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# Agricultural Practices and Nitrogen Cycling Microbes: The Impact of Sustainable Farming Practices on Nutrient Movement in Indianapolis Urban Farms

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Thesis adviser(s) Sean T Berthrong Date 5/4/22

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Date

Certified by

Director, Honors Program Date

**Agricultural Practices and Nitrogen Cycling Microbes: The Impact of Sustainable Farming Practices on Nutrient Movement in Indianapolis Urban Farms.**

A Thesis

Presented to the Department of Biological Sciences

College of Liberal Arts and Sciences

And Honors Program

Of

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In Partial Fulfillment

of the requirements for Graduation Honors

Margaret A. Davis

Advised by Dr. Sean T. Berthrong

**Abstract**

As population movement to urban centers and decreased availability of fresh foods becomes more common, the prevalence of food deserts is becoming far greater. Urban farming can potentially help address these issues by bringing healthy and fresh food sources directly to these areas that lack access to quality food. Urban farming is reliant on the implementation of sustainable practices, like the use of cover crops, to increase the amount of nutrients that are accessible to plants from the soil. Nitrogen is a vital nutrient but cannot be readily produced by plants, so it must be obtained by either an external or internal source. The internal supply of Nitrogen is obtained through a mutualistic, commensal, or symbiotic relationship between the plant and nitrogen (N)-cycling microbes found in soil. This study aims to quantify the number of N-cycling microbes that are present in urban farms throughout the city of Indianapolis. These factors indicate that N transformations between different chemical types are occurring within the soil, which will likely affect the immediate and long-term supply of N. This process is essential to increase the health of the soil.

The focus of this study is how sustainable farming practices and an increased N supply in the soil can lead to further success in urban farming in Indianapolis, therefore addressing some of the problems of food insecurity impacting many urban centers. It is hypothesized that sustainable practices will increase the amount of N-cycling occurring, making soil healthier for urban farming. I predict that soil samples retrieved from the growing beds of farms that implement sustainable practices will have higher quantity and diversity of N-cycling microbial genes than samples retrieved from outside the growing beds.

This study used samples from 4 urban farms in the Indianapolis area. The samples were gathered from inside growing beds and surrounding non-farmed background soil areas. The

samples were amplified using Polymerase Chain Reaction (PCR) and then quantified using Quantitative Polymerase Chain Reaction qPCR. Thought there was variability in our results, we found that the samples gathered from inside the growth beds had higher quantity of N-cycling genes than outside the growth beds.

**Introduction**

Nitrogen is one of the most common growth-limiting mineral nutrients for plants with regards to growth and development (Schlesinger & Bernhardt, 2013). Nitrogen cannot be readily created by plants, so it must be supplied by an external source or internal supply. Fertilizers and compost are two of the main external sources of nitrogen; they provide nitrogen directly to the soil so plants can pick it up through the roots. The internal supply of nitrogen is provided through a mutualistic, commensal, or symbiotic relationship between a plant and nitrogen (N)-fixing bacteria found in soil (Marschner, 2011).

Atmospheric nitrogen makes up 80% of the earth's atmosphere, however it cannot be used by plants until it is converted to ammonia. Nitrogen (N)-fixing microbes use an enzyme called nitrogenase to catalyze the reduction of atmospheric nitrogen into ammonia so plants can use it. Nitrogenase can be used as a genetic marker to identify the potential for nitrogen fixation in bacteria (Bernard, 2010). N-fixing bacteria are typically found living freely in farm soil and typically form symbiotic relationships with leguminous plants. Legumes are plants of the Fabaceae family that are an important source of nutrients. Within their symbiotic relationship, legumes will provide sugar for photosynthesis that is utilized by bacteria for nitrogen fixation. Therefore the plants provide carbohydrates for the bacteria, and the bacteria provide nitrogen for the plants (*Biological Nitrogen Fixation | Learn Science at Scitable*, n.d.). Because of their role in nutrient cycling, legumes also serve very important roles in agriculture as cover crops or fertilizers (Boston & Ma, 2019). Cover crops, like N-fixing legumes, do not produce fruits or vegetables but are commonly used in urban farming because they increase the concentration of N-fixing bacteria in the soil and hence plant-available nitrogen. Increasing the concentration of

N-fixing bacteria in the soil will increase the supply of usable nitrogen in the soil to assist in crop production (Ladha et al., 1992).

The N-cycle is the import, export, and chemical conversion of N throughout the earth's ecosystems. N-fixation is one of the major transformations that occurs within the nitrogen cycle and is also one of the most studied phases. There are many other phases of the N-cycle that can also be looked at to understand if N is cycling throughout the ecosystem. In this study, we will be focusing on ammonia oxidation and denitrification. Ammonia oxidation occurs when bacteria or archaea oxidize ammonia and nitrite to gain energy and produce nitrates. Nitrates are then readily able to be used by plants (Kirchman, 2018). Denitrification is the process of converting nitrate back into nitrogen gas. Once nitrogen gas is returned to the atmosphere, the process of nitrogen fixation and N-cycling can begin again. This study will specifically target 4 genes: NirK, NirS, AmoA-Archaea, and Chi. The presence of nitrite reductase genes (NirK and NirS) indicate that denitrification is occurring. We will also specifically target an archaeal gene involved in ammonia oxidation (AmoA- Archaea). Finally, we will target the chitinase gene (Chi); chitinase is an enzyme that breaks down chitin. Chitin is an insoluble polysaccharide that serves as a major source of carbon and nitrogen to many organisms (Delpin & Goodman, 2009).

By quantifying the number of N-cycling genes that are present in the soil, we will be able to understand the amount of N-cycling that is occurring and gain insight into how farming practices might synergistically increase these processes. Higher rates of nitrogen fixation, and cycling have been linked to higher abundance/quantity of N-cycling genes (Reed et al., 2010). Therefore, a higher abundance of N-cycling genes will be indicative of higher rates of N-cycling occurring within the different samples and farms. This is an indicator that all symbiotic

relationships, specifically bacterial-legume symbioses, are functioning effectively and this provides an environment that is best suited for crop growth.

It is important to understand rates of nitrogen cycling and the presence of N-cycling microbes so that urban farming can effectively use the benefits of symbiotic and commensal soil microbes. Urbanization is spreading rapidly as people everywhere are migrating to urban areas, and they are quickly becoming one of the world's main habitats. As of 2018, 55% of the world's population resided in urban areas (United Nations et al., 2019). Urban centers are beginning to be seen as their own ecosystems, the only issue is these ecosystems are often lacking nutrients that are essential for plant development. Urban farming is defined as "all forms of agricultural production (food and non-food products) occurring within or around cities," (Wagstaff & Wortman, 2015). With mass migration to urban centers, there is a lack of fresh produce that is readily accessible. Currently and historically, cities rely on transport of food from outside land and this is taking a very large toll on the ecological footprints of these cities (Deelstra & Girardet, 2000). The direct transport of food and supplies into cities increases their carbon footprint as well as pollution in general.

Most of these urban centers lack easy access to nutritious and fresh foods that are necessary for a stable diet. In 2019, 10.5% of American households were food insecure at least some time during the year (*The Prevalence of Food Insecurity in 2020 Is Unchanged from 2019*, 2021). This statistic is even higher for those who live in Indianapolis, with 22% of Indianapolis residents living in food deserts (Benson, 2019). The USDA defines a food desert as a region where the majority of the households have low incomes, inadequate access to transportation, and limited number of food retailers with nutritious and fresh food at affordable prices (Dutko et al., 2012). Most people within these food deserts suffer from food insecurity, or the lack of available

financial resources to acquire nutritious food or get to that food. Food insecurity is a real problem in the United States and something that needs to be addressed. Urban agriculture has been associated with greater dietary diversity and calorie availability. These are both measures of an improved diet and directly correlated with increased food security (Zezza & Tasciotti, 2010). Urban agriculture has the potential to eliminate the delivery issue by providing nutritious and fresh food within cities where people need it, and this will provide greater options to people who lack them.

Some very important benefits associated with urban agriculture include food and nutrition security, increased health, waste reduction, and decreased pollution. It has also been known to assist in the development of local economies, climate regulation, and social inclusion and gender relations (Orsini et al., 2013). There are a variety of limitations that will need to be overcome in order to successfully implement urban farming. One of these major limitations is the condition of the soil. Most urban centers struggle with polluted air, soil, and water due to greater housing frequency, high population density, industry, and traffic (Martínez-Bravo & Martínez-del-Río, 2019). Successful urban agriculture will need to accommodate for plant growth under strong stresses, determined by air and soil contamination legacies (Orsinsi et al., 2013). There is little research that looks at the nutrient cycling and soil functions that are essential to successful urban farming. This study will focus on the internal nutrient supply of nitrogen in soil from urban farms in the Indianapolis area.

Sustainable practices, like the use of cover crops, in urban farms can increase the levels of N accessible to plants in the soil. The number of N-cycling microbes in the soil and the diversity of such species will be a focal point of the study as they indicate that N-cycling is occurring in the soil. The increased nitrogen cycling will increase the immediate and long-term

supply of nitrogen within soil. It is hypothesized that sustainable practices will increase the amount of N-cycling occurring, making soil more adequate for urban farming. I predict that soil samples retrieved from the growing beds of farms that implement sustainable practices will have higher quantity and diversity of N-cycling microbial genes than samples retrieved from outside the growing beds.

**Materials and Methods**

Soil collected from farms within Indianapolis were used as samples for this study. These samples were gathered and stored during the summer of 2017. There were 4 farms samples, referred to as farms A, B, C, and D. The farms are all one acre or less in size and within Indianapolis city limits. Each farm utilizes annual crop rotation and sustainable practices including the use of compost in the soil, cover crop planting, and the absence of fertilizers and pesticides. 5 samples were collected from within the farming beds (Inbed) at each site as well as 5 samples from nearby unfarmed areas (background) for a comparison. The samples were collected from 0-3 inches (topsoil) and 3-6 inches (bottom soil). This variety of depths corresponded to common planting depths used in most agriculture. The soil samples were processed through a 2mm sieve to remove large debris and organic matter. The samples were then separated and stored for chemical and DNA analysis. For farm A, we examined 60 samples with 30 being from in the growing bed and 30 being outside the growing beds. For the other 3 farms, there were 20 samples examined with 10 being from in the growing beds and 10 being outside the growing beds. The final sample size was 120 samples.

*DNA Extractions: Methods and Analysis*

The DNA was stored and extracted using DNeasy Powersoil kits (Qiagen, Valencia, CA). 25mg of soil was vortexed with beads, detergents, and buffers to lyse the microbial cells. The fragments of the microbial cells were then centrifuged to separate the DNA by density and filtered to isolate the DNA. The DNA was then purified via ethanol precipitation.

*Polymerase Chain Reaction*

DNA samples were amplified using polymerase chain reaction. The 4 primers used were AmoA-Archaea, NirS, NirK, and Chi. Each run contained 15 microliters: 12.5 μl of mastermix, 1.25 μl of the forward primer, 1.25 μl of the reverse primer, and 10 μl of sample. The mastermix used was 2x QuantiNova SYBR Green PCR mastermix. For cycle parameters, refer to Qiagen QuantiNova SYBR Green PCR methods.

*Gel Electrophoresis*

The samples from PCR were then run using gel electrophoresis. Gel electrophoresis uses an electrical field to separate DNA, RNA or proteins based on molecular size. This study used a 1% agarose gel. The DNA strands amplified from PCR run towards the positive end of the agarose gel because DNA is a negatively charged molecule. The gels were photographed and analyzed to determine the presence of N-fixing microbial genes. The specific genes we targeted have known sizes, so were able to compare them to make sure we were seeing what was expected.

*Ethanol Precipitation*

Samples were extracted from the agarose gel using a UV transilluminator. They were then resuspended using the QIA quick PCR and Gel clean up kit. Ethanol precipitation was performed to further clean the samples. Samples were combined with 1/10 sample volume of 3M sodium acetate and 2-3 volumes of chilled 100% ethanol. The solution was mixed and placed in the -80 degree C freezer for an hour. The solution was then spun in a centrifuge at 4 degrees C for 30 minutes. The supernatant was decanted, and the pellet was dried at room temperature. Samples were finally resuspended in autoclaved water.

*Quantitative PCR: Methods and Analysis*

The quantity of N-fixing bacteria in the soil was determined using quantitative polymerase chain reaction (qPCR). qPCR creates identical strands of DNA using the original target strand of DNA. It also uses a fluorescent dye to determine the number of copies of a particular gene in a given sample. This study used primers for qPCR that target the nitrite reductase gene (NirS and NirK), an archaeal ammonia oxidation gene (Amo-Archaea), and the chitinase gene (Chi). The qPCR ran using QuantiNova SYBR green PCR reagents (Qiagen, Valencia, CA) and the number of copies of all genes was determined using the amplification of sample DNA. A standard curve of known concentrations of DNA from common N-cycling microbes was compared against the sample DNA.

The results from the qPCR were adjusted by standardizing to comparative abundance of a bacterial housekeeping gene- a vital gene in basic cellular functioning of a cell, and is essential for the existence of a cell. This counteracted the variation seen in N-fixing bacteria that may have been caused by external factors. This can include factors such as soil characteristics caused by environment. An analysis of variance test (ANOVA) was then performed. ANOVA is used to

identify the differences between group means. It can demonstrate the relationship between variables. This study will examine the following variables: the collection from a farmed or unfarmed area and the depth from which the soil was collected.

*Summer 2021*

Over the summer of 2021, I participated in the Butler Summer Institute. I was given the summer to continue my thesis work, and took the time to examine all original 60 samples from Farm A. The same samples were used as are outlined above (collected in 2017). The same methodology was followed as well, except a Student's T-Test was performed to determine significance rather than an ANOVA. The data from farm A was later combined with 20 samples from farms B, C, and D to gather information about urban farming in all of Indianapolis.

**Results**

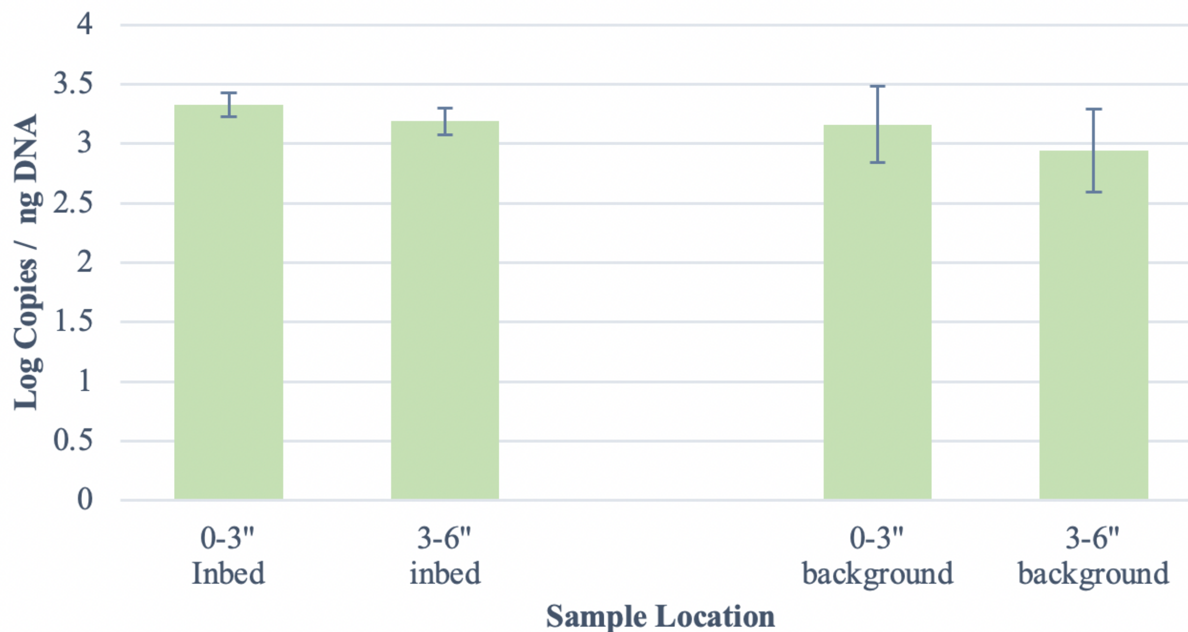


**Figure 1: qPCR results for NirK.** The graph represents the qPCR results for NirK. Data from each graph is comprised of 120 samples collected in 2017. There are 60 samples from farm A, and then 20 from farm B, C, and D. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. An ANOVA was performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that there is statistical significant difference between sample groups. Error bars denote standard error.

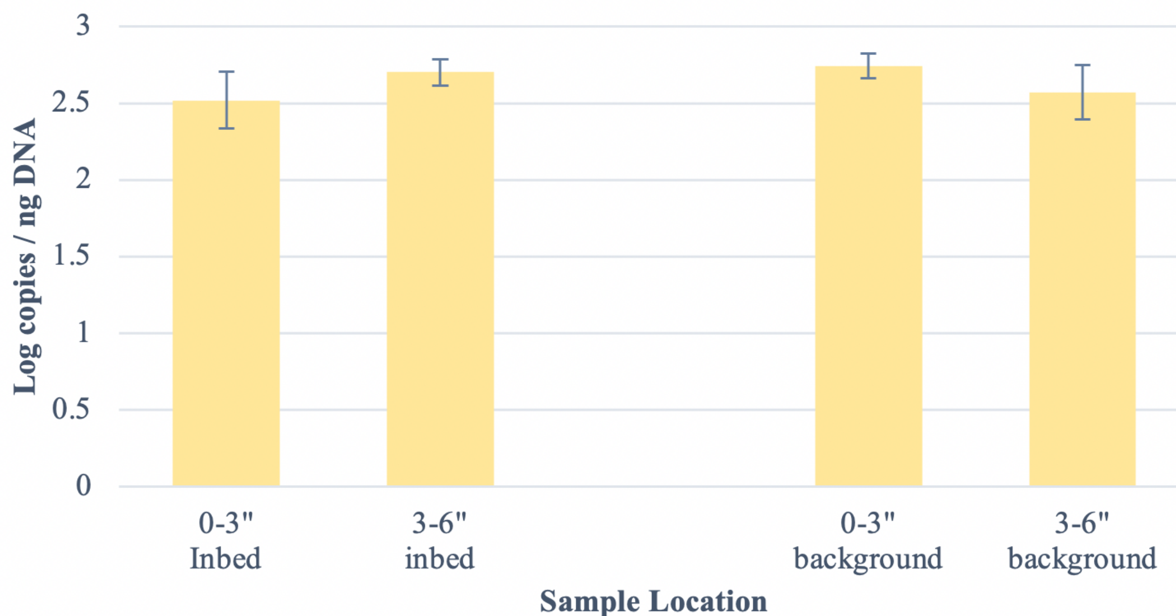


**Figure 2: qPCR results for AmoA-archaea.** The graph represents the qPCR results for AmoA-Archaea. Data from each graph is comprised of 120 samples collected in 2017. There are 60 samples from farm A, and then 20 from farm B, C, and D. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. An ANOVA was performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that there is statistical significant difference between sample groups. Error bars denote standard error.

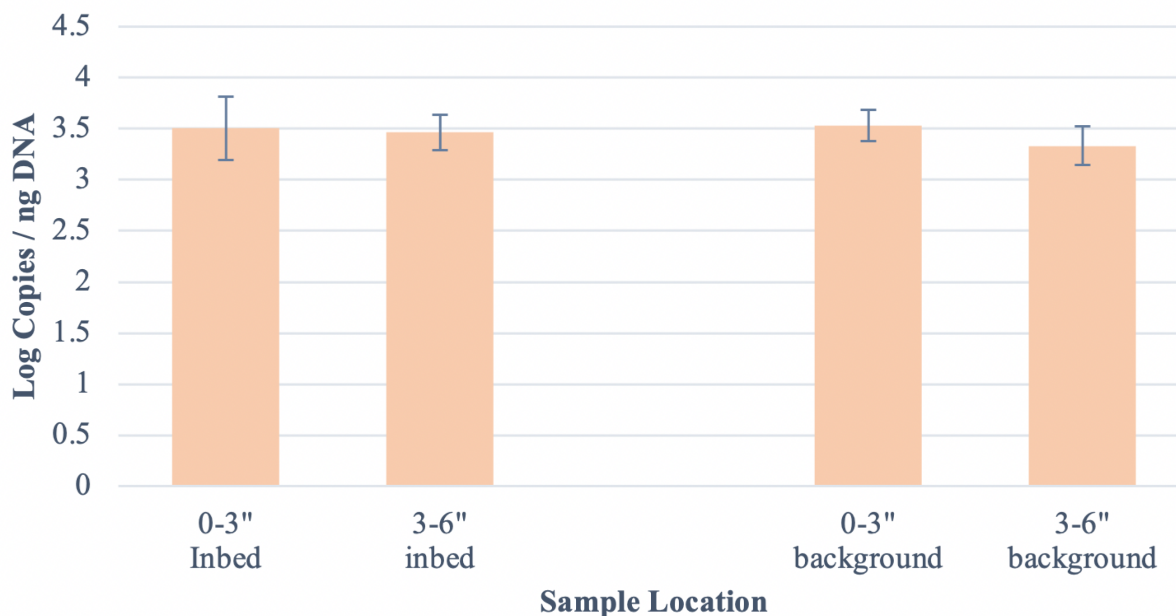


**Figure 3: qPCR results for Chitinase (Chi).** The graph represents the qPCR results for Chi. Data from each graph is comprised of 120 samples collected in 2017. There are 60 samples from farm A, and then 20 from farm B, C, and D. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. An ANOVA was performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that there is statistical significant difference between sample groups. Error bars denote standard error.

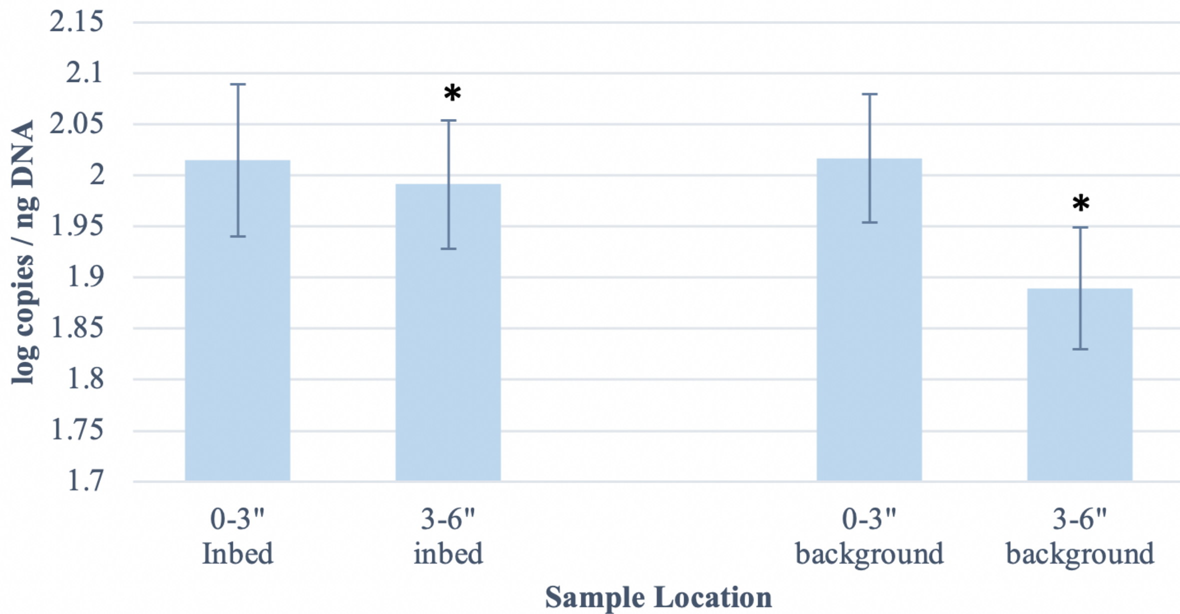


**Figure 4: qPCR results for NirS.** The different graphs represent the qPCR results for NirS. Data from each graph is comprised of 120 samples collected in 2017. There are 60 samples from farm A, and then 20 from farm B, C, and D. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. An ANOVA was performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that there is statistical significant difference between sample groups. Error bars denote standard error.

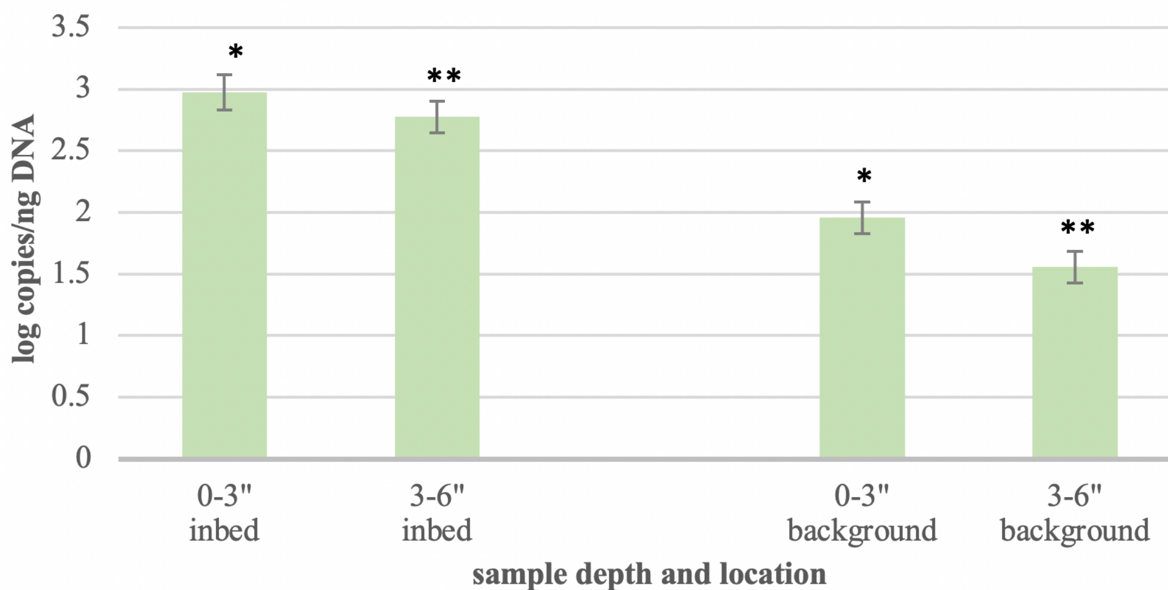


**Figure 5: qPCR results for NirK- Farm A only.** The different graphs represent the qPCR results for NirK. Data from each graph is comprised of 60 samples collected in 2017. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. T-Tests were performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that the data from the inbed data is significantly different from the background data at the same depth. Error bars denote standard error.

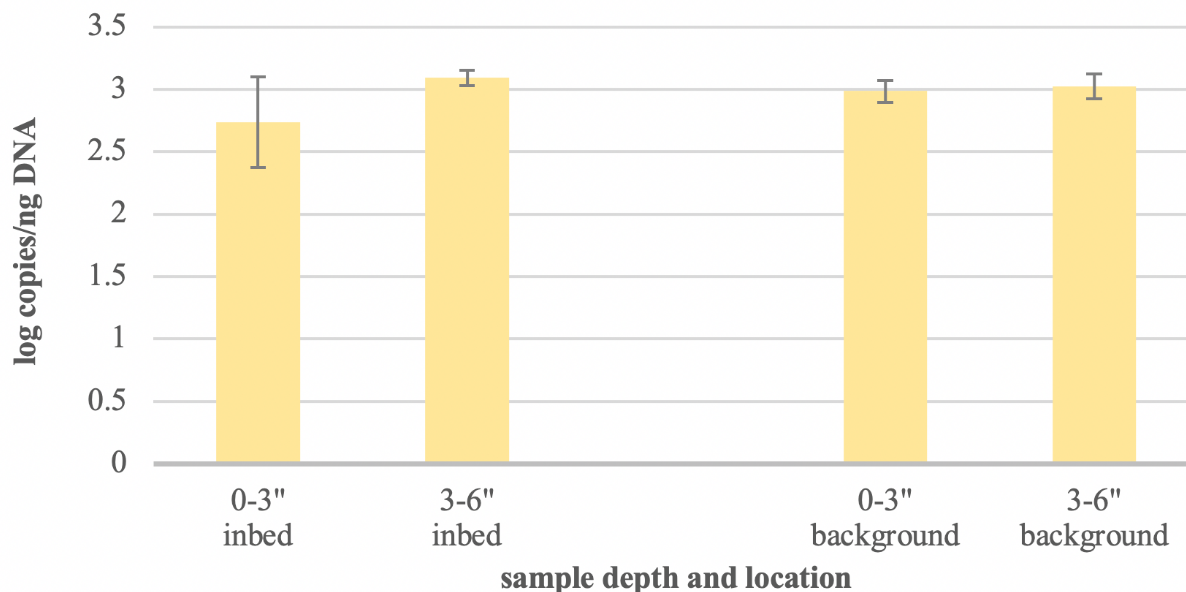


**Figure 6: qPCR results for AmoA- Archaea- Farm A only.** The different graphs represent the qPCR results for AmoA-Archaea. Data from each graph is comprised of 60 samples collected in 2017. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. T-Tests were performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that the data from the inbed data is significantly different from the background data at the same depth. Error bars denote standard error.

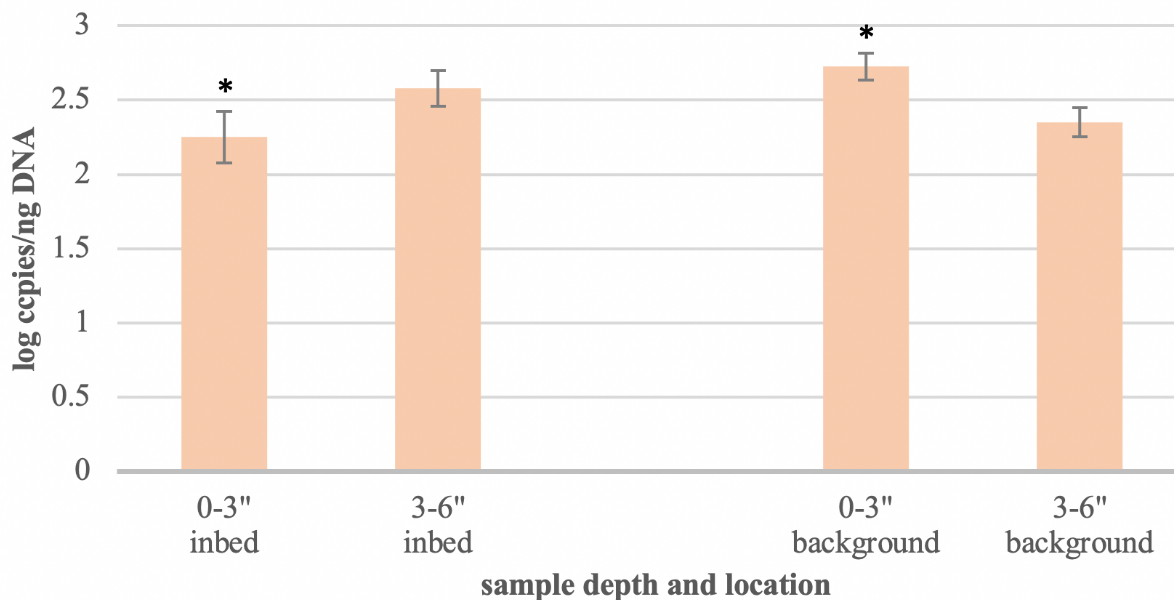


**Figure 7: qPCR results for Chitinase- Farm A only.** The different graphs represent the qPCR results for Chi. Data from each graph is comprised of 60 samples collected in 2017. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The “inbed” soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The “background” soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. T-Tests were performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that the data from the inbed data is significantly different from the background data at the same depth. Error bars denote standard error.

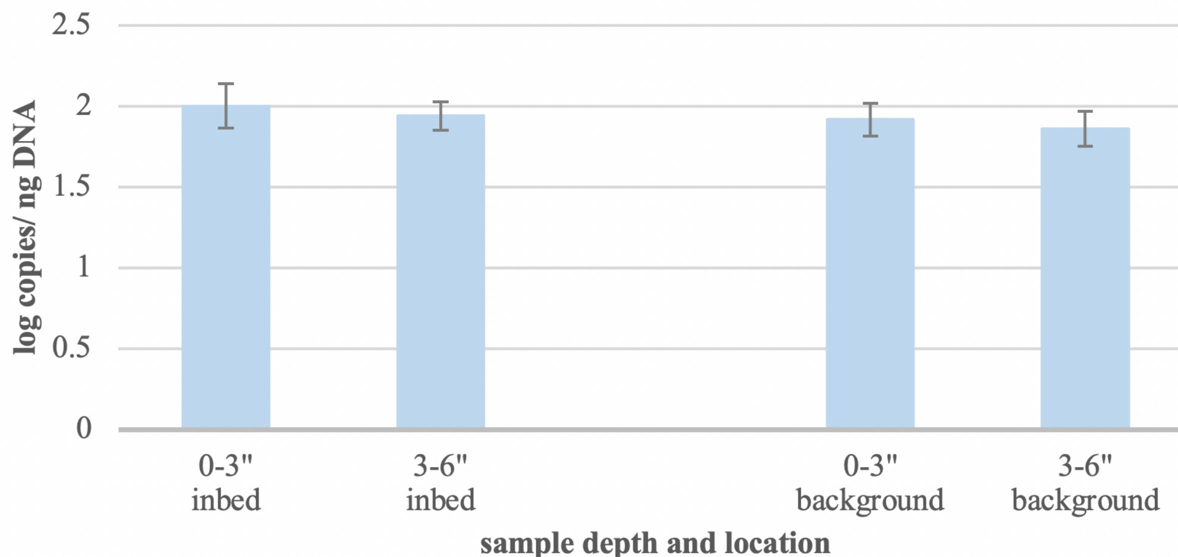


**Figure 8: qPCR results for NirS- Farm A only.** The different graphs represent the qPCR results for NirS. Data from each graph is comprised of 60 samples collected in 2017. The graphs represent the log number of copies/ng of DNA quantified from different soil depths and locations. The "inbed" soils include the samples collected from growth beds at depths of either 0-3 inches or 3-6 inches. The "background" soils include the samples collected from outside of the growth bed at depths of either 0-3 inches or 3-6 inches. T-Tests were performed to analyze statistical significance between the samples at the same depths between the different locations. An asterisk indicates that the data from the inbed data is significantly different from the background data at the same depth. Error bars denote standard error.

The data from the first four figures takes into account 60 samples from farm A, and 20 samples from farms B, C, and D. An Anova was performed for each of the different primers to compare the samples from in the growing bed and outside the growing bed at different depths. For NirS, a statistically significant difference was found between the samples at 3-6 inches in depth in the background versus the growing bed locations (p=.0005) (Fig. 1). There was no significant difference found for the topsoil. AmoA- Archaea, NirK, and Chi presented with no statistically significant difference between the samples taken from in the growing bed and background (Fig. 2, 3, &4).

Over the summer of 2021, a more extensive analysis was performed with a larger sample size for farm A. For the gene NirK, a statistically significant difference was found between the background and growing bed samples for both the top soil (p=1.15E-5) and bottom soil (p=2.69E-7). The results from Farm A aligned more closely with what we had expected to see; there was clearly a larger abundance of NirK genes in the samples taken from in the growing bed versus out of the growing bed at both soil depths (Fig. 5). While there were no significant results found for AmoA-Archaea or NirS, we did find that there was a significant difference in the topsoil for chitinase (p=.021)

**Discussion**

In general, our results did not detect significant differences in N-cycling enzymes in soil samples collected from within farmed growing beds and adjacent areas. The only exception was NirS. The data collected from the bottom soil (3-6 inches) showed significant results (Fig. 1). The isolated results from farm A were the most encouraging. We found significant results for the quantity of NirK genes in both the growing bed and background samples (Fig. 5). While a significant difference for the other genes were not detected, there is a visual difference that can be seen on the graphs. There are consistently more genes that were detected in samples from in the growing beds rather than outside the growing beds.

NirS and NirK are bacterial genes, and it is known that bacteria prefer a more neutral pH of soil. Sustainable farming practices produce a more neutral pH environment, which is consistent with our results that bacterial genes were better amplified and isolated than archaeal genes. We found that AmoA-Archaea was consistent in quantity in both the growing bed and background samples. Archaea are most successful in more acidic conditions. The pH of the background soil was consistently lower than the growing bed soil samples which would provide a more suitable environment for Archaea. I did not measure pH in this study, but I took the project over from another student who did such analyses (Lewis, 2019). This allowed us to make these connections between pH and bacteria and archaeal genes. It would be interesting to make comparisons of pH and look at the success of bacterial versus archaeal genes. This trend with regards to pH was not one we had expected to see, however it was consistent with what previous data and previous studies have shown. At the beginning of this project, we had the intention of quantifying the AmoA- Bacterial gene however we were unsuccessful. Once these issues are

sorted, the ammonia oxidation gene for bacteria and archaea could be analyzed to look at further differences between archaea and bacteria.

All of these results beg the question, why didn't we find anything more significant? There are a variety of factors that can have an impact on the affect of sustainable farming practices on soil health. One aspect to consider would be how long each of the farms have been participating in sustainable practices. Longer implementation of sustainable practices would lead to higher rates of N-cycling and healthier soil. It would also be interesting to look at what specific practices each farm uses and compare their impacts. Different practices might impact different stages of the nitrogen cycle which could have further impacts. Something else to consider is that these samples are currently 5 years old, which means that the farms have continued sustainable practices for the past 5 years. It would be interesting to look at the rates of nitrogen cycling now compared to before to see the impacts over a longer period of time. There is also a slight chance that the older samples have slowly degraded over time, so looking at new samples could be helpful in many ways.

The results we found are encouraging and of significant value to urban farming. Nitrogen is a dramatically important nutrient to plant growth, and the increased quantity of NirK in the growing bed samples leads us to believe that newer and more samples would support our hypothesis. Sustainable practices such as cover crops and crop rotation have the potential to increase intrinsic nitrogen levels as seen by the results for NirK and NirS. Continuing these practices over longer time scales may potentially increase the impact of such practices. Food insecurity is a major threat to urban centers, and that threat is only increasing. The addition of sustainable practices and increased nitrogen cycling can make urban farming potentially more

productive. This would help fight the issue of food deserts and food insecurity by bringing fresh, nutritious food directly to the areas that lack it.

There were many technical difficulties associated with this research. Initially, we struggled to isolate the DNA from our agarose gels. I performed many gels, and they would came back as expected when photographed. However, when it came time to isolate the DNA, vary small quantities were obtained. Originally, we had hoped to isolate 6 genes associated with N-cycling but were only successful with the 4 represented here. We were unable to isolate AmoA- Bacteria and 16S ribosomal subunit. We are not sure why we were unable to amplify these.

The next step in this project will be to continue to gather and expand the data collection. The first 60 samples from Farm A were examined in this project and only 20 samples from each of the other 3 farms were looked at. Another future step will be to increase the number of working primer sets. We began the project with 6 primer sets but were only able to isolate 4 out of those 6. Bacterial AmoA and bacterial 16S primers should also be used. As I mentioned previously, bacteria prefer neutral soil conditions while archaea can thrive in acidic soil conditions. Once AmoA- Bacteria can be successfully quantified, it would be interesting to compare the two ammonia oxidation genes and see how their abundance varies in different pH conditions. Once data collection is complete for all working primer sets, comparisons can be made between the different genes. While I will not be continuing this project, it is one that I hope others will be interested in continuing!

**Acknowledgements**

I would like to thank my advisor, Dr. Sean T. Berthrong, for supporting me throughout this entire process. We ran into quite a few obstacles along the way, but his constant support and enthusiasm was essential in bringing this project to a close. I would also like to thank Becca Lewis, who began this project in 2017. She provided the platform for my research and was an incredible resource throughout my entire process. I would also like to say thank you to Dr. Shelley Etnier, my second reader. She was always there to listen, and her encouragement and patience never went unnoticed. Finally, I would like to thank my family, the department of Biological Sciences, and the University Honors Program for making this all possible.

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