaminants leave the soil is via microbial decomposition. Many compounds that are potentially toxic to humans, plants, and animals are potential energy sources for soil microorganisms (Liu et al., 2012; Patterson et al., 2010; Semple et al., 2007). The exact nature of how the fate and transport pathways work for organic contaminants depends greatly on the contaminant in question. Some organic contaminants tend to remain in the soil for long periods of time because microbes are unable to degrade them. Contaminants with more complex structures and contaminants that are tightly adsorbed to soil humic substances are often difficult for microorganisms to break down (Liu et al., 2012; Patterson et al., 2010; Schlebaum, 1998; Semple et al., 2007).

Many inorganic contaminants are metals or metalloids and follow similar trends to Pb, Cd, and As. For these elements redox forms and sorption/desorption reactions are important in determining the dominant fate and transport pathways (McLaren et al., 2005; Sauve and Hendershot, 1997a; Schaider et al., 2014). It is important to remember that each element has its own unique reaction pathways that will determine how it will behave in a soil environment (McLaren et al., 2005; Sharma et al., 2009).

## *Bioavailability*

### *Lead*

Lead bioavailability to humans tends is unusually low and generally tied to Pb speciation (Attanayake et al., 2015; Hettiarachchi and Pierzynski, 2004). Even without the use of amendments, most lead in soils is highly adsorbed to soil minerals; it is not easily absorbed the human digestive tract (Chaney and Ryan, 1994; Freeman et al., 1996; Henry et al., 2015). Rat feeding tests (which is an acceptable model for human digestive bioavailability) often put the bioavailability of soil lead to the human gut as less than 10% (Freeman et al., 1994; Zia et al.,

2011). Lead phosphate minerals, which are readily formed, tend to have low human bioavailability (Henry et al., 2015; Sauve and Hendershot, 1997a; Scheckel et al., 2013). However, certain soil conditions can increase Pb bioavailability to humans, particularly low pH and the formation of certain easily dissolvable minerals. Low pH increases the solubility of lead which increases its bioavailability (Chaney and Ryan, 1994; Oliver et al., 1999; Sauve and Hendershot, 1997a; Yang et al., 2016). Some Pb minerals are more easily dissolved, particularly Pb oxides and Cerrusite (Hettiarachchi and Pierzynski, 2004; Smith et al., 2011; Zhang and Ryan, 1999).

Lead bioavailability also greatly depends on the organism in question. While human bioavailability is generally fairly similar to the bioavailability to other animals like rats or pigs, it is quite different from plant or microbe bioavailability (Attanayake et al., 2015; Chaney and Ryan, 1994; Hettiarachchi and Pierzynski, 2004). Even within the realm of human bioavailability there is considerable variation from person to person for a variety of reasons. Children tend to uptake Pb more than adults (Chaney and Ryan, 1994; Henry et al., 2015; McClintock, 2015; Mielke et al., 1983). People who are fasting when they consume the lead have higher Pb uptake. During fasting, the digestive tract pH decrease, which increases the solubility of any Pb in the digestive track. Even a seemingly small change in pH can significantly alter human Pb uptake (Brown et al., 2003; Chaney and Ryan, 1994; Oliver et al., 1999; Sauve and Hendershot, 1997b). Additionally, people who are not fasting will have, presumably food, in their digestive system; which often contains substances that can react with Pb. Lead can form complexes with P, Fe-oxides and organic matter even inside the digestive tract of an animal (Brown et al., 2003; Chaney and Ryan, 1994; Davis et al., 1994; Freeman et al., 1996; Hettiarachchi et al., 2003; Zhang and Ryan, 1999).

Since there are multiple ways of defining bioavailability there are multiple methods of accessing it. Bioavailability assessment can be broken down into two large groups; in-vitro and in-vivo. In-vitro is the use of proxy extraction solutions and chemical fractionation to make assumptions about bioavailability based on chemistry. In-vivo is the use of live organisms to determine actual bioavailability (Brown et al., 2003; Scheckel et al., 2009; Smith et al., 2011; Zia et al., 2011). In vivo is generally accepted as more accurate but is generally more difficult and expensive to perform. For human bioavailability the in-vivo model often uses rodents or pigs as proxies (Freeman et al., 1994; Hettiarachchi et al., 2003; Scheckel et al., 2009; Smith et al., 2011). Human in-vitro bioavailability is often accessed with artificial gastric methods like PBET (Physiologically Based Extraction Test) (Brown et al., 2003; Lestan and Finzgar, 2006; Smith et al., 2011). Plant and microbial bioavailability does not have a set model like with human bioavailability. Instead, bioavailability is often assumed based on chemical fractionation, Pb mineralogy or exchangeable Pb (Mao et al., 2016; McBride, 2013; Rooney et al., 2007). Plant bioavailability is also assessed using in-vivo methods much more frequently than with animal and human bioavailability as in-vivo methods for plants are much less expensive and less complicated to carry out (Attanayake et al., 2015; Hettiarachchi and Pierzynski, 2004; Mao et al., 2016; McBride et al., 2014).

### *Arsenic*

Arsenic bioavailability both to humans and to other organisms greatly depends on its oxidation state. Arsenic in the (As +III) has greater solubility and greater bioavailability than As in the (As +V) state (Mandal and Suzuki, 2002; Zhao et al., 2010b). Since As can be take up readily by plants under certain conditions it is important to consider human bioavailability from both direct soil consumption and from high-As crops (i.e. crops grown under saturated

conditions or irrigated with As containing water) (Bastías and Beldarrain, 2016). Most of the soils that are in urban garden areas are not regularly waterlogged or flooded. In this situation the human As bioavailability from direct soil exposure is typically fairly low (Garcia-Sanchez et al., 2010b; Kumpiene et al., 2009; Zhao et al., 2010b). Liu et al reported soil to human bioavailability of As from yard soil to be between 13.8 and 20% (Liu et al., 2016). Yoon et al. (2016) reported in-vitro bioavailability between 0.6 and 6% (Yoon et al., 2016). Much of the variability in human to soil bioavailability comes from differences in soil properties like pH, texture and mineralogy (Brown et al., 2012; Garcia-Sanchez et al., 2010b; Miller et al., 2010; Yang et al., 2007). While As bioavailability in terrestrial soils is generally low, As in plant tissues often has much higher human bioavailability and as a result most human As exposure comes from food grown in a high As environment (Bastías and Beldarrain, 2016; Clemente et al., 2016; Gillispie et al., 2015; Meharg and Rahman, 2003; Wu et al., 2011; Zhao et al., 2010b). As in plant tissues tends to be in the more bioavailable As +III form and in organo-As compounds (Clemente et al., 2016; Zhao et al., 2010b). Processing and cooking methods can make a difference in the bioavailability of As in food (Signes-Pastor et al., 2012; Yager et al., 2015). A study on cooking methods found that the most common rice cooking method (parboiling) can enhance As bioavailability by as much as 30% when compared to a non-parboil (Signes-Pastor et al., 2012). Clemente, 2016, reported that As bioavailability in white rice samples to be around 95% and in brown rice samples, 75% (Clemente et al., 2016). A similar range for rice bioavailability has been reported by others (Signes-Pastor et al., 2012; Yager et al., 2015). The differences in bioavailability reported here are likely due to the higher presence micronutrients like Fe and Mn in the brown rice, which can complex with As and reduce human bioavailability, even inside the human digestive tract (Bastías and Beldarrain, 2016; Clemente et al., 2016).

As bioavailability and uptake mechanisms to plants also depends on the oxidation state. Plants uptake both As+III and As+V, albeit with different mechanisms (Aryal and Reinhold, 2015; Gillispie et al., 2015; Wang and Mulligan, 2006b; Zhao et al., 2010b). In terrestrial systems, As+V enters plants via P uptake pathways; in anaerobic systems, As+III can enter the plant through Si and $NH_4^+$ channels. The active Si channels of rice combined with its cultivation in flooded fields greatly increases to rice As uptake (Sanglard et al., 2016; Zhao et al., 2010b).

Bioavailability measurement for As is similar to Pb; with in-vitro models being based on artificial gastric solutions (Bastías and Beldarrain, 2016; Clemente et al., 2016). Arsenic bioavailability can also be accessed via use of tissue cultures (Yoon et al., 2016). Finally, human As bioavailability can be studied via observational studies. While unable to determine exact causality, these studies measure As uptake in actual humans. One such study in Hunan Province, China, looked at As contents of soils, rice, and voluntary human hair samples. They found that 40% of people had elevated As in their hair samples (Wu et al., 2011).

***Cadmium***

Cadmium, can possibly be taken up by plants in fairly significant quantities. As such, it is important to understand the bioavailability of Cd in soils and of Cd in plants (Chaney, 2015; Chen et al., 2007; Franz et al., 2008; Qiu et al., 2011; Satarug et al., 2003). Direct soil consumption Cd bioavailability can be highly variable and is dependent on soil characteristics (Aziz et al., 2015; Balal Yousaf et al., 2016a; Xia et al., 2016; Zhao et al., 2016). Soils that are high in compounds that can sorb Cd tend to have lower bioavailability. Iron oxides, soil clays, soil carbonates, biochar, and Zn can all complex with Cd and reduce its bioavailability (Balal Yousaf et al., 2016a; Barrow, 2000; Chaney, 2015; de Livera et al., 2011; Fan MeiRong et al., 2012; Oliver et al., 1998). Bioavailability of Cd in food is typically low but can also be fairly

variable and can depend on the nutritional status of the person and on food preparation methods (Chaney, 2015; Fu and Cui, 2013; Reeves and Chaney, 2008). Iron, Zn, and Ca can all reduce Cd uptake inside the human body (Chaney and Ryan, 1994; Chaney, 2015; Reeves and Chaney, 2008). The method used to determine Cd bioavailability can also have large impacts on the results (Aziz et al., 2015; Reeves and Chaney, 2008). Most in-vitro models for Cd intake use a simple gastric solution that does not take into account (Aziz et al., 2015; Chaney, 2015; Reeves and Chaney, 2008). However, there are a few cases where human Cd bioavailability and human Cd exposure are higher than usual. First is with rice. Rice is particularly prone to accumulating Cd due to the way it is grown; additionally, the Cd in rice is typically more bioavailable due to the low Fe and Zn content of rice (Chaney and Ryan, 1994; Chaney, 2015; Reeves and Chaney, 2008; World Health Organization, 2010b). Cadmium is capable of bioaccumulation and can move up the food chain. When Cd contaminated soil is used to grow forages to feed livestock, the Cd will move from the soil to the plant to the animal where it will be accumulated in the liver and kidneys (Franz et al., 2008; Loganathan et al., 2003; World Health Organization, 2010b). As such organ meats can have high Cd contents; fortunately these are not a staple for most people and there is very little Cd accumulation in the muscle meats (Franz et al., 2008). This may be a slight concern for those wanting to raise urban livestock (usually chickens or goats), which is common in developing countries and like urban gardening, is a growing trend (Halloran and Magid, 2013; McClintock, 2015; Rogus and Dimitri, 2015). While not a food crop, tobacco is commonly consumed by humans, and is capable of accumulating high amounts of Cd in its tissues. Additionally, tobacco is generally not eaten, but smoked which greatly increases bioavailability (Satarug et al., 2003; World Health Organization, 2010b).

Cadmium bioavailability is typically determined with in-vitro gastric models (Fu and Cui, 2013; Intawongse and Dean, 2006; Reeves and Chaney, 2008). However these models often fail to completely capture Cd absorption processes in human bodies (Chaney, 2015; Reeves and Chaney, 2008). Investigations into better ways to model Cd uptake is needed (Chaney, 2015; Hutzell and Luecken, 2008b).

### *Polycyclic Aromatic Hydrocarbons*

Bioavailability of PAH's varies with the exact PAH compound (Chen et al., 2015; Plachá et al., 2009; Zohair et al., 2006). PAH's in soil tend to degrade over time and are often highly sorbed to SOC; and as a result direct soil contact is not a major pathway for human PAH exposure (Attanayake et al., 2015; Ounnas et al., 2009; Peters et al., 2015). Inhalation of PAH laden dust and consumption of PAH contaminated food are more important human exposure pathways for PAH's (Khan and Cao, 2012b; Lorenzi et al., 2011; Ramesh et al., 2004). Soil properties can also influence PAH bioavailability. Soils with higher organic carbon, tend to have more PAH's sorbed and thus not bioavailable (Khan and Cao, 2012a). Polycyclic Aromatic Hydrocarbon bioavailability can be accessed using both in-vitro and in-vivo models successfully (Attanayake et al., 2015; Costera et al., 2009; Ounnas et al., 2009; Peters et al., 2015; Ramesh et al., 2004).

## *Best Management Practices for Managing Mildly Contaminated Urban Soils*

Remediation of sites contaminated with heavy elements and polycyclic aromatic hydrocarbons can reduce risks to human and ecosystem health. There are two major categories of remediation methods; in-situ and ex-situ. In-situ remediation is the remediation of the impacted soil in place; while ex-situ is the removal of the soil to be remediated off site. This section will

be focusing primarily on in-situ methods. There are a number of methods that have been considered for in-situ remediation. One of the main benefits of in-situ remediation is that it is much more cost effective than ex-situ remediation – especially when the effected site is large in area and the contamination load is moderate (Hettiarachchi and Pierzynski, 2004). Remediation treatment can reduce human risks from exposure to contaminated soil and can improve soil ecological functioning. However, even with remediation, it is unlikely that affected sites will be as productive as sites that were never contaminated (de Mora et al., 2005; Hinojosa, 2004).

Most in-situ remediation methods involve using soil amendments and establishing plant growth on affect sites in order to stabilize them (chemical, physical, and phytostabilization). The idea behind stabilization efforts is to reduce mobility of hazardous metals and reduce bioavailability. The reduction of bioavailability and mobility makes the contaminants much less of a threat to humans and ecosystems, and prevents the contamination from spreading to other areas via erosion, leaching, or the food chain (Vangronsveld et al., 2009). Encouraging perennial vegetation on contaminated sites is phytostabilization. The roots help keep the soil in place and reduce erosion. Ideal plants for stabilization are ones that are well rooted, do not accumulate metals in their tissues, and are well suited to the area. Often on more highly contaminated sites, such as mine tailing disposal sites, the soil will have to be modified prior to planting as initial conditions are too harsh to allow for plant growth. (Mendez and Maier, 2008). Establishing plants on heavily contaminated sites can be challenging as many of these sites have properties that inhibit plant growth. Low organic carbon, low nutrients, and acidity often inhibit plant grown in mine waste sites and urban brownfields. In addition toxicity from whatever contaminant is impacting the site can also hamper plant growth. In order to establish plant

growth on contaminated sites, it is often necessary to amend the soil with organic matter (i.e. composts, manures), liming agents, and fertilizers (Mendez and Maier, 2008).

Another potential method is phytoextraction, which uses plants that uptake the metals, which are then harvested and then disposed of appropriately. Phytoextraction has a few difficulties associated with it. First is the tendency of most plants to accumulate low amounts of metals in their tissues, and second is that of the plants that do accumulate metals in their tissues, most are slow growing or produce low biomasses (Hettiarachchi and Pierzynski, 2004; McGrath and Zhao, 2003). The feasibility of phytoextraction depends on the contaminant in question and the degree of contamination. More soluble metals like Cd and Zn can possibly be remediated via phytoextraction in mild to moderately contaminated sites. However, other metals like Pb and Cr have limited feasibility for phytoextraction with current technologies (Hettiarachchi and Pierzynski, 2004; McGrath and Zhao, 2003). There is work on genetically modifying plants to create more ideal hyperaccumulators but this technology is not yet developed enough for use in widespread in-situ remediation (McGrath and Zhao, 2003).

A final possibility for reducing human exposure from food grown in contaminated soils (especially for contaminants that are more plant-available like Cd and As) is to breed crops that do not uptake contaminants of concern. Encouraging gardeners to plant low-accumulating plants and cultivars can reduce human transfer risk. From a future development perspective there is the possibility of specifically breeding plants that have very low metal uptake (Qiu et al., 2011).

### *Lead*

The most practical methods for remediating Pb in soils is to use plants and amendments to stabilize the soil and prevent the Pb from coming into contact with humans. Methods of

extraction and soil removal are generally impractical due to the high cost and the sheer number of sites impacted by Pb (Hettiarachchi and Pierzynski, 2004). While Pb cannot be truly removed from the soil with this method, it can be made immobile and non-bioavailable through changes in soil chemistry that encourage the adsorption of lead to SOC and soil minerals (Smith, 2009).

There are a number of soil amendments that can be added to reduce Pb availability and improve the ability of the soil to support stable plant growth. Organic amendments such as composts, manures, and biochar can help Pb impacted soils in a number of ways. Organic carbon compounds in native soils are capable of complexing Pb and reducing its availability. This process can be enhanced by adding organic matter to soils as part of the remediation process (Attanayake et al., 2015; Perez-de-Mora et al., 2006; Simmler et al., 2013; Yang et al., 2016). Organic amendments are often added in large enough volumes to also provide a dilution effect (Attanayake et al., 2015). Finally, organic amendments add nutrients and improve the ability of a soil to support plant growth; which can help establish permanent vegetation to prevent movement of Pb (Mendez and Maier, 2008). In addition to organic matter, agricultural lime can be very helpful in reducing risks from soil Pb. As Pb bioavailability, both to plants and to humans, is correlated with pH; raising the pH of soils can help reduce Pb related risks (Chaney and Ryan, 1994; Levonmäki et al., 2006b; Mendez and Maier, 2008). Lead readily forms unavailable complexes with P and thus the addition of P through rock phosphate or manures can decrease Pb availability (Hettiarachchi et al., 2003; Mendez and Maier, 2008; Scheckel et al., 2013). Finally, Fe amendments have also been shown to reduce the bioavailability of Pb in soils (Brown et al., 2012; Brown et al., 2003).

While organic amendments like composts are often great for remediating lead contaminated sites, it is important to keep in mind that these materials can also be a source of

lead. While Pb in composts is generally low, testing organic amendments before application can alleviate such concerns (Chaney and Ryan, 1994; Hettiarachchi and Pierzynski, 2004). More careful separation of compostable yard and food scraps from other wastes (i.e. metals and plastics) will help by creating safer composts (Smith, 2009).

### *Arsenic*

Much of the remediation of As contaminated sites uses similar principles to that of Pb; however since As chemistry is not the same as Pb, there are some differences. Of notable concern are sites that are co-contaminated with both Pb and As. Some of the Pb remediation strategies can actually increase As bioavailability, so care needs to be taken in sites that are co-contaminated (Henry et al., 2015). Phosphorus amendments can reduce Pb bioavailability in soils but they have the opposite effect on As (Brown et al., 2012; Henry et al., 2015). Organic amendments (composts, ect.) can reduce As bioavailability through forming organo-As complexes and by diluting As in the soil (Brown et al., 2012; Chen DanYan et al., 2011; de Mora et al., 2005; Huq et al., 2008). However, organic amendments can also increase the bioavailability of As; some forms of organo-As are bioavailable (Sizmur et al., 2011; Zhao et al., 2009). Poorly chosen organic amendments can also introduce significant amounts of P which out competes As for exchange sites; thereby increasing As availability (Brown et al., 2012; Henry et al., 2015). Iron mineral amendments generally reduce bioavailability of As by encouraging the formation of As-Fe complexes (Brown et al., 2012; Chen DanYan et al., 2011; Kumpiene et al., 2009; Sneath et al., 2013; Yang et al., 2007).

Since As is more bioavailable in its reduced form; management practices that reduce the incidence of water logging will reduce As bioavailability (Kumpiene et al., 2009; Zhao et al., 2010a). Ponded or waterlogged conditions can be prevented in garden soils by installing

drainage, using mulches, building raised beds, and avoiding gardening in poorly drained locations (Royal Horticultural Society, 2016). Plant uptake of As can also be managed by selecting plants and cultivars with low As uptake (Pillai et al., 2010; Xiao HouJun et al., 2012; Zhao et al., 2010a).

### *Cadmium*

Cadmium can be remediated using many of the same techniques as As and Pb. The addition of various soil amendments can reduce Cd bioavailability and thus reduce risks to human gardeners. Biochars are an organic amendment made from organic materials, such as straw, that are then charred in anoxic conditions. They have been shown to be effective at absorbing soils contaminants like Cd (Balal Yousaf et al., 2016b; Lu et al., 2014; Simmler et al., 2013; Sneath et al., 2013). Other organic amendments like composts can also be effective at absorbing Cd effectively; thus reducing bioavailability (Balal Yousaf et al., 2016b; Juang et al., 2012; Zeng et al., 2015). Controlling soil pH via liming is another cost effective way for urban gardeners to reduce the potential risks of soil Cd (Chaney and Ryan, 1994; El-Azeem et al., 2013; Hong et al., 2010). Mineral and inorganic amendments like silicate minerals have been shown to reduce Cd bioavailability as well (Gu JiaoFeng et al., 2016; Hou et al., 2014; Zhao et al., 2016).

Cadmium uptake by plants can vary considerably between different species and between different cultivars of the same species. This is often due to differences root structure and nutrient uptake. Gardeners can reduce their Cd related risks by selecting plant species and cultivars that have low Cd uptake (Daud et al., 2009; Li et al., 2005; Qiu et al., 2011; Xiao et al., 2015).

### *Polycyclic Aromatic Hydrocarbons*

Polycyclic aromatic hydrocarbons, PAH's, are organic contaminants, and they can be

mineralized by soil bacteria, and thus completely removed from the soil ecosystem. Much of PAH remediation is about encouraging the microbial activities that will enhance the breakdown of the PAH's (Boonchan et al., 2000b; Cerniglia, 1992; Chen et al., 2016). The rate that PAH's are degraded depends on the PAH in question and the soil conditions. As a general rule, lower molecular weight PAH's tend to degrade faster than larger PAH's (Boonchan et al., 2000b; Cerniglia, 1992; Plachá et al., 2009). Soil conditions that increase microbial activities will also increase PAH degradation. Actively growing plants exude compounds into the soil near the roots; creating a surge of microbial activity near plant roots. Not surprisingly, PAH degradation is greatly enhanced in the rhizosphere when compared to the bulk soil (Boonchan et al., 2000b; Chen et al., 2016; De Nicola et al., 2015; Kawasaki et al., 2012; Reilley et al., 1996). PAH's can also be sequestered and rendered non-available by absorbing to soil organic matter (Khan and Cao, 2012a; Ounnas et al., 2009; Wilcke, 2000; Zhang et al., 2010). Under optimal microbial growth conditions (Planted and ideal moisture/temperature), as much as 85 – 100 % of PAH's can be mineralized in a growing season (Chen et al., 2016).

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# Chapter 3 - Understanding Biochemical Contributions to the Resilience of Sequestered Soil Organic Carbon in Soils from Contrasting Agroecosystems


## Abstract

Climate change has the potential to alter soil microbial communities and biochemical processes in ways that impact soil carbon sequestration potential and crop performance. This study was conducted to determine how changes in temperature, moisture, and aggregation affect soil microbial communities from four different agroecosystems. Understanding how the changes in soil microbial properties influenced soil carbon and nutrient cycling was explored using a six month long incubation study. Four soils from long term agricultural experimental studies were selected for use in the incubation study. Two of the soils came from continuous corn plots in Manhattan, KS, USA. One of the KS soils was managed under no-till; and the other under conventional tillage. Two soils were from plots in Cruz Alta, Rio Grande do Sul, Brazil; both were managed under an intensive rotation cycle (soy/wheat/soy/oat+vetch/corn/oil radish). One plot was planted using no-till and other was managed with conventional tillage. Collected soils were wet sieved to <4mm and visible organic matter was removed. Half of each soil was set aside to be ground and further sieved to <0.25mm to simulation soil disruption. Each of the soils (both intact and ground) were separated out into sample cups and split into six incubation treatments. There were three incubation temperatures; 12°C, 24°C, and 36°C; and two moisture levels; field capacity and 80% of field capacity. Destructive sampling for biochemical work was take at the following days: 7, 30, 60, 120, and 180 days. Microbial groups were analyzed using phospholipid fatty acid analysis (PLFA). There were significant effects of temperature and soil preparation method on microbial groups. For the KS soil; the effects of temperature and moisture

were less prominent in the no-till soil for the all major microbial groups. For the Brazilian soil; the effects of moisture were less significant on the no-till soil. The effects of moisture overall were more prominent in the Oxisols. Overall this study has suggested that microbial resilience to changes in temperature and moisture patterns is dependent on tillage practices, soil aggregation, and soil mineralogy.

## Introduction

Climate change and agricultural production are intertwined in that agriculture is a major contributor to climate change yet agriculture is also vulnerable to climate change. Over the past 150 years the atmospheric $CO_2$ concentration has increased from 280 ppm to 401 ppm (Tans and Keeling, 2015). This is largely the result of fossil fuel burning and large scale land use changes. This has already increased the average temperature of the planet by about 0.75°C and has the potential to increase the temperature of the planet by 2.6° by 2050 given current economic and technological progress (Intergovernmental Panel on Climate Change, 2014; Shaftel et al., 2015). The current conventional agricultural systems are often sources of greenhouse gases and are not well adapted for climate change (Alvaro-Fuentes and Paustian, 2011; Farina et al., 2011; Schlesinger and Andrews, 2000). Increasing temperatures and altered precipitation patterns have the potential to affect crop growth – both directly through effects on the plants and indirectly through changes in soil ecological functioning (Intergovernmental Panel on Climate Change, 2007; Tissue et al., 2004; Waldrop and Firestone, 2004a).

Elevated soil temperature and decreased soil moisture due to climate change has the potential to alter SOC storage patterns and soil microbial communities (Boyero, 2014; McHale, 1998; Pold, 2015). This could very well decrease a soil's capacity for SOC storage (Davidson and Janssens, 2006b; McHale et al., 1998; Schindlbacher et al., 2010). Lost soil carbon is released into the atmosphere as $CO_2$, which further contributes to climate change (Intergovernmental Panel on Climate Change, 2007; Pold et al., 2015). Loss of soil carbon is also associated with a decrease in plant productivity. Soil organic carbon is not only important for carbon cycling, but it is important for soil quality and plant productivity. Organic carbon contributes to water holding capacity, nutrient holding capacity, water infiltration, provides a source of plant nutrients and supports the soil microbial community that is needed for nutrient

cycling (Brady and Weil, 2010; Shaftel et al., 2015). Practices that increase SOC not only mitigate climate change but increase plant productivity as well.

Changing agronomic management practices can help adapt to climate change while switching agricultural lands from being net carbon sources to net carbon sinks. Practices that increase soil carbon storage are practices that are important for mitigating greenhouse gases from agricultural production (Alvaro-Fuentes and Paustian, 2011; Farina et al., 2011; Intergovernmental Panel on Climate Change, 2007; Lal, 2004b). One of the most well studied management practices that improve soil carbon storage is no-till; which is especially effective in warmer climates (Farina et al., 2011; Lal, 2004b; West and Post, 2002). Other beneficial practices for increasing SOC are cover crops, organic matter additions (i.e. composts and manures) and reducing fallow (Alvaro-Fuentes and Paustian, 2011). Crop rotation is another practice that is known to increase soil carbon storage. Crop rotation increases the diversity of carbon compounds and soil microbial communities. Crop rotation is especially effective when combined with other practices like no-till or cover crops (West and Post, 2002; Balota, 2004; Farina, 2011).

The objective of this study was to determine the impact of temperature changes, moisture level changes, and level of aggregation on soil microbial communities and how that would influence soil carbon and nutrient cycling in soils from four different agroecosystems.

## Materials and Methods

Soils with different levels of aggregation, SOC, and different microbial communities were selected from two sites. The first site was at the Agronomy North Farm (Kansas State University Research Site) in Manhattan KS, USA. This site was part of a long term study (26 years) on the effects of tillage practices and fertilizer sources on soil carbon and greenhouse gas

balance under continuous corn. The soil at the KS site was a Mollisol with a silt loam texture (20% clay, 70% silt, 20% sand) and montmorillinite mineralogy. The climate is hot summer humid continental with 800 mm rain/yr and an average temperature of 11.4°C. From this site soil was collected from the no-till manure treatment and mixed (NTM). The same procedure was repeated to obtain a representative soil sample from soils under conventional-tilled manure (CTM) management.

The second site was at an Embrapa research site in Cruz Alta, RS, Brazil. This site is part of a long term study (30 years) on the effects of tillage practices and crop rotation on soil carbon stocks and greenhouse gas balance. The soil was an Oxisol with a clay texture (52% clay, 23% silt, and 25% sand) and mineralogy comprised of kaolinite, iron oxides, and aluminum oxides. The climate was humid subtropical with 1727 mm rain/year and an average temperature of 19.2°C. From this site soil was collected from various points under the no-till intensive crop rotation (NTR2) and mixed. The same procedure was repeated at the conventional tilled intensive crop rotation plots (CTR2). The intensive crop rotation was a 3 year cycle of soybean, oat, soybean, oat + vetch (intercropped), corn, oil radish, wheat.

The four collected soil samples (CTR2, NTR2, CTM, and NTM) were sieved to 4mm at field moisture conditions and visible plant matter removed. Each sample was divided in half. One half was left as it was after the 4mm sieving (intact soil) and the other was dried at 4°C in a walk-in cooler then it was ground it a ceramic mortar/pestle and sieved to 250 µm (crushed soil).

The moisture retention curve for each soil was determined using a pressure-plate method (Pierzynski et al., 2004). A subset of each of the 4mm-seived soils was used to develop these curves. Soils were packed into columns to a bulk density of 0.9 and then soaked on a ceramic pressure plate in a 0.05 *M* $CaSO_4$ solution. The soaked pressure plate with the columns was

placed in a pressure chamber set at 0.33 kPa. The soil was allowed to equilibrate on the pressure plate for a week and then moisture content was found. This process was then repeated at 0.5 kPa.

Gas sampling was done at the following days: 1, 3, 5, 7, 9, 14, 19, 25, 31, 37, 44, 51, 60, 80, 100, 120, 140, 160, and 180. Sample cups were placed in sealed mason jars and gas samples were collected every hour over a period of four hours to get a $CO_2$ flux. Released $CO_2$-C was analyzed using gas chromatography with a Bruker Scion 456-GC. Flux of $CO_2$ ($CO_2$-C $g^{-1}$ $hr^{-1}$) from soils was determined using the linear regression. Cumulative gas emission was also calculated using linear interpolation between sample points and integrating the curve.

Destructive sampling for chemical and biological analysis was done at the following days: 7 days (S1), 30 days (S2), 60 days (S3), 120 days (S4), and 180 days (S5).

A portion of the soil from each destructive sampling was freeze dried for PLFA analysis. Phospholipids were extracted using silica gel columns and chloroform, methane, and acetone. Following extraction the PLFA's are methylated with Methanolic KOH and then dissolved in hexane prior to analysis (White and Burton, 2007). Extracted PLFA's were analyzed using gas chromatography-mass spectrometry (GS-MS) using the Thermo Scientific Trace GC Ultra GC-MS. An internal standard of methyl nonadecanoate (C19:0) was used to calibrate the GC-MS and added to each sample as quality control.

Abundances for microbial groups was determined using specific fatty acids as biomarkers. Fungal abundance was determined using the fatty acid biomarker, *C18_2_9, 12* (Bossio and Scow, 1998; Ostle et al., 2004; Petersen et al., 2002; Potthoff et al., 2006; Ruess and Chamberlain, 2010). Actinomycete abundance was determined using the *10_methyl C:18*

biomarker (Bossio and Scow, 1998; Potthoff et al., 2006). General gram positive bacteria were determined using the following biomarkers: *i-C:15, a-C:15, i-C:16, a-C:16,* and *i-C:17* (Bossio and Scow, 1998; Ostle et al., 2004; Potthoff et al., 2006; Waldrop and Firestone, 2004b). General gram negative bacteria were determined using the following biomarkers: *C:10_0_2OH, C:12_0_2OH, C:12_0_3OH, C:14_0_2OH, C:14_0_3OH,* and *C:16_0_2OH* (Ruess and Chamberlain, 2010; Steinberger et al., 1999). Total bacteria was determined by summing all bacterial group and total biomass was determined by summing all biomarkers (Petersen et al., 2002; Potthoff et al., 2006; Waldrop and Firestone, 2004a). Labile C was determined as permanganate oxidizable carbon by Pitumpe Arachchige (2016).

A randomized complete block design was used; considering sampling times as blocks. Statistics were calculated using SAS 9.4 and a PROC MIXED model. A $\log_{10}$ transformation was used to achieve normal distribution. Differences were determined using Tukey's HSD test with $p<0.05$ as the level of significance.

## Results and Discussion

### *Total Biomarkers*

Total biomarkers were measured through the addition of all PLFA biomarkers. Total PLFA biomarkers have been shown to correlate well with total biomass, soil organic carbon, and carbon availability (Baath and Anderson, 2003; Giacometti et al., 2013; Steinberger et al., 1999; Yue Lin-yan et al., 2015).

For CTM there were three significant treatment effects on total biomarkers (Table 1 and Fig. 3-1). Overall the three trends suggest greater variability in the microbial population in the intact soil when compared to the crushed soil. The first significant treatment effect was an interaction effect of sampling time and aggregation. In the intact soil; total biomarkers increased

from the first sampling time (7 days) to the second (30 days). This was likely occurring as microbes began to colonize the soil. In the intact soil, there were more aggregates and a greater variety of micro-habitats that might take longer for microbes to fully colonize (Balota et al., 2004). In the crushed soil; total biomarkers decreased at the last sampling time (180 days). This was possibly due to the depletion of labile C substrates. With no growing plants and no additions of C; it was expected that microbial populations would decrease was labile C decreased. The crushed soil was more homogenized with less physical protection for the labile C; allowing it to be consumed quicker (Leifeld and von Lützow, 2014). Labile C from this study (Fig. 3-25) supports this hypothesis (Pitumpe Arachchige, 2016). The second significant treatment effect on the CTM soil was an interaction effect between sampling time and temperature. There was an increase in total biomarkers at the second sampling time (30 days) for 24°C that did not change significantly for the rest of the experiment. This might be due to microbial populations increasing to colonize the intact soil container in the beginning of the experiment (Balota et al., 2004). The third significant effect of treatments on CTM was an effect of moisture on total biomarkers. There were more biomarkers in the wetter treatment. Higher soil moisture has been associated with greater total PLFAs (Garten et al., 2009; Gray et al., 2011). In contrast with NTM there was only one significant treatment effect on total biomarkers (Table 1 and Fig. 3-2). There was an interaction effect between aggregation and sampling time. There was an increase in microbial population with time in the intact soil and a decrease in the crushed soil; which is similar to the trend observed in CTM. However, the lack of significance of temperature or moisture is different from CTM and could suggest greater resilience to changing climate in the no-till soil when compared to the conventional tilled soil. No-till soils tend to have more stable

aggregates which have been shown to decrease the sensitivity of soil microbes to changes in temperature and moisture (Jiang et al., 2013; Trivedi et al., 2015).

In CTR2 there were two significant trends for total biomarkers (as seen in Table 1 and Fig. 3-3). First there was a significant interaction between sampling time and temperature. There was a decrease in total biomarkers at 36°C treatment for the last sampling time (180 days). Labile C (Fig. 3-27) declined in the 36°C treatment by the end of the study; which would explain the decline in soil microbes (Pitumpe Arachchige, 2016). *In-situ* studies of soil microbes have found that microbial populations decline when labile C declines; which happens faster in warmer systems (Giardina and Ryan, 2000; Hartley et al., 2007). There is also the possibility of an effect of pH on the CTR2 microbial population. Over the course of the experiment, pH declined in all soils, however, in both Oxisols the pH went below 6.0 (Fig. 3-33), which has been cited as a threshold for microbial stress (Fierer and Jackson, 2005). Soil pH declined the most in the higher temperature treatments as higher $CO_2$ emissions led to greater formation of carbonic acid (Pitumpe Arachchige, 2016). Lastly, like in CTM, there were more biomarkers in the wetter soil; which is a trend that other authors have reported (Garten, 2011; Gray et al., 2011). Drier soil moisture conditions are anticipated for much of the continental U.S. as part of climate change (Lofgren et al., 2013; Milly and Dunne, 2011; Tissue et al., 2004). This has the potential to alter SOC storage patterns on large scales, especially when combined with the higher temperatures that are also anticipated with climate change (ArchMiller and Samuelson, 2016; Garten et al., 2009; Gray et al., 2011; Vesterdal et al., 2012).

For NTR2 there were two significant treatment effects on total biomarkers (Table 1 and Fig. 3-4). First, there was a significant effect of temperature; there were significantly less biomarkers at the highest temperature. Typically microbial activity increases with temperature;

however it is dependent on the availability of a labile C. When substrate is limited there is often no increase in microbial populations with elevated temperatures (Giardina and Ryan, 2000; Hartley et al., 2007; Nadelhoffer et al., 1991). Labile C declined at 36°C (Pitumpe Arachchige, 2016). Second; there was a significant effect of sampling time on total biomarkers; there was a decrease in total biomarkers at the last sampling time (180 days); indicating a decline in microbial populations. Labile C data showed a decline in labile C 180 days for this soil; which is likely the reason for the decline in microbial biomass (Pitumpe Arachchige, 2016). Like in CTR2, the pH in NTR2 declined below 6.0 in the higher temperature treatments can could also be a reason for the decline at 36°C (Fierer and Jackson, 2005; Pitumpe Arachchige, 2016).The lack of effect of moisture on NTR2 could be due to increased aggregate stability, which has been shown to increase resilience to changing environmental conditions (Jiang et al., 2013; Trivedi et al., 2015). The lack of effect of crushing on NTR2 when compared to CTR2 was possibly due to mineral stabilization. In Oxisols the complexation of organic C with soil minerals (often Fe and Al oxides) is an important SOC stabilization mechanism that does not rely on aggregation (Pitumpe Arachchige, 2016; Siqueira Vendrame et al., 2011; Thaymuang et al., 2013).

In both the Oxisol and the Mollisol, there was less of an effect of moisture on the no-till soil when compared to the conventional tilled soil. No-till soils have higher organic carbon contents that facilitate better adsorption of water to soil particles, reducing sensitivity to lower water contents. Part of this resilience is due to better aggregate structure (Balota et al., 2004; Jiang et al., 2013; Rusu et al., 2013). Additionally there were less interactive effects on the no-till soils. The reduced impact of altered climate on no-till soils possibly indicates better climate resilience. Enhanced resilience to changing climate in no-till soils has been reported by others (Alvaro-Fuentes and Paustian, 2011; Farina et al., 2011; Jiang et al., 2013).

### *Fungi*

Fungi are an important part of soil ecosystems. They typically consume more recalcitrant C (i.e. lignin) and are important in the formation of soil aggregates. Elevated fungal populations are linked to enhanced soil carbon storage through their improvement of soil structure and their production of recalcitrant by-products (chitins) (Chaer et al., 2009; Smith et al., 2014). Measuring fungal populations allows for insight into soil carbon storage and soil aggregate structure (Bossuyt et al., 2001; Majumder Bidisha et al., 2010; Wilson et al., 2009b).

In the CTM soil, there was a significant three way interaction effect between temperature, aggregation, and sampling time on fungal biomarkers. In the crushed soil, there was no change in fungi over time or between temperature treatments. In the intact soil, there were several spikes in fungal biomarkers. There was an increase in fungal biomarkers at 30 days (primarily 12°C and 24°C) and at 180 days (primarily 36°C). Fungal populations are typically highly sensitive to soil disturbance; which follows that none of the increases in fungal biomarkers were in the crushed soil (Rice, 2013; Swedrzynska, 2013). Kladivko (2001) found that the sensitivity of soil organisms to disturbances was correlated with the size of the organism. Fungi are eukaryotes and often multicellular, and thus much larger than bacteria (Kladivko, 2001). The delay in effect with the 36°C temperature treatment could also be due to an inherent temperature sensitivity of fungi. Several authors have found fungi to be more sensitive to changes in temperature and moisture than bacteria (Gray et al., 2011; Swedrzynska, 2013; Waldrop and Firestone, 2004b).

With NTM, there was an effect between sampling time and aggregation on fungal biomarkers. There was a spike in fungal biomarkers at 60 days in the intact soil. Once again; the sudden increase was in the intact soil; which is expected given the sensitivity of fungi to disturbance. The suddenness of the spike may also be due to issues with random sampling of soil. Mummey et al (2009) found a high degree of spatial variability in fungal population; which

means that the dramatic rise in fungi seen in this soil could be due to sampling error (Mummey et al., 2010). While there was no quantitative measurement; spots of fungal growth were observed in the intact soils during the incubation process (see Fig. 3-36 in Supplementary Materials). Although soils were well mixed prior to sub-sampling for PLFA analysis and precautions (such as mixing soil well before and after freeze drying); that does not make this issue impossible. Finally, as observed with total biomarkers, there was no effect of temperature or moisture in the no-till soil; which is possibly indicating greater resilience to changing temperature or moisture conditions.

In CTR2, there were two significant treatment effects on fungal biomarkers. First was an interaction effect between sampling time and aggregation. There was a spike in fungal populations at 60 days in the intact soil. Like in the previous two soils, this increase in fungal populations only occurs in the intact soil. The second significant difference was an interaction effect between temperature and sampling time. There was an increase in fungal biomarkers in the 24°C treatment at 60 days; and a decrease in fungal biomarkers at 180 days for the 36°C treatment. Fungal populations have been shown in the literature to be sensitive to higher temperatures; so it follows that fungal populations would respond more positively to the cooler treatments. The lower treatments were more within the ideal range for soil fungi species (Gray et al., 2011; Swedrzynska, 2013; Waldrop and Firestone, 2004a).

For NTR2 there were two significant effects of treatments on fungal biomarkers. First was an interaction effect between sampling time and aggregation. There was a spike in fungal biomarkers in the intact soil at 120 days. It is interesting to note that in both no-till soils the spike in fungal population happened a time period later than in conventional tilled counterpart. The second significant difference was another interaction effect; this time between aggregate and

temperature. There was a decrease in fungal population at 36°C for the intact soil; which follows the above observations on fungi and elevated temperatures.

Across all soils there was a general trend of increases in fungal populations in the intact soil and of decreases at 36°C. Fungi are known to be sensitive to both aggregation and temperature (Chaer et al., 2009; Gray et al., 2011; Smith et al., 2014; Swedrzynska, 2013; Waldrop and Firestone, 2004a; Wilson et al., 2009b). This is important because fungi are important for soil aggregate structure and soil carbon storage. Destruction of fungal populations via fungicides has been shown to decrease soil aggregate structure and soil carbon storage potential (Bossuyt et al., 2001; Wilson et al., 2009c) . Practices that decrease fungal populations reduce the resilience of soil carbon by decreasing aggregate protection.

### *Actinomycetes*

Actinomycetes are a group of colony forming gram positive bacteria that are important in the decomposition of more recalcitrant C compounds (Abdulla, 2007; Helfrich et al., 2015; Krsek and Wellington, 2001).

In CTM, there were opposing trends in the intact versus crushed soil. In the intact soil there was an increase in actinomycete biomarkers in the beginning of the study. Conversely, in the crushed soil there was a decrease in actinomycete biomarkers at the end of the study. Since actinomycetes typically consume less labile C; the reason for this oppositional effect is likely not changes in the labile C; overall C reductions in the soil. There was also an interaction effect between temperature and sampling time; there was a decrease in actinomycete biomarkers at 180 days for the 36°C treatment. The decrease in actinomycetes at higher temperatures has been reported by others; possibly indicating temperature sensitivity (Pold et al., 2015; Waldrop and Firestone, 2004a). This may also be indicative of changes in the availability of actinomycete

substrate. Analysis with Nuclear Magnetic Resonance (NMR) revealed a decrease in the amount of aliphatic C over the course of the experiment; which could correlate to use of SOC by actinomycetes (Pitumpe Arachchige, 2016).

With NTM, there was a single interaction effect, between sampling time and aggregation. In the intact soil only, there was an increase in actinomycetes over time; with no temporal changes in the crushed soil. Despite the potential for temperature sensitivity, which was observed in CTM, there was no effect of temperature in the NTM soil. The lack of response in microbial populations to elevated temperature was reported in another study. A study in China looking at seasonal changes in microbial populations in a rice-canola system on a high OM Anthrosol (WRB classification system; highly degraded due to long-term agricultural use. Based on OM and pH data this soil would likely be considered a Mollisol under U.S. Soil Taxonomy System) found that in no-till soils there was less change in actinomycete populations with the season. Further analysis found that the least amount of change with season in microbial populations was inside macroaggregates and the most change in the microaggregates (Jiang et al., 2013). While not looking directly at the mechanisms responsible, a number of other studies have found enhanced and more stable microbial populations in no-till soils (Balota et al., 2004; Jia et al., 2016; Kladivko, 2001) This was found to be especially true for larger soil organism, like fungi, earthworms, and actinomycete colonies (Kladivko, 2001).

In CTR2, there were two significant treatment effects on actinomycete biomarkers. First was an interaction effect between temperature and sampling time. There was a decrease in actinomycete biomarkers at 180 days for the 36°C treatment only; which was also reported and discussed for the CTM soil. There was a significant decrease in labile C and actinomycetes at 180 days and at 36°C. It is likely that similar mechanisms were responsible for the same trend in

both CTM and CTR2. As discussed in the section on total biomarkers, there was a decline in pH at 36°C by the end of the experiment; which could also have impacted the actinomycete population. Actinomycetes tend to be more sensitive to changes in pH than other microbial groups (Fierer and Jackson, 2005) However, the second significant difference was not found in CTM or NTM and that was an effect of moisture. More actinomycetes were present in the wetter soil. The elevated count of actinomycete biomarkers in wetter soils has been reported in the literature (Manucharova et al., 2007; Zenova et al., 2007; Zvyagintsev et al., 2007) Higher water contents are preferable for actinomycetes to enter reproductive growth in (Zenova et al., 2007; Zvyagintsev et al., 2007). The clays in this soil are primarily 1:1 clays (i.e. kaolinite) which often have a lower water holding capacity than the 2:1 clays (i.e. montmorillonite) that are found in the KS soil (Bradford and Blanchar, 1999; Thomas and Moody, 1962). This may explain why there was no trend with moisture observed in the KS soil. Water content at field capacity for CTR2 was actually higher than that of CTM yet there was an effect with CTR2 and not CTM.

For NTR2 there were two significant interaction effects on actinomycete biomarkers. First, there was an interaction effect between temperature and sampling time; there were less actinomycete biomarkers at 36°C, at the end of the study. This is similar to what was observed and discussed in CTM and CTR2. Like in CTR2, the pH of NTR2 went below 6.0 at 36°C which could also explain the decline in actinomycetes (Fierer and Jackson, 2005). While the no-till KS soil had less response to temperature, this was not true for the Brazil soil. There was an interaction effect between soil moisture and sampling time; the drier moisture condition had more actinomycetes at the end of the study. More actinomycetes in the drier moisture condition was not expected and was opposite to what occurred in CTR2. While actinomycetes usually prefer moist conditions, changes in soil moisture storage in the no-till soil (due to higher SOC)

could alter what is considered ideal moisture content. No-till soils tend to store and transport water more effectively than their conventional tilled counterparts (Balota et al., 2004; Panettieri et al., 2015; Rusu et al., 2013). This difference in water storage and transport may be responsible for the changes seen in NTR2 when compared to CTR2.

Actinomycetes response differed the most between the Mollisol and the Oxisol. In the Mollisol, there was no effect of soil water in either the no-till or the conventional tilled soil. In the Oxisol, both the no-till and the conventional till were affected by soil water conditions. This is likely due to differences in soil mineralogy (Information on the mineralogy of these soils can be found in supplementary materials (3-36, 3-37)) and the sensitivity of actinomycetes to soil water (Bradford and Blanchar, 1999; Thomas and Moody, 1962; Zenova et al., 2007; Zvyagintsev et al., 2007).

### *Gram Positive Bacteria*

Gram positive bacteria are a large and diverse group of microorganisms that include actinomycetes (Mackie et al., 2015; Rollins and Joseph, 2004). Gram positive bacteria consume a wide variety of C compounds – both labile and more recalcitrant (Mbuthia et al., 2015; Rollins and Joseph, 2004). These bacteria are generally not sensitive to physical disturbance (Rollins and Joseph, 2004). Most of the responses in gram positive bacteria to changing soil conditions is due to changes in availability of C substrates (Ahmad et al., 2016; Creamer et al., 2015a; Mackie et al., 2015; Mrozik et al., 2014).

The first significant difference with CTM was an interaction effect between sampling time and aggregation. There was a decrease in gram positive biomarkers at the end of the study (180 days) for the crushed soil. Conversely, in the intact soil, there was an increase in biomarkers in the beginning of the study (30 days). The change in abundance over time in the two soils was

likely more to do with availability of substrate (as seen in labile C data) than with the disturbance. Greater availability of labile C in the crushed soil likely led to faster bacterial growth at the beginning of the study (Pitumpe Arachchige, 2016). However, without additions of new labile C; this substrate eventually is depleted and microbial populations decline; as was observed with total biomarkers. It makes sense that there would be similar trends between gram positive bacteria and total biomarkers as gram positive bacteria were the largest microbial group. Second, there was an interactive effect between sampling time and temperature. There was an increase in biomarkers at 30 days for the cooler treatment. There was also less gram positive bacteria in the 36°C treatment at 180 days when compared to other treatment combinations. Gram positive bacteria are generally not sensitive to changes in temperature (Gray et al., 2011; Wang et al., 2013; Wei et al., 2014). Once again, the decrease in gram positive bacteria at the 36 °C treatment was likely due to substrate availability rather than an effect of elevated temperature (Creamer et al., 2015b). This can be supported by cumulative $CO_2$ results. Cumulative $CO_2$ were greater at higher temperatures (Pitumpe Arachchige, 2016). Greater release of $CO_2$ indicates greater utilization of substrate; which leads to a decline in bacterial populations as the substrate declines. Labile C collaborates this hypothesis (Pitumpe Arachchige, 2016).

In NTM, there was an interaction effect between sampling time and aggregation. In the intact soil there was an increase in biomarkers from the beginning of the study to the end of the study. However, in the crushed soil, there was no significant change over time. This was likely because the crushed soil was mixed and homogenized more evenly and had a uniform distribution micro-habitat sites. The intact soil had a much wider range of aggregates and pores that possibly took a bit longer for bacteria to colonize; however the crushed soil also had a flush of labile C that would cause faster initial microbial growth. In the intact soil, labile C is made

available more slowly and over a longer period of time (Balota et al., 2004). The lack of effect of temperature on gram positive bacterial population might indicate greater resilience in the NTM soil as similar trends were seen with the NTM soil in other microbial groups.

The first significant difference for CTR2 was an interaction effect between aggregation treatment and sampling time. In the intact soil, there was an increase in gram positive biomarkers over time; while with the crushed soil, there was a decrease in biomarkers over the course of the experiment. This was likely due to similar trends in substrate availability and microbial colonization as observed and discussed for CTM and NTM. Second, there was an interaction effect between temperature and sampling time. There was a decrease in total biomarkers at 180 days for 36°C. This was also seen in CTM and once again this decrease in gram positive biomarkers was collaborated by a decrease in labile C (Pitumpe Arachchige, 2016). Third, there was an effect of moisture treatment on gram positive bacterial biomarkers; there were less biomarkers in the drier soil. Gram positive bacteria tend to be resistant to dry conditions, never the less, the drier treatment resulted in less gram positive bacteria for CTR2 (Rollins and Joseph, 2004). Labile C declined in the drier treatment in the latter part of the study (Pitumpe Arachchige, 2016).

The first significant difference with NTR2 was an effect of aggregation. There were more gram positive bacteria in the crushed soil. Gram positive bacteria are typically thought of as being resistant to physical disturbance but respond to increases in available labile C (Creamer et al., 2015b; Rollins and Joseph, 2004; Wang et al., 2013). Labile C was more available in the crushed soil; this explains the rise in gram positive bacteria in that soil.(Pitumpe Arachchige, 2016). There was also a decrease in gram positive biomarkers at the highest temperature; which

was accompanied by a decrease in labile C (Pitumpe Arachchige, 2016). For the microbes in NTR2; labile C availability was the greatest predictor of gram positive bacterial population.

Across all four soils the greatest predictor of gram positive bacteria was the availability of labile C. The moisture treatment was only significant in the conventional tilled Oxisol; most likely as a result of both weaker aggregates due to tillage practices and the kaolinite and oxy(hydr)oxides dominating mineralogy (typically low water absorption capacity minerals) found in that soil (Bradford and Blanchar, 1999; Colozzi et al., 2004; Lal, 2004a; Schlesinger and Andrews, 2000; Thomas and Moody, 1962).

### *Gram Negative Bacteria*

Gram negative bacteria are another major bacterial group. These bacteria are distinguished by their thin, yet complex cell walls. Gram negative bacteria tend to have very fast growth rates when labile C is available and there is minimal disturbance to their environment; both physical disturbances and changes in temperature and moisture regime (Gray et al., 2011; Rollins and Joseph, 2004; Wang et al., 2013; Wei et al., 2014).

There was a significant three way interaction on gram negative bacterial biomarkers in the CTM soil; the effect was between sampling time, aggregation, and temperature. In the intact soil, there was an increase in gram negative biomarkers between 60 and 120 days for the 24°C treatment. In the crushed soil there was a decrease in gram negative bacteria at 180 days in the 24°C treatment. Gram negative bacteria tend to be sensitive to both changes in temperature, disturbance, and substrate availability (Gray et al., 2011; Rollins and Joseph, 2004; Wang et al., 2013; Wei et al., 2014). The increase in the intact soil likely occurred as gram negative bacteria colonized the soil and made use of labile C as it became available (Balota et al., 2004). The

decrease in gram negative bacteria towards the end of the experiment in the crushed soil was likely a result of declining labile C (Pitumpe Arachchige, 2016).

There was an interaction effect between sampling time and aggregation on gram negative bacterial biomarkers in the NTM soil. In the intact soil, there was no change in gram negative biomarkers over time. In the crushed soil, there was an increase in gram negative biomarkers from 7 days until 60 days that was then followed by a decrease between 60 and 180 days. This appears to be due to shifts in labile C availability. Crushing the soil was observed to increase labile C; however as no new labile C is added, labile C declines as microbes use it for growth (Pitumpe Arachchige, 2016). As gram negative bacteria do not readily consume other less labile substrates, there is a die-off as the labile C runs out in the crushed soil (Rollins and Joseph, 2004).

CTR2 had a non-interactive effect of aggregation on gram negative bacteria. There were more gram negative biomarkers in the intact soil compared to the crushed. While there was greater availability of labile C in the crushed soil, there was still a decline in gram negative bacteria with crushing (Pitumpe Arachchige, 2016). Gram negative bacteria tend to be sensitive to changes in their habitat, such as the disturbance brought on by crushing and sieving the soil to <0.25mm (Rahman et al., 2008; Rollins and Joseph, 2004; Wei et al., 2014). With NTR2 the effects of physical disturbance were less pronounced. In NTR2, there were two significant treatment effects. There was a significant three way interaction effect between moisture, temperature, and aggregation and a two way interaction effect between sampling time and temperature. In the intact soil, in the drier moisture treatment, there was a decrease in gram negative bacterial biomarkers at 36°C. Second, there was an interaction effect between sampling time and temperature. There was a decrease in gram negative bacterium at 36°C and 180 days.

Both of these trends correspond to decreases in labile C, the main substrate for gram negative bacteria (Pitumpe Arachchige, 2016). Gram negative bacteria are sensitive to substrate type and environmental conditions and drop offs in their populations like this are somewhat expected (Rollins and Joseph, 2004).

Gram negative bacteria as a group were sensitive to both crushing and labile C availability across all four soils. There was a difference in how tillage practices interacted with gram negative bacteria in the Mollisol when compared to the Oxisol. In the Mollisol, no-till decreased the effect that soil conditions had on gram negative bacteria. Conversely, in the Oxisol there was a greater effect on gram negative bacteria in the no-till soil; which was unique for gram negative bacteria.

### *Total Bacteria*

Measuring total bacteria as a sum of all bacteria groups allowed for the observation of bacterial population trends as a whole. Comparing the effects of experimental variables on total bacteria to the effects on fungi is a common way to interpret results (Gray et al., 2011; Swedrzynska, 2013; Waldrop and Firestone, 2004b; Wan et al., 2015). The main purpose behind looking at total bacteria was to provide a summary on how the experimental variables affected all three bacterial groups (i.e., gram positive, gram negative and actinomycetes) that were studied.

For CTM; there were two significant treatment effects on total bacterial biomarkers. First, there was a significant interaction effect between sampling time and aggregation. For the intact soil, there was an increase in total bacterial biomarkers between 7 days and 30 days. While for the crushed soil, there was a decrease in bacterial biomarkers between 120 and 180 days. At 7 days and again at 60 days there were more bacterial biomarkers in the crushed soil. The labile C

data shows that there is greater labile C in the crushed soil until the end of the experiment (Pitumpe Arachchige, 2016). Second, there was an interaction effect between temperature and sampling time. There was an increase in bacteria between 7 and 30 days for the 12°C treatment. Microbial growth is often slowed at low temperatures, which is a probable cause for this trend (Conant et al., 2011; Davidson and Janssens, 2006d; Leifeld and von Lützow, 2014). These trends were a reflection largely of the gram positive biomarkers, as gram positive bacteria made up a large majority of bacterial biomarkers.

For NTM there was a single significant effect; an interaction effect between sampling time and aggregation. In the intact soil, there was an increase in biomarkers over the course of the experiment (7 to 180 days). In the crushed soil there was no change in biomarkers over time. In the first two sampling times (7 days and 30 days) there were more bacteria in the crushed soil. Crushing homogenizes the soil and makes labile C easily accessible, which fuels quick microbial growth. The lack of mixing and protection of SOC in the intact soil lead to a steady increase in microbial population as labile C becomes available more slowly. Aggregation has been shown to protect soil organic carbon from microbial attack and a portion of this is possibly due to the physical protection of labile C (Balota et al., 2004; Majumder Bidisha et al., 2010; Wang et al., 2014b). Labile C increased with crushing (Pitumpe Arachchige, 2016). The slower increase in the intact soil was likely due to the more complex habitat that aggregation provides; allowing for slower microbial growth that protects soil organic carbon (Balota et al., 2004).

In CTR2, there were three significant treatment effects on total bacterial biomarkers. First, there was a significant interaction between sampling time and aggregation. In the intact soil, there was an increase in biomarkers between 7 and 120 days. In the crushed soil, the inverse occurred; there was a decrease in biomarkers between 7 days and 180 days. Second,

there was an interaction effect between sampling time and temperature. At 36°C and 180 days there was a decrease in total bacteria. Third; there was a decrease in total bacteria in the drier moisture treatment. All three of these effects follow along with trends seen in labile C data (Pitumpe Arachchige, 2016). Bacterial populations tend to change fairly quickly with changes in availability of labile C (Creamer et al., 2015b; Rahman et al., 2008; Wang et al., 2013). There was no effect of moisture on NTR2; as seen in previous microbial groups this is possible an indication of greater resilience.

For NTR2, there were two treatment effects on total bacterial biomarkers; there was a decrease in biomarkers at the highest temperature, and there was an increase in bacteria in the crushed soil. Labile C data explains both trends; there was more available labile C in the crushed soil when compared to the intact soil and less at the highest temperature (Pitumpe Arachchige, 2016).

Overall we observed more changes in microbial populations in the tilled soils when compared to the no-till soils. This decreased influence of experimental variables on the no-till soils is indicative of greater resilience. No-till improves the structure and strength of soil aggregates; particularly macroaggregates. The increased prevalence of macroaggregates provides greater protection of soil organic carbon from the elements and from microbial attack (Balota et al., 2004; Jiang et al., 2013; Majumder Bidisha et al., 2010; Wang et al., 2014b).

## Conclusions

In general across biomarkers (except for gram negative bacteria) there seems to be a trend of greater influence of temperature and/or moisture conditions on the conventional-tilled soils when compared to the no-till soils. Part of this seems to be due to the influence of the increased aggregate stability in the no-till soils that has a stabilizing effect on microbial populations (Jiang

et al., 2013; Majumder Bidisha et al., 2010). No-till management improves soil quality in ways that promotes resilience to changes in temperature and moisture. Adopting no-till can be a useful tool for farmers to adapt to climate change (Alvaro-Fuentes and Paustian, 2011; Balota et al., 2004; Farina et al., 2011).

When comparing the Oxisol to the Mollisol; it appears that the resilience in the microbial population to changing climate was less influenced by tillage practices in the Oxisol. There also appears to be a greater influence of moisture on the microbial populations in the Oxisol when compared to the Mollisol. A difference in the response of microbial populations from different soils to changing environments has been reported by other authors (Smith et al., 2014; Wang et al., 2014a). These soils have very different mineralogy that is possibly causing differences in response to the same treatments.. Another possible explanation is the difference in total nitrogen (as seen in Table 1); Wan et al (2015) noted that C:N ratio had a significant impact on soil microbial populations (Wan et al., 2015). The lower C:N ration in the KS soils could also explain some of the differences observed.

## Acknowledgements

We thank the National Science Foundation for their funding (Award No. NSF EPS-0903806-Sub award KCUR NSF73698). We would like to thank Oshadhi Athukorala Arachchige and Caleb Gravesen for help with sample preparation and analysis. We appreciated the assistance of Andrew McGowan with GC-MS analysis and with the PLFA data analysis. We are grateful for Dr. Nora Bello's statistical consultation.

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# Tables

**Table 3-1: General properties for pre-incubation soils. Adapted from (Pitumpe Arachchige, 2016).**

| General Soil Properties | | | | | |
|---|---|---|---|---|---|
| **Soil** | **pH** | **TOC (%)** | **Labile C (mg/kg)** | **Water content (g/g)** | **Total N (%)** |
| CTM | 7.58 | 2.78 | 1142 | 0.091 | 0.33 |
| NTM | 7.46 | 5.05 | 1843 | 0.124 | 0.63 |
| CTR2 | 5.58 | 1.77 | 604 | 0.115 | 0.16 |
| NTR2 | 6.44 | 2.93 | 1261 | 0.18 | 0.25 |

**Table 3-2: Statistical information for total PLFA biomarkers. Data was transformed using log base 10 for statistical operations to achieve a normal distribution. Values significant to p < 0.05 are highlighted and discussed.**

| p-values for Total Biomarkers | | | | |
|---|---|---|---|---|
| **Effect** | **CTM** | **NTM** | **CTR2** | **NTR2** |
| Temperature | 0.0012 | 0.0612 | <.0001 | **<.0001** |
| Moisture | **0.0345** | 0.6093 | **0.0138** | 0.7742 |
| Temperature * Moisture | 0.6374 | 0.3741 | 0.5339 | 0.4526 |
| Aggregate | 0.809 | 0.5359 | 0.6537 | 0.9993 |
| Temperature * Aggregate | 0.0741 | 0.8143 | 0.634 | 0.5077 |
| Moisture * Aggregate | 0.6534 | 0.5454 | 0.3219 | 0.3569 |
| Temperature * Moisture* Aggregate | 0.3718 | 0.8276 | 0.1243 | 0.5748 |
| Sampling Time | 0.0009 | 0.0006 | <.0001 | **0.0066** |
| Temperature * Sampling Time | **0.014** | 0.5255 | **0.0018** | 0.1832 |
| Moisture* Sampling Time | 0.8892 | 0.9576 | 0.1793 | 0.5081 |
| Temperature * Moisture * Sampling Time | 0.5465 | 0.5033 | 0.2717 | 0.9786 |
| Aggregate * Sampling Time | **<.0001** | **<.0001** | 0.1358 | 0.0763 |
| Temperature * Aggregate * Sampling Time | 0.1245 | 0.3867 | 0.6483 | 0.9953 |
| Moisture * Aggregate * Sampling Time | 0.4825 | 0.8834 | 0.4414 | 0.9251 |
| Temperature * Moisture * Aggregate * Sampling Time | 0.6386 | 0.7379 | 0.8214 | 0.8147 |

**Table 3-3: Statistical information for fungal PLFA biomarkers. Data was transformed using log base 10 for statistical operations to achieve a normal distribution. Values significant to p < 0.05 are highlighted and discussed.**

| p-values for Fungal Biomarkers | | | | |
|---|---|---|---|---|
| **Effect** | **CTM** | **NTM** | **CTR2** | **NTR2** |
| Temperature | 0.0119 | 0.7029 | <.0001 | <.0001 |
| Moisture | 0.0764 | 0.6093 | 0.7878 | 0.3895 |
| Temperature * Moisture | 0.8467 | 0.345 | 0.3981 | 0.5545 |
| Aggregate | 0.0001 | 0.0078 | 0.0003 | <.0001 |
| Temperature * Aggregate | 0.8909 | 0.6341 | 0.0531 | **0.0422** |
| Moisture * Aggregate | 0.1014 | 0.3925 | 0.3892 | 0.7015 |
| Temperature * Moisture* Aggregate | 0.342 | 0.4039 | 0.2612 | 0.9385 |
| Sampling Time | 0.0003 | <.0001 | <.0001 | <.0001 |
| Temperature * Sampling Time | 0.0373 | 0.1961 | **0.0218** | 0.1841 |
| Moisture* Sampling Time | 0.3617 | 0.6225 | 0.4845 | 0.2805 |
| Temperature * Moisture * Sampling Time | 0.4208 | 0.08 | 0.7415 | 0.777 |
| Aggregate * Sampling Time | <.0001 | **<.0001** | **0.0015** | **<.0001** |
| Temperature * Aggregate * Sampling Time | **0.0296** | 0.5831 | 0.1566 | 0.6309 |
| Moisture * Aggregate * Sampling Time | 0.8878 | 0.907 | 0.4141 | 0.9543 |
| Temperature * Moisture * Aggregate * Sampling Time | 0.283 | 0.5601 | 0.8923 | 0.4763 |

**Table 3-4: Statistical information for actinomycete PLFA biomarkers. Data was transformed using log base 10 for statistical operations to achieve a normal distribution. Values significant to p < 0.05 are highlighted and discussed.**

| p-values for Actinomycete Biomarkers | | | | |
|---|---|---|---|---|
| **Effect** | **CTM** | **NTM** | **CTR2** | **NTR2** |
| Temperature | 0.0002 | 0.3953 | <.0001 | <.0001 |
| Moisture | 0.5026 | 0.1278 | **0.0378** | 0.2763 |
| Temperature * Moisture | 0.4695 | 0.8648 | 0.2532 | 0.5626 |
| Aggregate | 0.0001 | 0.8298 | 0.1958 | 0.0092 |
| Temperature * Aggregate | 0.0669 | 0.7698 | 0.7556 | 0.4839 |
| Moisture * Aggregate | 0.8477 | 0.6032 | 0.4923 | 0.0598 |
| Temperature * Moisture* Aggregate | 0.2891 | 0.9483 | 0.1144 | 0.784 |
| Sampling Time | <.0001 | <.0001 | <.0001 | 0.097 |
| Temperature * Sampling Time | **0.0133** | 0.6629 | **<.0001** | **0.0086** |
| Moisture* Sampling Time | 0.6017 | 0.9763 | 0.075 | **0.0061** |
| Temperature * Moisture * Sampling Time | 0.4006 | 0.6188 | 0.079 | 0.905 |
| Aggregate * Sampling Time | **<.0001** | **<.0001** | 0.3117 | 0.3223 |
| Temperature * Aggregate * Sampling Time | 0.0859 | 0.4606 | 0.7142 | 0.966 |
| Moisture * Aggregate * Sampling Time | 0.2561 | 0.8516 | 0.1982 | 0.4271 |
| Temperature * Moisture * Aggregate * Sampling Time | 0.4477 | 0.8399 | 0.8981 | 0.6247 |

**Table 3-5: Statistical information for gram positive bacterial biomarkers. Data was transformed using log base 10 for statistical operations to achieve a normal distribution. Values significant to p < 0.05 are highlighted and discussed.**

| p-values for Gram Positive Bacterial Biomarkers | | | | |
|---|---|---|---|---|
| **Effect** | **CTM** | **NTM** | **CTR2** | **NTR2** |
| Temperature | 0.079 | 0.3286 | <.0001 | **0.0094** |
| Moisture | 0.0842 | 0.6451 | **0.0351** | 0.7367 |
| Temperature * Moisture | 0.4979 | 0.6242 | 0.4387 | 0.403 |
| Aggregate | 0.0004 | 0.2606 | 0.0004 | **0.0099** |
| Temperature * Aggregate | 0.1034 | 0.7562 | 0.9484 | 0.6317 |
| Moisture * Aggregate | 0.9646 | 0.4379 | 0.3709 | 0.1567 |
| Temperature * Moisture* Aggregate | 0.2286 | 0.8721 | 0.2722 | 0.7821 |
| Sampling Time | 0.0187 | 0.0054 | 0.0324 | 0.5022 |
| Temperature * Sampling Time | **0.0188** | 0.4007 | **0.0009** | 0.3672 |
| Moisture* Sampling Time | 0.7165 | 0.9626 | 0.1086 | 0.1654 |
| Temperature * Moisture * Sampling Time | 0.4308 | 0.4969 | 0.254 | 0.6206 |
| Aggregate * Sampling Time | **<.0001** | **<.0001** | **0.0213** | 0.4279 |
| Temperature * Aggregate * Sampling Time | 0.0653 | 0.4483 | 0.3122 | 0.9954 |
| Moisture * Aggregate * Sampling Time | 0.2691 | 0.6904 | 0.2908 | 0.8303 |
| Temperature * Moisture * Aggregate * Sampling Time | 0.6035 | 0.846 | 0.8264 | 0.7348 |

**Table 3-6: Statistical information for gram negative bacterial biomarkers. Data was transformed using log base 10 for statistical operations to achieve a normal distribution. Values significant to p < 0.05 are highlighted and discussed.**

| p-values for Gram Negative Bacterial Biomarkers | | | | |
|---|---|---|---|---|
| **Effect** | **CTM** | **NTM** | **CTR2** | **NTR2** |
| Temperature | 0.2924 | 0.1584 | 0.1895 | 0.0004 |
| Moisture | 0.8378 | 0.8098 | 0.08 | 0.3948 |
| Temperature * Moisture | 0.2498 | 0.4425 | 0.0644 | 0.8919 |
| Aggregate | 0.1984 | 0.0736 | **0.0094** | 0.421 |
| Temperature * Aggregate | 0.0505 | 0.6295 | 0.2869 | 0.0481 |
| Moisture * Aggregate | 0.1803 | 0.6196 | 0.1037 | 0.227 |
| Temperature * Moisture* Aggregate | 0.6302 | 0.9343 | 0.1349 | **0.0088** |
| Sampling Time | 0.0003 | 0.1016 | 0.1003 | 0.0018 |
| Temperature * Sampling Time | 0.0522 | 0.9339 | 0.727 | **0.0045** |
| Moisture* Sampling Time | 0.9135 | 0.9997 | 0.2681 | 0.9459 |
| Temperature * Moisture * Sampling Time | 0.4878 | 0.7715 | 0.5268 | 0.4691 |
| Aggregate * Sampling Time | 0.0075 | **0.0008** | 0.3316 | 0.7242 |
| Temperature * Aggregate * Sampling Time | **0.0023** | 0.0639 | 0.6011 | 0.2054 |
| Moisture * Aggregate * Sampling Time | 0.1819 | 0.7755 | 0.4014 | 0.8909 |
| Temperature * Moisture * Aggregate * Sampling Time | 0.8908 | 0.153 | 0.2376 | 0.9484 |

**Table 3-7: Statistical information for total bacterial biomarkers. Data was transformed using log base 10 for statistical operations to achieve a normal distribution. Values significant to p < 0.05 are highlighted and discussed.**

| p-values for Total Bacterial Biomarkers | | | | |
|---|---|---|---|---|
| **Effect** | **CTM** | **NTM** | **CTR2** | **NTR2** |
| Temperature | 0.0651 | 0.3045 | <.0001 | **0.0085** |
| Moisture | 0.0829 | 0.6852 | **0.0355** | 0.7276 |
| Temperature * Moisture | 0.4949 | 0.5658 | 0.5936 | 0.4081 |
| Aggregate | 0.0004 | 0.3045 | 0.0004 | **0.0094** |
| Temperature * Aggregate | 0.0857 | 0.7516 | 0.9399 | 0.6239 |
| Moisture * Aggregate | 0.9294 | 0.4195 | 0.3153 | 0.1582 |
| Temperature * Moisture* Aggregate | 0.2394 | 0.8718 | 0.2267 | 0.7765 |
| Sampling Time | 0.0139 | 0.0079 | 0.0462 | 0.4998 |
| Temperature * Sampling Time | **0.0155** | 0.3885 | **0.0029** | 0.3684 |
| Moisture* Sampling Time | 0.7273 | 0.9464 | 0.1732 | 0.1734 |
| Temperature * Moisture * Sampling Time | 0.437 | 0.533 | 0.2997 | 0.6255 |
| Aggregate * Sampling Time | **<.0001** | **<.0001** | **0.0235** | 0.48 |
| Temperature * Aggregate * Sampling Time | 0.0548 | 0.4335 | 0.2935 | 0.9945 |
| Moisture * Aggregate * Sampling Time | 0.2558 | 0.737 | 0.3137 | 0.8323 |
| Temperature * Moisture * Aggregate * Sampling Time | 0.6147 | 0.8324 | 0.7704 | 0.7492 |

# Figures

## *PLFA Data*

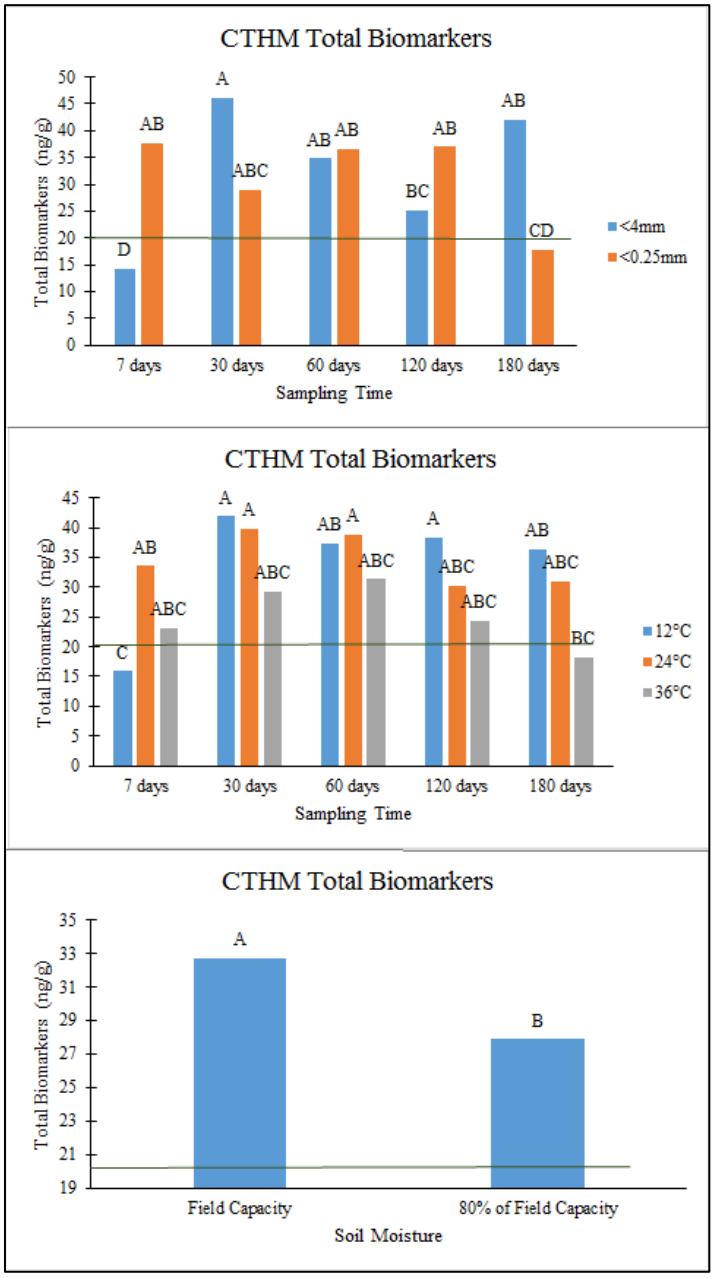


**Figure 3-1: Total biomarker trends with CTM. Green line indicates value in initial soil. Top: Effect of sampling time * aggregation interaction. Letters indicate differences significant to p<0.05 across sampling times. Middle: Effect of sampling time * temperature interaction. Letters indicate differences significant to p<0.05 across sampling times. Bottom: Effect of moisture on total biomarkers. Letters indicate differences significant to p<0.05.**

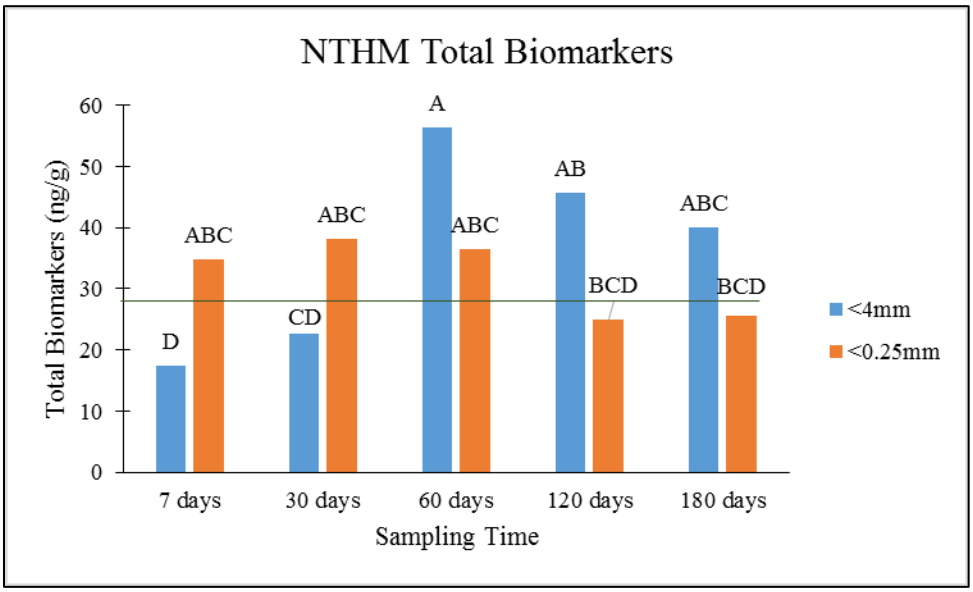


**Figure 3-2: Total biomarker trends with NTM. Letters indicate differences significant to $p<0.05$ across sampling times. Green line indicates value in original soil.**

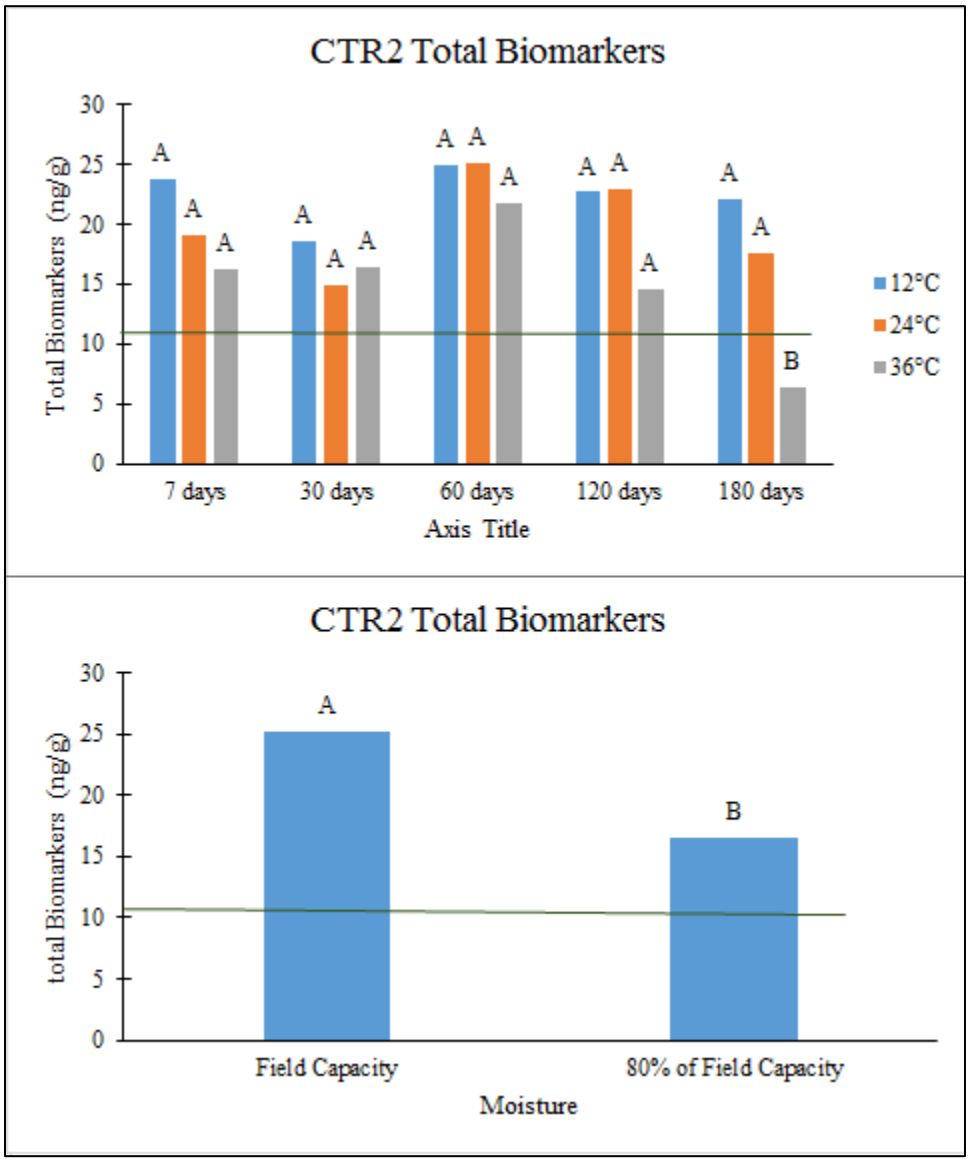


**Figure 3-3: Total biomarker trends for CTR2. Green line indicates value in initial soil. Top: Effect of sampling time * temperature interaction. Letters indicate differences significant to $p<0.05$ across sampling times. Bottom: Effect of moisture. Letters indicate differences significant to $p<0.05$.**

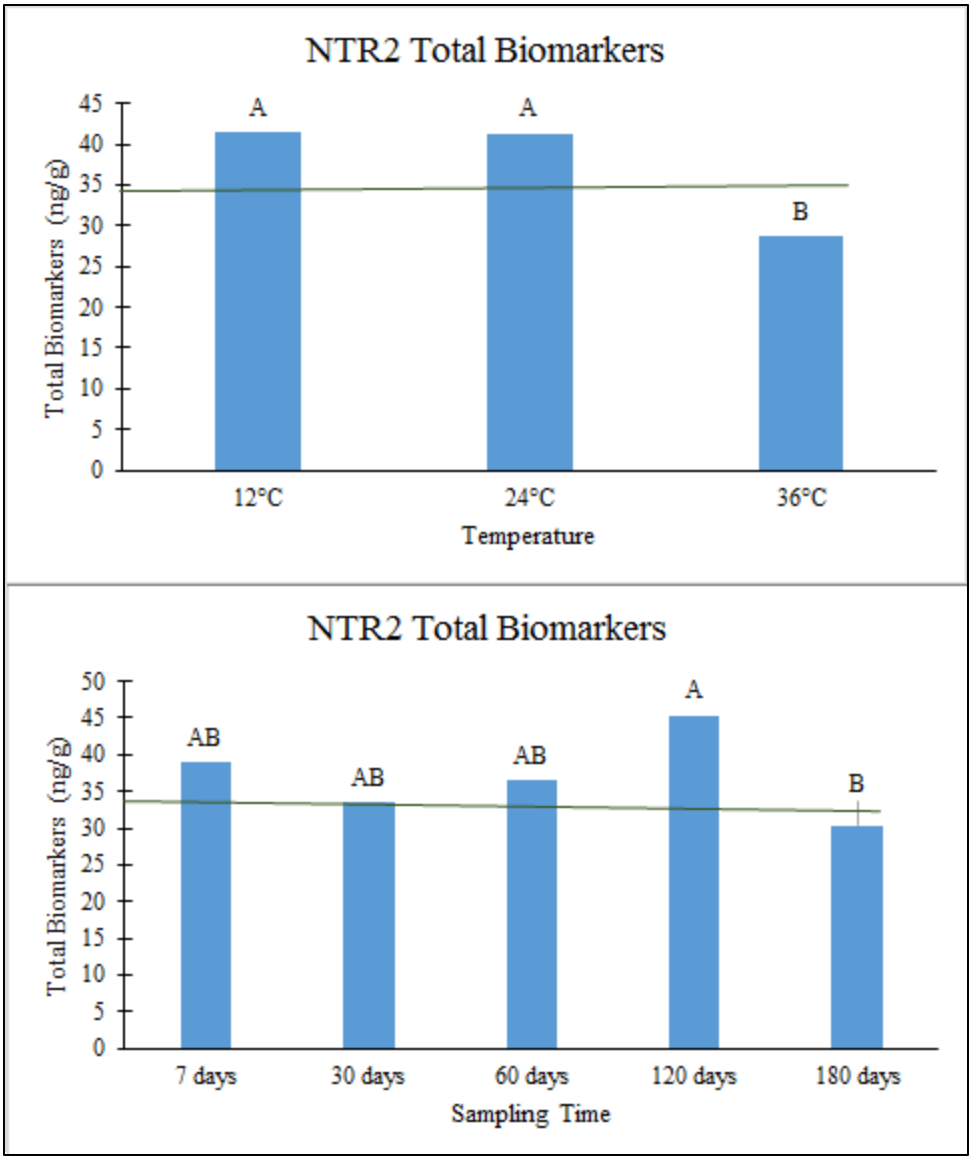


**Figure 3-4: Total biomarker trends for NTR2. Green line indicates value in initial soil. Top: Effect of temperature on total biomarkers. Letters indicate differences significant to $p<0.05$. Bottom: Effect of sampling time on total biomarkers. Letters indicate differences significant to $p<0.05$ across sampling times.**

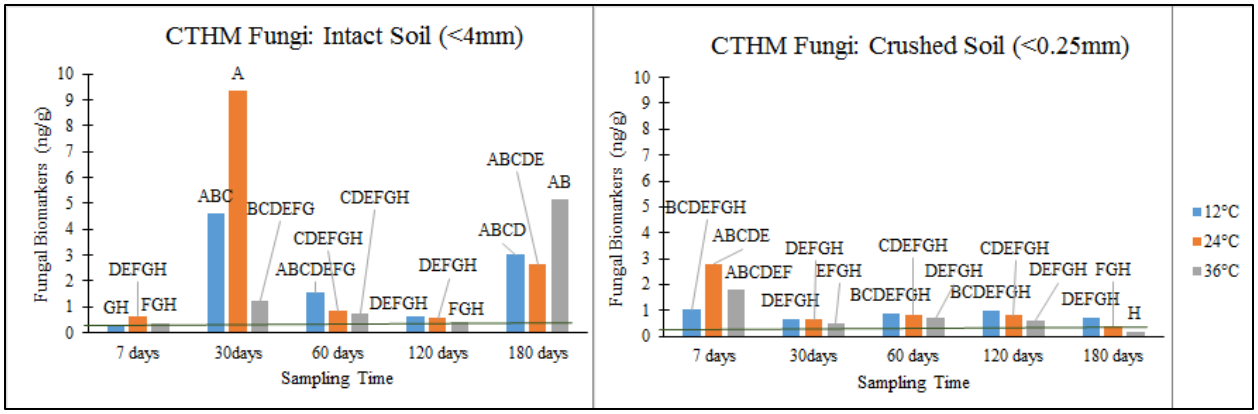


**Figure 3-5: Fungal biomarkers for CTM had a three way significant interaction effect. Green line indicates value in initial soil. Left: Intact Soil; Right: Crushed. Letters indicate differences to $p<0.05$ across both intact and crushed soils and across all sampling times.**

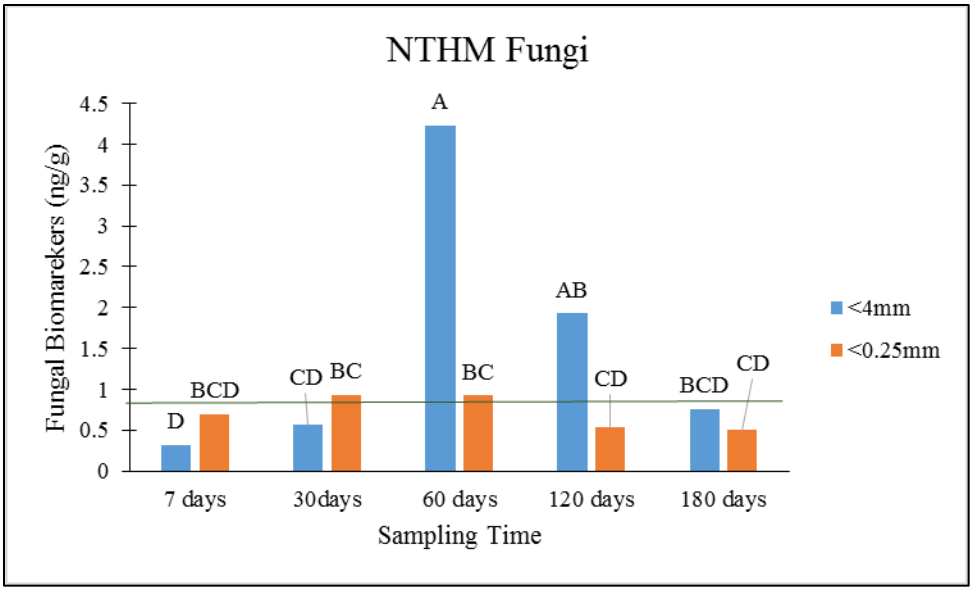


**Figure 3-6: There was a significant interaction effect between sampling time and aggregation on NTM for fungal biomarkers. Green line indicates value in initial soil. Letters indicate differences significant to $p<0.05$ across sampling times.**

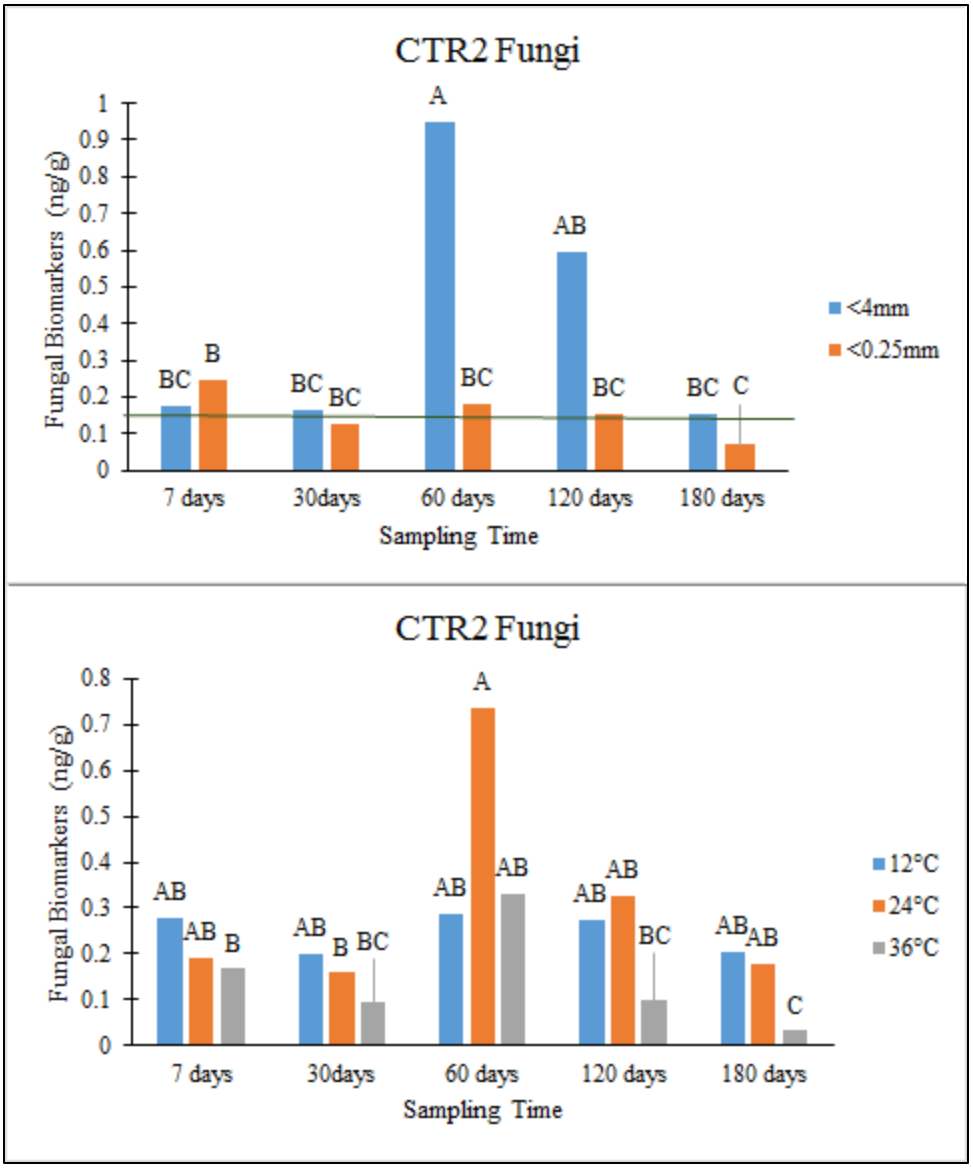


**Figure 3-7: Effects of treatments on fungal biomarkers for CTR2. Top: Interaction effect between sampling time and aggregation. Letters indicate differences significant to p<0.05 across sampling times. Bottom: Interaction effect between temperature and sampling time. Letters indicate differences significant to p<0.05 across sampling times.**

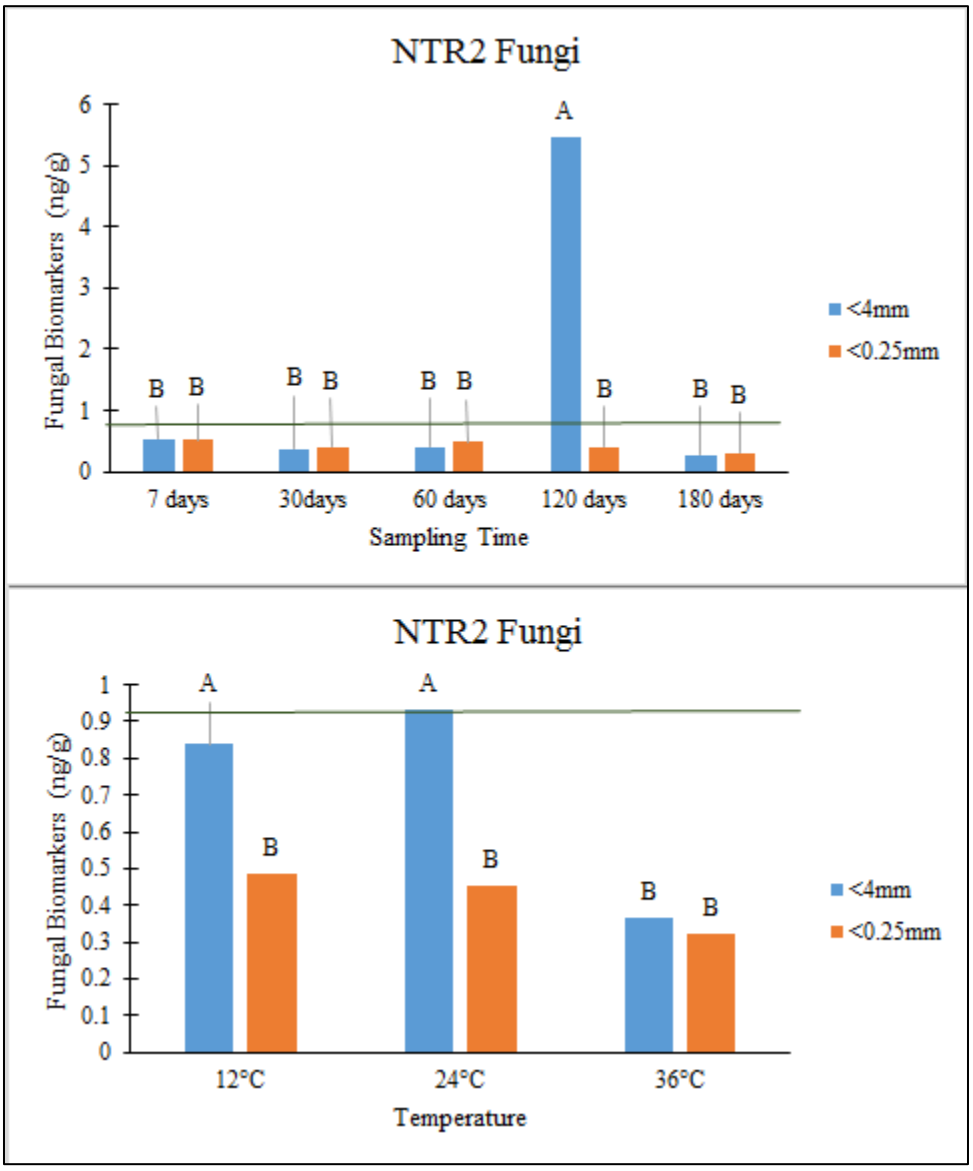


**Figure 3-8: Effects of treatments on fungal biomarkers for NTR2. Green lines indicate value in initial soil. Top: Interaction effect between sampling time and aggregation. Letters indicate differences significant to $p<0.05$ across sampling times. Bottom: Interaction effect between aggregate and temperature. Letters indicate differences significant to $p<0.05$ across temperatures.**

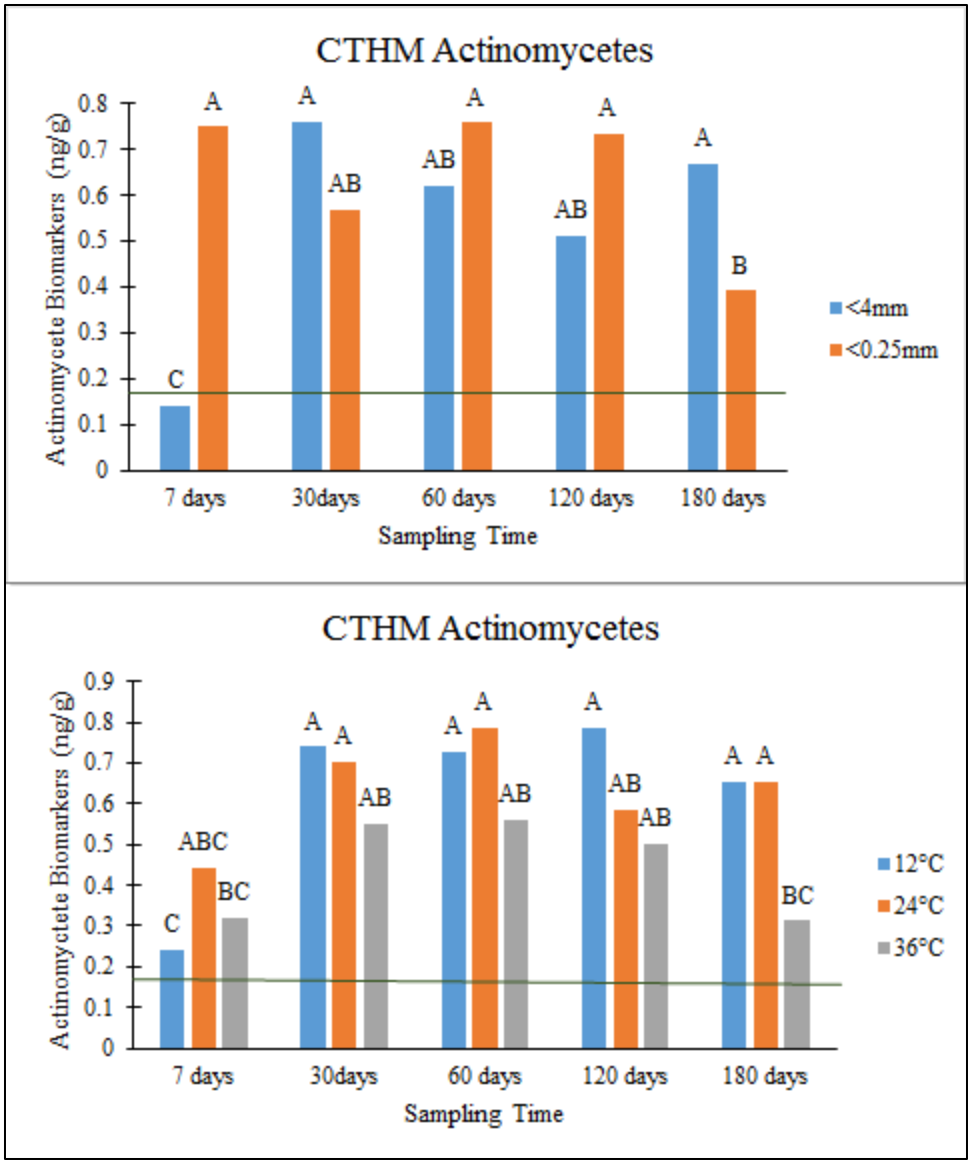


**Figure 3-9: Effects of treatments on CTM actinomycete biomarkers. Green lines indicate value in initial soil. Top: Interaction effect between sampling time and aggregation. Letters indicate differences significant to p<0.05 across sampling times. Bottom: Interaction effect between temperature and sampling time. Letters indicate differences significant to p<0.05 across sampling times.**

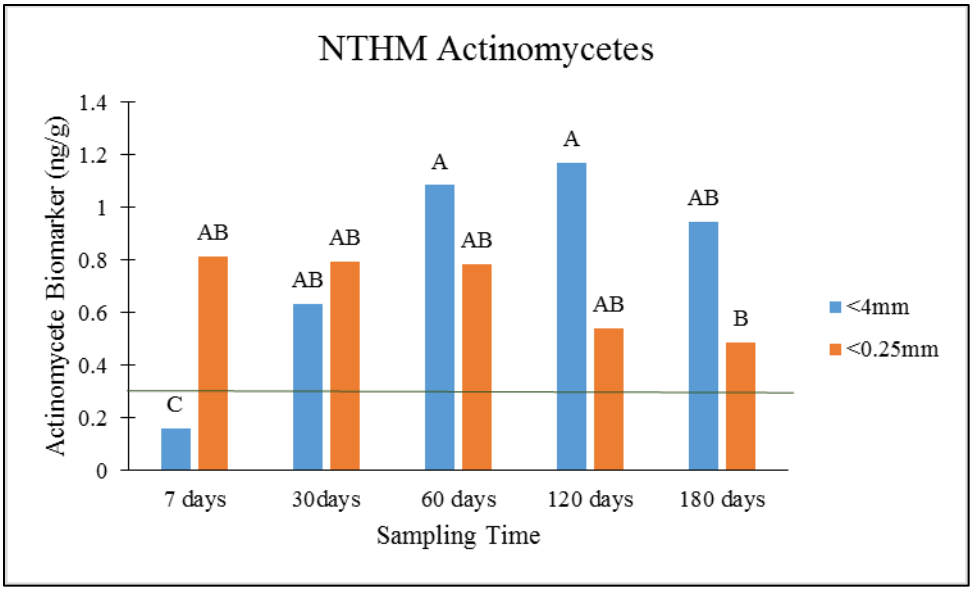


**Figure 3-10: Interaction effect between sampling time and aggregate on NTM actinomycete biomarkers. Green line indicates value in original soil. Letters indicate differences significant to $p<0.05$ across sampling times.**

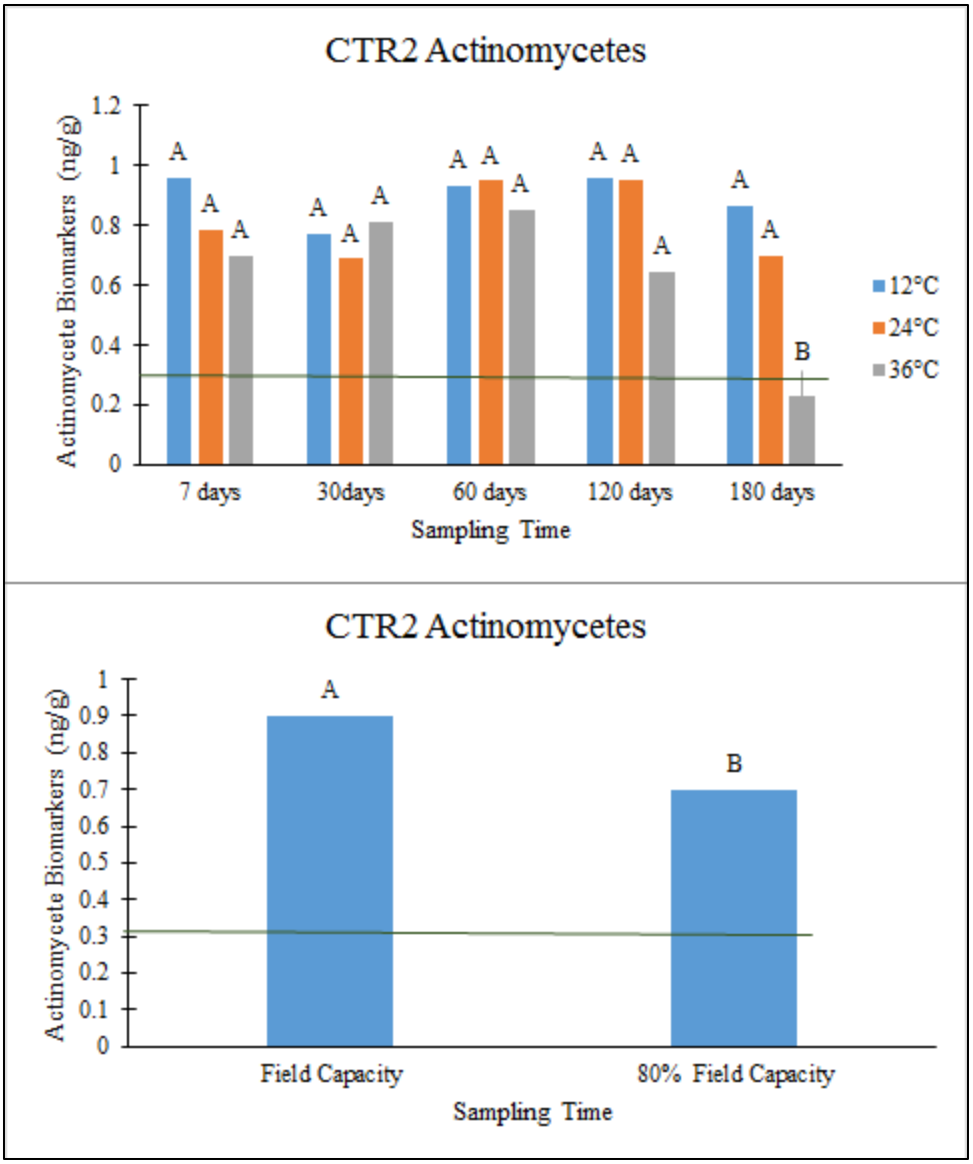


**Figure 3-8: Effects of treatments on actinomycete biomarkers for CTR2. Green lines indicates value in original soil. Top: Interaction effect between temperature and sampling time. Letters indicate differences significant to $p<0.05$ across sampling times. Bottom: Effect of moisture; letters indicate differences significant to $p<0.05$.**

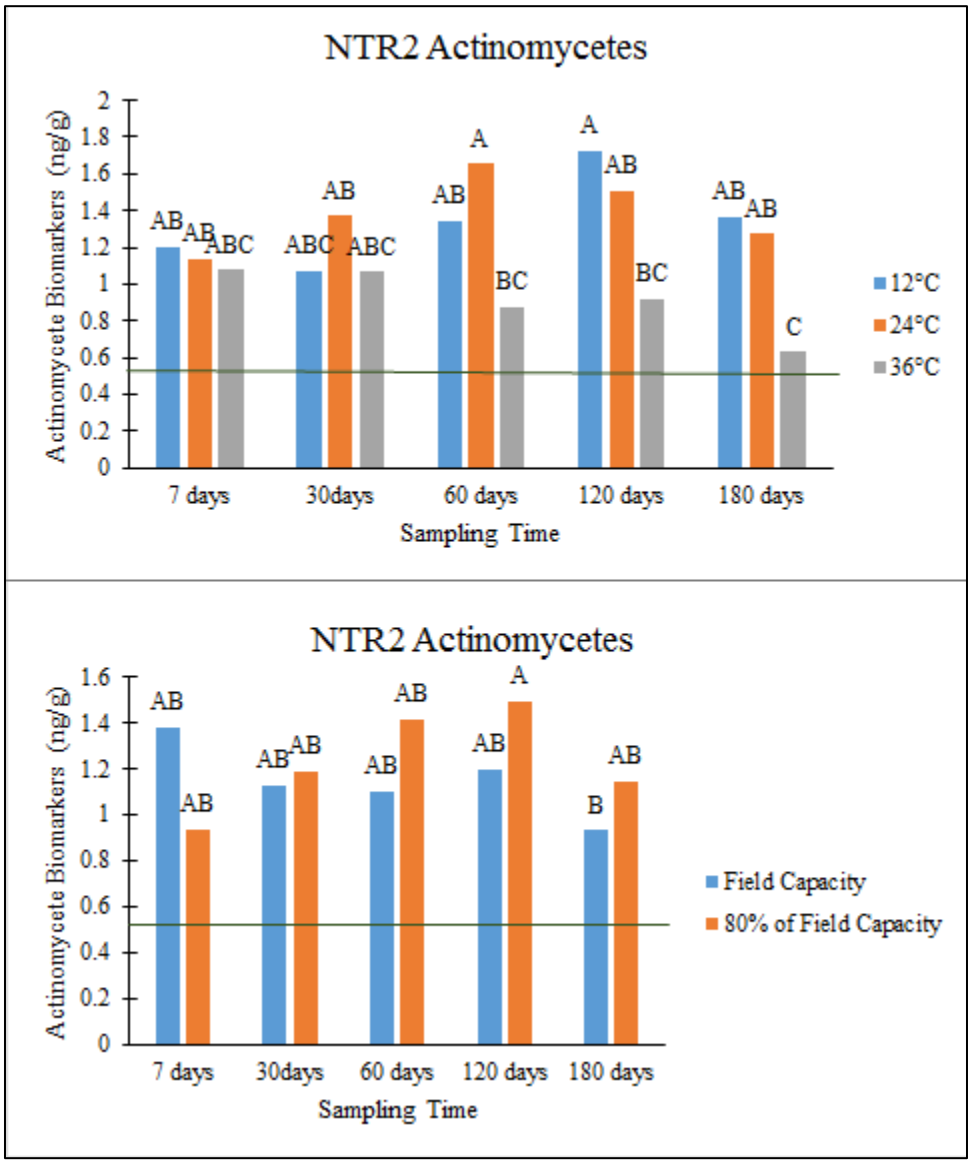


**Figure 3-9: Effects of treatments on actinomycete biomarkers for NTR2. Green lines indicates value in original soil. Top: Interactive effect between temperature and sampling time on NTR2 biomarkers. Letters indicate differences significant to $p<0.05$ across sampling times. Bottom: Interaction effect between soil moisture and sampling time on actinomycete biomarkers. Letters indicate differences significant to $p<0.05$ across sampling times.**

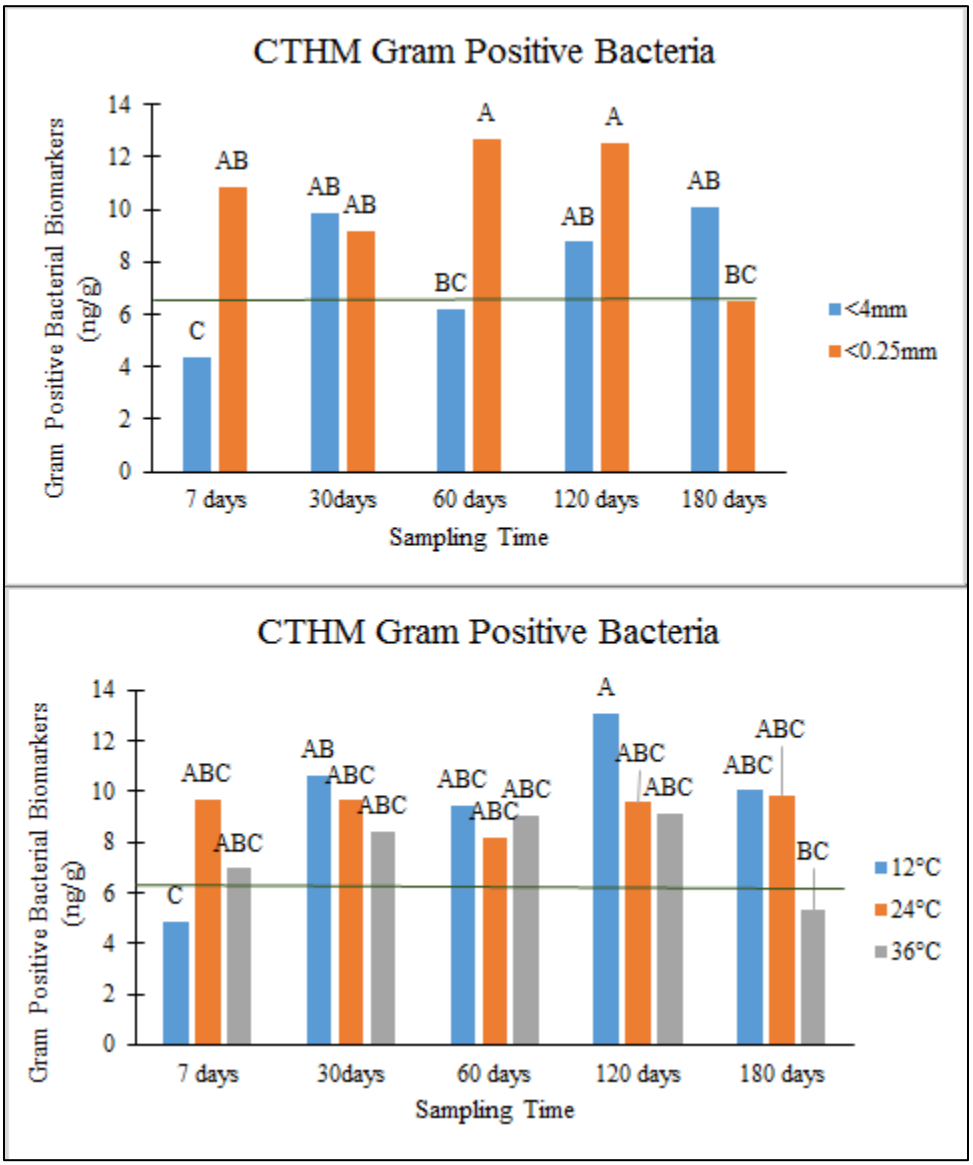


**Figure 3-10: Effects of treatments on gram positive bacterial biomarkers for CTM. Green lines indicate values in original soil. Top: There was an interaction effect between sampling time and aggregation. Letters indicate differences significant to $p<0.05$ across sampling times. Bottom: Interaction effect between temperature and sampling time. Letters indicate differences significant to $p<0.05$ across sampling times.**

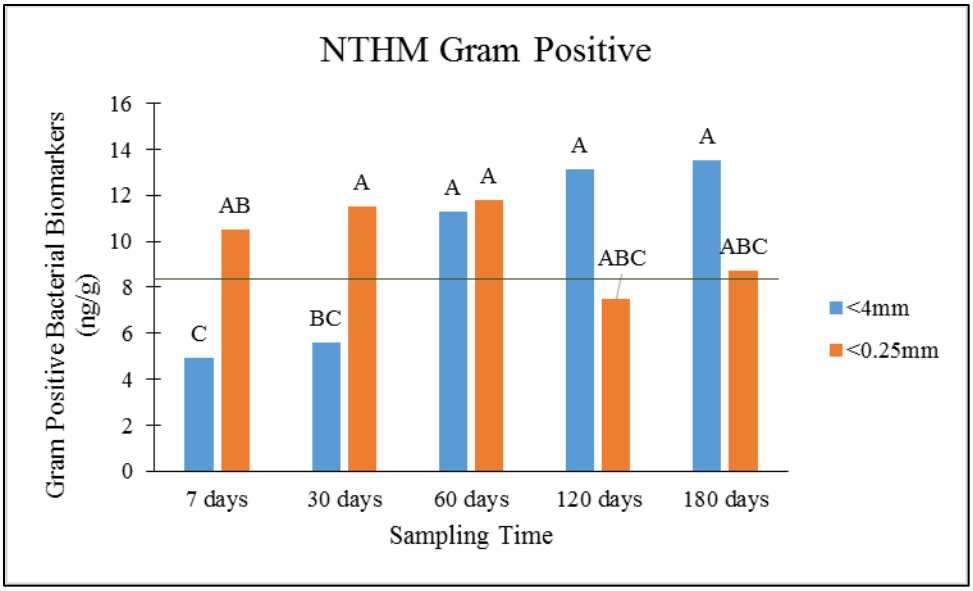


**Figure 3-11: Effect of the interaction between sampling time and aggregation on gram positive bacterial biomarkers. Letters indicate differences significant to $p<0.05$ across sampling times.**

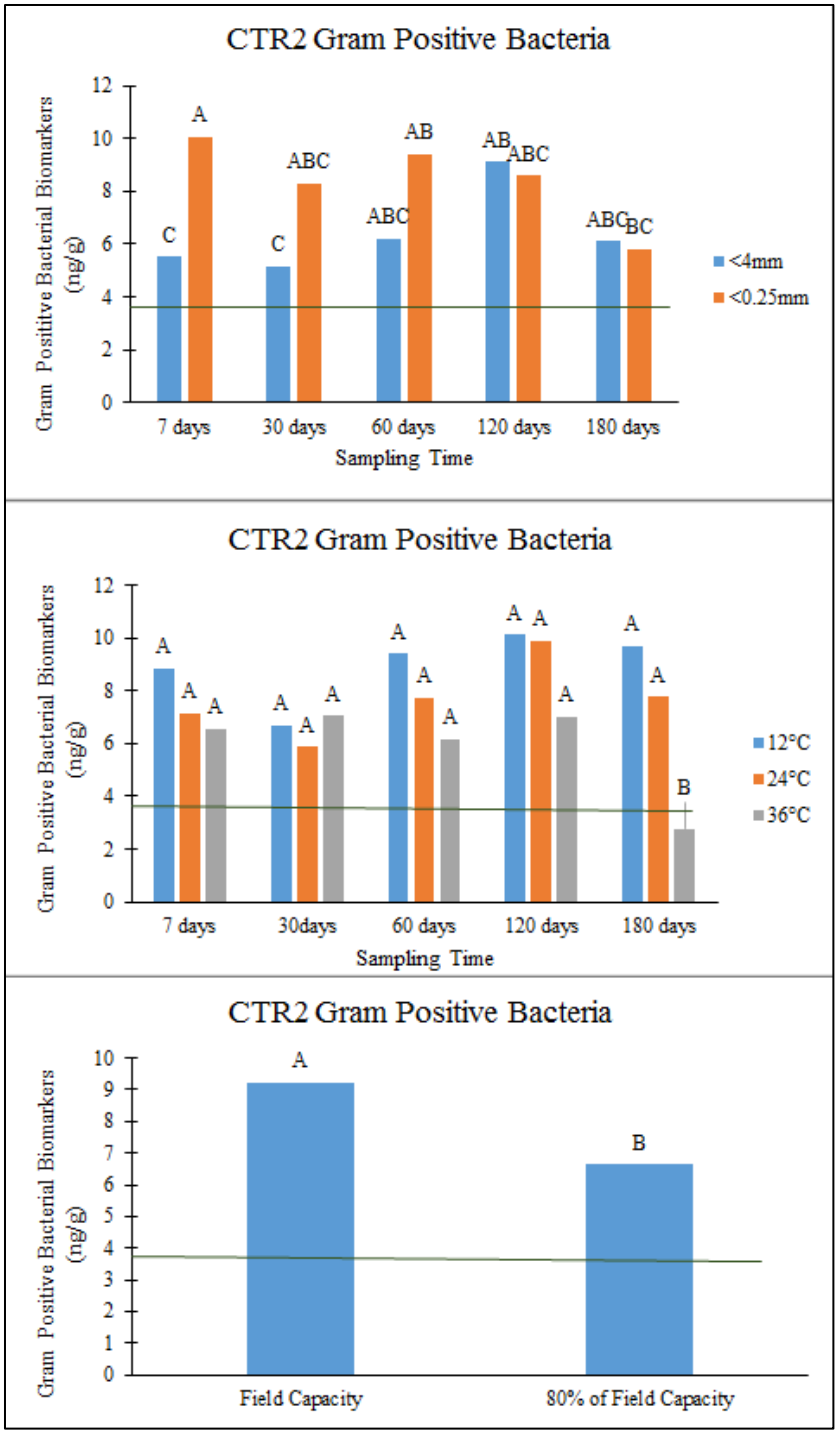


**Figure 3-12: Effects of treatments on gram positive bacterial biomarkers for CTR2. Green lines indicate values in original soil. Top: Interaction effect between aggregation and sampling time. Letters indicate differences significant to $p<0.05$ across sampling times. Middle: Interaction effect between temperature and sampling time. Letters indicate differences significant to $p<0.05$ across sampling times. Bottom: There was an effect of moisture on gram positive bacterial biomarkers; letters indicate differences significant to $p<0.05$.**

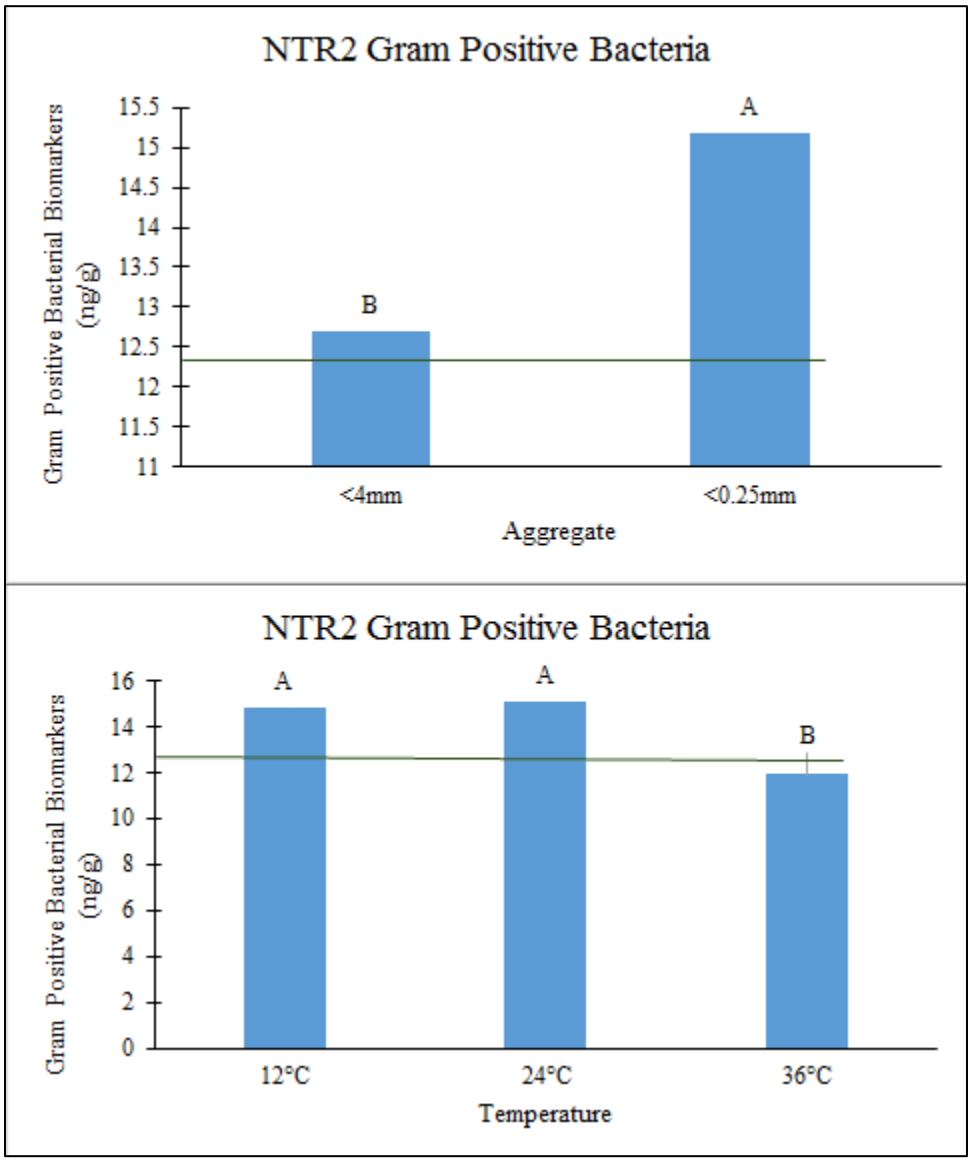


**Figure 3-13: Effect of treatments on gram positive bacterial biomarkers for NTR2. Green lines indicate values in original soil. Top: the effect of aggregation on gram positive bacteria. Letters indicate differences significant to p<0.05 across sampling times. Bottom: The effect of temperature on gram positive bacteria. Letters indicate differences significant to p<0.05 across sampling times.**

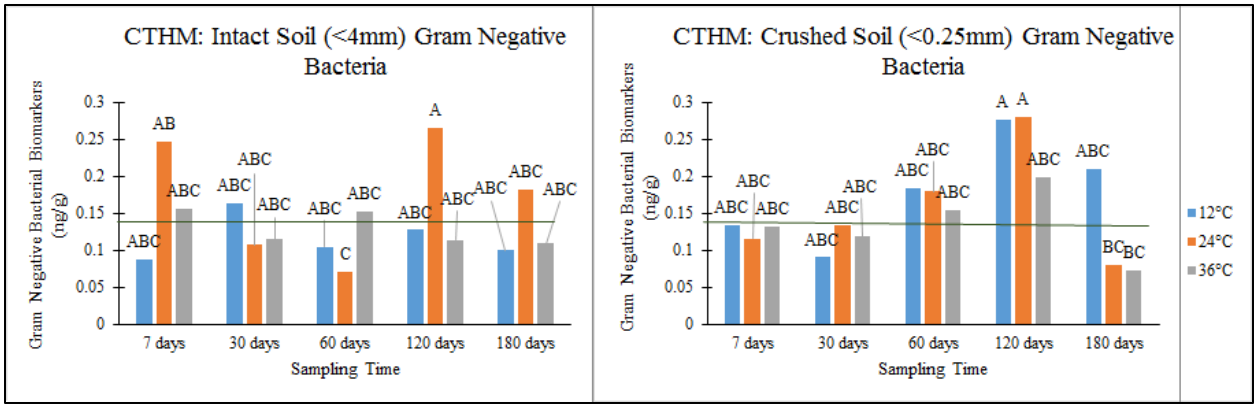


**Figure 3-14: There was a significant three way interaction between sampling time, aggregation, and temperature on gram negative bacterial biomarkers for CTM. Green lines indicate values in initial soil. Left: Intact Soil; Right: Crushed. Letters indicate differences to $p<0.05$ across both intact and crushed soils and across all sampling times.**

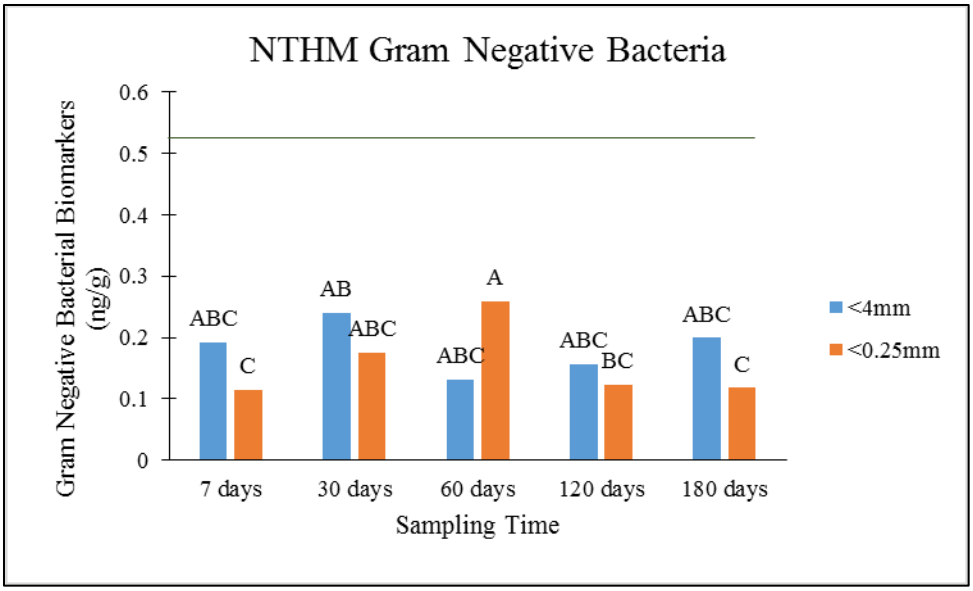


**Figure 3-15: There was an interaction effect between sampling time and aggregation on gram negative bacterial biomarkers for NTM. Green line indicates value in initial soil. Letters indicate differences to $p<0.05$ across all sampling times.**

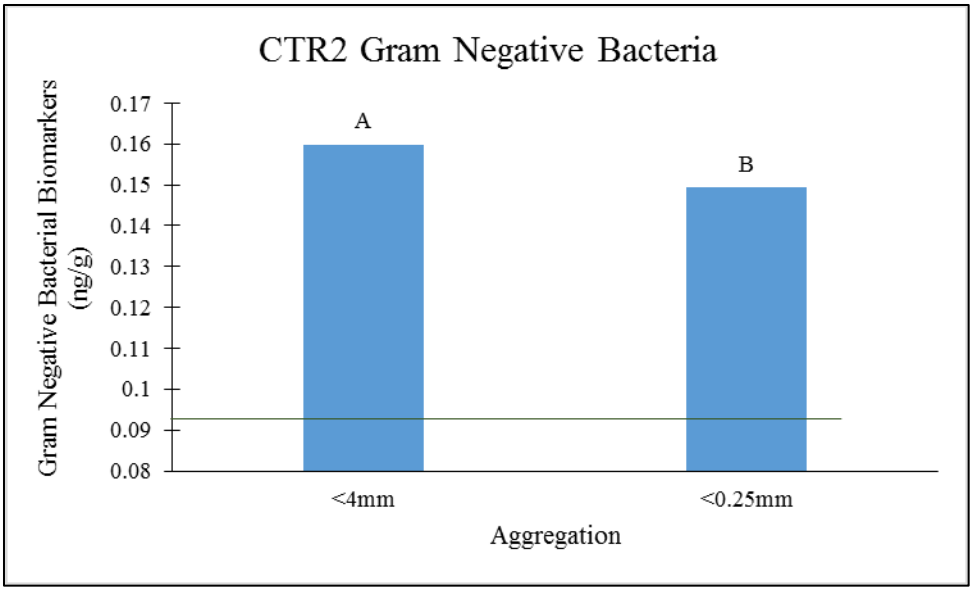


**Figure 3-16: There was a significant effect of aggregation on gram negative bacterial biomarkers. Green line indicates value in initial soil. Letters indicate differences to p<0.05.**

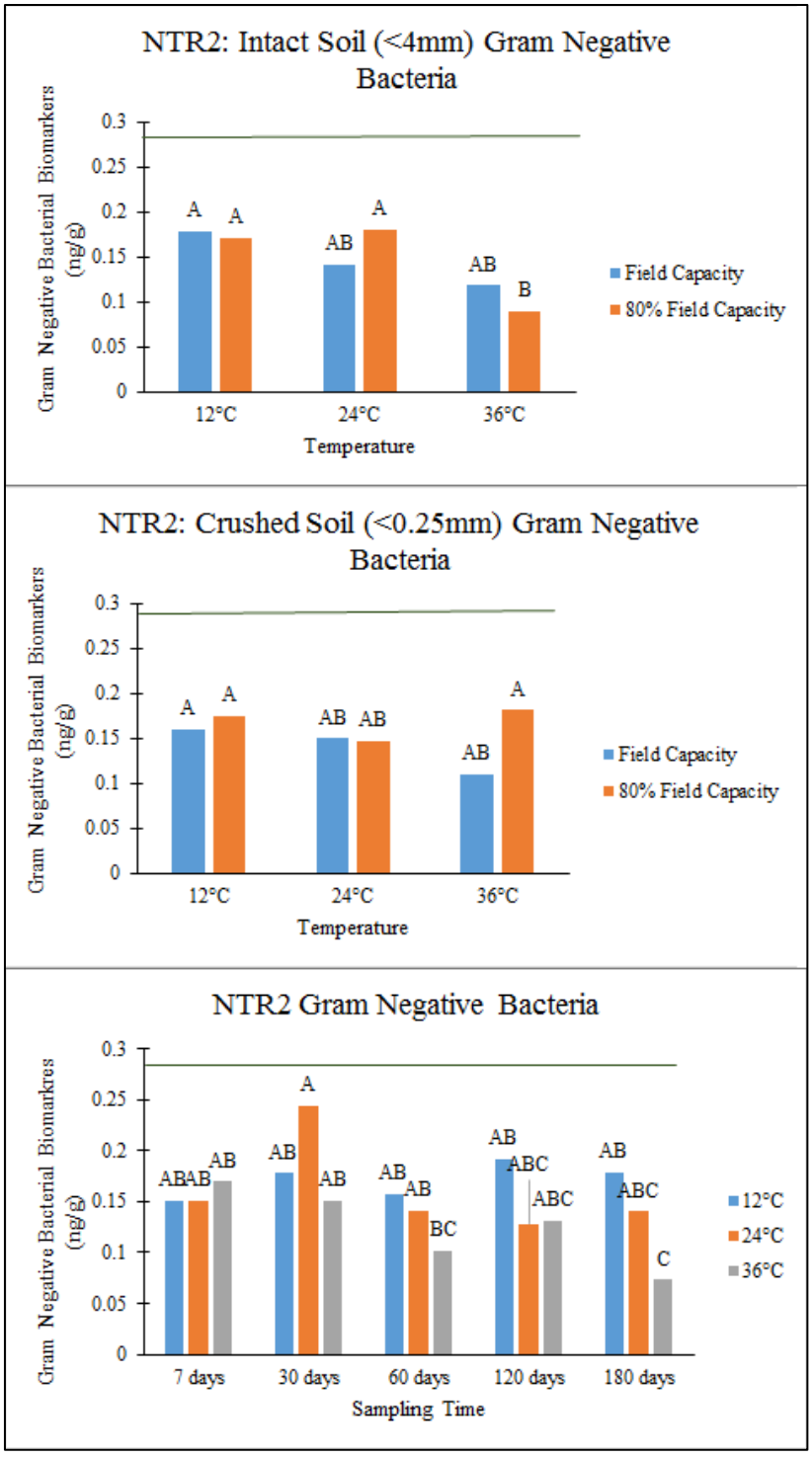


**Figure 3-20: Effects of treatments on gram negative bacteria for NTR2. Green lines indicate values in initial soil. Top/Middle: there was a three way interaction effect between moisture, temperature, and aggregation. Letters indicate differences to $p<0.05$ across both intact and crushed soils and across all sampling times. Bottom: interaction effect between temperature and sampling time. Letters indicate differences to $p<0.05$ across all sampling times.**

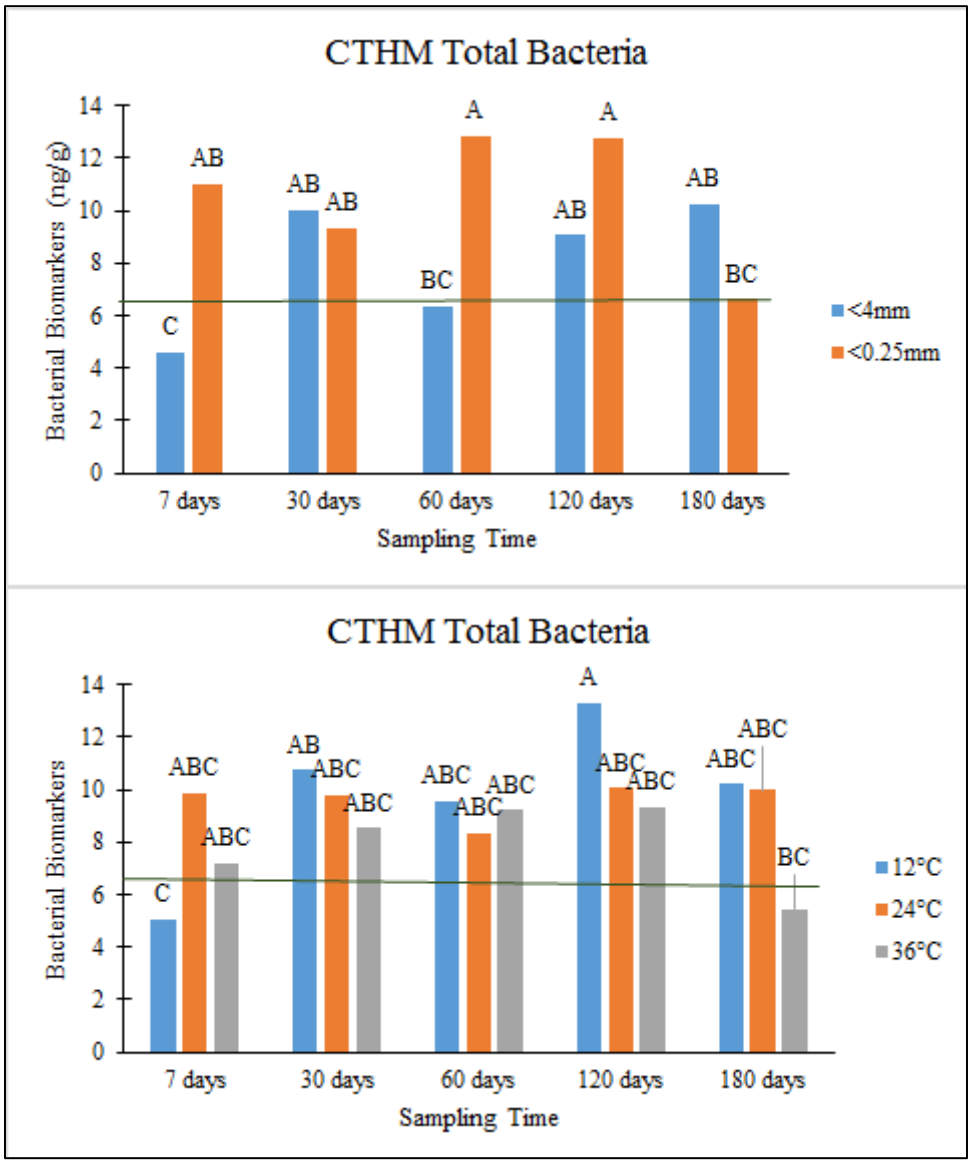


**Figure 3-17: Effects of treatments on total bacterial biomarkers for CTM. Green line indicates value in original soil.Top: There was a significant interaction effect between sampling time and aggregation. Letters indicate differences to $p<0.05$ across all sampling times. Bottom: The effect of temperature and sampling time on total bacteria. Letters indicate differences to $p<0.05$ across all sampling times.**

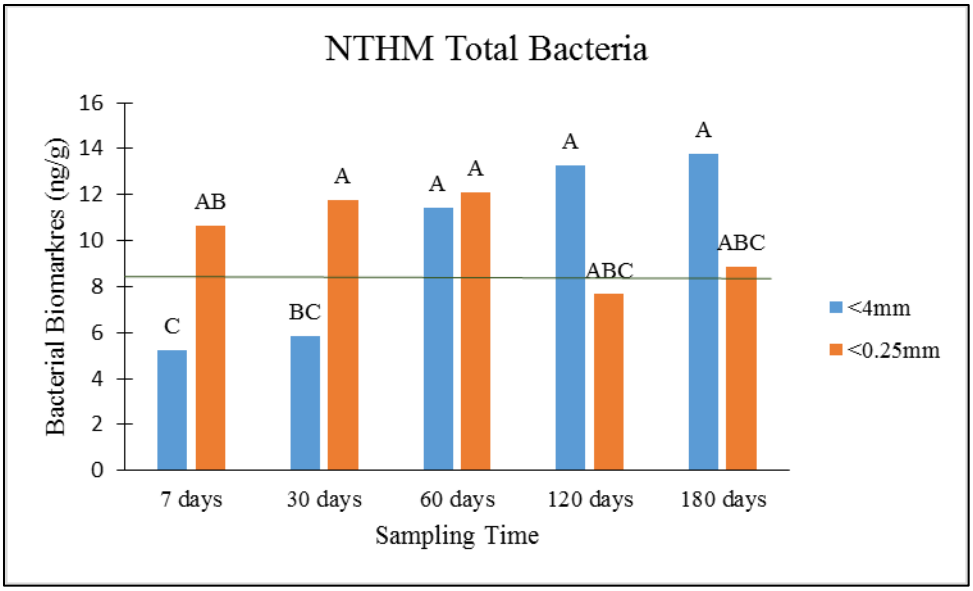


**Figure 3-18: Effect of the interaction between sampling time and aggregation on bacterial biomarkers for NTM. Green line indicates value in original soil. Letters indicate differences to $p<0.05$ across all sampling times.**

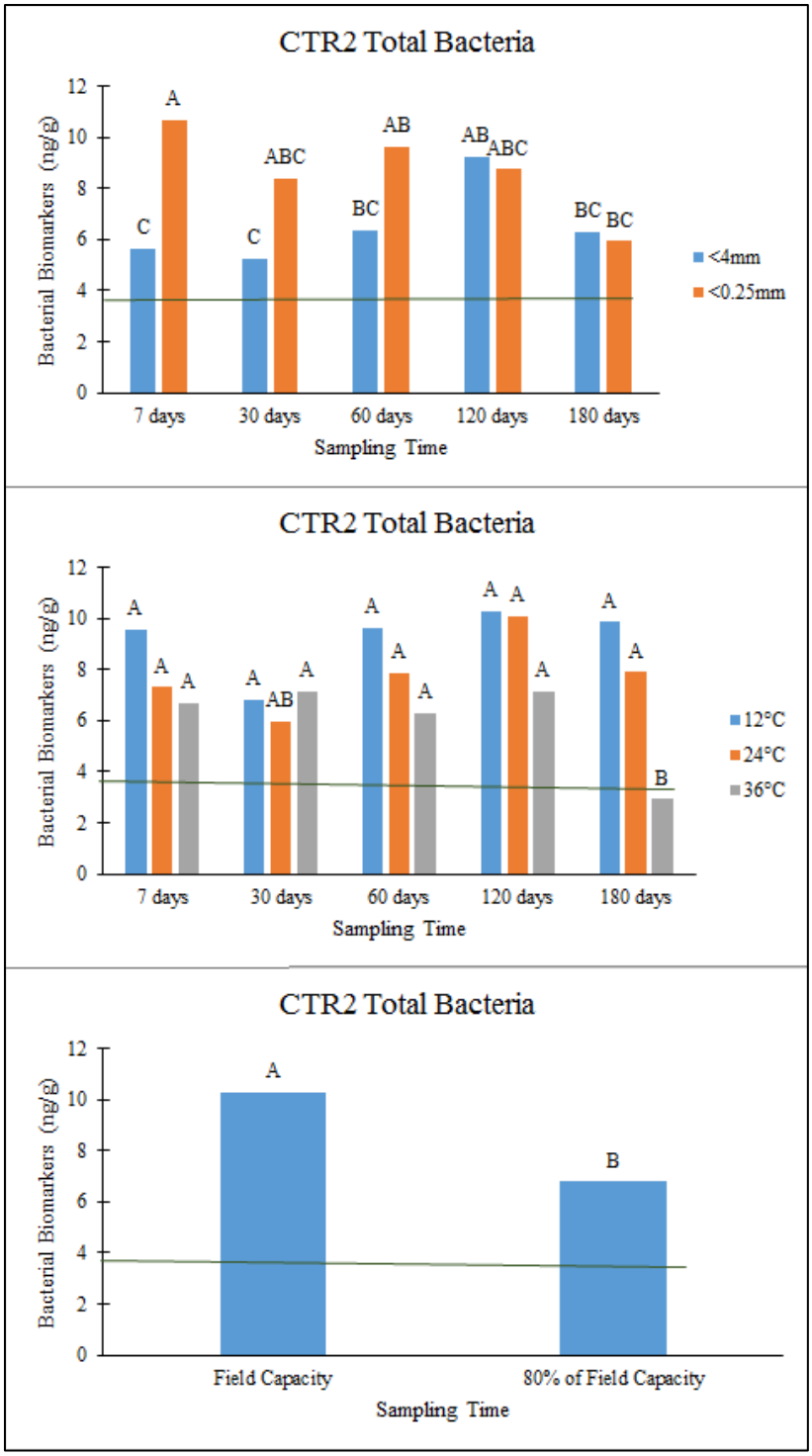


**Figure 3-19: The effects of treatments on total bacterial biomarkers for CTR2. Green lines indicate values in original soil. Top: Interaction effect between sampling time and aggregation. Letters indicate differences to $p<0.05$ across all sampling times. Middle: Interaction effect between sampling time and temperature. Letters indicate differences to $p<0.05$ across all sampling times. Bottom: Effect of moisture treatment on total bacteria; letters indicate differences to $p<0.05$.**

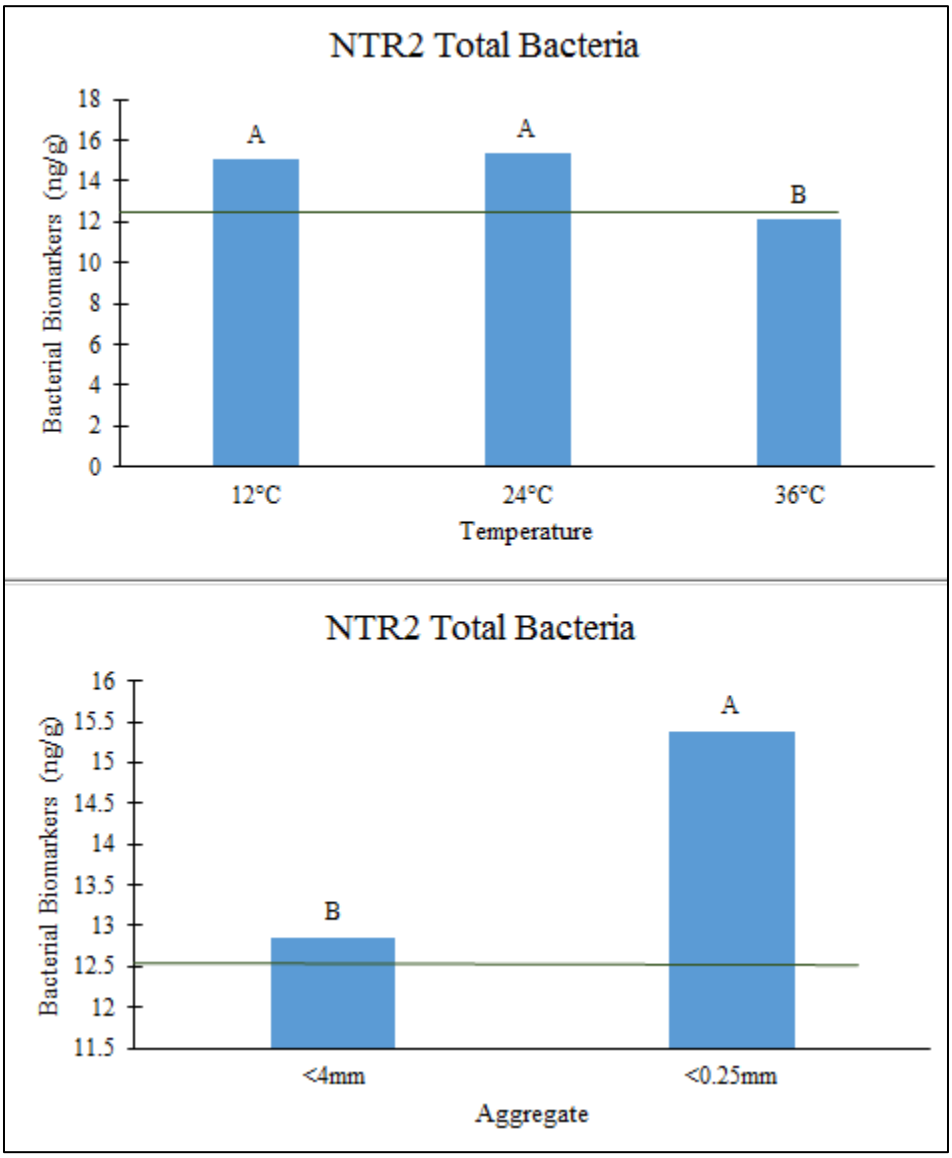


**Figure 3-20: Effects of treatments on total bacterial biomarkers for NTR2. Green lines indicate values in original soil. Top: Effect of temperature on total biomarkers. Letters indicate differences to p<0.05. Bottom: Effect of aggregation on total biomarkers. Letters indicate differences to p<0.05.**

*Labile C Information*

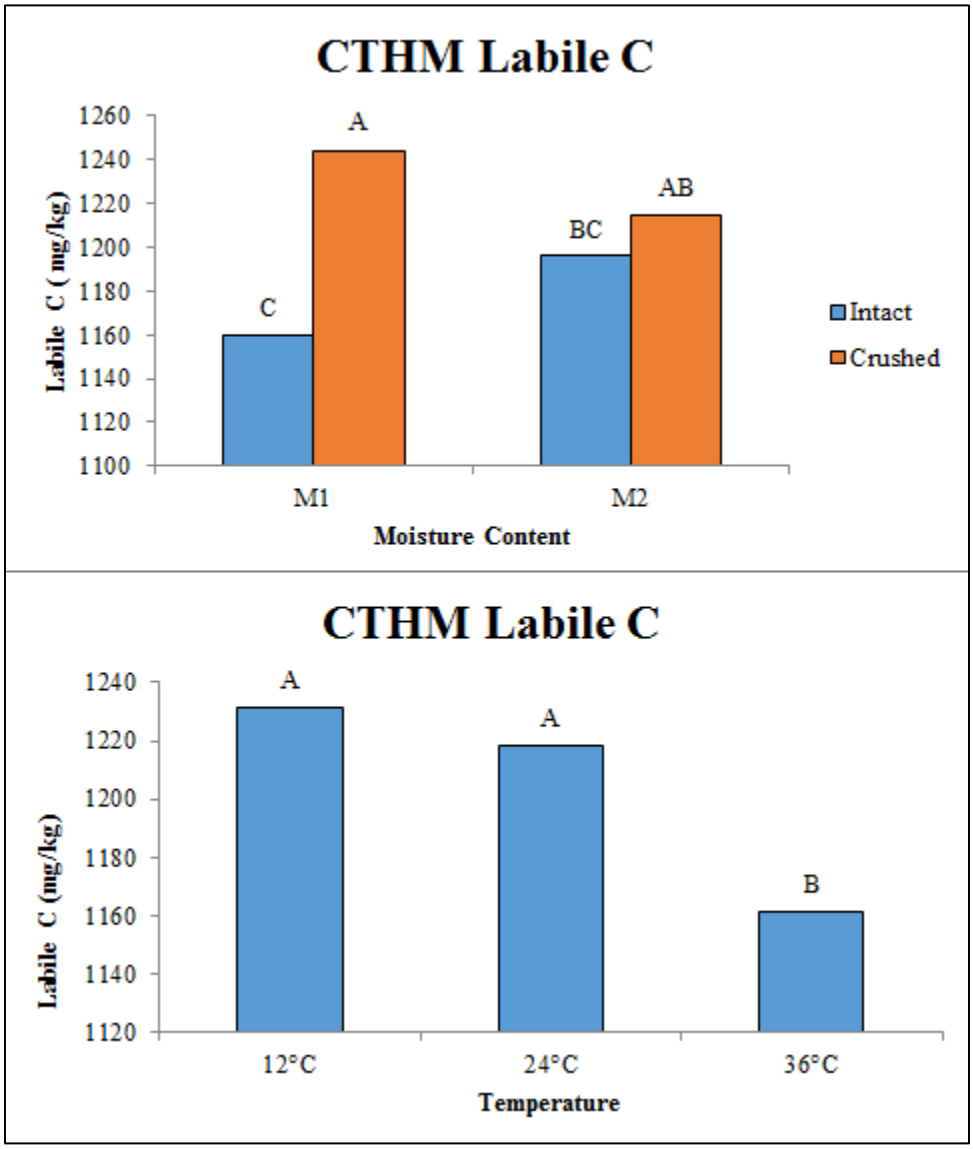


**Figure 3-21: CTM Labile C. Top: Differences in whole v crushed soil for labile C. Bottom: Differences across temperature treatments. Labile C was determined as permanganate oxidizeable C. Data adapted from (Pitumpe Arachchige, 2016). Letters indicate differences to $p<0.05$.**

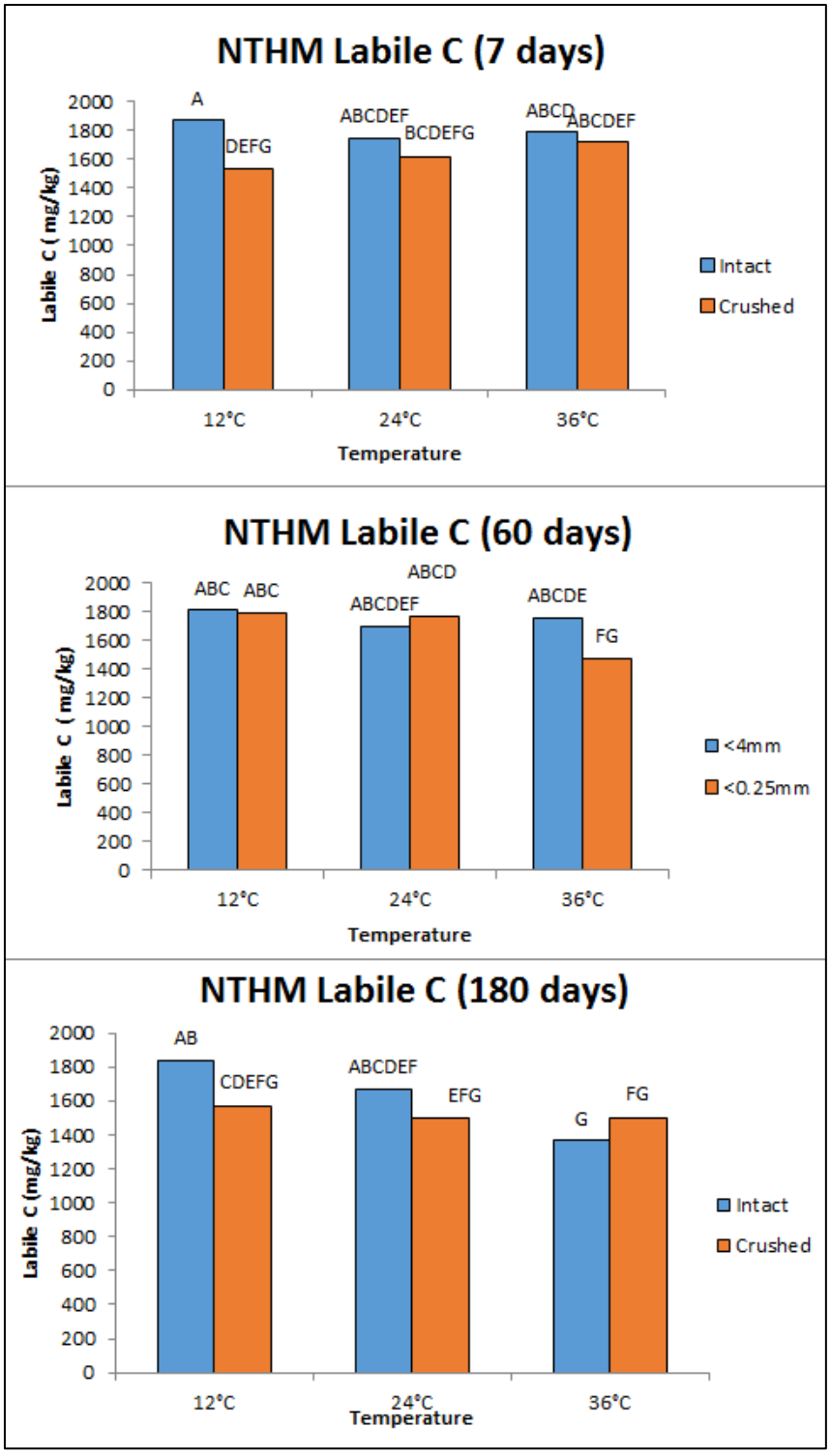


**Figure 3-22: Labile C data for NTM. Top: Labile C at 7 days; Middle: Labile C at 60 days; Bottom; Labile C at 180 days. Letters indicating significant difference carry through all three time periods (3 way interaction). Labile C was determined via permanganate oxidizeable C. Data adapted from (Pitumpe Arachchige, 2016).**

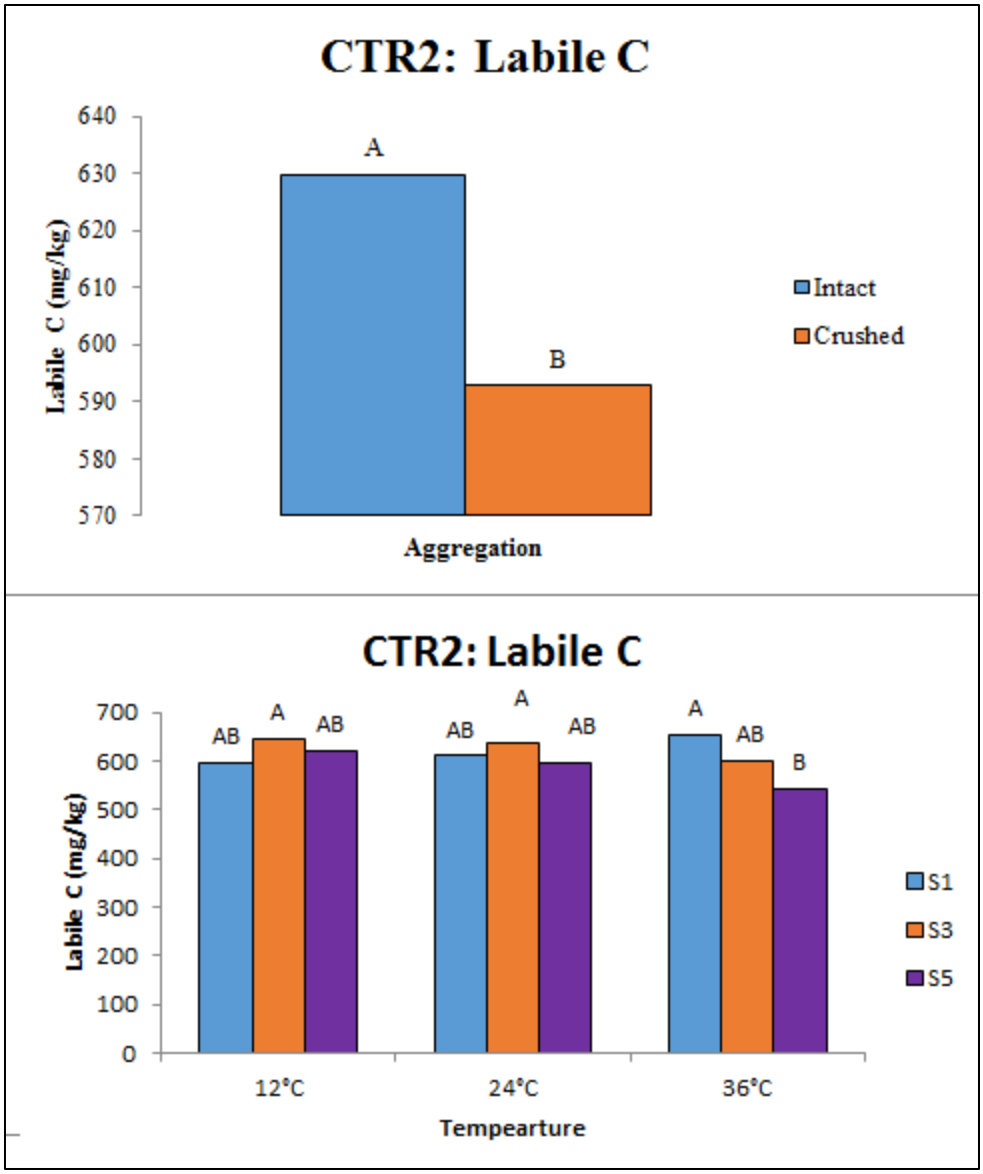


**Figure 3-23: Labile C data for CTR2. Top: Effect of aggregation; letters indicate significant differences to p<0.05. Bottom: Interaction between sampling time and temperature. Letters indicate differences to p<0.05 across temperature treatments. Labile C was determined via permanganate oxidizeable C. Data adapted from (Pitumpe Arachchige, 2016).**

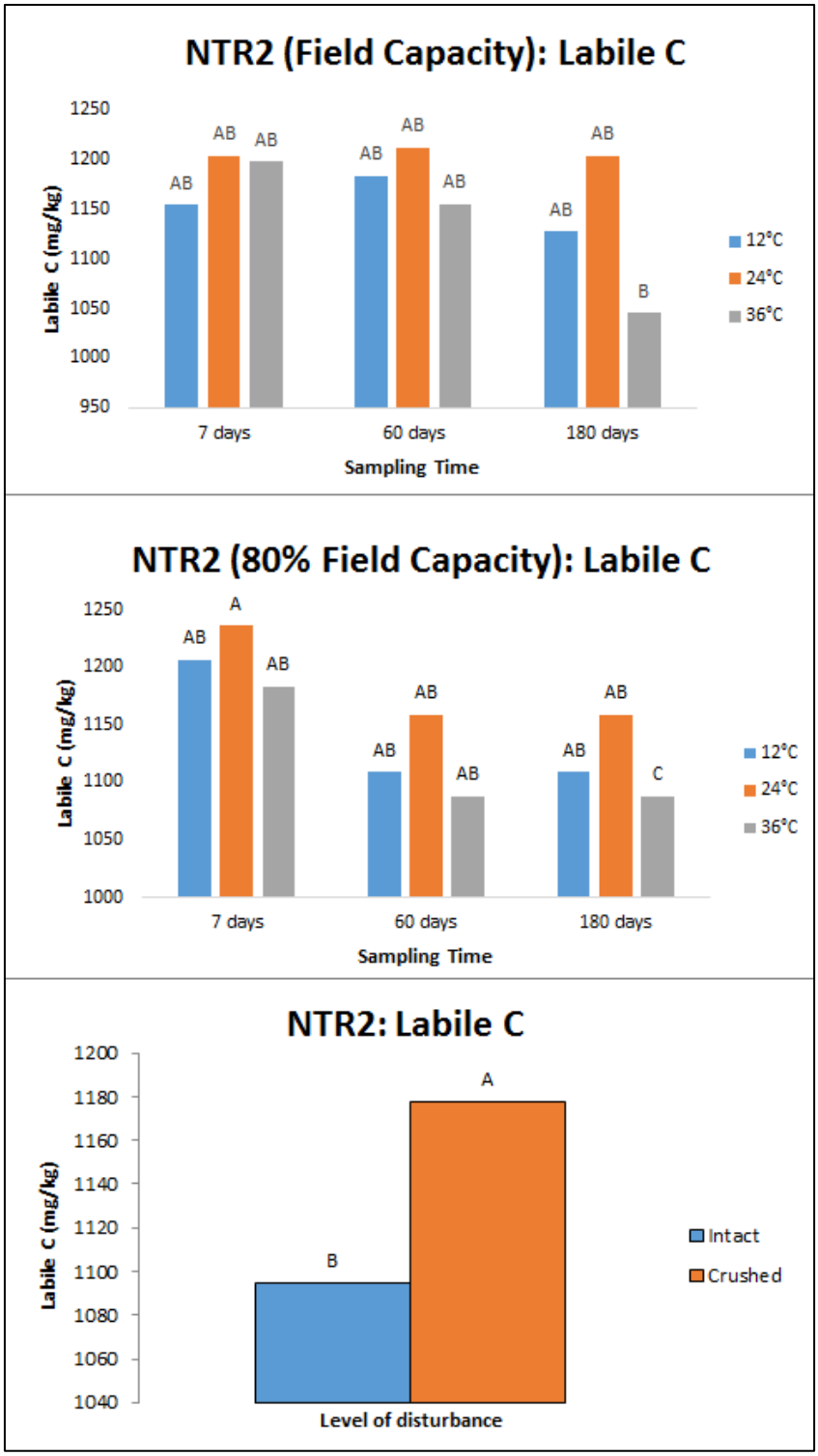


**Figure 3-24: Labile C data for NTR2. Top & Middle: Effect of temperature, moisture, and sampling time; letters indicate significant (to $p>0.05$) differences cross both figures. Bottom: Effect of aggregation; letters indicate differences to $p<0.05$. Labile C was determined via permanganate oxidizeable C. Data adapted from (Pitumpe Arachchige, 2016).**

*Cumulative $CO_2$ Emissions*

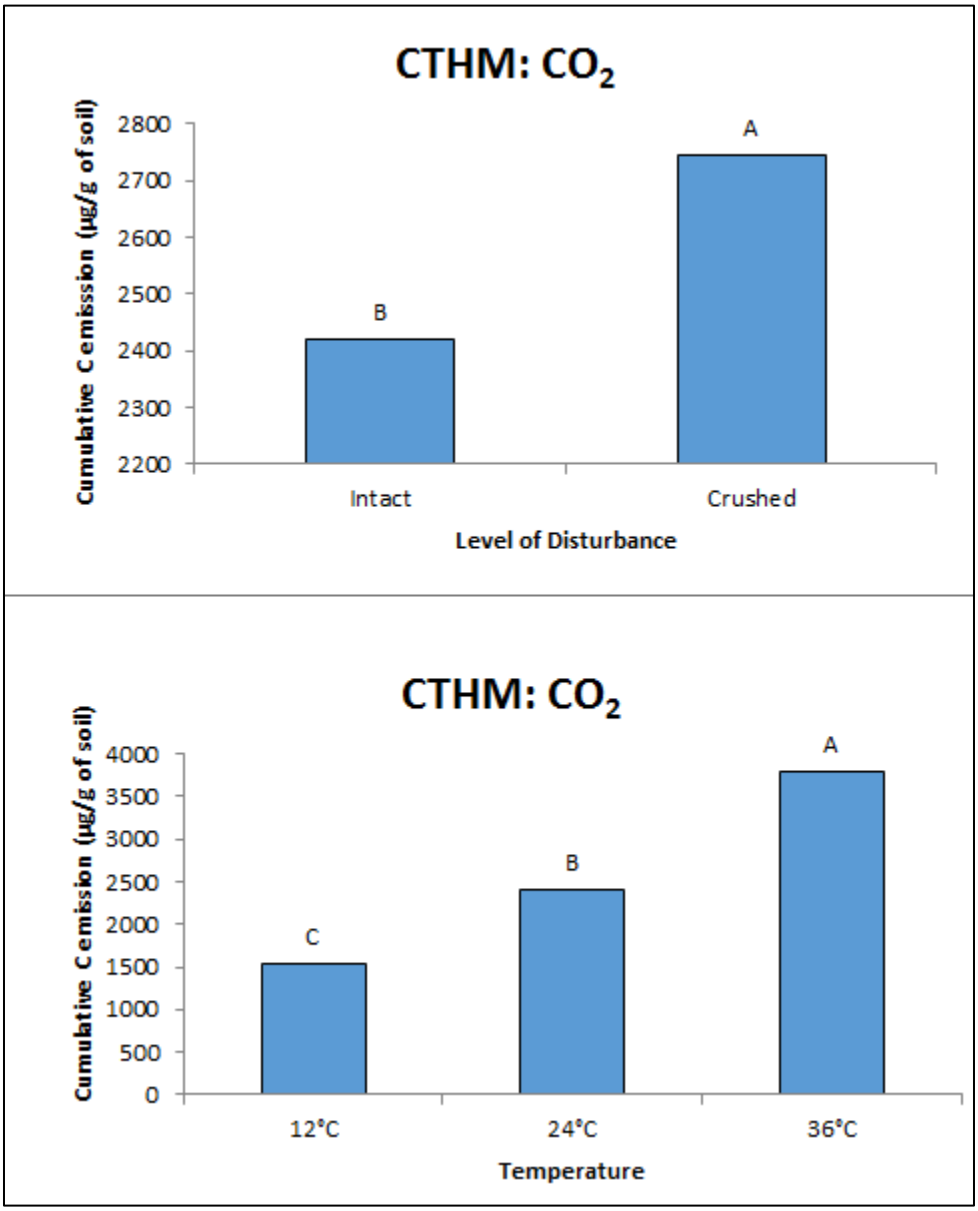


**Figure 3-25: Cumulative $CO_2$ emissions for CTM. Top: $CO_2$ by aggregation; letters indicate differences to $p<0.05$. Bottom: $CO_2$ by temperature; letters indicate differences to $p<0.05$. Data adapted from (Pitumpe Arachchige, 2016).**

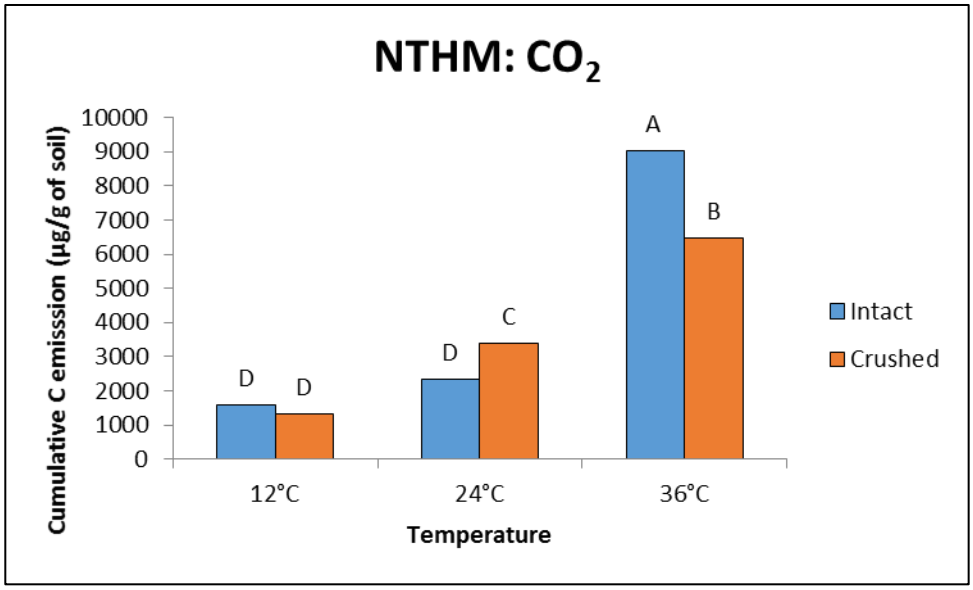


**Figure 3-26: Cumulative $CO_2$ emissions for NTM as effected by aggregation and temperature; letters indicate differences to $p<0.05$ across all temperatures. Data adapted from (Pitumpe Arachchige, 2016).**

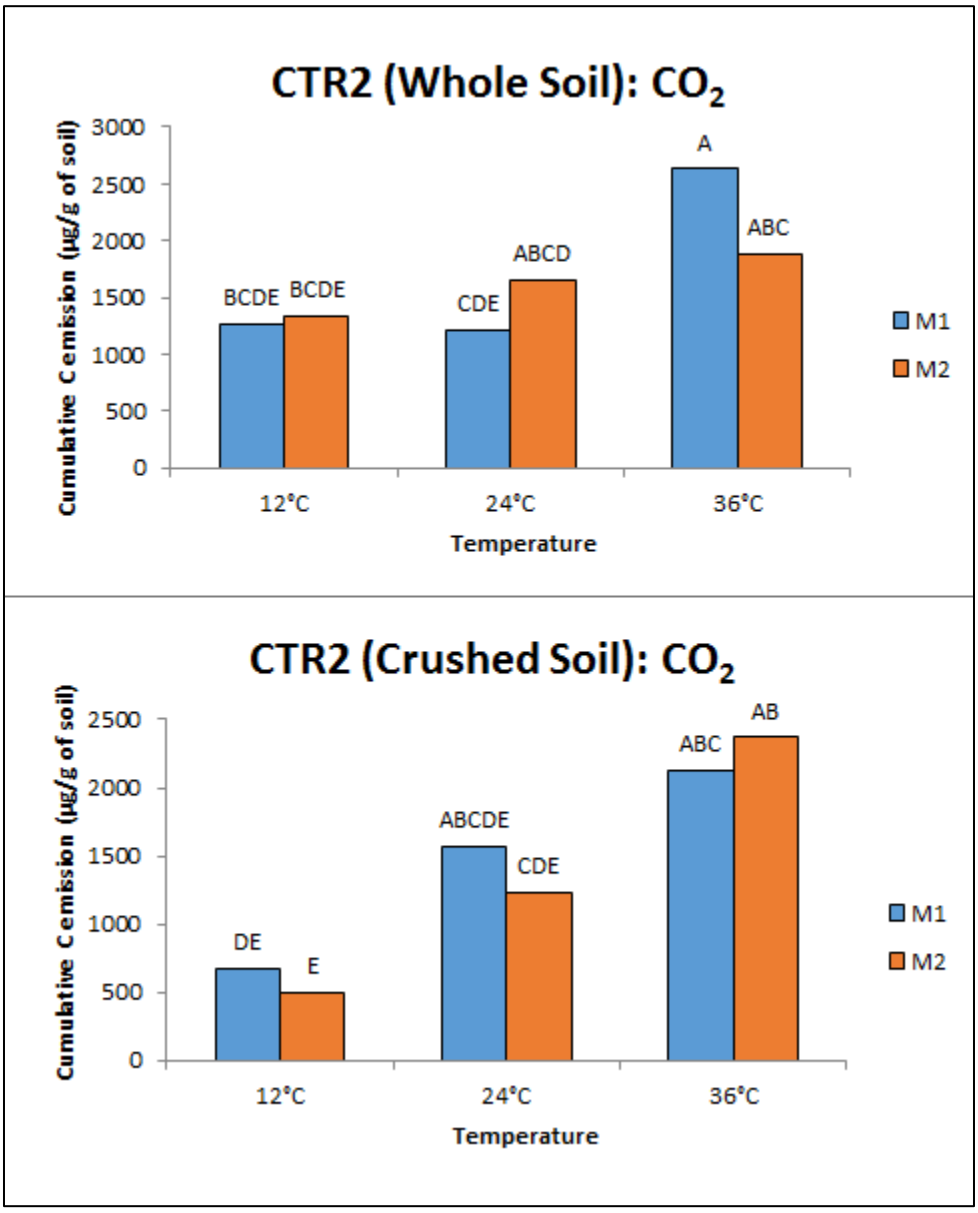


**Figure 3-27: Cumulative $CO_2$ in CTR2; effect of temperature, moisture, and aggregation. Letters are significant (p>0.05) across both images. Data adapted from (Pitumpe Arachchige, 2016).**

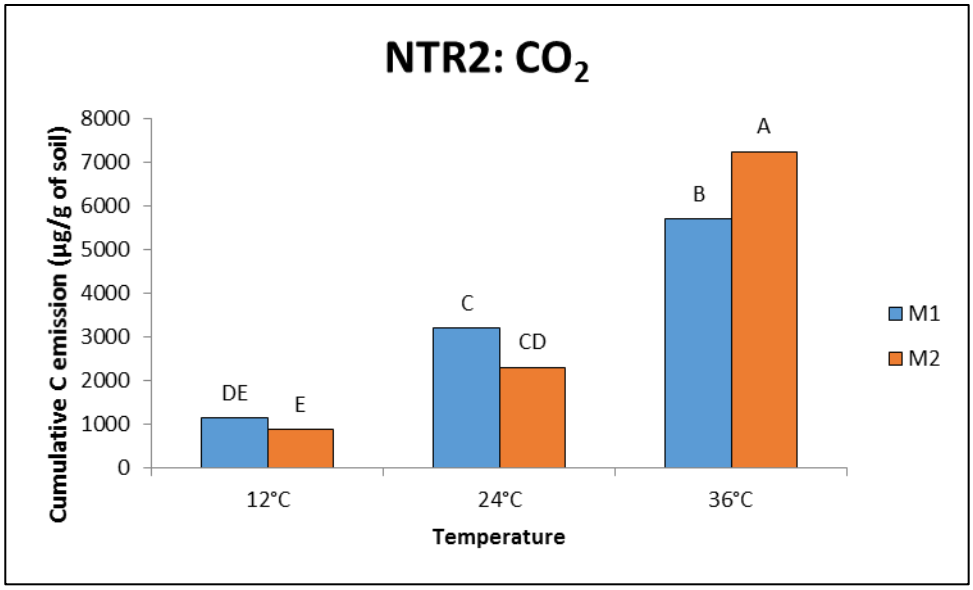


**Figure 3-28: Effect of temperature and moisture on cumulative $CO_2$ on NTR2; letters indicate differences to $p<0.05$ across all temperatures. Data adapted from (Pitumpe Arachchige, 2016).**

***pH differences***

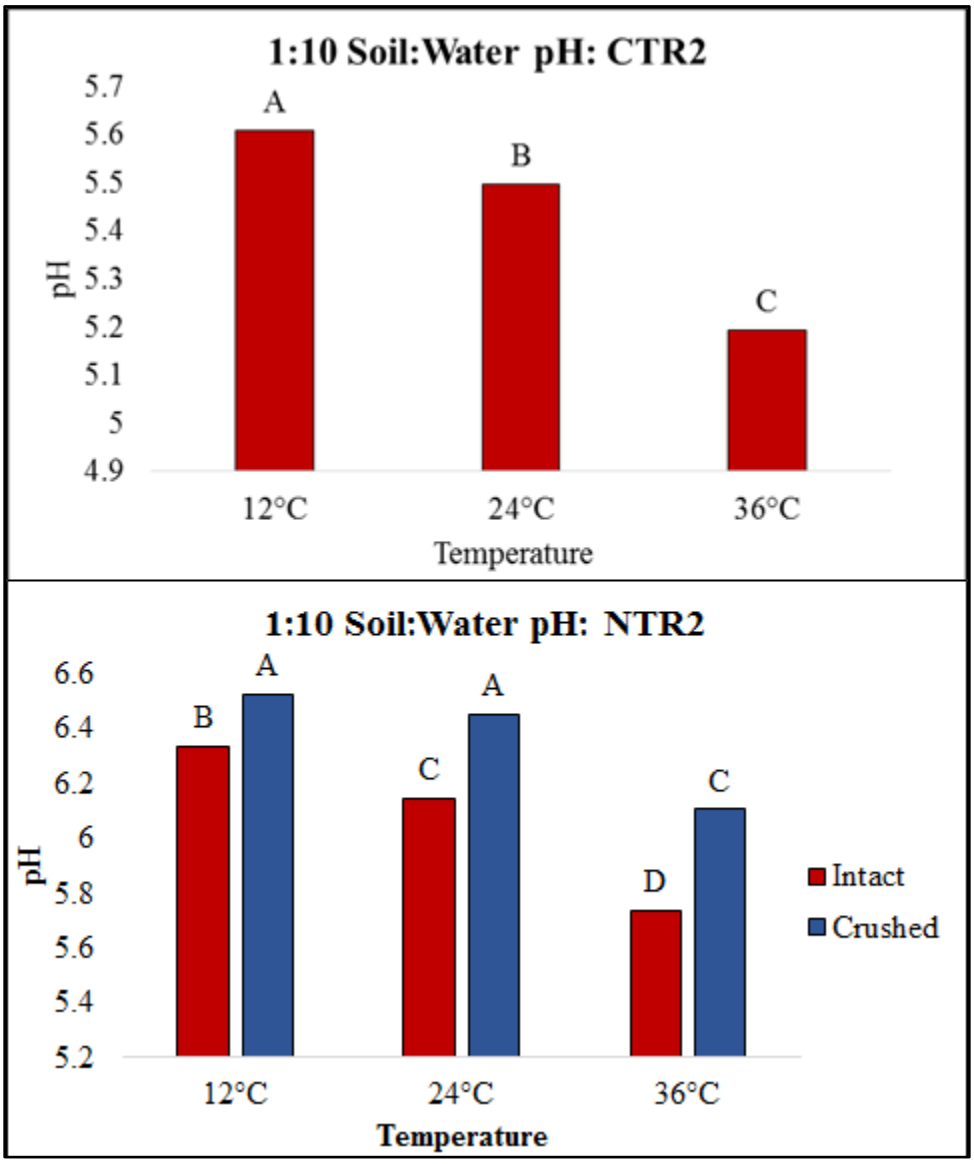


**Figure 3-29: Soil pH compared to temperature treatment for the two Oxisols. pH differences were seen in the Mollisols, however pH remained above 6.0. Data adapted from ((Pitumpe Arachchige, 2016).**

## Supplementary Materials

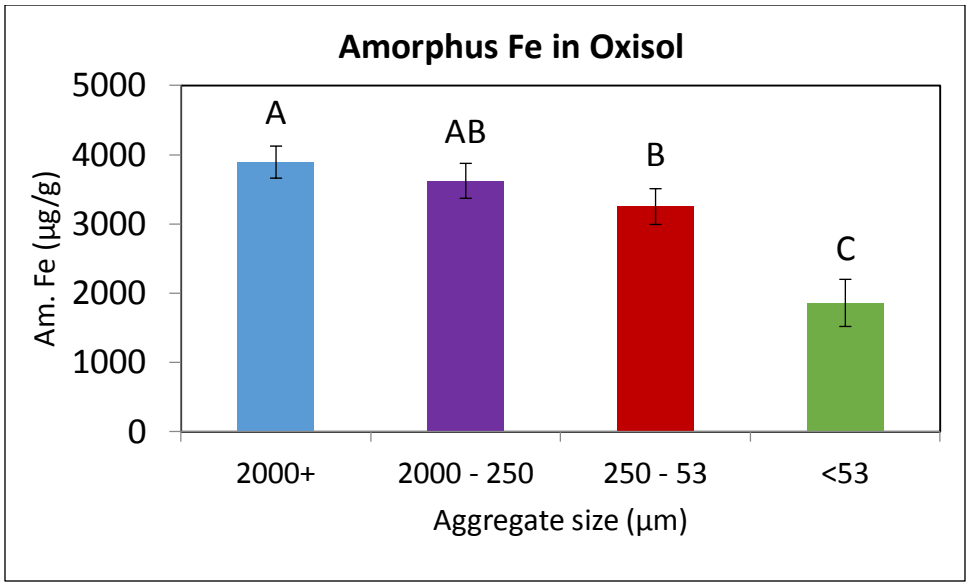


**Figure 3-30: The effect of aggregate size on mineralogy in an Oxisol. This is from a previous study of mine using the NTR2 soil. Letters indicated differences significant to $p<0.05$**

**Figure 3-31: Fungal growth in intact NTM soil. This type of growth was common in the intact soils.**

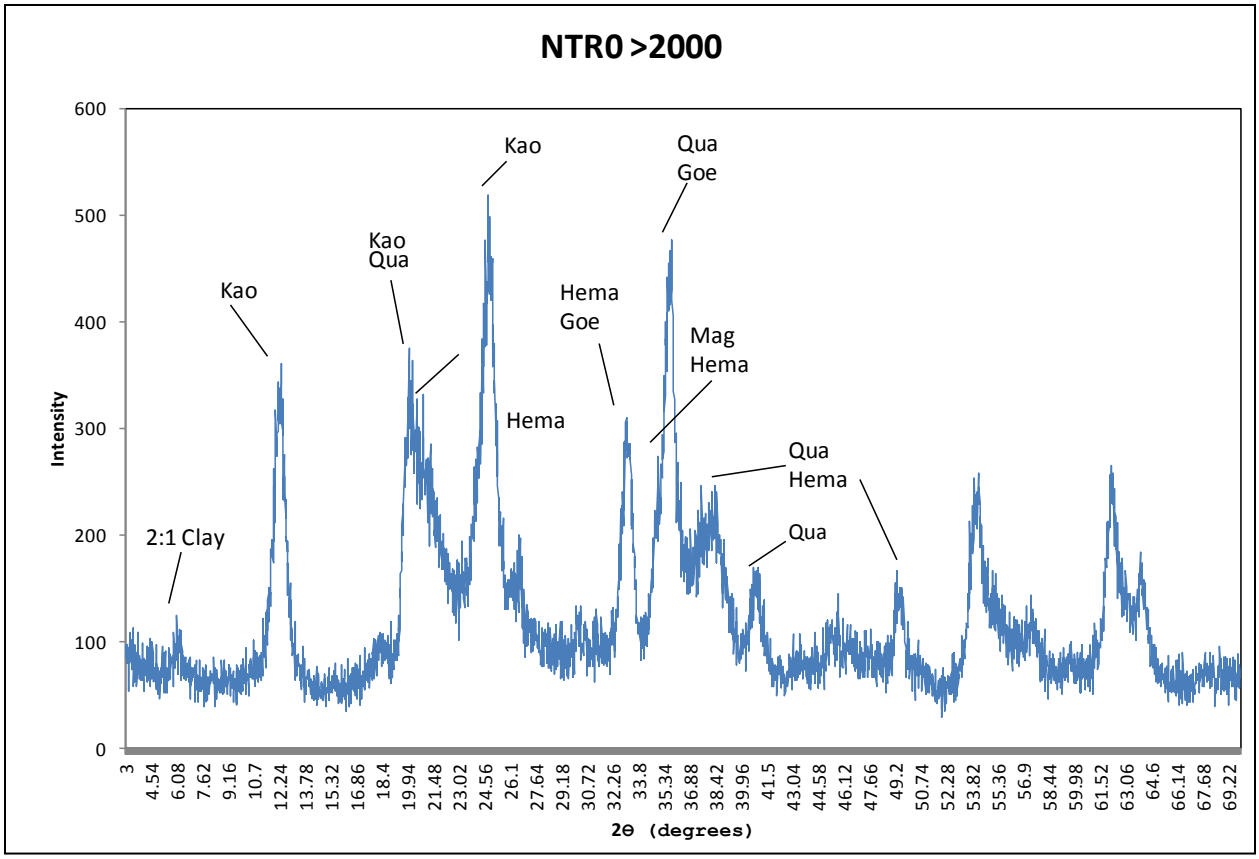


**Figure 3-32: Sample mineralogy work by X-ray Diffraction work on no-till Oxisol. This is from a soil at the same research site as CTR2 and NTR2 that is being managed under no-till and a soy/wheat rotation. Kao = Kaolinite; Qua = Quartz; Hema = Hematite; Goe = Goethite; Mag = Magnetite.**

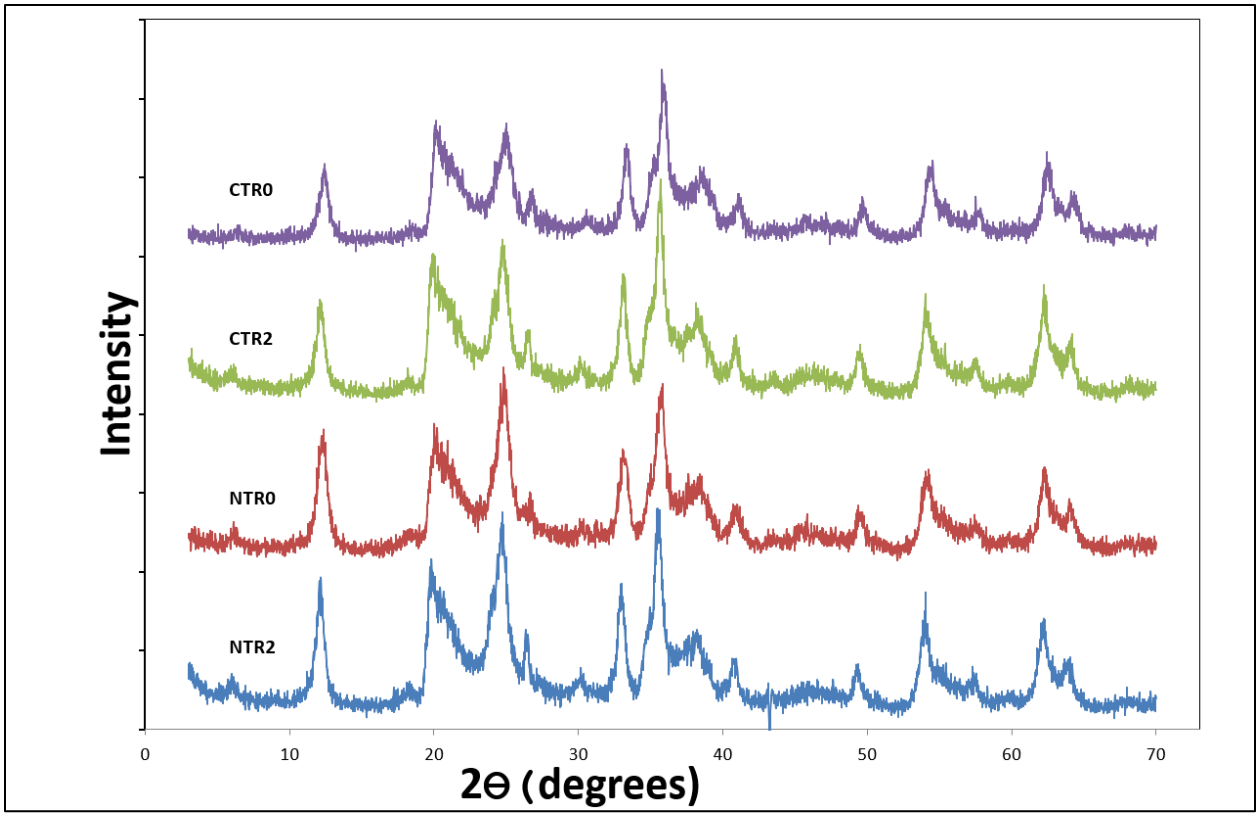


**Figure 3-33: XRD results for four Oxisols; NTR2 and CTR2 are included in this set.**

# Chapter 4 - Experiment 2 – Differences in Microbial Community Structure and Enzyme Activities in a Contaminated Urban Soil Amended with Organic Matter


## Abstract

The rise in public interest in urban gardening and the widespread issues of mild to moderate levels of urban soil contamination have prompted research into managing urban soils for the purpose of vegetable production. The primary objective of this study was to understand the effects of common urban soil contaminants on soil microbial community and soil nutrient cycling; and to understand how organic soil amendments can affect the extractability of soil contaminants influencing microbial processes. Soil samples from a contaminated site in Indianapolis, Indiana were used in this study. The site was located near a former railroad (Monon rail) and has elevated lead (Pb), arsenic (As), and polycyclic aromatic hydrocarbons (PAHs). Soils studied were collected 484 days after the application of organic amendments (composted biosolids and mushroom compost) to the soil. Soils were incubated for 1 month at 24°C at 60% of maximum water holding capacity to re-stimulate microbial activity. Enzyme activities were measured after the incubation. Phospholipids fatty acids were used to characterize the microbial community. Metal speciation was done using a sequential extraction procedure. Both the extractable portion of soil contamination and the use of organic amendments had significant ($p<0.05$) effects on microbial populations. The use of organic amendments generally improved microbial community composition and enzyme activities indicative of soil nutrient cycling and soil health; however, the mushroom compost caused a decline in actinomycete populations. This was most likely due to an increase in the amount of extractable Pb; and the actinomycetes were

most sensitive to soil Pb. Aside from the issue with the reduction in actinomycetes in the mushroom compost, organic amendments were beneficial to contaminated urban garden soil microorganisms.

# Introduction

Urban gardening is a resurging trend in agriculture that has a number of potential benefits for urban communities. Urban gardens provide green-space, improve food security, and increases employment opportunities. Urban garden also provides an opportunity to recycle food waste via composting and reduces the need for transporting food into the city. As such there is a growing interest in using vacant lots (often called brownfields) as community gardens (Brown et al., 2016; Halloran and Magid, 2013; Lee-Smith, 2010; Rogus and Dimitri, 2015; Wortman and Lovell, 2013). Despite the benefits of urban gardening, there are a few problems with urban soils to be addressed. Most soils in urban areas are poor quality; low organic matter, low nutrient, and high in contaminants like Pb, As, and polycyclic aromatic hydrocarbons (PAHs). Additionally, urban areas often have microclimates that are different than the surrounding countryside. These problems can make gardening in urban areas more challenging (Chen et al., 2014; Clark et al., 2006; Mendez and Maier, 2008; Rice, 1999; Wortman and Lovell, 2013; Yang, 2010). Of the many potential urban contaminants, Pb is the most common problem in urban soils. Much of the Pb in urban environments comes from historical use of leaded gasoline, leaded paints, and from metal processing industrial activities like smelting (Brown et al., 2016; Hettiarachchi and Pierzynski, 2004; McBride et al., 2014; McClintock, 2015; Wortman and Lovell, 2013). Much of the As in urban soils comes from historical use of As containing household pesticides and preservatives as well as from metal processing (i.e. smelting); some soil As also comes from the use of contaminated water for irrigation (Gillispie et al., 2015; Han et al., 2003; Mandal and Suzuki, 2002; Wang and Mulligan, 2006a). Polycyclic Aromatic Hydrocarbons are formed primarily during the combustion of fossil fuels but also in the combustion of other organic compounds. Heavy traffic and energy us in cities has resulted in higher PAH concentrations in

urban soils (Canadian Council of Ministers of the Environment, 1999; Li et al., 2014b; Rengarajan et al., 2015).

These problems can impact the microbial communities that are necessary for soil processes. Cadmium, As and Pb contamination has been shown to decrease the activity of enzymes that are responsible for N and P cycling. Cadmium, As and Pb contamination decreases the total biomass in the soil with actinomycetes, N-fixing bacteria and gram positive bacteria being most affected (Abaye et al., 2005; Gai et al., 2011; Khan et al., 2010; Liao et al., 2007; Oliveira and Pampulha, 2006; Wang et al., 2007; Yang, 2010). The problems with soil quality can also impact the ability of the soil to grow plants. Contamination with metals and PAHs can reduce plant biomass and yield (Claassens, 2006; Kawasaki et al., 2012; Szakova et al., 2007; Yeates et al., 1994).

Soil contamination issues common to urban areas can also be a potential hazard to human health. Low-level exposure to common soil contaminants can build up over time, leading to chronic health problems. Lead exposure is particularly dangerous for young children. Elevated blood Pb is associated with the development of learning disabilities and reduced IQ (Henry et al., 2015; Hettiarachchi and Pierzynski, 2004; Lourenco et al., 2010; McClintock, 2015). Some of the PAH compounds are carcinogenic (Canadian Council of Ministers of the Environment, 1999; Lorenzi et al., 2011). Arsenic is carcinogenic and can lead to liver and skin problems (Environmental Protection Agency, 2012; Mandal and Suzuki, 2002). As a result there has been recent effort into understanding these problems and developing cost-effective remediation strategies (Hettiarachchi and Pierzynski, 2004; Scheckel et al., 2013). The application of organic amendments is a common remediation method that has the potential to both decrease bioavailability of metals and increase plant productivity (Mendez and Maier, 2008). Organically

bound Pb is usually immobile and not bioavailable to humans or plants (Smith, 2009). Organic amendments are often successful in reducing the bioavailability of Pb by forming organo-Pb complexes (Attanayake et al., 2015; Brown et al., 2012; Chen DanYan et al., 2011; Yang et al., 2016). Organic amendments (biochar and compost) have also been shown to reduce Cd and As bioavailability through the formation of metal-complexes (Balal Yousaf et al., 2016a; Juang et al., 2012; Lu et al., 2014; Simmler et al., 2013; Zeng et al., 2015). However, organic amendments can also increase metal availability through the formation of soluble organo-metal compounds. This is especially a problem with As, but occurs with other metals like Cd as well (Balal Yousaf et al., 2016a; Huq et al., 2008; Zhao et al., 2010a). The main objective of this study was to understand how urban contaminants affect soil microbial properties and to understand how organic amendments affect microbial properties.

The microbial properties were assessed using enzyme activities and phospholipid analysis. Enzyme activities are important indicators of soil microbial activity. Three enzymes studied in this experiment; Beta-D-glucosidase, alkaline phosphatase, and arylsulfatase. Rather than measuring microbes themselves, the enzymes they produce were measured. These enzymes are produced mostly by bacteria and are important in soil nutrient and carbon cycling (Acosta-Martinez et al., 2000; Aon and Colaneri, 2001; Fitzgerald, 1976). Beta-D-glucoside is a common soil enzyme that is responsible for the breakdown of more complex carbon compounds; it does this by cleaving glucosides off of larger C compounds to produce glucose. This enzyme is sensitive to metal contamination and is well correlated with microbial population and organic carbon availability (Acosta-Martinez et al., 2000; Claassens, 2006; Makoi and Ndakidemi, 2008). Arylsulfatase is a common soil enzyme that cleaves sulfur from organic compounds; resulting in mineralized S and a remainder organic C compound. This enzyme is important in the

mineralization of sulfur, which is an important plant nutrient (Acosta-Martinez et al., 2000; Fitzgerald, 1976; Makoi and Ndakidemi, 2008). Alkaline phosphatase is a soil enzyme that breaks down organic P containing compounds into organic carbon and mineralized P. Since P is a very important plant nutrient, this enzyme is an important indicator of soil ecological health (Acosta-Martinez et al., 2000). The activity of this enzyme depends in part on the organic-P concentration of the soil, when soil inorganic P is high, this enzyme will be less common. Alkaline phosphatase functions at higher soil pH (Aon and Colaneri, 2001; Makoi and Ndakidemi, 2008). Decreased enzyme activity is an indicator of soil stress; often from disturbance or from the effects of metal contamination (Aon and Colaneri, 2001; Claassens, 2006; Khan et al., 2010; Wang et al., 2007).

Phospholipid analysis allows for the characterization of the microbial community. Phospholipids are an essential part of cell membranes and the exact chemistry of the cellular phospholipids varies from organism to organism. By characterizing the phospholipids in the soil, it is possible to characterize the types of microbes that are present in the soil. The main objective of this study is to understand how soil contaminants influence soil microbial properties and how organic amendments can improve microbial processes in a contaminated soil.

## Materials and Methods

An incubation study was conducted using soils from an urban garden site in Monon, Indianapolis, Indiana that was contaminated with lead, arsenic, and polycyclic aromatic hydrocarbons (PAH's). The site was in a former industrial area, near the Monon railroad; which is likely the main source of the contaminants. The garden was established in 2011 as part of a research project on urban soil contamination (Attanayake et al., 2015). Four replicate plots were delineated at the site and each plot was split into subplots of soil amendments and crop type. Soil

amendments were: mushroom compost (compost used for mushroom production before field application), leaf compost (from yard waste), composted biosolids, non-composted biosolids, and a control. The control was amended with NPK as per soil testing results. Soils collected 484 days after establishment from the collard greens plots that were amended with the mushroom compost, composted biosolids, and the control were selected for use in the study. The mushroom compost is spent mushroom-growing media (which if often horse manure and other organic materials like wood chips or sawdust, the mushroom growing media is used for one growing season and then sold as fertilizer) that was purchased from Tiffany's garden store. The composted biosolids were donated to the study by Soil Solutions Co., Roanoke, IN, and it is a product of the municipal water treatment plant of the city of Fort Wayne, Indiana. Composted biosolids were produced and inspected according to U.S. EPA standards and regulations.

Maximum water holding capacity (MWHC) under free gravity of the soil was determined (Jenkinson and Powlson, 1976). Dry soil was weighed into plastic sample cups (50 grams per sample) and soils adjusted to 60% of MWHC. Moisture adjusted soils were then incubated at 24°C for 30 days to stimulate microbial activity. Following the incubation the soils were stored at 4°C prior to biochemical analysis. The first procedure was the extraction and measurement of the soil microbial enzymes: β-D-glucoside, arylsulfatase, and alkaline phosphatase. The enzymes were extracted using a mixture of toluene and organic acids as described by (Tabatabai, 1994). Extracted enzymes were measured with colorimetry at 405 nm using a Beckman Coulter DU 800 spectrophotometer. Phospholipid fatty acids (PLFAs) were extracted using organic solvents and silica gel columns as described by (White et al., 2007). Extracted PLFA's were analyzed using gas chromatography-mass spectrometry (GS-MS) using the Thermo Scientific Trace GC Ultra

GC-MS. An internal standard of methyl nonadecanoate (C19:0) was added to all samples and used to calibrate the GC-MS.

Microbial groups were determined using different fatty acid biomarkers. Fungal abundance was determined using the *C18_2_9,12* biomarker (Bossio and Scow, 1998; Ostle et al., 2004; Petersen et al., 2002; Potthoff et al., 2006; Ruess and Chamberlain, 2010). Actinomycete abundance was determined using the *10_methyl C:18* biomarker (Bossio and Scow, 1998; Potthoff et al., 2006). General gram positive bacteria were determined using the following biomarkers: *i-C:15, a-C:15, i-C:16, a-C:16,* and *i-C:17* (Bossio and Scow, 1998; Ostle et al., 2004; Potthoff et al., 2006; Waldrop and Firestone, 2004b). General gram negative bacteria were determined using the following biomarkers: *C:10_0_2OH, C:12_0_2OH, C:12_0_3OH, C:14_0_2OH, C:14_0_3OH,* and *C:16_0_2OH* (Ruess and Chamberlain, 2010; Steinberger et al., 1999). Total bacteria was determined by summing all bacterial groups and total biomass was determined by summing all biomarkers (Petersen et al., 2002; Potthoff et al., 2006; Waldrop and Firestone, 2004a).

Soil pH was determined in a 1:1 soil-to-water ratio. Total organic carbon was determined by the Walkley-Black method. Available N ($NO_3$ and $NH_4$) was found via a 1.0 *M* KCl extraction and measured using colorimetry (University of Missouri Agricultural Experiment Station, 1998). Available P was measured using the Mehlich-3 method (University of Missouri Agricultural Experiment Station, 1998). Available K was extracted using 1 *M* ammonium acetate and then measured with Flame Atomic Absorption (University of Missouri Agricultural Experiment Station, 1998). Available Pb was extracted in 0.01 *M* $Sr(NO_3)_2$ (Kukier and Chaney, 2001) and analyzed using atomic adsorption spectroscopy (Varian AA240Z). A sequential extraction of Pb was performed using progressively more reactive reagents using the Zwonitzer

et al. (2003) modification of the method by Tessier et al. (1979). Statistics for PLFA, enzymes, and exchangeable metals were done using a randomized complete block design and a PROC MIXED model and SAS 9.4. Linear correlation between actinomycetes and exchangeable Pb was done using R 3.14 (Kutner, 2013).

## Results and Discussion

### *Influence of soil amendments on microbial communities*

Phospholipid fatty acid were calculated using both relative abundance and total biomass. For relative abundance (proportion of group to total microbes) there were three significant differences between treatments for gram positive bacteria, actinomycetes, and total bacteria. For total biomass there was a significant difference between treatments for actinomycetes. There were no significant differences for total microbes, fungi, or gram negative bacteria in either method (Table 4-5).

Fungi and gram negative bacteria have been noted to be resistant to soil metal contamination (Gao et al., 2010; Liao et al., 2007; Oliveira and Pampulha, 2006; Wang et al., 2010; Wase, 1997). It is still somewhat surprising that there was no response to treatments with the gram negative bacteria as the addition of organic matter (i.e. composts) has been shown in the literature to elevate the abundance and activity of gram negative bacteria (Creamer et al., 2015b; Nottingham et al., 2009; Watzinger et al., 2014). Fungi tend to consume more recalcitrant soil C and are thus less responsive to the addition of organic amendments (Busse et al., 2009; Watzinger et al., 2014). The lack of response from the fungi was expected. Meanwhile, the lack of response from the gram negative bacteria might be due to the long time period between the application of the organic amendments and the time of soil sampling and (484 days); as most of the easily labile C was likely consumed. Dissolved organic C (DOC) from this site supports this

notion. The comparison of DOC between 7 days after compost addition and 106 days after compost addition already showed a major decline in DOC; which is largely labile and easily accessible C. Composted biosolids had declined by 67%, mushroom compost by 83% and the control by 100% at 106 days after compost addition (Attanayake et al., 2015). It has probably been too long since compost addition to observe changes in the gram negative bacterial population.

The relative abundance of gram positive bacteria (Fig. 4-1) was greatest in the mushroom compost treatment and lowest in the control treatment. Gram positive bacteria have been shown to respond positively to the addition of organic carbon which is likely the main driving reason behind the increase in gram positive bacteria in the mushroom compost (Creamer et al., 2015b). Unlike gram negative bacteria, which are more responsive to labile C; gram positive bacteria consume a wide variety of C compounds that were likely remaining in the soil after 484 days (Mbuthia et al., 2015; Rollins and Joseph, 2004). While gram positive bacteria have been shown to be negatively affected by soil metals; this was unlikely the case in this study as the mushroom compost amended soils had the highest exchangeable lead. Multiple studies have shown a decline in gram positive bacteria in association with soil Pb (Abaye et al., 2005; Liao et al., 2007; Wang et al., 2010; Wase, 1997). It was possible that the population benefits of additional organic carbon in the mushroom compost were outweighed by the costs to the gram positive population. The addition of organic amendments to metal contaminated soil has been shown in other experiments to increase gram positive bacteria (Ahmad et al., 2016; Mackie et al., 2015; Mrozik et al., 2014). Gram positive bacteria are a large and diverse group of microorganisms and some species show more resistance than others (Mackie et al., 2015).

With actinomycetes, there was a significant treatment effect both on total biomass and on relative abundance (Fig. 4-2). The total biomass of actinomycetes was highest in the composted biosolids, and lowest in the control and the mushroom compost. The relative abundance of actinomycetes (Fig. 4-2) was greatest in the composted biosolids and lowest in the mushroom compost; with the control being in-between the two. Actinomycetes are a group of gram negative bacteria that have been shown in the literature to be highly sensitive to soil metals (Kaloyanova, 2007; Oliveira and Pampulha, 2006; Wang et al., 2010; Yang, 2010). As such a negative correlation between exchangeable Pb and actinomycete biomass was found in this study (Fig. 4-3). The mushroom compost had more exchangeable Pb than the other treatments (Fig. 4-8); so despite the higher organic carbon content; it had reduced actinomycete biomass. The composted biosolids had the greatest actinomycete population due to both lower available Pb and higher organic carbon; which is the main substrate for actinomycetes. The reduction of actinomycetes with Pb can have negative impacts on soil C cycling as actinomycetes are important in breaking down recalcitrant C and plant residues (Watzinger et al., 2014). Actinomycetes have similar role to fungi in soil ecosystems and are capable of breaking down more recalcitrant residues like chitin and lignocelluose; and Pb has been found to inhibit these enzymes that attack recalcitrant C (Brzezinska et al., 2013). Conditions that enhance actinomycete populations lead to faster decomposition of resistant C residues (Abdulla, 2007; Helfrich et al., 2015; Krsek and Wellington, 2001).

The relative abundance of all bacteria (Fig. 4-4) was greatest in the mushroom compost and lowest in the control. Given the greater Pb availability in the mushroom compost; this difference was likely driven largely by the greater abundance of organic carbon in the mushroom

compost (Table 4-1). Most soil bacteria are heterotrophic and use organic carbon as their main substrate (Busse et al., 2009; Creamer et al., 2015b).

There was a difference in the response of the soil microbial communities between the two organic amendments. The composted biosolids had no effect on fungi or gram negative bacteria and a positive effect on actinomycetes, gram negative bacteria, and total bacteria at 484 days after compost application. The mushroom compost had no effect on fungi or gram negative bacteria, had a positive effect on gram positive bacteria and total bacteria, and had a negative effect on actinomycetes. Metal speciation revealed that the mushroom compost amended soils had elevated exchangeable Pb; which was correlated linearly with the decline in actinomycetes (Figure 4-3). This is consistent with other studies finding actinomycetes to be especially sensitive to soil metals (Kaloyanova, 2007; Oliveira and Pampulha, 2006; Yang, 2010). It is important to think of Pb ecotoxicity in terms of available or labile Pb instead of as total Pb. Available Pb fractions (i.e. $KNO_3$ extractable or NaOH extractable) have been shown to be better indicators of microbial response than total Pb (Sun Bo et al., 2004; Zalaghi and Safari-Sinegani, 2014).

### *Enzyme Activity*

Enzyme activity is important for understanding how changes in microbial communities are affecting soil processes. The activity of microbial enzymes is correlated well with microbial biomass, organic carbon, nutrient cycling and soil health (Acosta-Martinez et al., 2000; Aon and Colaneri, 2001; Claassens, 2006; Hinojosa, 2004; Makoi and Ndakidemi, 2008). Results from enzyme activity analysis revealed that there was greater enzyme activity in the compost amended plots compared to the non-amended plots (Table 4-4). As enzymes are important for soil

processes, understanding how these organic amendments have affected them will be important for making recommendations for urban gardeners.

The activity of beta-D-glucosidase (Fig. 4-5) was greatest in the plots amended with the mushroom compost, lowest in the control plots, and in-between in the plots amended with composted biosolids. Beta-D-glucosidase removes glucose groups from larger organic carbon molecules and is important in the general breakdown of SOC and the stimulation of microbial activity. Many microbial groups produce this enzyme, and it is a good indicator of general microbial activity (Acosta-Martinez et al., 2000; Claassens, 2006; Makoi and Ndakidemi, 2008). The greater activity of beta-D-glucosidase in the mushroom compost was likely due to the higher level of organic carbon in the mushroom compost (Table 4-1). Organic carbon compounds are the main substrate of beta-D-glucosidase and greater concentration of organic carbon substrate has been shown to lead to greater production of this enzyme by microbes (Bandick and Dick, 1999; Garcia-Gil et al., 2000; Song et al., 2012; Taylor et al., 2002). Beta-D-glucosidase has also been shown to be sensitive to inhibition by soil metals (Burgos et al., 2002; Kahkonen et al., 2008; Makoi and Ndakidemi, 2008). The use of organic amendments has been shown to increase beta-D-glucosidase activity even in Pb contaminated soil (Burgos et al., 2002; Garcia-Gil et al., 2000; Perez-de-Mora et al., 2006; Tejada et al., 2007). The increase in this enzyme with the amendments was a positive indicator that the organic amendments were improving soil functioning in the Pb contaminated garden soil.

The activity for alkaline phosphatase (Fig. 4-6) was equally higher with both organic amendment treatments than the control. This enzyme is part of the breakdown of organic P containing compounds; phosphate groups are cleaved from organic C compounds resulting in mineralized P and a remainder organic C compound (Acosta-Martinez et al., 2000; Makoi and

Ndakidemi, 2008). The addition of P that was largely in the organic-P form with the composts (Table 4-1) was likely responsible for the increase in alkaline phosphatase with the treatments. Alkaline phosphatase activity is generally increased in soils with higher SOC and decreased in soils with large amounts of mineralized P (i.e. from rock phosphate) (Claassens, 2006; de Mora et al., 2005; Makoi and Ndakidemi, 2008; Perez-de-Mora et al., 2006). As P is an important nutrient; the elevated alkaline phosphatase in the amended soils indicates a greater ability to support the growth of plants in the urban garden.

The activity of arylsulfatase (Fig. 4-7) was highest in the composted biosolids treatment, lowest in the control treatment, and in-between in the mushroom compost. Arylsulfatase is important in the breakdown of organic sulfur compounds; sulfate groups are cleaved from organic C compounds resulting in mineralized S and a remainder organic C molecule (Acosta-Martinez et al., 2000; Fitzgerald, 1976; Makoi and Ndakidemi, 2008). Sulfur data from the amendments showed higher S in the mushroom compost compared to the composted biosolids. Since most soil S is organic-S, this indicates inhibition by available Pb (Attanayake et al., 2015).

The addition of both compost amendments enhanced all three soil enzymes. This overall increase in enzyme activity that was observed with the addition of composts was expected and is a good indicator of soil ecological functioning. The degree that the enzymes were enhanced in the soil depends in part on the type of compost and micro/macro nutrients that it contains. Composts are a useful tool for urban gardeners wanting to stimulate soil microbial activity.

### *Contaminant availability*

Plant growth data on this site revealed that As and PAHs, while elevated in the soil, were not detected in the plant tissues in any significant amount; indicating that the majority of the

ecotoxicity risks were with Pb (Attanayake et al., 2015). As such, the plan for this study was to focus on Pb. The extraction of Pb and Zn with 0.01 *M* $Sr(NO_3)_2$ was not significantly different between treatments (Table 4-5). However, the sequential extraction of Pb that was performed on the soils in this study showed a significant correlation with compost treatment (unpublished data from K-State Soil and Environmental Chemistry Laboratory). The first step of the sequential extraction was to measure the Pb that was exchangeable (i.e., non-specific sorption); and this exchangeable Pb was significantly elevated in the mushroom compost amended soil (Fig. 4-8). There was no significant difference in exchangeable Pb between the control and the composted biosolids amended soils; both were equally lower than the mushroom compost in exchangeable Pb. This increase in exchangeable Pb in the mushroom compost is likely responsible for the decline in actinomycete activity in that soil (Fig. 4-2, 4-3). The lack of pH differences between soil plots suggests that decreased pH was not the main mechanism for the increased Pb availability in the mushroom compost (Table 4-6). A possible explanation for this was ligand complexation; however further research would be required to determine if this was indeed the case. Soluble organo-Pb complexes can form and are often bioavailable (Attanayake et al., 2015; Marcano-Martinez and McBride, 1989; Sauve and Hendershot, 1997a; Weng et al., 2002). Greater concentrations of soluble organic compounds generally lead to increased prevalence of soluble organo-Pb compounds (Sauve and Hendershot, 1997a; Weng et al., 2002). Previous work on the soils at this site did show significantly greater dissolved organic carbon in the mushroom compost amended soil at 7 and 106 days post application; however whether or not that remains the case is yet to be determined (Attanayake et al., 2015). Mushroom compost is typically composted of horse manure mixed with other organic wastes like saw dust that is used for 1 production cycle of mushroom production and then sold as a fertilizer. The amount of time

that the material is composted for is far shorter than that of the composted biosolids, which are produced according to EPA regulation (2-3 year composting and drying process). The organic carbon in the mushroom compost is 'fresher' and therefore more labile than that in the composted biosolids. In addition to showing no enhancements in exchangeable Pb, the composted biosolids treatment clearly indicated that soil Pb chemistry was changed by the amendment additions. As a result, soil arsenic speciation is planned for future studies.

## Conclusions

From the results of the study it appears that the organic amendments largely increase the activity of soil microorganisms. Organic amendments generally improve the microbial indicators of contaminated soils via three main mechanism; the dilution of contaminant via the application of large volumes of material, the increase availability of nutrients and organic substrate from the amendment, and finally through the potential to form insoluble complexes with soil contaminants (Ahmad et al., 2012; Attanayake et al., 2015). However, the mushroom compost increased the exchangeability of Pb enough to cause a decline in actinomycete populations. While the mushroom compost itself had very little Pb; the chemical changes it brought about in the soil environment appears to have increased the ecological toxicity of soil Pb to sensitive soil microbial population. Understanding the chemical speciation and transformation pathways of contaminant is important for understanding impacts of soil contaminants on microbiological communities. For urban farmers working on mildly contaminated soils, the use of more mature composts and organic amendments are recommended above fresher amendments. Mature

organic amendments have little soluble carbon and thus are less likely to form soluble organo-metal complexes.

## Acknowledgements

We thank the US Environmental Protection Agency for providing funding for this research (Grant No. TR-83416101). We also thank USDA National Institute of Food and Agriculture Multistate project W2170 for providing funding for this research. We would like to thank Chammi Attanayake for allowing us to use her soil samples for this work. We would like to acknowledge Oshadhi Athukorala Arachchige for helping with the laboratory analysis and Pavithra Pitumpe Arachchige for her help with data analysis. We would also like to thank Madhubhashini (Buddhika) Galkaduwa for the GF-AAS analysis and Andrew McGowan for his help with PLFA data analysis and interpretations.

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# Tables

**Table 4-1**: **General properties of the organic amendments used in this study. Adapted from Attanayake, 2015**

| Property | Mushroom Compost | Composted Biosolids |
|---|---|---|
| Organic Matter (g/kg) | 606 | 212 |
| C to N ratio | 13.6 | 13.8 |
| pH | 7.91 | 6.18 |
| Total Lead (mg/kg) | 2.5 | 50.8 |
| Total Arsenic (mg/kg) | <1.4 | 6.2 |
| Total P (mg/kg) | 5540 | 7160 |
| Total S (mg/kg) | 27100 | 3550 |

**Table 4-2: Properties of initial soil at the garden site by the Monon railroad in Indianapolis, Indiana.**

| Soil Properties (Adapted from Attanayake et al. 2015 and unpublished data from K-State Soil and Environmental Chemistry Laboratory) | | | | |
|---|---|---|---|---|
| **Property** | **Initial Soil** | **Composted Biosolid Amended** | **Mushroom Compost Amended** | **Control** |
| Total Pb - mg/kg | 475 | 505.8±15.0 | 616.0±19.26 | 671.7±20.7 |
| Total As - mg/kg | 95 | 44.1±1.4 | 65.4±2.41 | 60.8±1.1 |
| EPA priority PAHs - mg/kg | 23–50 | - | - | - |
| Texture | Sandy Loam | Sandy Loam | Sandy Loam | Sandy Loam |
| pH (1:10 soil:water) | 7.48 | 7.39 | 7.38 | 7.50 |
| Percent Organic Matter | 5.4% | 9.84±0.42 | 9.16±0.61 | 7.39±0.64 |
| Available N – mg/kg | 5.2 | 101.8±27.2 | 50.4±32 | 36.7±11.3 |
| Available P – mg/kg | 61.2 | 508.6±43.6 | 214.4±50.3 | 145.8±42.6 |
| Available K – mg/kg | 50 | 181.8±12.7 | 185.6±40.8 | 123.8±17.7 |

**Table 4-3: Statistical information for PLFA analysis. Values that are significant to p<0.05 are bolded and discussed.**

| p-values for PLFA Analysis | | |
|---|---|---|
| **Group** | **Relative Abundance** | **Biomass** |
| Total Microbes | 0.1010 | 0.1010 |
| Fungi | 0.1209 | 0.0851 |
| Gram Positive Bacteria | **0.0328** | 0.0920 |
| Gram Negative Bacteria | 0.6492 | 0.9339 |
| Actinomycetes | **<.0001** | **0.0467** |
| Total Bacteria | **0.0248** | 0.0942 |

**Table 4-4: Statistical information for enzyme activities. Values significant to p<0.05 are highlighted and discussed.**

| p-values for Enzyme Analysis | |
|---|---|
| **Enzyme** | **p-value** |
| Beta-D-Glucosidase | **<.0001** |
| Alkaline Phosphatase | **<.0001** |
| Arylsulfatase | **<.0001** |

**Table 4-5: Statistical information for available Pb. Values significant to p<0.05 are bolded and discussed. Exchangeable Pb information is unpublished data from (Gravensen, 2016).**

| p-values for Available Pb Analysis. | |
|---|---|
| **Procedure** | **p-value** |
| 0.01 *M* $Sr(NO_3)_2$ Extractable Pb | 0.2864 |
| 0.01 *M* $Sr(NO_3)_2$ Extractable Zn | 0.5006 |
| Exchangeable Pb – Unpublished data from Gravensen, 2016. | **<.0001** |
| Correlation Between Exchangeable Pb and Actinomycete Biomass | **0.037** |

**Table 4-6: pH of soils; pH differences between soils were not statistically significant at p = 0.05.**

| Soil pH | |
|---|---|
| **Soil treatment** | **pH** |
| Control | 7.50± 0.07 |
| Mushroom Compost | 7.38± 0.03 |
| Composted Biosolids | 7.39± 0.08 |

# Figures

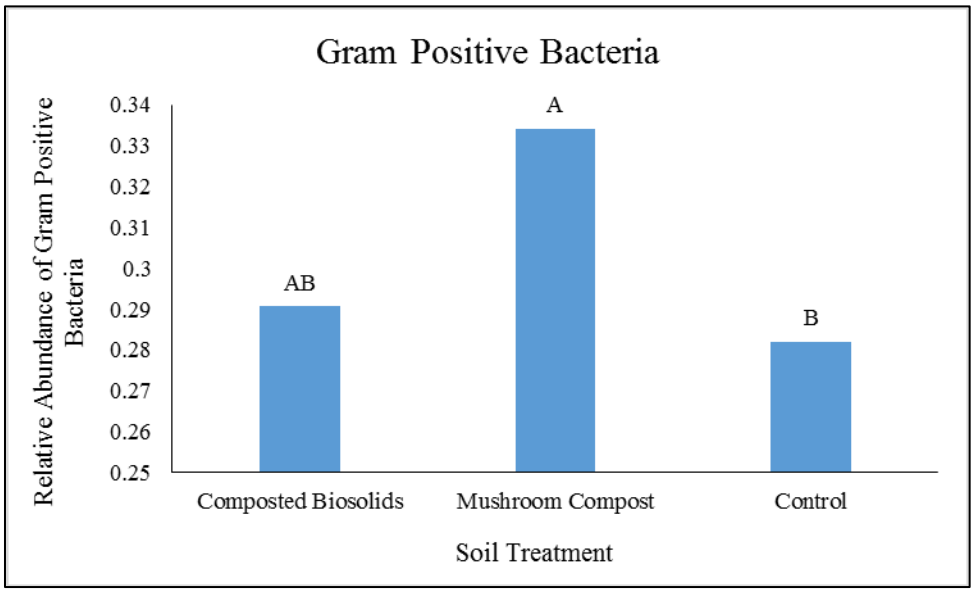


**Figure 4-1: Relative abundance of gram positive bacteria across soil treatments. Letters indicate significant differences at the $p<0.05$ level.**

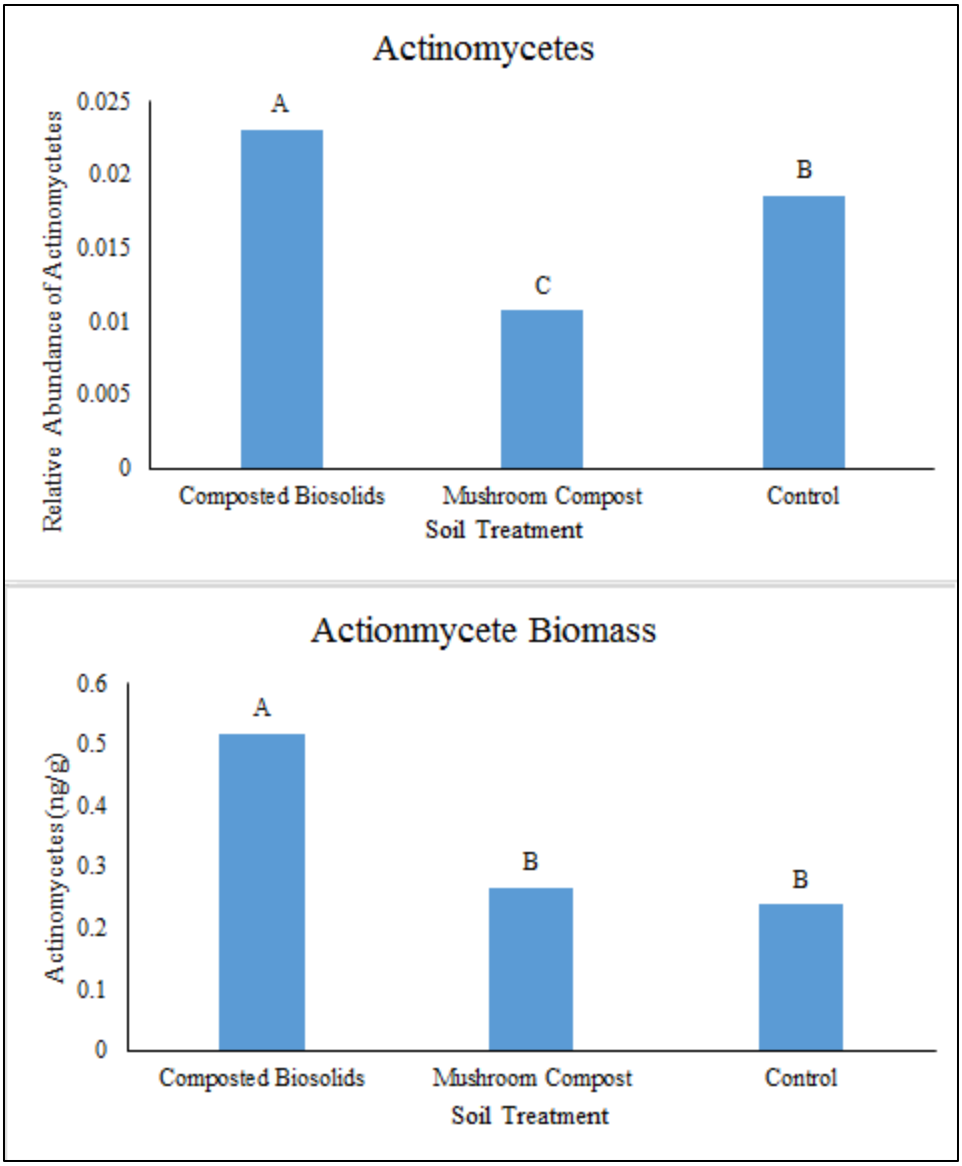


**Figure 4-2: Effect of treatments on actinomycetes. Top: actinomycete relative abundance compared to treatments. Letters indicate significant differences at the p<0.05 level. Bottom: actinomycete biomarker biomass compared to treatment. Letters indicate significant differences at the p<0.05 level.**

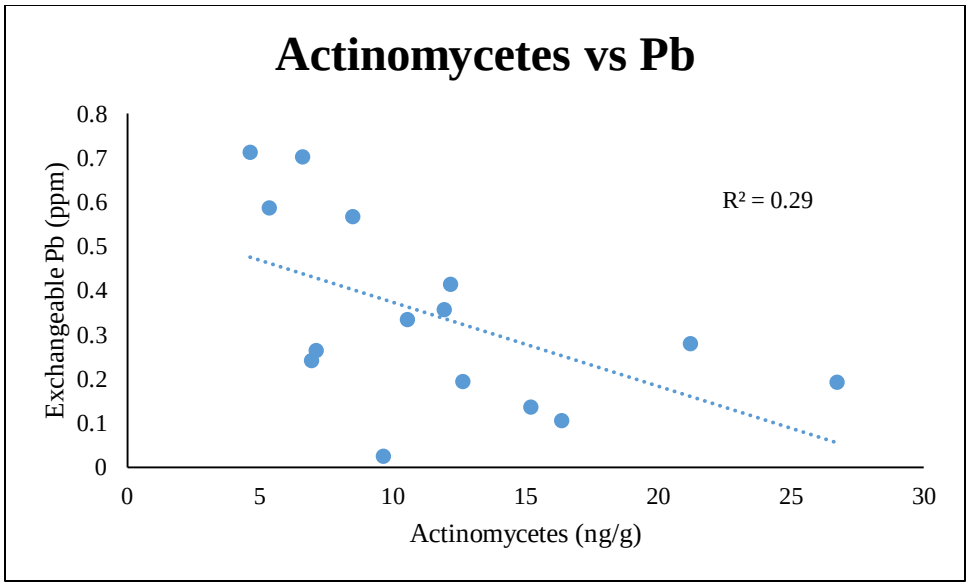


**Figure 4-3: Linear relationship between actinomycetes and exchangeable Pb. This relationship is significant to the p<0.05 level. Exchangeable Pb data is unpublished data from K-State Soil and Environmental Chemistry Laboratory**

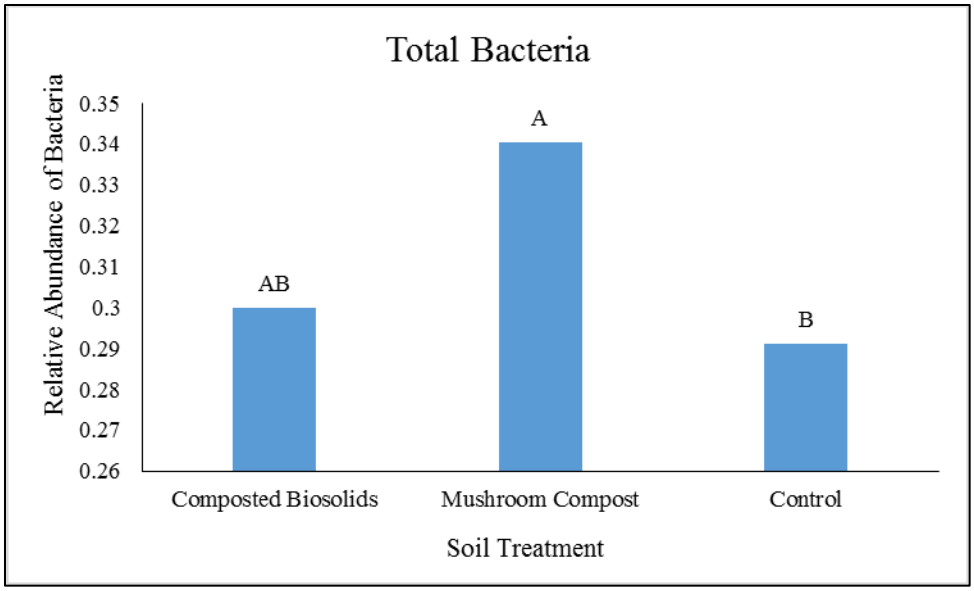


**Figure 4-4: Relative abundance for all bacteria as compared to soil treatment. Letters indicate significant differences at the $p<0.05$ level.**

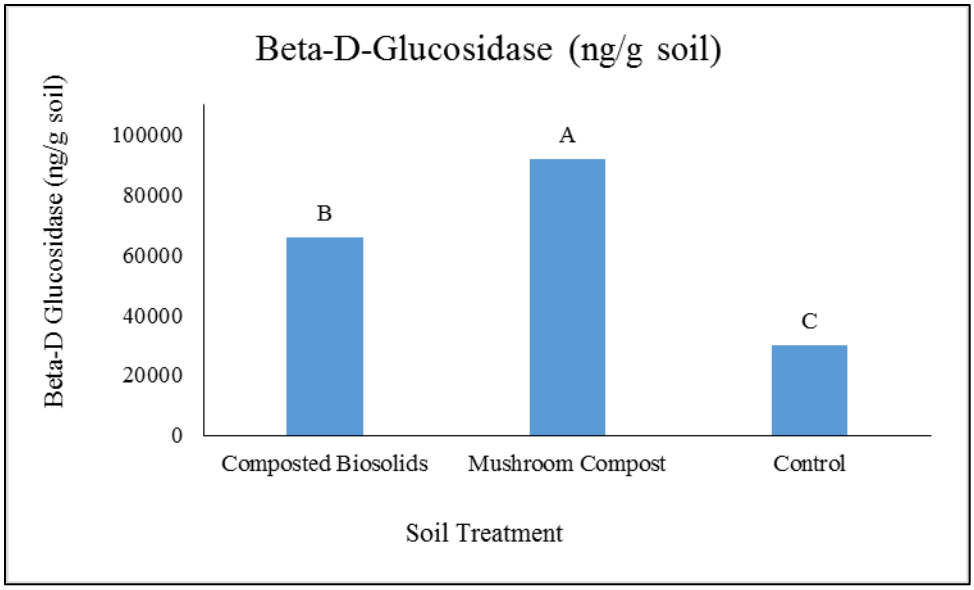


**Figure 4-5: Beta-D-Glucosidase activity compared to soil treatment. Letters indicate significant differences at the p<0.05 level.**

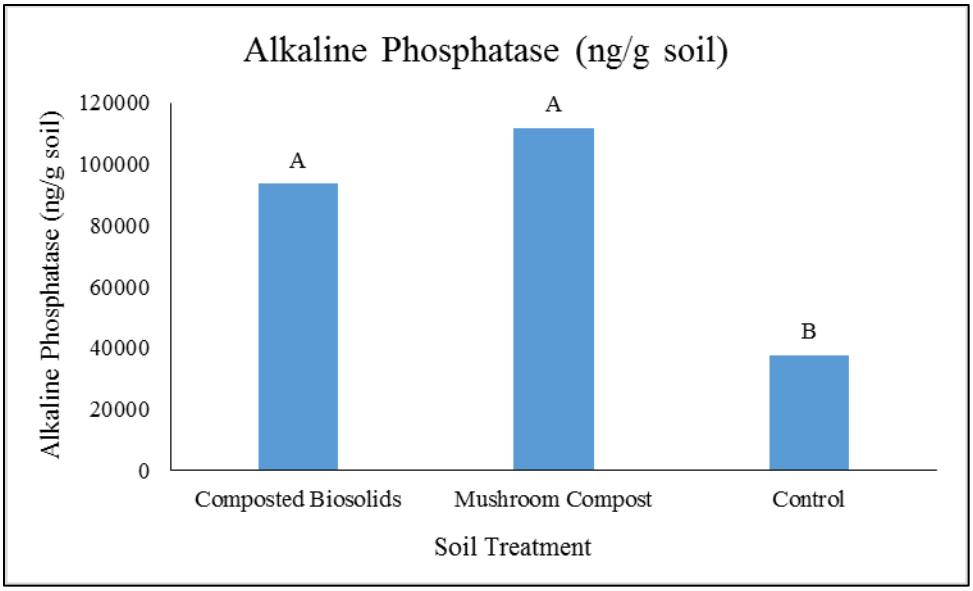


**Figure 4-6: Alkaline phosphatase activity compared to soil treatment. Letters indicate significant differences at the $p<0.05$ level.**

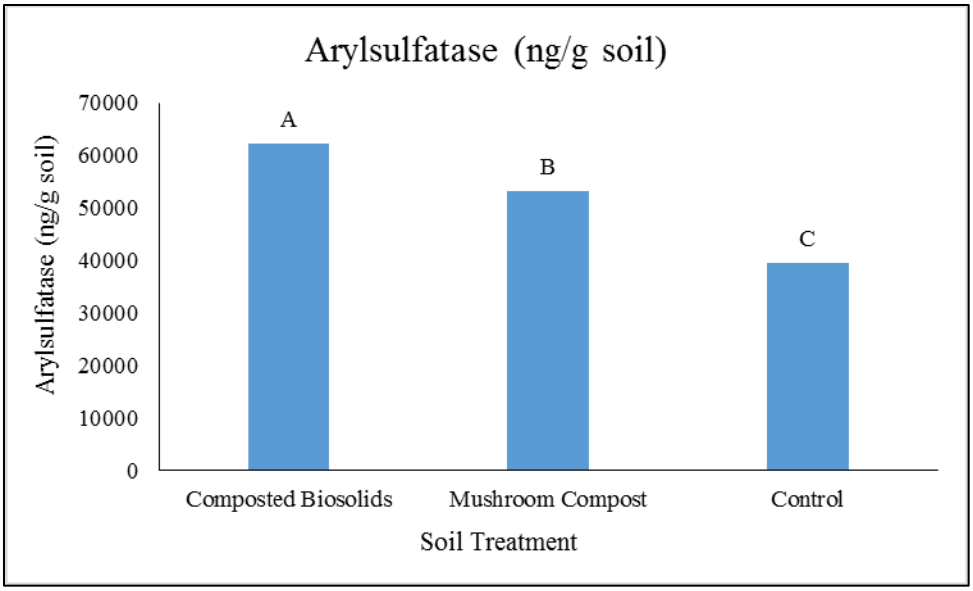


**Figure 4-7: Effects of soil treatments on arylsulfatase activity. Letters indicate significant differences at the $p<0.05$ level.**

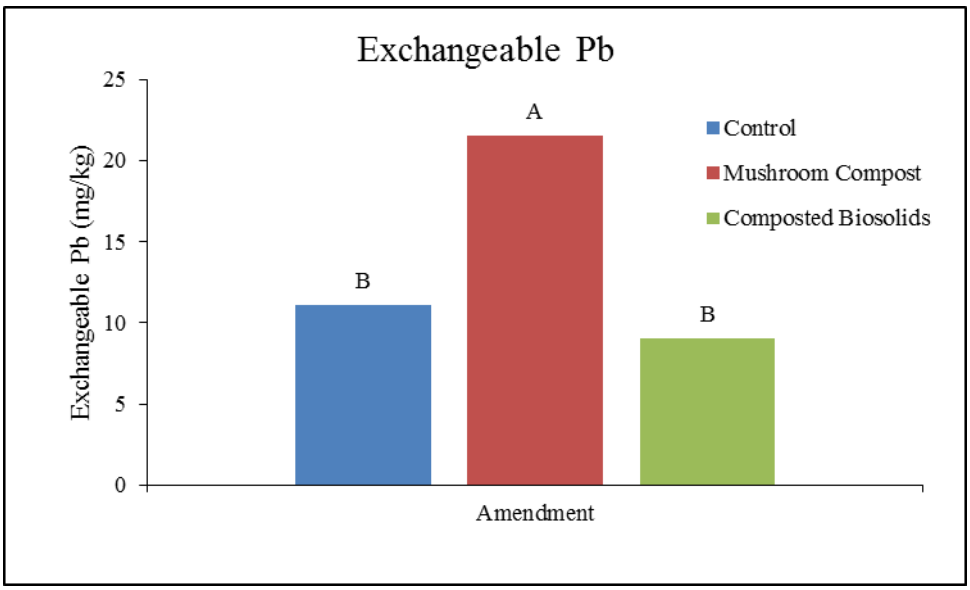


**Figure 4-8: Exchangeable Pb by soil treatment. Letters indicate significant differences at the p<0.05 level. Exchangeable Pb data is unpublished data from K-State Soil and Environmental Chemistry Laboratory (2016)**

# Supplementary Information

**Table 4-7: Enzyme Activity Averages**

**Enzyme Activity Averages; letters reflect differences at the 0.05 level.**

| Treatment | Beta-D-Glucosidase (ng/g soil) | Alkaline Phosphatase (ng/g soil) | Arylsulfatase (ng/g soil) |
|---|---|---|---|
| Composted Biosolids | $65985_{B}$ | $93501_{A}$ | $62245_{A}$ |
| Mushroom Compost | $91855_{A}$ | $111908_{A}$ | $53115_{B}$ |
| Control | $30064_{C}$ | $37799_{B}$ | $39462_{C}$ |

**Table 8: PLFA Averages; both relative abundance (ratio of group biomass to total biomass) and group biomass.**

| PLFA Averages; letters reflect differences at the 0.05 level. | | | | |
|---|---|---|---|---|
| **Treatment** | **Gram Positive Bacteria Abundance (ratio)** | **Actinomycetes Abundance (ratio)** | **Total Bacteria Abundance (ratio)** | **Actinomycete Biomass (ng/g)** |
| Composted Biosolids | $0.2906_{AB}$ | $0.02303_{A}$ | $0.3000_{AB}$ | $0.518391_{A}$ |
| Mushroom Compost | $0.3341_{A}$ | $0.01072_{C}$ | $0.3405_{A}$ | $0.264797_{B}$ |
| Control | $0.2820_{B}$ | $0.01851_{B}$ | $0.2910_{B}$ | $0.238127_{B}$ |

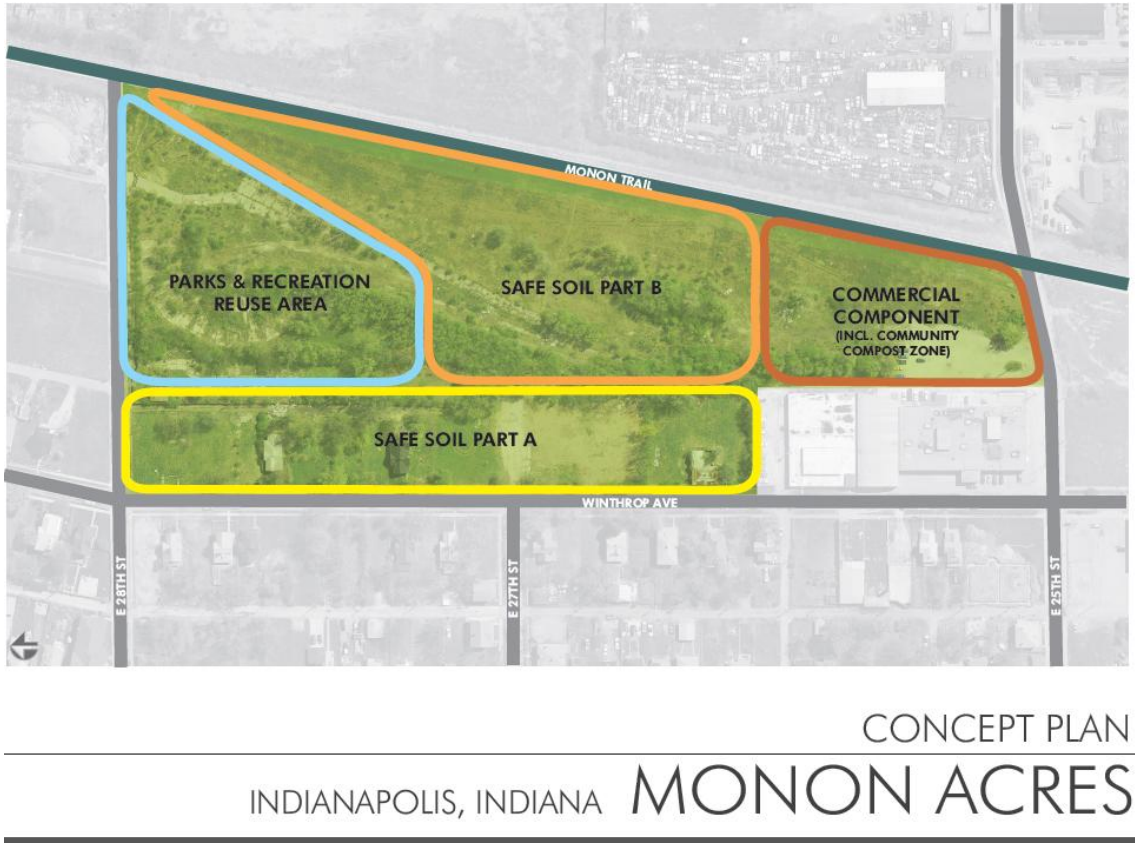


**Figure 4-9: Garden site map.**

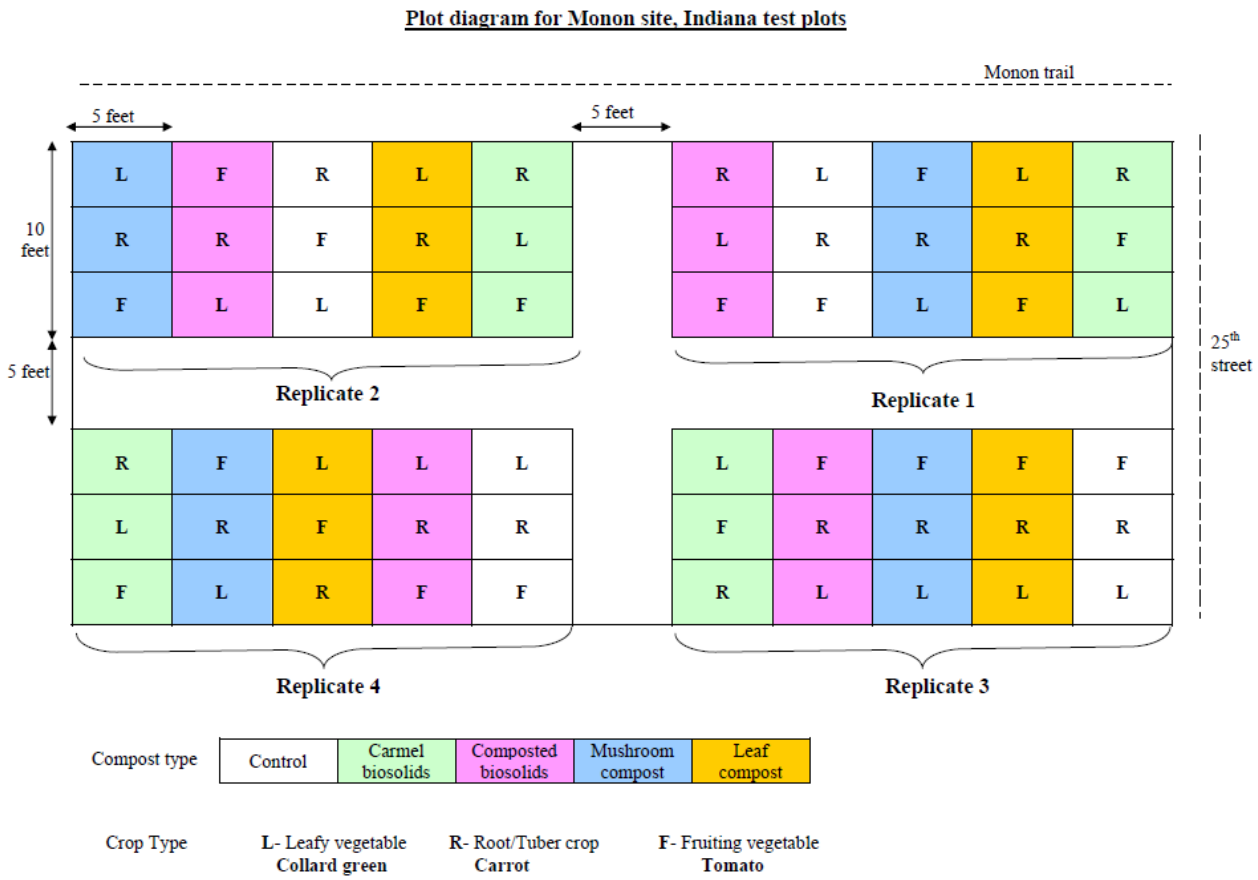


**Figure 4-10: Plot map for Indianapolis site. Map from Attanayake et al (2015).**

# Chapter 5 - Overall Summary and Recommendations

The main goal of this thesis was to achieve a better understanding of how anthropogenic alterations on the soil environment affect microbial community structure and activity. The two major anthropogenic influences studied were climate change and soil contamination. Climate change from elevated atmospheric greenhouse gases (CO2, CH4, and N2O) will alter soil temperature and moisture regimes. Warming the soil increases the rate of chemical reactions, including the ones inside of soil microbes, possibly leading to greater mineralization of SOC. Soil microbes also need moisture to carry out their activities and they are typically at peak activity around field capacity; deviations (both wetter and drier) from field capacity can result in lower microbial activity and biodiversity. Soil contamination is very prevalent in urban areas as a result of historical use of hazardous products, industrial processing (i.e. smelting), and fossil fuel combustion. The most common soil contaminant is Pb, which has been shown to have deleterious effects on human health and on ecosystem processes.

In order to study the effects of climate change and soil contamination on soil microbial processes, two incubation studies were carried out. The first study looked at the effects of changes in temperature, moisture and physical protection of soil organic on soil microbial communities and consisted of three temperature treatments (12°C, 24°C, and 36°C), two moisture treatments (field capacity and 80% of field capacity), and two aggregation treatments (<4.0mm "intact", and <0.25mm "crushed"). These treatment combinations were applied to four different soils. For each combination of treatments, there were three replicates in each soil. Two of the four soils were KS Mollisols (one no-till and the other conventional tilled), and two were BR Oxisols (one no-till and the other conventional tilled). The microbial community was analyzed via phospholipid and fatty acid biomarkers at the following sampling times: 7 days, 30

days, 60 days, 120 days, and 180 days. The second incubation study looked at the effects of organic amendments on the soil microbial communities and processes; and how contaminant speciation affects microbial communities and processes. Garden test plots at a site contaminated with Pb, As, and PAH's in Indianapolis, IN were selected for this study. Soils were collected 484 days after the addition of organic amendments from plots amended with; composted biosolids, mushroom compost, and a NPK added control. Each amendment type/control had four field replicates. Collected soils were adjusted to 60% MWHC and incubated for 30 days at 24°C to stimulate microbial activity. Following incubation, the microbial community was analyzed using phospholipid and fatty acid biomarkers as well as enzyme activity. In addition to characterizing the microbial community, indirect speciation of soil Pb was also performed on the soils.

In the first study, changes in temperature, moisture, and aggregation had significant effects on the soil microbial communities. Soil microbes across multiple groups declined towards the end of the study (180 days) especially in the crushed and 36°C. Decline in labile C (no new C was added over the course of the experiment) was responsible for most of the decline in microbial populations. Microbial populations in the no-till soils were less affected by the experimental variables, likely due to higher starting SOC, better aggregate structure, and better water holding capacity (due to higher SOC). These differences were more apparent in the Oxisols, which were more affected by changes in soil moisture. Practices that increase soil carbon storage and improve soil aggregate structure improve the resilience of soil microbes to changing temperature and moisture conditions. No-till, organic matter additions (manure, compost, etc.), crop rotation and cover crops can all contribute to increasing SOC and improving soil structure.

In the second study, available carbon and exchangeable Pb had significant effects on soil microbial populations and processes. In the mushroom compost amended soils (a fresher or younger carbon source) labile C appeared to be forming complexes with Pb and increased solubility and thus bioavailability. Actinomycetes and S cycling microbes were inhibited by available Pb. In soils contaminated with metals like Pb, the use of mature organic amendments (like composted biosolids) with high Fe contents can reduce bioavailability and improve soil ecological functioning. As such, mature organic amendments and/or organic amendments with high Fe content, capable of reducing contaminant availability, are recommended for mildly contaminated urban soils.