EFFECT OF COMPOST TEA ON PLANT GROWTH PERFORMANCE AND THE FATE OF MICROBIAL COMMUNITIES IN SOIL

by

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DEDICATION

This is dedicated to my parents, Noel and Leo Valdes, for their constant support in allowing me to pursue my education, and Sarah for always being a great big sister. I also want to dedicate this to my grandparents, Pamela, John, Angelita, and Valdemar, who always pushed me to do what I wanted and let nothing stop me.

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ABSTRACT

Compost tea is a popular amendment used to improve soil quality and to control soil-borne diseases in plants. With proper brewing, compost tea contains many of the beneficial microbes and nutrients of compost, but is more easily applied to plants. The purpose of this study was to (i) analyze the fate of microbial communities in spent mushroom substrate compost tea applied to soil microcosms planted with corn, and (ii) determine if growth of corn is influenced by specific constituents from compost tea, including microbes only, nutrients only, or a combination of both (i.e. the complete compost tea). Two trials were performed, one with anaerobic soil conditions and a second with aerobic soil conditions. Bacteria and Eukarya were quantified over the 30 days with sampling events on days 0, 1, 2, 5, 10, 20, and 30, as were plant growth performance characteristics like root and sprout length or their biomass. Results demonstrated a significant drop (70-90%) in abundance of microbes after application of compost tea, without recovery during the 30-day incubation period. Plant growth performance characteristics were not statistically significantly different for corn on soil receiving compost tea or separated components (i.e. microbes or nutrients) only, or a water control. While these results cannot support assumptions on beneficial effects of compost tea on plant growth performance and microbial communities in soil after application, further scientific research should consider long-term studies with different plant species and soils to further investigate potential beneficial effects of compost tea.

I. INTRODUCTION

Growing food for the world's burgeoning population is a constant struggle for agriculturalists. Dwindling land and fresh water resources are constantly challenging the boundaries between availability versus necessity. In 1798, Thomas Malthus wrote a series of papers on population, food trends, and our resources describing that the rates of food demand and production do not match (Landsburg, 2008). Soon we will reach a point where the world's population will be too high and food availability will not meet consumption (Landsburg, 2008). He predicted that we should have suffered famine and mass starvation, on a global scale, years ago. However, the development of genetically engineered crops as well as the help of chemicals to increase growth or kill off pests have enabled food production to match demand well beyond Malthus' predictions. Farmers grow enough food, but at a devastating cost to the environment. The switch to a more sustainable way of producing crops has been difficult and delayed through policy and mistrust. In 1981, the secretary of Agriculture decreed that 'millions would starve if farmers switched from inorganic to organic fertilization and farming' (Heckman, 2005). Politics have always caused confusion among the public regarding these two approaches to agricultural production (Heckman, 2005). Chemicals are easy to apply and cheap, therefore, more available. The switch to organic farming would be more time consuming and requires more maintenance on the part of the farmer, however, would potentially be more sustainable for agriculture in the long term.

Many pests and weeds have now become resistant to chemicals. Fertilizers and pesticides cause pollution to our ground and surface waters (Higa & Parr 1995). Justus

von Liebig (1803-1873) supported a theory; "The Law of the Minimum" that described replacing nutrients in order to boost plant production, which would eventually become the beginning of using artificial inorganic fertilizers to supplement plant's needs (Heckman, 2005). Chemicals used on crops end up in the soil, and move to our water systems by way of runoff. This can cause massive algal blooms that can spread for miles (Diaz & Rosenberg, 2008). A mixture of warmer water temperatures and the availability of fertilizers support the algal blooms (Diaz & Rosenberg, 2008). The fertilizers prompt massive growth, which might then lead to the formation of dead zones when bacteria mineralize algal biomass and concomitantly decrease oxygen concentrations. Dead zones form when microbial mineralization has removed all oxygen, thus creating a hypoxic environment beneath the surface of the water (Diaz & Rosenberg, 2008). With no oxygen available, fish cannot survive and die off quickly (Diaz & Rosenberg, 2008). There are several of these dead zones established or forming seasonally around the world; one major dead zone lies off the coast of China and another in the Gulf of Mexico (Diaz & Rosenberg, 2008).

Between the years 1940 and 1978, there were large differences in opinions among those who supported the use of inorganic fertilizers and those arguing for organic farming (Heckman, 2005). Sir Albert Howard (1873-1947) was a writer and agriculturalist and became a leader and promoter of the organic farming movement (Heckman, 2005). Known as the father of the organic movement, he studied in India and brought back the things he learned to Great Britain (Heckman, 2005). He illustrated the "Law of Return" that describes returning organic waste material back to the soil to improve soil quality (Heckman, 2005). Although he introduced organic farming in the early 1930's, it did not

gain general popularity until the 1990's as part of the sustainability initiative. Since more information has become widely available, consumers are now debating the quality of traditionally grown commercial fruits and vegetables compared to that of those from organic farming. Consumers are now more inclined to buy organic fruits and vegetables due to the lack of industrial pesticides and fertilizers used in their production (Hargreaves, 2009). Awareness has raised a 20% increase in demand for organically grown produce (Heckman, 2005). Government incentives have helped increase organic farm acreage to 2.3 million in a now $8 billion industry (Heckman, 2005).

Sustainable farming has been around for hundreds of years, though there are many interpretations of what constitutes sustainable farming. Sustainable agriculture and organic farming are not interchangeable terms (Heckman, 2005). Organic farming represents one approach to sustainable farming. Considered the most extreme type of sustainable farming, it has very strict rules in what constitutes true organic farming. The best sustainable agricultural developments are able to enhance and improve fertility without a devastating aftermath, and the best place to start is from the ground up (Higa & Parr, 1995).

Soil is a highly diverse environment that limits quantitative scientific analyses and thus our knowledge on soils and its constituents (Zhao et al., 2011). Soil is a mixture of minerals, organic matter, water, and air (Lowenfels & Lewis, 2010). Soils and their underlying rock materials are constantly exposed to physical or chemical weathering processes, which together with the basic composition of rock material determines the physiochemical properties of the soil (Lowenfels & Lewis, 2010). Soil quality is not only a function of these physiochemical conditions, but also of nutrient availability that is

promoted by a delicate balance of organic and inorganic material and microorganisms. When soils are farmed with no time to recover, the nutrients are exhausted and microorganisms begin dying off or become inactive, causing an imbalance of nutrient demand and availability (Ingham, 2005). Supplementing depleted soils is one obvious solution, with compost representing one alternative to industrial fertilizers. However, heavy metals from specific composts or fertilizers can also disrupt soil ecology as well as the plants ability to take in food (Karak et al., 2014). As one example, the presence of high levels of chromium in municipal solid waste compost was studied to determine if there were any toxic side effects (Karak et al., 2014). The two-year study concluded that the chromium levels were not high enough to produce side effects (Karak et al., 2014). It remains possible, however, that chromium levels could accumulate to toxic levels after many years of municipal solid waste compost amendments (Karak et al., 2014). In contrast to amendments with municipal solid waste compost, however, there are several types of compost or soil amendments that should not cause heavy metal accumulation over time.

Amendments of soil with organic material improves plant productivity, fertility, quality, and yield (Higa & Parr, 1995). Soil, untouched by pesticides or inorganic fertilizers, does not cause pollution to ground water from leaching of inorganic compounds, even though inorganic compounds are present at low concentrations within the soil (Ingham, 2005). Healthy soil does not become saturated with nutrients since nutrient availability is a function of microbial activity releasing inorganic components from the mineralization of organic material (Lowenfels & Lewis, 2010). Concentrations of chemical fertilizers and pesticides applied to soil can be too high to be retained entirely

and thus a portion can pass right through to the ground water, wash away, or turn into vapors (Ingham, 2005). Traditional industrial farming also does not allow soil to rest and recover from having crops repeatedly planted which might accumulate crop-specific pests or reduce specific nutrients (Ingham, 2005).

Microorganisms in soil are important because they maintain homeostasis. They decompose organic material, recycle nutrients, remove some pollutants, stave off pathogens, and release some potentially unavailable nutrients (Thies, 2008). Soil ecosystems are the most diverse compared to any other systems. Soil can contain more than 10^9 microbial cells per gram and harbor up to 10^6 different bacterial species per gram (Zhao et al., 2011). Other studies estimate that there may be close to 277,000 bacterial genomes per gram of soil (Thies, 2008). It has been shown that bacterial populations found in soils are positively influenced (i.e. found in greater abundance) from management practices that include residue incorporation compared to those in soils that are left alone (Davari et al., 2012). There have been debates on what is actually more beneficial for productivity, the bacteria or the organic residues (Higa & Parr, 1995). Many believe that adding residues to the soil will trigger growth for those microbes already present in the soil, while others believe that adding bacteria to the soil will help increase numbers and outcompete pathogenic microorganisms that might be present (Higa & Parr, 1995).

Compost is one of the original ways to introduce microbial populations back into the soil. Composting methods practiced for hundreds of years are known not only for the fertility effects on crops and soil but also for disease reducing capabilities (Higa & Parr, 1995). Composting is the process of decomposition of particulate organic matter.

Bacteria and fungi are the major players of this three-step process, which includes a mesophilic, a thermophilic, and then a mesophilic "curing" or maturation phase (Mehta et al., 2014). The initial heating of the compost is the first stage or mesophilic stage which is characterized by bacteria consuming the readily digestible sugars, starches, fats and proteins causing the temperature to rise (Baldwin & Greenfield, 2009). The farmer or other organic waste manager ensures the environment, maintaining safe production of the final product, compost by controlling the factors effecting compost include temperature, pH, moisture, carbon to nitrogen, and particle size (Schaub & Leonard, 1996; Baldwin & Greenfield, 2009). Composting works best when the microorganism growth is maintained (Rynk et al., 1992). It is the operators' job to watch and monitor the compost heap by watching temperature and moisture, adding water, turning the pile, or using artificial airflow to increase oxygen (Baldwin & Greenfield, 2009).

The second phase is the thermophilic stage, lasting several weeks, begins with a shift in the microbial community from mesophilic to the thermophilic bacteria that start breaking down material faster, further increasing temperatures (Baldwin & Greenfield, 2009). Bacteria decompose readily digestible material while fungi break down more difficult material such as cellulose and lignin (Schaub & Leonard, 1996). The thermophilic stage is characterized by a high rate of decomposition and kills pathogens, weeds, and other undesireables, but must be maintained for 3-15 days (Schaub & Leonard, 1996). The temperature during this stage must be maintained and monitored because of it proclivity to overheat. Overheating can cause bacterial loss and possibly spontaneous combustion (Baldwin & Greenfield, 2009). Turning compost heaps before 160°F will prevent this as well as adding moisture to the heap (Baldwin & Greenfield,

2009). The compost heap will begin to cool down, despite turnings, signaling a return to the mesophilic or "curing" phase while also shrinking in size by 25-50% (Baldwin & Greenfield, 2009). Once the compost pile reached the final "curing" phase, decomposition slows significantly and the compost pile no longer has to be turned (Rynk et al., 1992). Temperatures fall again and mesophilic microorganisms take over again. The curing period follows where stabilization of microbial communities levels off and can take a few months to 2 years depending on the raw materials (Schaub & Leonard, 1996).

There are three types of composting methods; passive piles, windrows, and aerated static pile method (Schaub & Leonard, 1996; Baldwin & Greenfield, 2009). Windrow is creating a long narrow pile that is turned frequently and a certified organic production method (Baldwin & Greenfield, 2009). Windrows require an active oxygen flow created by large fans or through active turning (Schaub & Leonard, 1996).

Bacteria dominate in the composting cycle, however, there are many other microbes present within the compost including fungi, yeasts, nematodes, protozoa, and viruses (Mehta et al., 2014). Actinomycetes, a group of bacteria often present in filamentous form are considered highly favorable for their ability to produce enzymes that can break down debris such as newspaper and bark that others cannot (Mehta et al., 2014). Higa and Parr (1995) explained the importance and benefits of microorganisms if used properly. There are effective bacteria, which are specific and known, and there are beneficial bacteria, which are not defined but work beneficially within the soil (Higa & Parr, 1995). Bacteria are essential in soil because of their ability to release nutrients slowly, which in turn feeds other organisms and plants as well as keeps nutrients in the

soil (Lowenfels & Lewis, 2010). Effective microorganisms consist of yeasts, lactic acid bacteria, photosynthetic bacteria, as well as actinomycetes (Higa & Parr, 1995). Survival of microorganisms that are inoculated into the soil depends on maintaining stable physicochemical conditions including pH, nutrients, temperature, and water (Higa & Parr, 1995). The food web in compost is a biodiverse system that includes a wide range or other organisms including isopoda, myriapoda, acari, collembola, and nematoda, to name a few, which can be found in any composts including mushroom composts (Steel & Bert, 2011). These food webs shift with the phases of compost, for instance, there is a rise in nematodes and other bacteria-feeders upon returning to the cooler mesophilic stage (Steel & Bert, 2011).

Compost can be added as soil amendment directly, or be used as a substrate for additional manipulations. Compost is generally beneficial for soil, however, composting needs careful monitoring for age and maturity. Compost provides beneficial organisms, however, the effects of the compost depends on the type of compost and the raw materials used to make it. Immature compost and potentially harmful pathogens from organic waste compromise those good effects (Seneviratne et al., 2011). Immature compost can actually be harmful to a plant and have adverse effects, such as stunted growth (Zucconi et al., 1981). An example of commercially available compost is spent mushroom substrate (SMS) compost which is the byproduct of the commercialized production of mushrooms (Gea et al., 2014). SMS is produced in large volumes, 1.2 billion cubic feet, in the United States and has been considered an environmentally friendly product (Gea et al., 2014). SMS has an abundance of macronutrients including carbon, nitrogen, and phosphorous, and micronutrients such as calcium, potassium, and

magnesium (Romaine & Holcomb, 2001). There are two types of spent mushroom substrate compost: fresh and weathered (Landschoot & McNitt, 2000). Fresh SMS taken straight after mushroom harvest is fibrous and light brown. Fresh SMS can have a relatively high salt content when compared to other composts such as municipal solid waste compost or even the weathered SMS compost (Romaine & Holcomb, 2001). A higher salt content can have negative effects on the crops depending on certain crops needs. Fresh mushroom compost has an average pH of 6.6, C:N ratio of 13:1, organic matter content of 25.86%, containing nitrogen, phosphorous, potassium with an average 13.30 mmho/cm salt content based on a study sampling thirty SMS compost farms in Pennsylvania (Fidanza et al., 2010). Weathered SMS is left in the yard and allowed to further decompose for weeks or even months which reduces some of the adverse effects (Landschoot & McNitt, 2000).

Currently, the availability of SMS greatly overwhelms the demand for its use as organic fertilizer, and thus producers try to find adequate applications to promote the use of their product (Romaine & Holcomb, 2001). One such application is the production of compost tea. Compost tea has been studied for various uses. There are many names that are attributed to the soaking of compost in water that include compost tea, aerated compost tea, non-aerated compost tea, organic tea, compost extracts, slurries, and others (Scheuerell & Mahaffee, 2002). Compost tea can be made aerobically or anaerobically (Hargreaves, 2009). Aerobic compost tea maintains aerobic conditions with an aerator that adds oxygen to the slurry with a maturation time of 24-72 hours (Scheuerell & Mahaffee, 2002). Anaerobic compost tea is soaking compost in water and allowing it to mature undisturbed for a longer amount of time (around 10 days) (Scheuerell &

Mahaffee, 2002). Compost extract is anaerobic compost tea, when compost soaks in water for many weeks, producing a noxious smell, and little aerobic activity (Lowenfels & Lewis, 2010). Manure tea is similar to compost extract, but instead of compost, manure is soaked in water for a few weeks with a similar outcome (Lowenfels & Lewis, 2010). Our understanding of compost tea and its potential applications remain limited (Scheuerell & Mahaffee, 2002). However, studies have shown that aerated compost tea can increase performance of some crops, e.g. strawberries (Hargreaves, 2009). Compost tea is intended to create a noticeable greening of plants by providing nutrients (Gershuny, 2011). Compost tea advocates boast its uses in disease suppression, the production of healthier plants under safe conditions, the extension of root systems, the lack of over-fertilization, the reduction of needs for chemicals, as well as the enhancement of the taste (Hussey, 2015). In 2010, the price of professional grade compost in Vermont ranged from $40- $65 per cubic yard (Gershuny, 2011). While large scale producers can get a cost reduction to $20-$45 per yard, the commercial value of compost tea can be appreciated here (Gershuny, 2011). The above price only accounts for the compost itself and does not include labor and equipment costs (Gershuny, 2011). Compost tea can turn a small amount of compost into a large amount of tea that can cover a wider area, provided that the quality of the soil to be supplemented is not completely void of nutrients (Diver, 2001). Five to ten gallons can cover an acre depending on application rate and whether it is being applied as a soil drench or foliar (Diver, 2001). For small-scale farmers or the average person wanting to help their lawn or small garden, compost tea could be great tool to have and know if its benefits outweigh the costs associated with its use.

For the production of compost tea, it has been proven that adding certain amendments such as molasses or even small amounts of inorganic nutrients, creates environments that are more conducive to growing bacteria (Higa & Parr, 1995). However, there is debate over the function and effects of addition of such amendments. Molasses, an additive of compost tea has been shown to support proliferation of bacteria (Duffy et al., 2004). However, there has been debate on whether or not it also increases the populations of human pathogens including *Salmonella* and *E. coli* O157:H7 (Duffy et al., 2004). The fear of pathogens comes from an *E. coli* O157:H7 outbreak from Odwalla juice and from the detection of *S. enterica* Thompson in contaminated cilantro (Duffy et al., 2004). In the study performed by Duffy et al. (2004), they concluded that molasses when applied as an additive to compost tea, did favor the growth of these human pathogens; higher concentrations of molasses created increasingly higher numbers of the human pathogen (Duffy et al., 2004). Other examples of additives to compost tea that provide food sources to microbes include kelp powder, fish powder, humic acid, and rock dust (Diver, 2001). Usually, the composting process kills all or at least most of the pathogenic bacteria due to the high temperature during the thermophilic stage of the composting cycle, however, it is thought that compost tea, more specifically the additives, can result in re-occurrence of those pathogens (Kannangara et al., 2006). Carrot juice was previously studied and was concluded to be an inhibitor of *E. coli O157:H7*, but not of other pathogens (Kannangara et al., 2006). The addition of molasses does increase the concentration of *E. coli* in compost tea (Kannangara et al., 2006), and thus it was concluded that additives to compost tea do promote growth of human pathogens (Durham, 2006).

Soil borne pathogens affecting crops are a major concern because of their ability to cause disease with 50-70% loss in crop yield (Mokhtar & El-Mougy, 2014). Compost tea applied to foliage can reduce some diseases (Hirzel et al., 2012). Compost provides a long-term protection and fertilization though it acts slowly (Mokhtar & El-Mougy, 2014). In contrast, compost tea acts quickly and effectively, but only for a short duration of time (Mokhtar & El-Mougy, 2014). Compost tea is a microbial biofertilizer or rather a product containing microorganisms applied to plants or soil having direct and indirect effects (Seneviratne et al., 2011). Both compost and compost tea work efficiently by providing beneficial and effective microbes that can outcompete and create antibiotics that kill pathogens (Mokhtar & El-Mougy, 2014). However, successful application of organisms applied to soil depend on their adaptability and persistence (Van Overbeek et al., 1997). Opponents to aerobic compost tea believe that using amendments, such as molasses, can stimulate the growth of pathogens, while concerns with anaerobic compost tea are that it may promote the growth of pathogens, cause a foul odor, and cause phytotoxic symptoms (Scheuerell & Mahaffe, 2002). Not very many studies evaluate the effects of compost tea on soil fertility (Hargreaves, 2009; Hirzel et al., 2012). Positive results are not always guaranteed using or other organic methods of soil amendments due to improper methods or applications (Seneviratne et al., 2011).

Corn is tolerant of compost maturity; therefore, immature compost will not inhibit plant growth (Zucconi et al., 1981). Corn (*Zea mays*) is also a staple crop and is used in human and animal consumption as well as industrial uses (Ilea et al., 2013). It is resistant to drought, heat, has few pests that effect it and is very adaptable to environments (Ilea et al., 2013). Corn is tolerant to biotic and abiotic stresses (Chen et al., 2014). Modern

hybrids of corn are more tolerant to changes in nitrogen availability within the soil (Chen et al., 2014). In a study performed by Okoboi et al. (2013), they tested how different corn seeds reacted to varying treatments including fertilizers. It was concluded that differences were seen in only the highest market seed available, but no differences were seen in other seeds (Okoboi et al., 2013). Corn was grown to measure biomass and the differences in growth were compared to corn grown using inorganic fertilizers (Weber et al., 1997). The study concluded that there were no significant differences between growth of corn grown with SMS compost and inorganic fertilizer (Weber et al., 1997). The authors did conclude, however, that there was more leaching of nitrates when using the inorganic fertilizer making SMS compost a better sustainable and environmentally friendly alternative for growing corn (Weber et al., 1997). The only downside was that because nitrogen was the most limiting nutrient, over the years, there would be an excess of potassium and phosphorous, so implementing a crop rotation system that allowed other crops that readily use those nutrients would be more economical as well as sustainable (Weber et al., 1997). The accumulation of phosphorous in the soil can reach levels high enough to cause concern (Heckman, 2005).

Approaches to quantifying responses to compost tea

Modern microbial ecology provides several approaches that can enable specific evaluations of compost tea and its effects on soil and plant responses. There are both classical and molecular microbiology tools that are now widely used to evaluate quantity and composition of microbes in soils. This is valuable as bacteria are most readily abundant in compost (Mehta et al., 2014). Nevertheless, the task of characterizing soil ecology is difficult because of the intricate network of microorganisms in soil (Thies,

2008). Quantification of cells within the soil is essential to determine the actual numbers of microbes that are active in the soil at any given time. There are two quantification methods, culture based and molecular based, to analyze microbial populations (Mehta et al., 2014). A culture based quantification method is based on growing bacteria in vitro using growth media and counting cell colonies (Mehta et al., 2014). While effective for some populations, it is not effective for analyzing most microbial populations that exist in soil (Mehta et al., 2014). Plating techniques will not work on many organisms (Bohlool & Schmidt, 1980), because of an inability to culture them (Hahn et al., 1992). Bacteria that are abundantly present in soil are often times not culturable (Mehta et al., 2014), in fact, about 95-99% of bacteria cannot be isolated and cultured under laboratory conditions (Torsvik et al., 1990). This is a major setback for this method when trying to quantify diverse populations and >99% of bacteria seen under a microscope cannot be isolated using plating methods (Mehta et al., 2014).

Several molecular methods of quantifying, describing, and distinguishing microbial diversity in soil have been studied and deemed successful (Zhao et al., 2011). Stable Isotope Probing has been successful in the identification of microbes in soils (Zhao et al., 2011). Microarray, random amplified polymorphic DNA, and DNA fingerprinting focuses on the distinguishing the genetic makeup and variations (Zhao et al., 2011). DNA extracted from the soil, specific genes being amplified and cloned, and then sequenced can give a better idea of the diversity within the soil (Thies, 2008).

Quantitative PCR (*q*PCR) is a molecular tool that amplifies specific gene targets. *q*PCR has been used to achieve an approximate quantification of microbes in composting environments, but it is very time consuming and costly (Mehta et al., 2014). It is also not

appropriate for the enumeration of large numbers of several different microbial populations (Mehta et al., 2014), and biased by a large range of gene copy number abundances in different microbes. Although *q*PCR has been used to quantify microbial populations in compost environments, compost tea has not yet been analyzed. Also, *q*PCR usually determines presence of specific RNA gene sequences and detects sequences from both active cells and cells that are inactive.

Fluorescent *in situ* hybridization (FISH) is advantageous in specifically targeting and visualizing active whole cells (Zhao et al., 2011). It has been cited in previous studies that *in situ* hybridization is successful in quantifying soil-borne bacteria (Hahn et al., 1992). FISH targets ribosomal RNA (rRNA) sequences in whole cells using fluorescently labeled oligonucleotides as probes (Mehta et al., 2014). This tool not only allows quantification of active cells, but can also evaluate morphology and spatial arrangement (Mehta et al., 2014). The effective use of probes can also target a broad or narrow range of microbes (Mehta et al., 2014).

Problems faced with FISH can include specificity issues, autofluorescence, nonspecific binding (Bohlool & Schmidt, 1980), poor cell permeability, and a low throughput (Mehta et al., 2014). Being fully competent in retrieving accurate estimations of microbes in soil is difficult because it is largely reader biased and it is hard to obtain a standard (Bohlool & Schmidt, 1980). Repetition is important in getting an accurate reading. In order to be successful, the technician must have a level of skill in order to accurately read and assess an accurate portrayal of the targeted probe seen (Bohlool & Schmidt, 1980).

II. OBJECTIVE

The goal of this research was to assess whether compost tea has beneficial effects on plant growth performance, and if so, which component (i.e. microbes or nutrients in the water phase) was associated with this beneficial effect. This research question included monitoring the development of soil microbial communities established in compost tea though compost tea inoculation (i.e. application of compost tea into soil) over time.

My expectation was that compost tea had beneficial effects on plant growth performance by both enhancing inorganic nutrients availability in soil after application, and by supporting the development of larger communities of indigenous microbes, most likely bacteria, after application. Thus, my primary null hypothesis was that there were no net benefits to plant growth by the addition of compost tea.

One objective of this study was to determine how microbial populations behaved in a spent mushroom substrate (SMS) compost tea when applied to a low nutrient soil. I used molecular tools to determine the changes over time, if any, in these microbial communities.

The second objective of this study was to determine if constituents in SMS compost tea, including microbes, nutrients, or a combination of both by experimental testing and statistical evaluation of the plant growth, soil nutrient and microbial community datasets, influenced corn growth.

III. MATERIALS AND METHODS

Compost

The compost used in this study was spent mushroom substrate originally composed of straw, dried poultry waste (DPW), brewer, cottonseed (CS) Burrs, urea, chicken litter, CS meal, and gypsum, that was used for the production of mushrooms in year 2013 and afterward stored outside exposed to the elements to "weather" for 3-6 months. The Texas Plant and Soil Lab tested the weathered compost for total nutrient composition on January 9, 2012, prior to first use for mushroom production. The compost had a C:N ratio of 9:1. The compost was made in open windrows that were turned every other day for roughly two months. The weathered compost was obtained from Kitchen Pride Mushroom Farm, Inc, located in Gonzalez, Texas in July 2014.

Compost tea was made and administered to corn plants to determine whether the addition causes a significant difference in plant growth performance, and if microbial communities within the compost tea contribute to overall performance of the corn. Compost tea was made by the bucket-bubbler method mixing 500 grams of compost and 5 liters of distilled water since a 1:10 ratio of compost to water had been promoted and used before (Ingham, 2005; Palmer et al., 2010). At least 500 grams of compost was necessary to avoid sampling error (Scheuerell & Mahaffee, 2002). The bucket-bubbler method uses an aquarium sized pump and air stone for aeration, mixing, and agitating (Diver, 2001). Five liters of distilled water was placed in a clean 10 gallon bucket with a large air stone connected to an air pump, which aerated the water for the entrie production time. Oxygen levels were checked with a D.O. (dissolved oxygen) meter and

were maintained by the air pump to between 6.2-6.5 mg oxygen (or 66.8% oxygen saturation) per liter. The compost was weighed and placed -covered in cheesecloth- in the water above the air stone. This method allowed the water and oxygen to permeate through the compost. The 10-gallon bucket was left covered at ambient temperature at around 72 degrees Fahrenheit (22°C) for 48 hours (Ingham, 2005). The compost tea that was created had a favorable smell, meaning it smelled earthy rather than putrid, which signified that an aerobic state was maintained and that the compost tea did not turn anaerobic. The bucket bubbler method takes anywhere from 24-48 hours to produce a good product (Lowenfels & Lewis, 2010), therefore, for this experiment, it was left to "brew" for 48 hours to allow maximum time for bacteria to grow. The compost tea was used for inoculation immediately after production. The compost tea was applied to the plants indoors at ambient temperatures (Lowenfels & Lewis, 2010).

After the 48 hour incubation period, the compost tea was separated into three treatments; the compost tea, the bacterial load after centrifugation, and the supernatant that was filter-sterilized. To generate the sterile supernatant, 50 mL samples taken from the fresh compost tea were centrifuged for 15 minutes at 4000 rpm. The supernatant, or nutrient-rich broth, was removed from the bacterial pellet to be filter sterilized and added to one batch of soil. The bacterial pellet then underwent two washing steps with 45 mL of deionized water, vortex-mixed, and centrifuged at 4000 rpm for 15 minutes. This process separates the compost tea into a bacterial mixture (bacterial load) and the nutrients available (supernatant).

Corn Production

Three corn seeds were planted in triplicate in 50 mL tubes for three different inoculation experiments (supernatant, bacterial load, and the compost tea) and one control (water only). A sandy loam soil, obtained from Bastrop, Texas, was used because of its low nutrient content and small numbers of microbes. The soil used in this experiment is a sandy soil however, any moisture present is available to the root system (Killham, 1994). Sand has almost no charge and therefore does not hold on to nutrients as well as clay soil, with the consequence that it can hardly provide nutrients to the plants (Lowenfels & Lewis, 2010). Light colored sand has a cation exchange capacity of 3-5 MEQ/100 g which is the lowest of all soil types (Lowenfels & Lewis, 2010). Bacteria must have moisture in order to operate their daily functions including reproduction and nutrient exchange (Lowenfels & Lewis, 2010). Using sand as the growing medium eliminates some problems that can arise from the use of top soil or soils containing clay that are much more nutrient rich (Ellis, 2004). The nutrient poor sand was meant to provide stronger effects of the inoculum on plant growth performance and microbial communities than soils richer in nutrients. Soils that are mostly sand also use less liquid for suspension when compared to clay-based soils (Hahn et al., 1992).

Sixty ± 1 grams of soil were weighed and added to the tubes. Introducing a soil microcosm has its advantages because outside influences such as temperature and light are more easily controlled (Ellis, 2004). The seeds germinated after four to five days in a damp paper towel under medium sunlight condition at a maintained temperature of 22 ± 2°C to allow the seeds to soften and start a taproot. Once the seedlings germinated, the endosperms that provide nourishment to the seedlings were removed in order to create

nutrient deficient conditions (Mader, 2001). Three seeds were weighed and planted about 2 cm into the soil in the Falcon tubes and 10 ml of inoculate was then added to the soil. Samples were procured on days 0, 1, 2, 5, 10, 20, and 30. Around 3 ml of distilled water were added every two to three days to maintain moisture levels after the initial 10 ml inoculation on day 0.

Microcosms were analyzed in two separate trials with different environmental conditions. Trial 1 was performed in 50 ml falcon tubes that became water-saturated quickly and thus anaerobic conditions should have been established soon after setup. Lack of oxygen was not determined analytically, however, at sampling time fermentation products were noticed indicating anaerobic conditions. Trial 2 used a similar setup, except that tubes were perforated at the bottom to allow water leakage to occur. This prevented water-saturation and thus kept microcosms aerobic. No fermentation products were noticed at sampling time.

Measurements

Sprout and root length as well as sprout, root, and overall biomass were measured (in centimeters and grams, respectively). The samples were measured by first carefully extracting the three plants from the soil and washing in distilled water to remove any excess soil. Root and sprout length were measured and recorded. After another thorough washing, the plants were left to dry for approximately 10-15 minutes to allow any excess water to evaporate before obtaining a total wet weight of the samples. This measurement was taken to ensure that there has been growth from the initial starting weight that was obtained in the previous step. The samples were dried in an oven at 200°F for 2-4 hours.

After a thorough drying, plants looked dry, crunchy, and lacked any moisture. The entire plant was measured for total biomass, there was a separation at the base where the roots meet the start of the sprouts, each set was measured separately after a shaking to remove any excess soil.

Washing

The soil was placed in a plastic bag and mixed thoroughly, then two samples of 250 mg of soil were placed into 2-ml screw cap tubes. Two-hundred-fifty µl of 4% paraformaldehyde (PFA) was added to the screw cap tube with 250 mg of soil. The samples were put into the refrigerator (4°C) to fix for 16 hours. The following morning, the samples were centrifuged at 10,000 rpm for 3 minutes. The supernatant was discarded, and the pellet was washed twice with 250 µl of 0.1% sodium pyrophosphate. After the second wash, 250 µl 95% ethanol and 250 µl 0.1% sodium pyrophosphate was added and the suspended sample stored in the -20°C freezer. Once fixed the samples can be stored for many years. Extra soil and dried plants were bagged and stored at -20°C.

Method evaluation

Two methods of quantification were initially investigated. These included fluorescent *in situ* hybridization (FISH) and quantitative polymerase chain reaction (*q*PCR). FISH is a method in which labeled oligonucleotides binding to target-specific sequences on ribosomal RNA (rRNA) are detected by epifluorescence microscopy. Quantitative polymerase chain reaction (*q*PCR) is a method in which target-specific rRNA gene fragments are amplified. Amplification was compared to that of standards, which allows quantification of rRNA genes that, however, were related to cell numbers in

the environmental sample. The usefulness and accuracy of both quantification methods was assessed against numbers of organisms determined by epifluorescence microscopy after DAPI staining. DAPI is a DNA intercalating dye that is commonly used to detect all organisms in an environmental sample with highest accuracy.

In situ hybridization

The *in situ* hybridization slides were prepared beforehand by first prewashing the slides in 95% ethanol. 0.1% gelatin and 0.001g $KCr(SO_4)_2$ were dissolved in 50 ml water by heating in the microwave in 15-20 second intervals until the liquid is hot but not boiling. The prewashed slides were dipped into the solution and dried vertically. Once the slides dried, they were stored in the refrigerator at 4°C. A mixture of 10 µl of sample and 990 µl of 0.1% sodium pyrophosphate was prepared to disperse cells evenly, and 10 µl were spread over each well using the pipette tip horizontally to carefully cover the entire well. The slides were dried for 10 minutes in the 37°C incubator. Once fully dried, the slide was washed in 50%, 70%, and 95% ethanol for 3 minutes each, respectively. Upon removal from the 95% ethanol wash, the slides were air-dried. While the slides were washing, hybridization buffers for each probe were made. EUB338III requires a 30:70% formamide to buffer ratio, while the EUK516 requires a 20:80% formamide to buffer ratio. Amounts equal to 1000 µl were made. 80 µl of this solution was placed in a separate tube and 8 µl of 10x block solution were added to create the hybridization buffer.

Once the slides dry, 10 µl of hybridization buffer was smeared over each well, in the same fashion to that of the original sample. The slide was then placed into a

hybridization chamber and incubated at 37°C for 30 minutes. The hybridization chamber was made by placing a Kim wipe into a 50 ml Falcon tube and pouring the remaining hybridization buffer into the chamber. After the 30 min incubation, 1 µl of probe was added to each well; the slide was again inserted back into the chamber, and placed in the incubator. The slide labeled by the EUB338III was left in the incubator overnight for optimum hybridization (16 hours). The slide labeled with the EUK516 probe hybridized in the incubator for two hours. After the allotted timeframe, the slide was washed in the hybridization washing solution for 20 minutes. The slide was rinsed with distilled water, backside up, and then air-dried. Two drops of citifluor were added to the center of the slide and a cover slide placed on top. Pressure was added to ensure the cover slide was in place and no air bubbles formed. Drops of immersion oil were added on top of the cover slide before placement under the microscope at 100x magnification. Each well was observed and microbe populations were visually counted for either EUB338III or EUK516 using a 10 x 10 grid read in 10 different placements on the well. Detections of either were based on size, shape, and fluorescence as well as its presence on both DAPI and CY3 light fields. Both DAPI and CY3 numbers were recorded for further calculations. DAPI is 4',6-diamidino-2-phenylindole used to stain all cells containing DNA (Hahn et al., 1992). Cy3 is a sulfoindocyanine dye used to label the specific oligonucleotides (Daims et al., 1999). The EUB and EUK results found under the CY3 light field would theoretically sum to the numbers recorded for the DAPI light field. The original sample was also tested for Archaea, but there were none present in the sample and no subsequent tests for Archaea were conducted.

The hybridization solution was made by taking a 100 ml wide mouthed glass bottle and heating 40 ml of distilled water to 55-65°C in the microwave, then adding 150 µl of 1 N NaOH and 2 grams of paraformaldehyde and allowing it dissolve for about 2 minutes. 5 ml of 10 x phosphate binding saline (PBS) was added. Distilled water was added to make the total volume 50 ml. pH was adjusted to between 7.2 and 7.4.

Quantitative-polymerase chain reaction (*q*PCR)

DNA was extracted from a 250 mg sample of compost using the SurePrep Soil DNA Extraction Kit. 250 mg of glass beads were added to the soil inside the 2 ml screw cap tube,which was placed in a bead beater for 5 minutes. Following several washes based on the instructions of the SurePrep Soil DNA Extraction Kit, DNA was separated from remaining material and then eluted twice with elution buffer. The samples were stored in sterile tubes inside a -20°C freezer. DNA was quantified by spectrophotometric analyses (NanoDrop). Quantitative-polymerase chain reaction (*q*PCR) was used to enumerate bacteria by targeting and amplifying the 16S rRNA gene fragment using primers Bact 1369f and Prok 1492r, and fast mix with 1 µl of the template sample. Ten-fold dilutions of freshly prepared and quantified *E. coli* cells were used as standards. Amplification conditions were 50°C for 2 minutes, 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, and 60°C for 30 seconds on an Eco real time PCR (Illumina).

Statistical analyses:

Differences in plant growth performance parameters per day between the four samples were determined using an ANOVA (analysis of variance test) to prove there was

a statistical differences between the four samples with a significance value set at $p<0.05$. If the null was rejected, i.e. $p<0.05$, then multiple pairwise comparisons were performed in order to determine where the significance lies. The significance value was adjusted to avoid Type 1 error according to the Bonferroni correction (α/# of comparisons) to the adjusted significance value of $p<0.00833$.

IV. RESULTS

Method Evaluation

Quantitative PCR and FISH probes both target 16S rRNA sequences, though PCR uses DNA and FISH rRNA as target. Comparative analyses revealed about ten-fold differences in bacterial numbers between methods, with *q*PCR based enumeration providing higher values than FISH based enumeration. FISH based enumeration, however, results in very similar values as those retrieved after DAPI-staining which are considered to reflect the most accurate numbers for all organisms.

Table 1. Comparative analyses. Comparative analyses of cell numbers in compost determined after DAPI-staining, *in situ* hybridization (FISH) and *q*PCR.

	Number of cells (x 10^9)
DAPI	7.71 ± 0.74
FISH (EUB338III)	5.58 ± 0.64
*q*PCR	39.10 ± 6.7

*q*PCR uses 16S rRNA genes as template for amplification. Number of genes per cell or genome, however, can be highly variable with copy numbers generally ranging between 1 and 11 copies. Our standard for *q*PCR based analyses was *E. coli*, which typically has seven copies of the 16S rRNA gene. The average copy number determined by analyzing all available genome sequences in the EMBL database is 4.2 copies for the 16S rRNA. Compared to FISH that visualizes individual cells, *q*PCR results will

therefore typically be larger and thus often overestimate abundance of bacteria. Estimations showed that a gram of soil has a carrying capacity of up to about 10^{10} microbial cells (Ellis, 2004). Anything above this threshold is an indicator of inaccuracy. As seen in the chart above, the *q*PCR result is much larger than the DAPI results while the EUB FISH results in fewer counts of cells. This is consistent as our analysis was targeting Bacteria only and excluded Eukarya and Archaea.

Fluorescent *in situ* hybridization provides a more accurate enumeration of the active cells present. The large number of Bacteria detected in our samples (i.e. the high percentage of Bacteria detected as a portion of the DAPI-stained organisms) indicates active cells and thus FISH provides an adequate tool to analyze Bacteria in our experimental setup. Using this technique, one is able to visualize and count a narrow or broad range of targets based on the probe used. In our study, we focused on the analyses on the Domain level. i.e. Bacteria, Eukarya and Archaea using probes EUB338III, EUK516, and Arch915, and compared these numbers to those obtained after DAPI staining as the most accurate detection strategy to analyze microbial community shifts. Problems faced with using FISH include autofluorescence, low dispersion of cells (i.e. clumping), and reader error (Hahn et al., 1992).

Figure 1. Corn setup. This picture shows the typical setup of the microcosms and corn (*Zea mays*) plant growth on day 5 before plants are removed for measurements and soil extracted for sample preparation of *in situ* hybridization. The microcosms are held in Styrofoam containers that only cover the bottom 1/5th of the microcosm, but provide support so the microcosms do not move and can be held upright.

Plant growth performance in trial 1 and trial 2

Trial 1 was performed by creating an anaerobic environment within the soil. The 50 ml Falcon tubes in which the soil was placed and corn was grown were not perforated and thus water was not allowed to drain creating an anoxic environment within the tube and soil. Plant growth performance data for this trial are shown in figures 2 to 6. Trial 2 was performed by creating an aerobic environment within the soil. By creating holes in the bottom of the 50 ml Falcon tube, water does not pool in the pores of the soil allowing oxygen to penetrate. The following graphs describe the data collected from the measurements taken on the sampling events over the course of 30 days. Plant growth performance data for this trial are shown in figures 7 to 11.

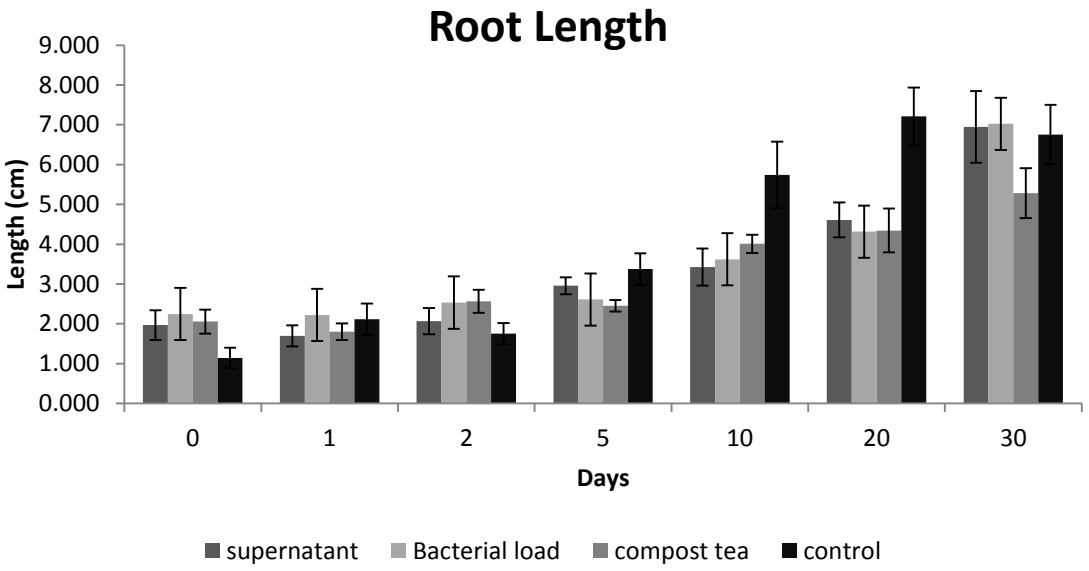

Figure 2. Root length (anaerobic conditions). This graph is a representation of the average growth for root length (cm) for sampling events over the 30 day period. There is no statistical difference between the four groups on any of the days ($p<0.05$).

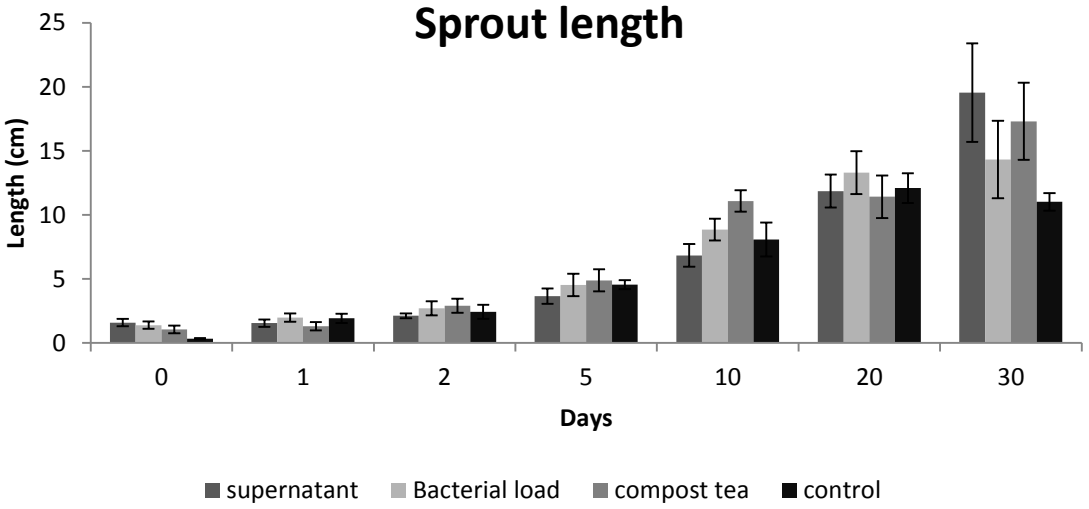

Figure 3. Sprout length (anaerobic conditions). This graph is a representation of the average growth for sprout length (cm) for sampling events over the 30 day period. Differences ($p<0.05$) between the four treatments were observed on day 0 (control is different than the other three treatments) and on day 30 (difference between compost tea and the control).

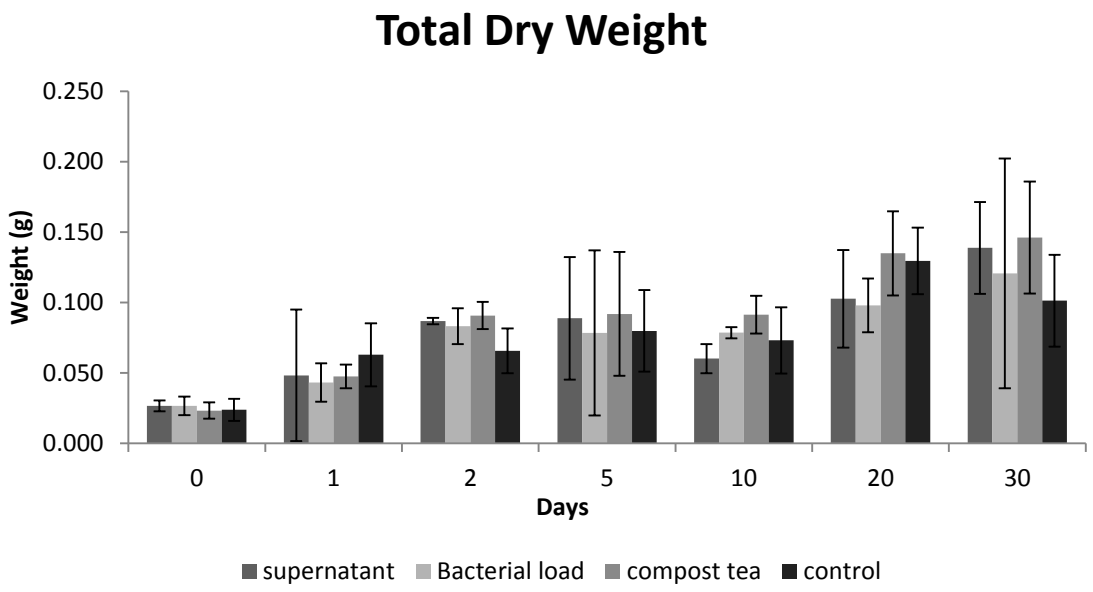

Figure 4. Total dry weight (anaerobic conditions). This graph shows total dry weight (g) of all plant material taken at the sampling events over a 30 day period. Differences ($p<0.05$) between the four treatments were observed on day 10 (supernatant different from the bacterial load and compost tea).

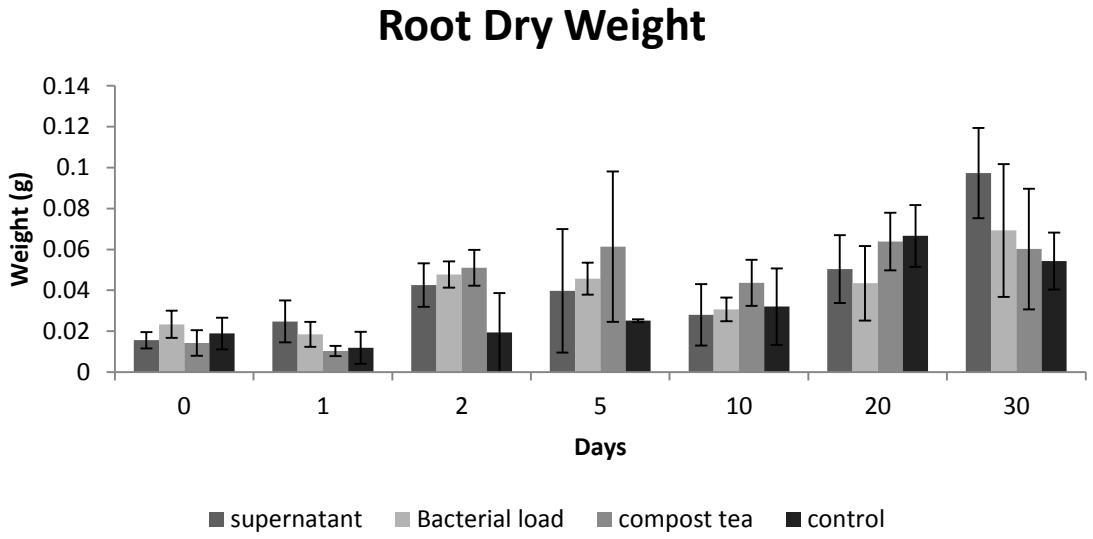

Figure 5. Root dry weight (anaerobic conditions). This graph shows root dry weight taken at the sampling events over a 30-day period. There were no differences between the four treatments on any day during the trial ($p<0.05$).

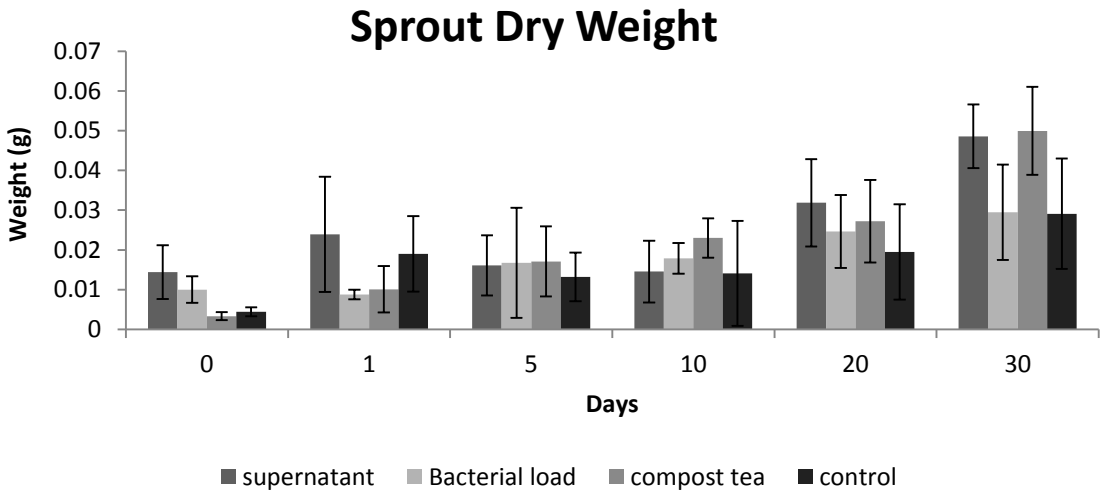

Figure 6. Sprout dry weight (anaerobic conditions). This graph shows sprout dry weight taken at the sampling events over a 30-day period. Dataset from day 2 was removed due to outliers. No statistical differences were found between the four treatments on any given day.

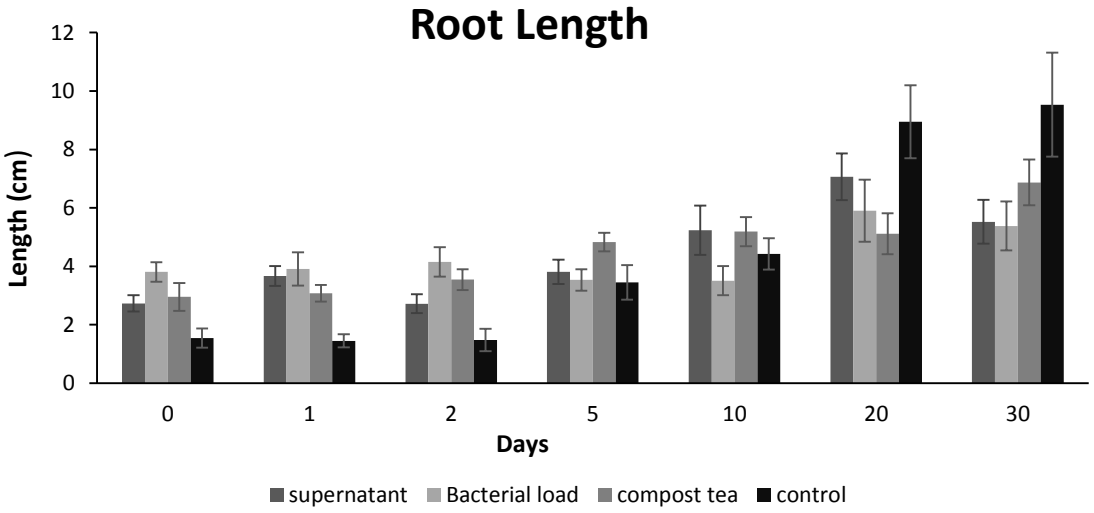

Figure 7. Root length (aerobic conditions). This graph is a representation of the average growth for root length (cm) for sampling events over the 30-day period. Statistical analysis was performed for each day to determine differences across the four treatments. Differences observes on day 0 ($p<0.05$) were found between the supernatant and the control as well as the bacterial pellet and the control. On day 1, the control was statistically different than the three other treatments. Day 2, the control was statistically different than both the bacterial pellet and compost tea.

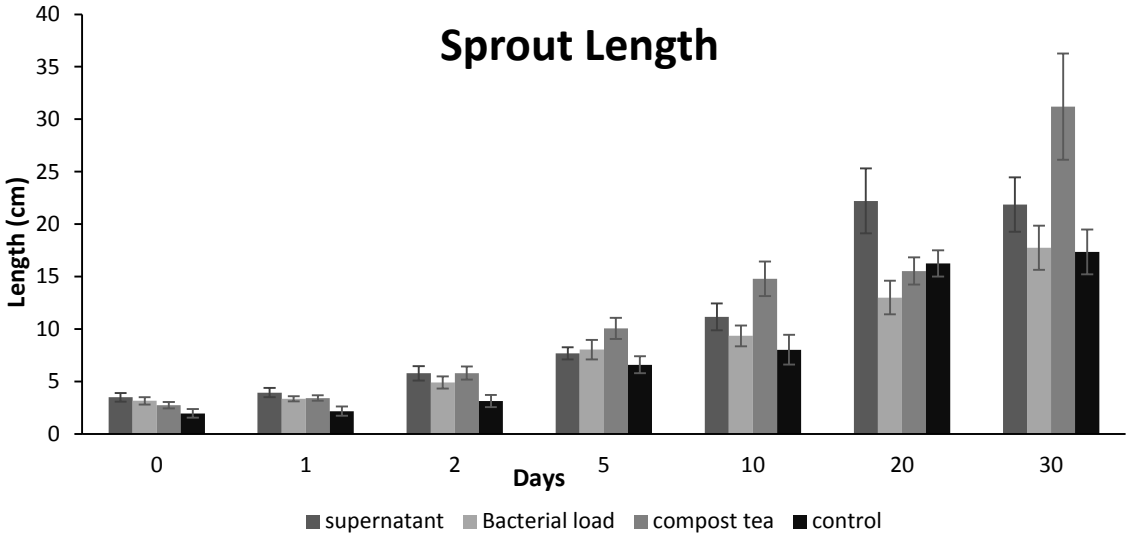

Figure 8. Sprout length (aerobic conditions). This graph represents the sprout length taken at the sampling events over the 30-day period of corn (*Zea mays*) growth. On day 1, the control was statistically lower than the supernatant and compost tea treatments. Day 2 shows a significant difference between the control and compost tea. Day 10 shows a significant difference between the bacterial pellet and the compost tea ($p<0.05$). Day 20 shows a significant difference between the supernatant and the bacterial pellet ($p<0.05$).

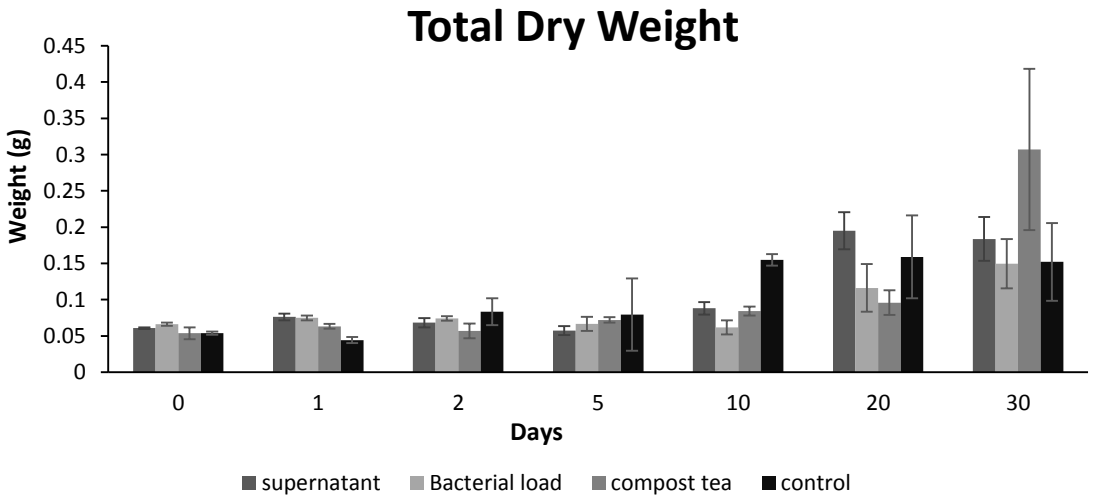

Figure 9. Total dry weight (aerobic conditions). This graph shows total dry weight taken at the sampling events over a 30-day period. Day 1 showed a statistical difference ($p<0.05$) between the control and both the bacterial load and the supernatant.

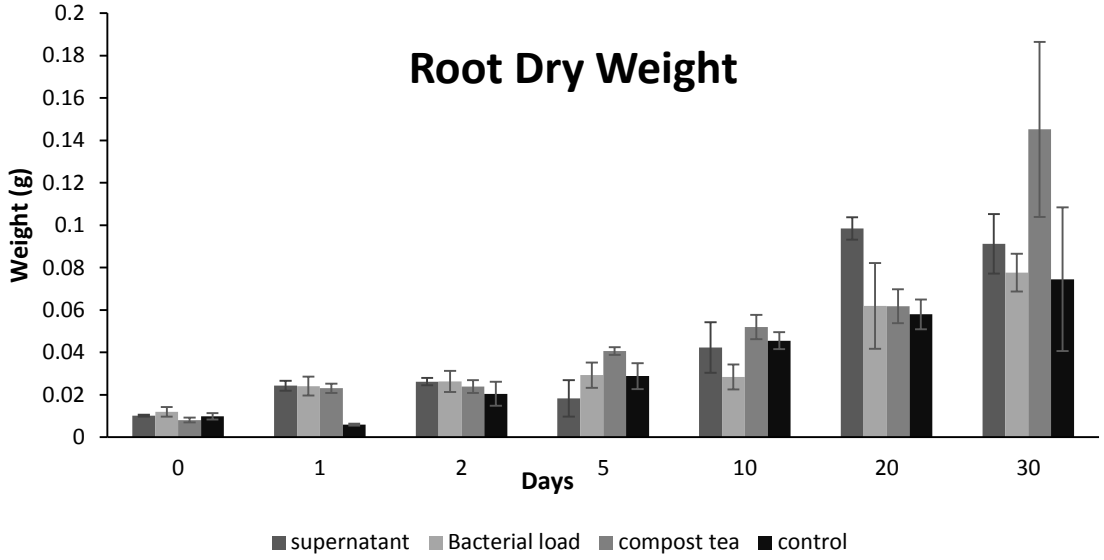

Figure 10. Root dry weight (aerobic conditions). This graph shows root dry weight taken at the sampling events over a 30-day period. Day 1 showed a significant difference between the control and both the compost tea and the supernatant ($p<0.05$).

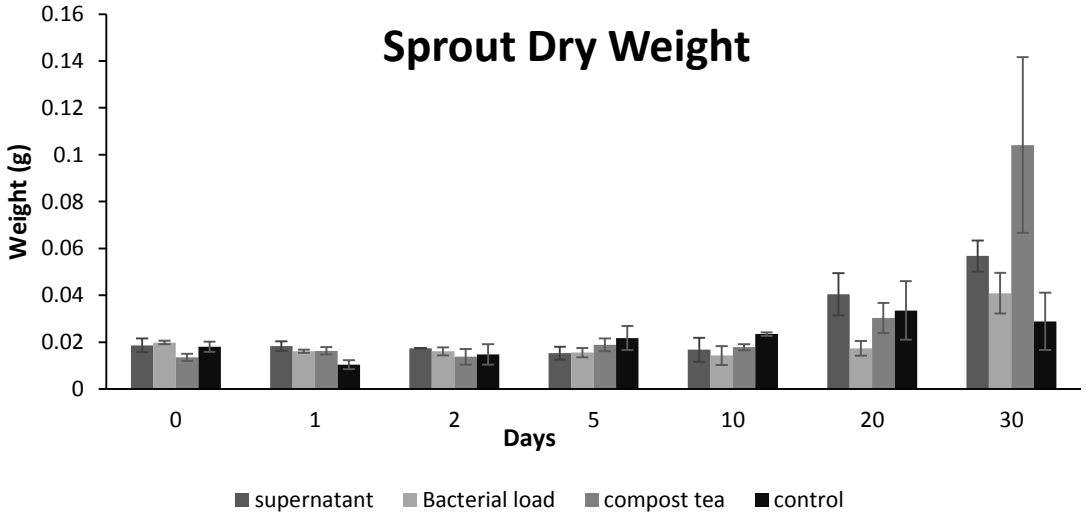

Figure 11. Sprout dry weight (aerobic conditions). This graph shows sprout dry weight taken at the sampling events over a 30-day period. No statistical differences were discovered throughout the samples on any given day.

Measurements were taken throughout the experiment to determine if there were any differences in plant growth based on the four inoculants supernatant, bacterial pellet, compost tea, and water as a control. Root length, sprout length, root dry weight, sprout

dry weight, and total dry weight were taken on days 0, 1, 2, 5, 10, 20, and 30. The three plants for each tube were each measured and the results recorded. A one-way ANOVA (Analysis of variance) was performed for each day and measurement for the four treatments. By day 30, there were no differences between any treatment for all of the measurements that were taken based on an adjusted significance rate of $p<0.05$. The only statistical difference seen on day 30 for the anaerobic trial was the sprout length measurement between the compost tea and the control. In the aerobic trial, no statistical differences were observed on day 30 between the four treatments. The statistical differences seen early on in the experiment (i.e. days 1-5) can be attributed to the initial variations of the growth sizes of the seedling. The experiment did not take into account the varying rates at which the seedlings germinated. Three germinated seedlings were chosen at random for each microcosm to try to establish a random sample. However, this could have led to statistical errors in the first few days in terms of growth rates. The endosperm was removed in order to circumvent any additional supplementation (sugars) to the seedling, so that any growth observed would be from the nutrients provided by the inoculants. Based on the data collected, it shows that addition of compost tea does not result in better corn (*Zea* mays) plant performance when compared to the addition of either microbes only, the nutrient- rich supernatant, or the water control. It remains possible that the experiment did not last long enough to see statistical differences between treatments in plant growth performance. However, the experiment only allowed one dose of the inoculant on day 0 to test microbial populations that compost tea could contribute to the soil over time and its impact on plant growth. Future experiments could include longer growing period with increased inoculant doses throughout.

Fate of microbial communities in trial 1 and trial 2

The following series of graphs depicts the abundance of microbial communities (i.e. DAPI-stained cells) and the Domains Bacteria and Eukarya determined after *in situ* hybridization and their dynamics throughout the sampling period. The initial value represents numbers determined in the inoculum, and presented as inoculum per gram of soil.

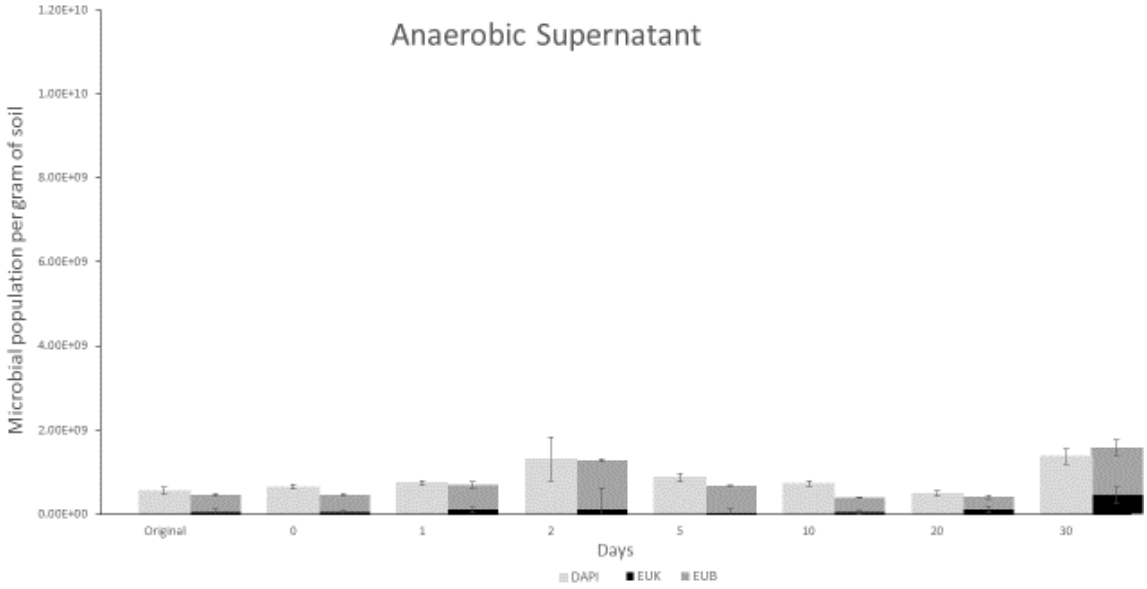

Figure 12. Anaerobic supernatant microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the supernatant treatment under anaerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338III, respectively.

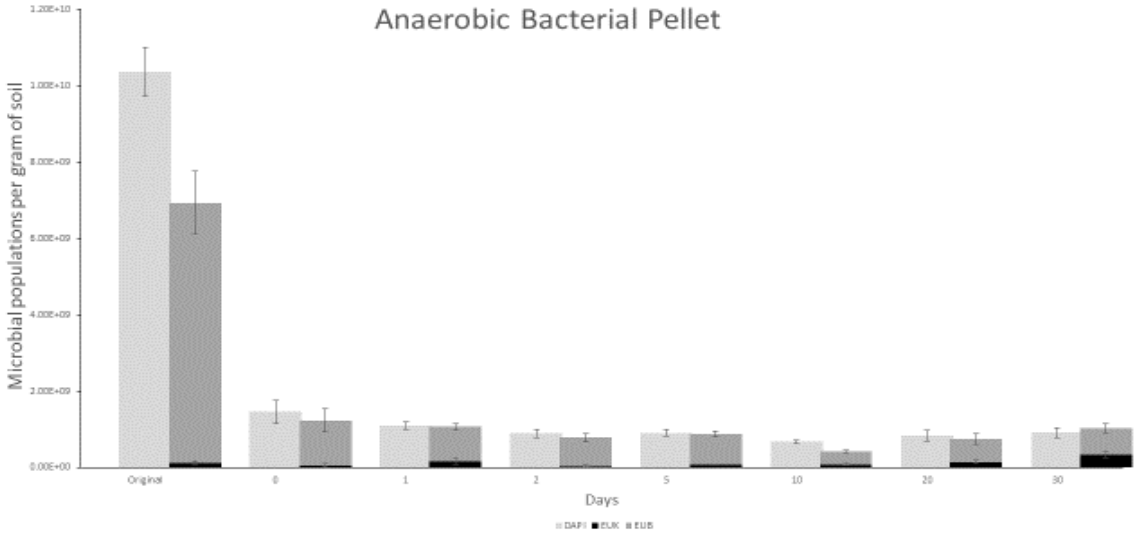

Figure 13. Anaerobic bacterial pellet microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the bacterial pellet treatment under anaerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

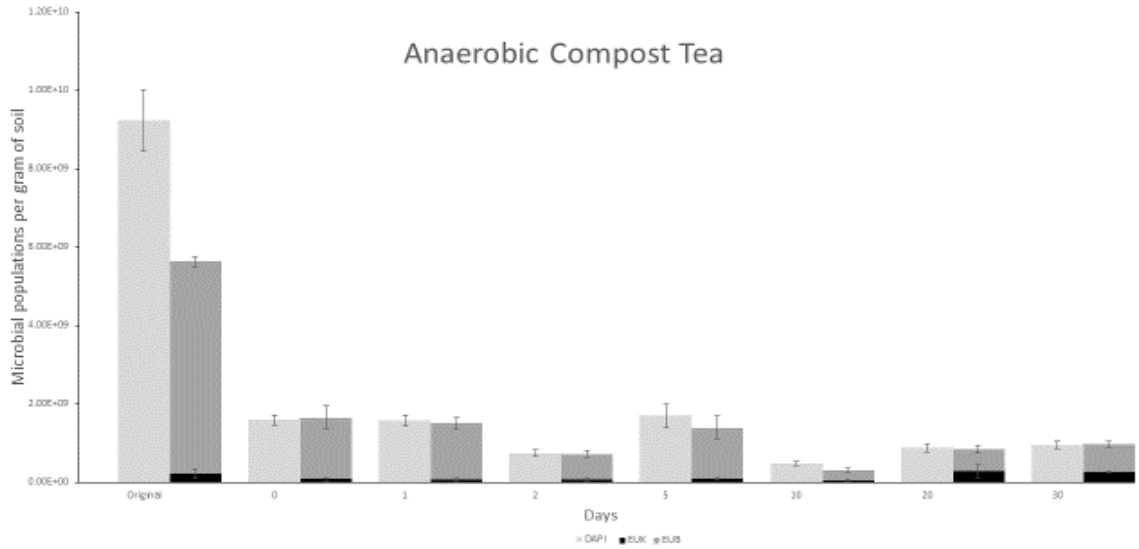

Figure 14. Anaerobic compost tea microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the compost tea treatment under anaerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

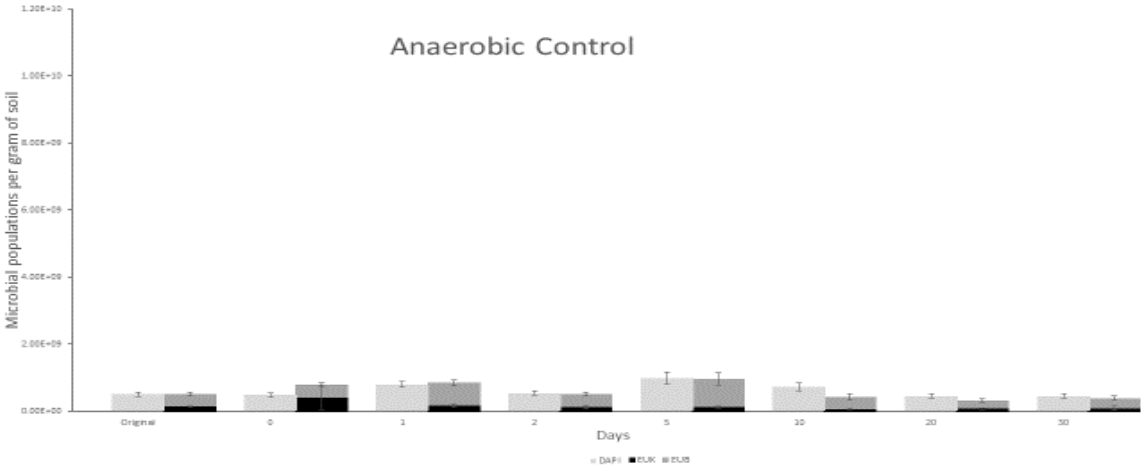

Figure 15. Anaerobic control microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the control treatment under anaerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

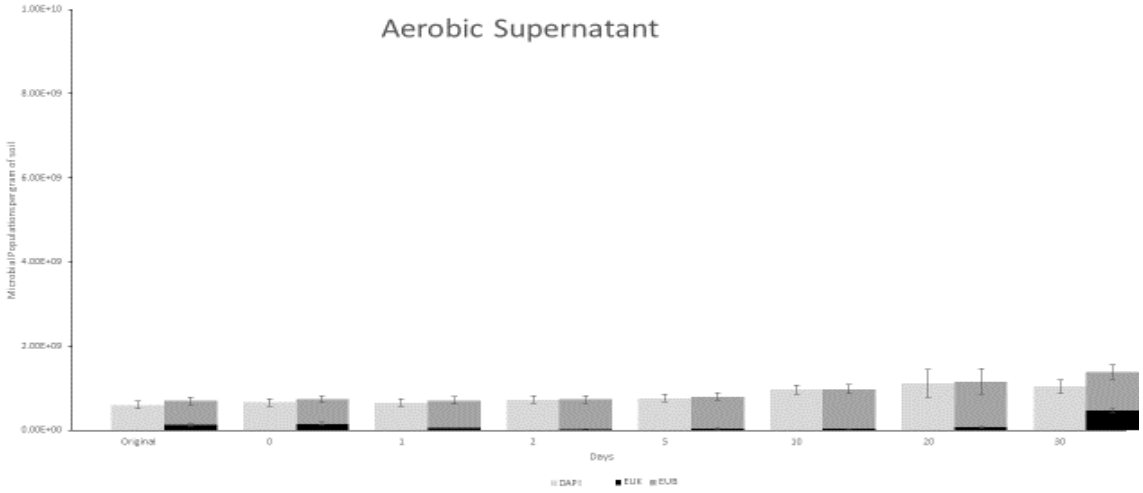

Figure 16. Aerobic supernatant microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the supernatant treatment under aerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey (right side) show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

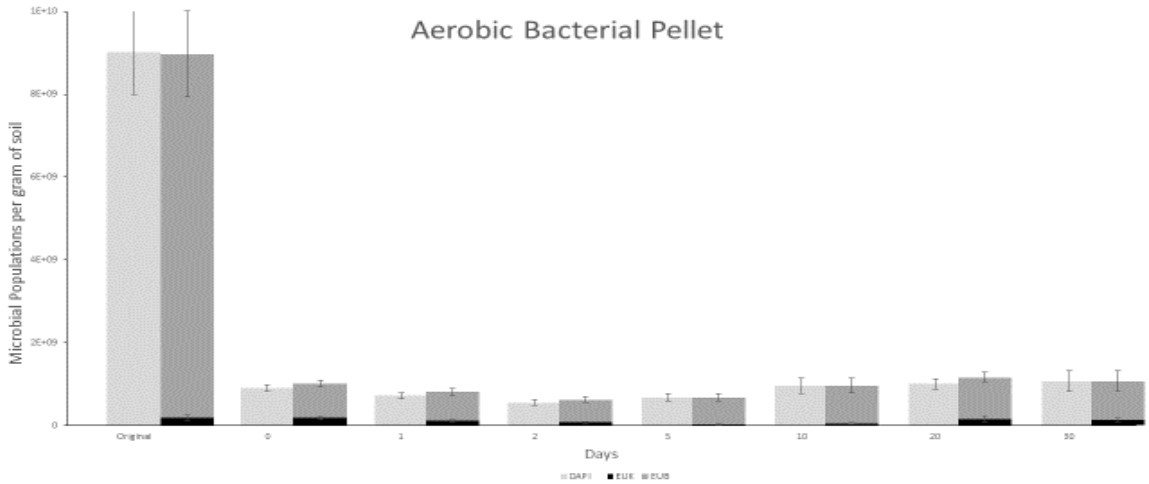

Figure 17. Aerobic bacterial pellet microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the bacterial pellet treatment under aerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

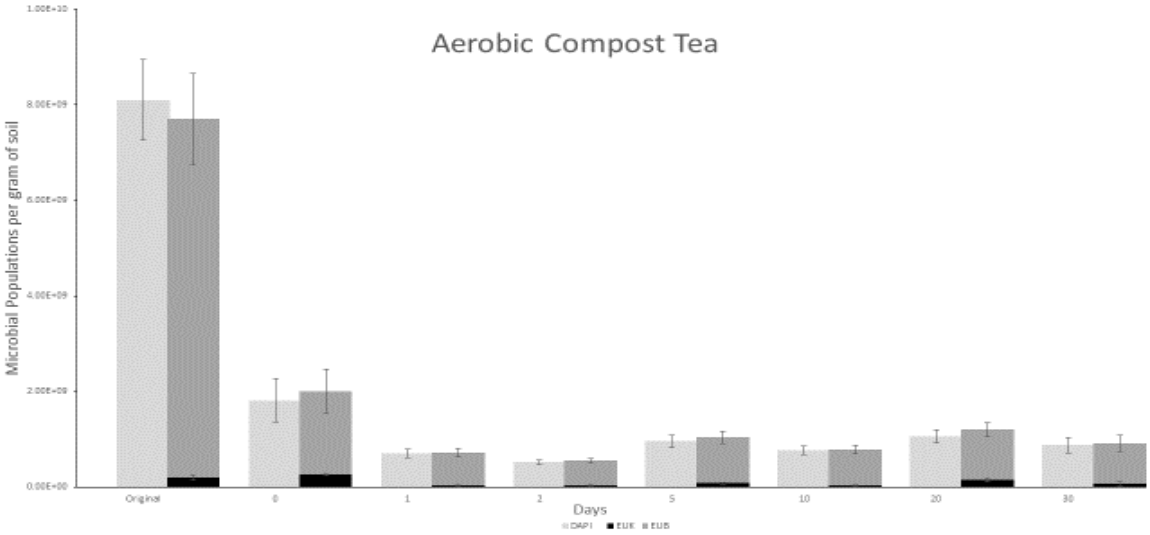

Figure 18. Aerobic compost tea microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the compost tea treatment under aerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

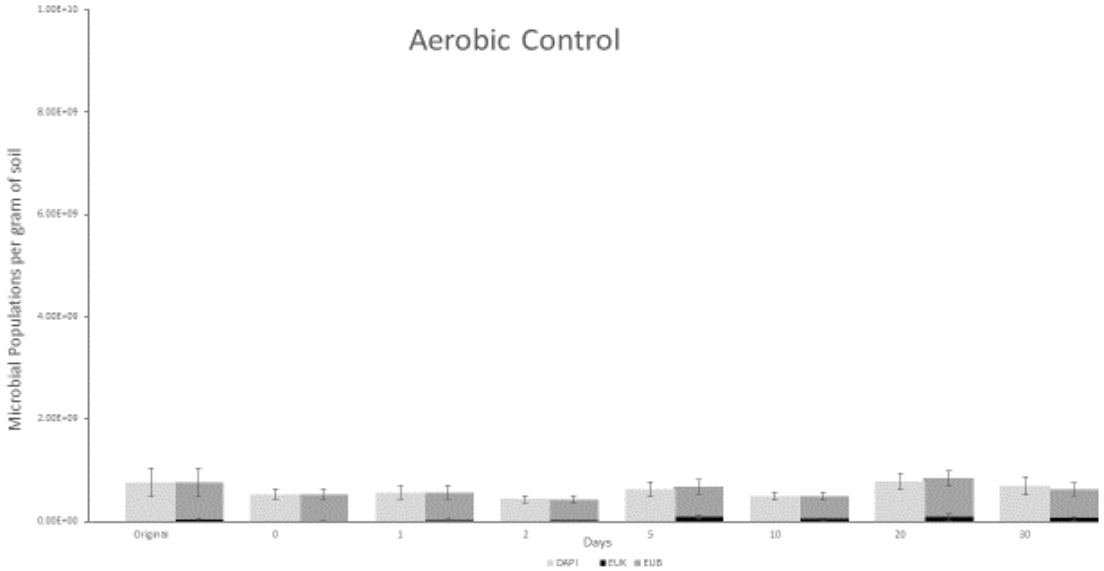

Figure 19. Aerobic control microbial populations. The figure above depicts populations of Bacteria and Eukarya, taken at sampling events over a 30-day period that were determined through fluorescent *in situ* hybridization, under the control treatment under aerobic microcosm conditions. The bars in light grey (left side) represent DAPI-stained cells (i.e. all cells containing DNA). The bars in black and dark grey show populations of Eukarya and Bacteria, respectively, that were counted after hybridization with probes EUK516 and EUB338, respectively.

Each day a sample was taken and *in situ* hybridization was performed to determine populations of both classes of Bacteria, using the EUB338 probe, and Eukarya, using the EUK516 probe, along with a DAPI counterstain. The DAPI numbers represent all cells containing DNA and should approximate the sum of cells detected after hybridization with probes EUB338III and EUK516, considering that members of the third Domain, the Archaea, were not detected in any of the samples. Independent of anaerobic or aerobic growth conditions, inoculation of samples that harbored large numbers of cells like compost tea or the microbial pellet did not result in accumulations of cells. Usually a reduction of cell counts by about 90% was observed immediately (i.e. at the sampling immediately following inoculation). These numbers corresponded to

those of samples that did not harbor large numbers of cell such as the sterile-filtered supernatant and the control with a sterile water control. Incubation afterwards did not change numbers over time by large values: over the 30 day period, numbers of microbes, with Bacteria being the major component of the community, remain between 10^7-10^8 per gram of soil, compared to the hypothetical value of about 10^9 cells of microbes or Bacteria after inoculation of compost tea. It is know that inoculation of laboratory grown organisms into soils usually results in a massive die off due to changes in environmental conditions (Wise et al., 2000). Soil conditions, including pH, soil water content, temperature, and oxygen, are a factor that influence the survivability of added microorganisms (Wise et al., 2000). Organic matter and clay content can also effect survivability (Vogel, 1996).

Table 2. Nutrient analysis This table shows the nutrient analysis of the supernatant, bacterial pellet, compost leachate, and compost tea depicting the analyzed numbers for nitrate, soluble reactive phosphorous, ammonia, and dissolved organic carbon. The bacterial pellet has low amounts of nutrients while the supernatant and compost tea contain higher amounts. The compost leachate was taken by pouring water over the compost and collecting the liquid, it contains the highest amount of nitrate and ammonia. The supernatant contains the highest amount of soluble reactive phosphorous.

Sample Name	NO_3 (µg/L)	SRP (µg/L)	NH4 (µg/L)	DOC (mg/L)
Supernatant	9678.33	765.57	237.87	4.66
Bacterial pellet	191.08	78.70	46.55	0.66
Compost leachate	28928.75	539.41	414.69	18.50
Compost tea	3685.48	324.44	160.03	20.90

In a study where soil was amended with compost, cultivable bacterial count showed slight decreases in CFU (Cherif et al., 2009). Microbial populations also varied

within treatment replicates confirming that microbial populations show great variability (Cherif et al., 2009). Another study concluded that using mature compost as an inoculating agent, while it maintained microbial diversity, overall biomass decreased (Kato & Miura, 2007). The addition of compost as an amendment to forest soil that had suffered a wildfire caused a decline in soil microbial populations for the first 30-60 days (Guerrero et al., 2000). However, after three months, there was an increase of bacterial populations (Guerrero et al., 2000). It is possible that high salt content of the soil or compost could have caused the massive decline of microbial populations (Borken et al., 2002). While not all microbes are able to cope with salt or water stress, most can endure greater stresses and are able to survive harsh conditions (Killham, 1994). The soil that was used in our study originated from Bastrop, Texas, an area that had experienced a massive wildfire along with extreme drought conditions for the years prior to collection. It is possible that compost tea could have eventually led to an increase in overall microbial populations over time, though an increase was not evident during the time frame of 30 days used in our study. It has been suggested that the sand collected may have possible nutrients that contribute to growth, however, none of our results supports that suggestion. Negatively charged clay attracts positively charged nutrient ions (Killham, 1994). Since the soil used contained no clay, it is unlikely that the soil contained substantial nutrients. Compost tea, while sharing common traits with compost, does not act the same. It is a variable product which in its liquid form, seeps through the soil. Compost tea also requires frequent applications in order to gain the required results. This experiment took compost tea and applied only a single dose to measure microbial populations over the span of 30 days.

The environment created during the process of brewing the compost tea leads to a rapid population growth of R-strategists, or rather the opportunistic microorganisms that have major population booms when conditions are right (e.g. nutrient availability is high) (Cycoń et al., 2013). When the environment changes rapidly to one that is less optimal, these type of bacteria die off leaving the remaining K-strategists behind. This group of microorganisms survive in environments with less favorable conditions (e.g. low nutrient availability), just in relatively smaller numbers (Cycoń et al., 2013). When introduced to nutrient limiting environments, an initial stop on growth is observed (Bren et al., 2013). This can be an explanation as to what is happening here. Calculations were made in order to normalize the data so that they could be compared to one another. This calculation was based on the DAPI data. A possible error could have been made in the normalization calculation which contributed to the high increase seen in the anaerobic EUK control group upon introduction to the soil. Another possible mistake could be reader error in terms of the fluorescent *in situ* hybridization. Reader error could be contributed to a high degree of autofluoresence, although a block was introduced to limit this, but it was still an issue.

The differences between the two experiments was the environment the soil microcosm created. While minute differences can be seen between the two experiments, it is hard to put any value on what those differences actually mean. Two separate batches of compost tea were created for each experiment. Unfortunately, the chemical and biological composition of compost tea is variable, thus replication of data is near impossible. One batch of compost tea can produce an entirely different set of microbes

than another (Chalker-Scott, 2007). However, trends were observed on the nature of how populations of microorganisms fluctuate through time.

The process of creating compost tea can include the addition of additives. This experiment opted out of using additives. Whether or not the use of additives enhances the growth of microorganisms during the brewing process is not in question for this experiment. The purpose of this experiment was to create a compost tea using quantities and procedures commonly recommended for its production. As well as observe populations within the soil over time while measuring plant production. When using compost tea in its most basic form, the majority of microorganisms die upon application as seen in this study. This observation does not take into account the food sources that those dead microorganisms contribute to the overall health of the plant of diversity within the soil; however, our data do also not support significant effects of inoculation on plant growth performance. Further testing could measure the total bacterial mass (live and dead) and the amount of nutrient pay off that it can contribute. A more effective experiment would be to use varying soils, some that are more favorable to the microorganisms to see any differences in the resulting microbial communities as well as plant productivity.

V. CONCLUSION

The interactions between the soil, microbes and plant are complex and range in outcomes from neutral, to harmful, and to beneficial (Jeffries et al., 2003). Compost with its huge amount of organic material and low C:N ratio provides the perfect environment and source of microbial communities for soil health (Seneviratne et al., 2011). Aerated compost tea is the product of extracting nutrients and microorganisms from compost while enhancing mineralization by active aeration. This aeration provides oxygen as terminal electron acceptor which allows microbes to efficiently mineralize organic material and release nutrients like NH_4^+ that can further be oxidized to NO_3^- while promoting growth of these organisms. Released nutrients are easily dissolved in water and thus be present in compost tea. There are other ways of creating compost tea that include anaerobic incubation, which is leaving compost in water without aeration for an extended amount of time. There is much debate over the use of anaerobic compost teas and whether the process facilitates the growth of human pathogens. While there are other ways of making compost tea, compost tea should not be confused with other compost products. Compost leachate is the brown liquid created when water runs through compost and extracts nutrients and organic components (Lowenfels & Lewis, 2010). This product is supposed to have lower nutrient value or microbial communities than compost tea after active aeration (Lowenfels & Lewis, 2010). Our ion analyses (Table 2) do not corroborate these speculations on nutrient values since leachate has about 10-fold higher NO_3^- concentrations, 3-fold higher NH_4^+ concentrations and similar amounts of dissolved organic carbon as compost tea after 2 days of aeration. The speculative hypothesis behind compost tea is that the product is putting the microbiology back into the soil without having to haul massive amounts of compost (Lowenfels & Lewis, 2010). Not only does it

put microorganisms into the soil, but also it adds to the microbial fauna of the plant itself, thus potentially warding off pathogenic invaders or diseases (Lowenfels & Lewis, 2010).

Although it has been stated by Lowenfels & Lewis (2010), that compost tea can have unlimited applications and that it can be used in place of watering, other studies have shown that the nutrients created in compost tea actually can pass through the soil and into the water in the same fashion as chemical fertilizers. It has been suggested that the nutrients released in compost tea may not be absorbed by the plants fast enough and instead add to water pollution (Chalker-Scott, 2007). This represents a future direction for experiments measuring the amount of nutrient runoff after a compost tea application.

Compost tea is a product whose true effectiveness lies in the eye of the beholder. There are many advocates who believe that works and makes a difference in disease suppression, amending damaged soils, or improving plant productivity, while there are those who believe it is a fad. Compost tea is a product that is very different from one batch to the next. Not only are there many ways to make it, there is also the main component, compost, that ranges greatly in terms of raw ingredients and how those have varying effects on plants especially when turned into a tea.

The results compiled from this experiment indicate that compost tea is ineffective as a plant growth promoting amendment when used as a soil drench for sand. It is also not supporting the development of a large microbial community. Results from a careful, statistically designed experiment specifically applied to testing compost tea for plant growth of corn (*Zea mays*) when used as a soil drench for sand and the quantification of microbial populations in the soil, provides no evidence of functional value. Plant growth

performance was assessed for five separate measurements and statistical differences were not found by day thirty. Fluorescent *in situ* hybridization results showed that for the compost tea and bacterial pellet, bacterial populations dropped 70-90% with only small numbers of Eukarya detected for both anaerobic and aerobic soil microcosm environments upon inoculation, and by day thirty had not developed more abundant cell numbers. Compost tea may provide some microbial life into the soil, but the numbers represented in the compost tea in its true form, do not relate to what transfers to and establishes in the soil.

Table 3. Anaerobic population data. The table above shows the total populations of both Bacteria and Eukarya, using EUB and EUK probes respectively, compared to the DAPI counterstain, which stains whole cells under aerobic microcosm conditions. It compares populations and the percent that represent each in respect to the total DAPI population.

Anaerobic Data												
	Supernatant			Bacterial Pellet			Compost Tea			Control		
Days	DAPI	EUB	EUK	DAPI	EUB	EUK	DAPI	EUB	EUK	DAPI	EUB	EUK
0	6.46(±0.59) E+08	4.27(±0.48) E+08 (62±4%)	3.69(±1.44) E+07 (11±4%)	1.46(±.30) E+09	1.16(±0.30) E+09 (75±3%)	5.37(±4.43) E+07 (5±3%)	1.59(±0.12) E+09	1.56(±0.30) E+09 (92±11%)	9.01(±4.01) E+07 (7±3%)	4.88(±0.66) E+08	3.77(±0.58) E+08 (65±7%)	2.26(±1.85) E+08 (23±7%)
1	7.37(±0.5)E +08	5.83(±0.56) E+08 (72±4%)	1.13(±0.72) E+08 (9±3%)	1.1(±0.097)E+09	8.92(±0.87) E+08 (81±3%)	1.63(±.77)E +08 (19±5%)	1.57(±0.14) E+09	1.42(±0.14) E+09 (86±3%)	8.49(±3.14) E+07 (9±3%)	7.97(±0.87) E+08	6.84(±0.80) E+08 (81±4%)	1.94(±0.59) E+08 (30±6%)
2	1.32(±0.52) E+09	1.18(±0.52) E+09 (81±4%)	9.97(±2.94) E+07 (16±5%)	8.98(±1.08)E+08	7.40(±1.07) E+08 (73±5%)	3.96(±1.55) E+07 (7±3%)	7.44(±0.88) E+08	6.50(±0.91) E+08 (78±5%)	7.71(±3.31) E+07 (15±4%)	5.43(±0.61) E+08	3.92(±0.57) E+08 (61±5%)	1.49(±0.53) E+08 (34±6%)
5	8.67(±1.05) E+08	6.43(±0.93) E+08 (68±5%)	3.23(±1.01) E+07 (11±4%)	9.11(±0.79)E+08	7.91(±0.80) E+08 (83±4%)	7.68(±1.77) E+07 (10±2%)	1.7(±0.30)E +09	1.29(±0.30) E+09 (68±4%)	9.70(±2.33) E+07 (12±3%)	9.88(±1.89) E+08	8.44(±1.87) E+08 (75±4%)	1.50(±0.31) E+08 (26±5%)
10	7.17(±0.64) E+08	3.38(±0.41) E+08 (46±5%)	4.64(±1.44) E+07 (7±2%)	6.73(±0.5) E+08	3.35(±0.42) E+08 (44±5%)	8.26(±2.84) E+07 (14±4%)	4.76(±0.53) E+08	2.55(±0.51) E+08 (45±5%)	5.83(±2.34) E+07 (16±4%)	7.34(±1.24) E+08	3.69(±0.95) E+08 (47±6%)	9.42(±2.39) E+07 (24±5%)
20	4.89(±0.73) E+08	3.02(±0.74) E+08 (53±6%)	1.02(±0.50) E+08 (22±5%)	8.34(±1.39)E+08	6.00(±1.38) E+08 (59±5%)	1.46(±0.40) E+08 (25±6%)	8.78(±1.05) E+08	5.66(±0.93) E+08 (56±5%)	2.85(±1.6)E +08 (25±5%)	4.59(±0.70) E+08	2.35(±0.53) E+08 (36±6%)	1.08(±0.17) E+08 (41±6%)
30	1.37(±0.18) E+09	9.33(±1.83) E+08 (77±5%)	4.62(±1.93) E+08 (38±6%)	9.15(±1.24)E+08	6.97(±1.14) E+08 (79±4%)	3.27(±0.73) E+08 (46±6%)	9.55(±0.93) E+08	7.14(±0.76) E+08 (78±4%)	2.68(±0.40) E+08 (45±6%)	4.56(±0.63) E+08	3.02(±0.60) E+08 (50±6%)	1.22(±0.68) E+08 (22±5%)

Table 4. Aerobic population data. The table above shows the total populations of both Bacteria and Eukarya, using EUB and EUK probes respectively, compared to the DAPI counterstain, which stains whole cells under aerobic microcosm conditions. It compares populations and the percent that represent each in respect to the total DAPI population.

Aerobic Data												
	Supernatant			Bacterial Pellet			Compost Tea			Control		
Days	DAPI	EUB	EUK	DAPI	EUB	EUK	DAPI	EUB	EUK	DAPI	EUB	EUK
0	6.60(±0.86) E+08	5.80(±0.83) E+08 (75±5%)	1.46(±0.38) E+08 (31±6%)	9.05(±0.75) E+08	8.28(±0.80) E+08 (86±4%)	1.76(±0.38) E+08 (31±6%)	1.81(±0.46) E+09	1.74(±0.46) E+09 (92±2%)	2.46(±0.14) E+08 (33±6%)	5.29(±1.07) E+08	5.16(±1.08) E+08 (73±6%)	2.15(±0.93) E+07 (6±3%)
1	6.57(±0.89) E+08	6.43(±0.90) E+08 (88±4%)	5.00(±1.28) E+07 (20±5%)	7.20(±0.70) E+08	7.00(±0.72) E+08 (91±3%)	1.07(±0.25) E+08 (27±6%)	7.00(±0.94) E+08	6.77(±0.94) E+08 (92±3%)	4.38(±1.09) E+07 (12±4%)	5.60(±1.35) E+08	5.39(±1.36) E+08 (81±5%)	4.09(±2.02) E+07 (10±4%)
2	7.27(±0.88) E+08	7.00(±0.90) E+08 (94±2%)	2.03(±0.71) E+07 (10±4%)	5.53(±0.67) E+08	5.46(±0.68) E+08 (82±5%)	7.18(±1.59) E+07 (23±5%)	5.19(±0.52) E+08	5.13(±0.52) E+08 (84±4%)	4.27(±1.05) E+07 (19±5%)	4.32(±0.72) E+08	4.12(±0.73) E+08 (80±5%)	3.70(±1.15) E+07 (17±5%)
5	7.64(±0.89) E+08	7.51(±0.90) E+08 (96±2%)	3.77(±1.25) E+07 (11±4%)	6.77(±0.89) E+08	6.53(±0.90) E+08 (93±3%)	1.88(±0.92) E+07 (6±3%)	9.58(±1.31) E+08	9.55(±1.31) E+08 (95±3%)	8.71(±1.75) E+07 (15±5%)	6.20(±1.44) E+08	5.90(±1.45) E+08 (76±5%)	1.01(±0.39) E+08 (11±4%)
10	9.65(±1.06) E+08	9.42(±1.08) E+08 (93±3%)	2.32(±0.77) E+07 (11±4%)	9.58(±1.80) E+08	9.15(±1.81) E+08 (92±3%)	4.28(±1.42) E+07 (11±4%)	7.74(±0.87) E+08	7.44(±0.87) E+08 (87±4%)	4.28(±0.98) E+07 (15±5%)	4.89(±0.76) E+08	4.49(±0.78) E+08 (74±6%)	6.43(±2.15) E+07 (20±5%)
20	1.12(±0.33) E+09	1.08(±0.30) E+09 (100±0%)	6.09(±1.78) E+07 (12±4%)	1.01(±0.13) E+09	9.98(±1.22) E+08 (98±2%)	1.51(±0.70) E+08 (28±6%)	1.06(±0.14) E+09	1.05(±0.14) E+09 (98±2%)	1.55(±0.41) E+08 (22±5%)	7.74(±1.55) E+08	7.47(±1.56) E+08 (78±5%)	1.07(±0.53) E+08 (12±4%)
30	1.04(±0.16) E+09	9.01(±1.67) E+08 (79±5%)	4.62(±0.43) E+08 (41±6%)	1.07(±0.25) E+09	9.45(±2.50) E+08 (82±5%)	1.20(±0.44) E+08 (23±5%)	8.75(±1.67) E+08	8.54(±1.68) E+08 (92±3%)	6.82(±4.08) E+07 (16±5%)	6.87(±1.74) E+08	5.56(±1.24) E+08 (81±5%)	8.28(±3.57) E+07 (16±5%)

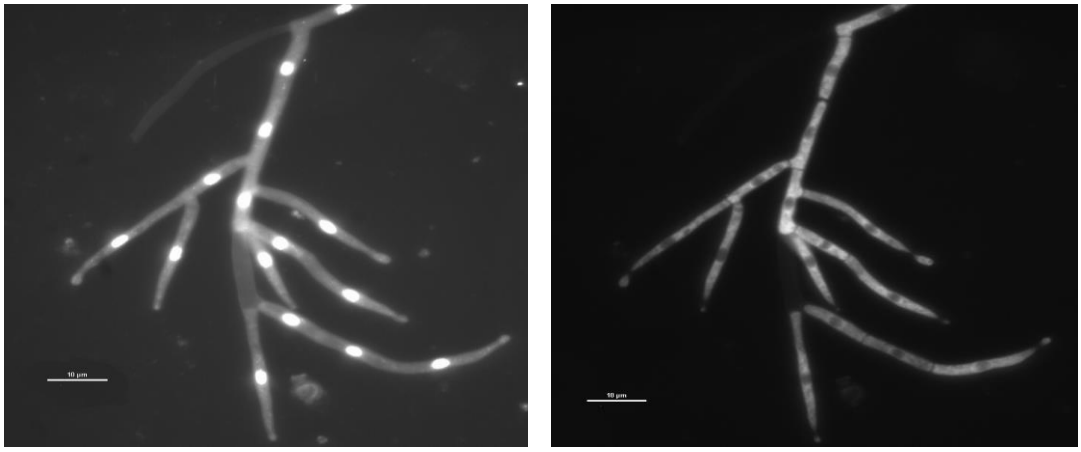

Figure 20. FISH images (EUK). The images are Fluorescent *in situ* hybridization pictures. Both pictures are images of Eukarya taken under two different lenses accentuating the two different target probe or stain. The picture on the left is stained with DAPI while the picture on the right is the same image under EUK516 probe. Pictures were taken with a Nikon Eclipse 80*i*-microscope camera.

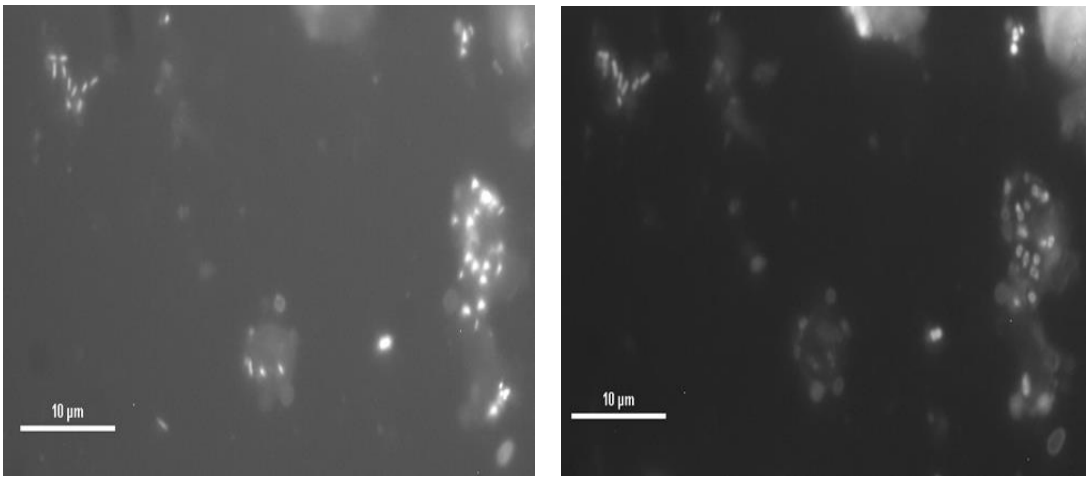

Figure 21. FISH images (EUB). The images are Fluorescent in situ hybridization pictures. Both pictures are images of Bacteria taken under two different lenses that fluoresce differently depending on the stain or probe. The sample was made using both the DAPI stain and the EUB338III probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera.

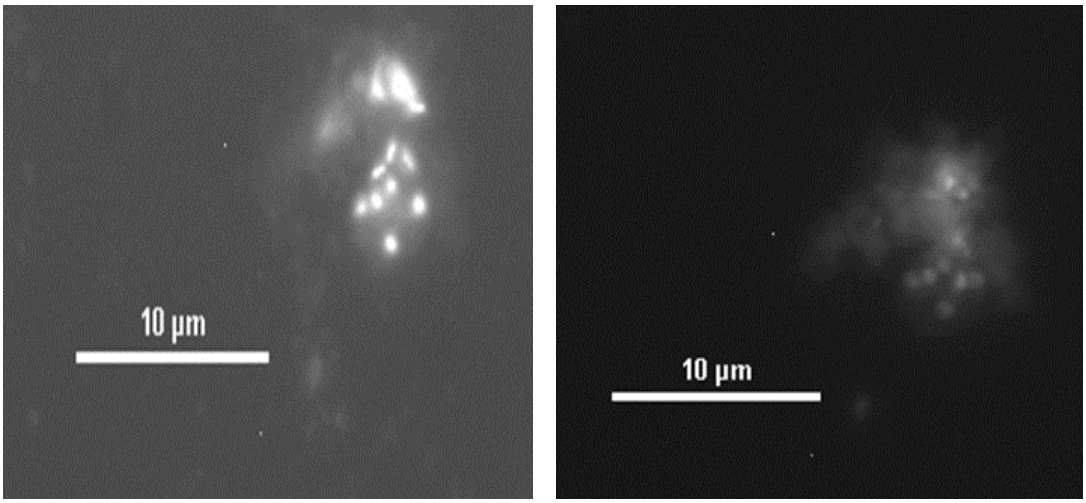

Figure 22. FISH images EUB (compost tea). The images are Fluorescent in situ hybridization pictures of Bacteria present in compost tea on day 0. Both pictures are images of Bacteria taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of compost tea was prepared using both the DAPI stain and the EUB338III probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. There is a slight problem with the fluorescence under the Cy3 light. DAPI was used as a countermeasure.

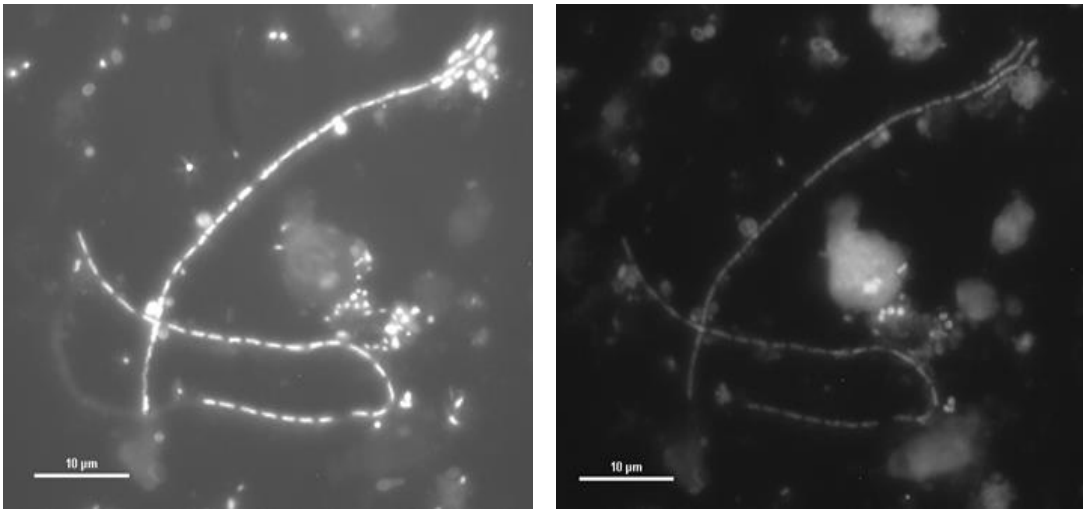

Figure 23. FISH images EUB (bacterial pellet). The images are Fluorescent in situ hybridization pictures of Bacteria present in the bacterial pellet on day 0. Both pictures are images of Bacteria taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of bacterial pellet was prepared using both the DAPI stain and the EUB338III probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera.

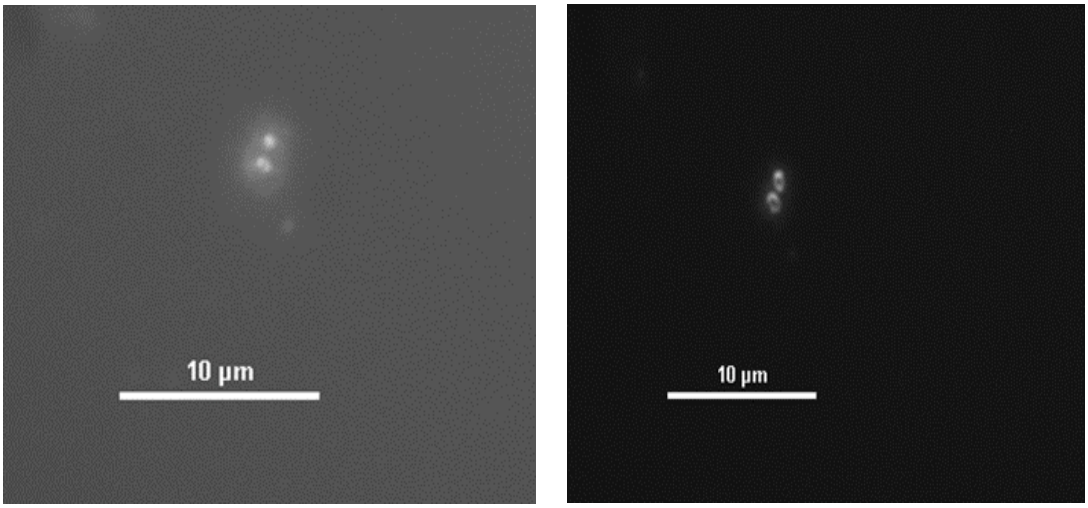

Figure 24. FISH images EUB (control). The images are Fluorescent in situ hybridization pictures of Bacteria present in the water control on day 0. Both pictures are images of Bacteria taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of water control was prepared using both the DAPI stain and the EUB338III probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. The water used for the control did not contribute many Bacteria.

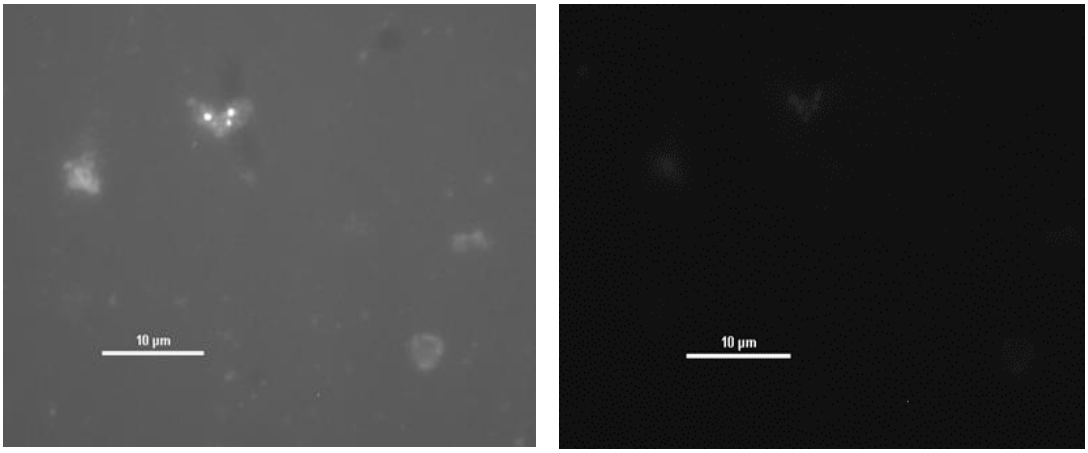

Figure 25. FISH images EUB (sand). The images are Fluorescent in situ hybridization pictures of Bacteria present in the sand used for the soil microcosms. Both pictures are images of Bacteria taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of sand was prepared using both the DAPI stain and the EUB338III probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. The soil used for the soil microcosm did not contribute many Bacteria.

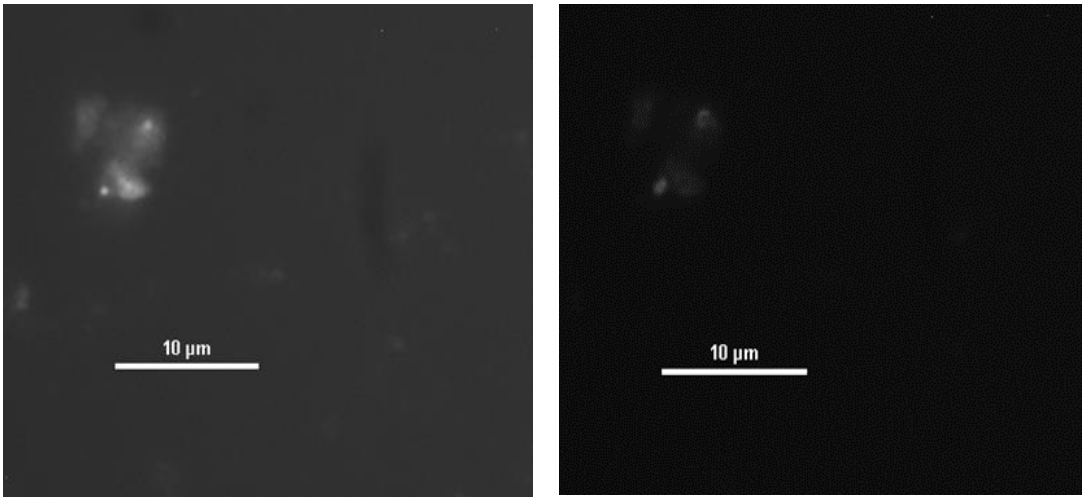

Figure 26. FISH images EUB (supernatant). The images are Fluorescent in situ hybridization pictures of Bacteria present in the supernatant treatment. Both pictures are images of Bacteria taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of supernatant was prepared using both the DAPI stain and the EUB338III probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. The supernatant was separated from the bacterial pellet.

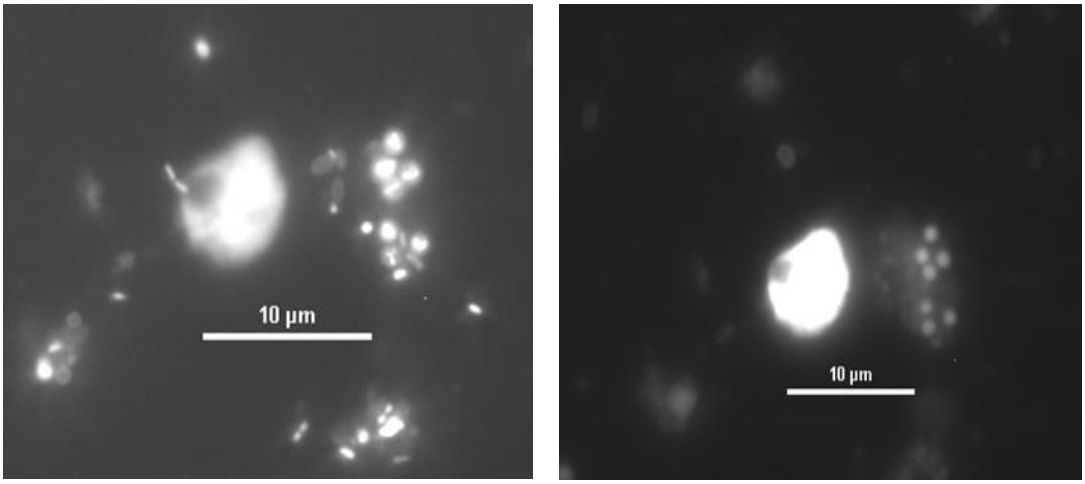

Figure 27. FISH images EUK(compost tea). The images are Fluorescent in situ hybridization pictures of Eukarya present in the compost tea treatment. Both pictures are images of Eukarya taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of supernatant was prepared using both the DAPI stain and the EUK516 probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera.

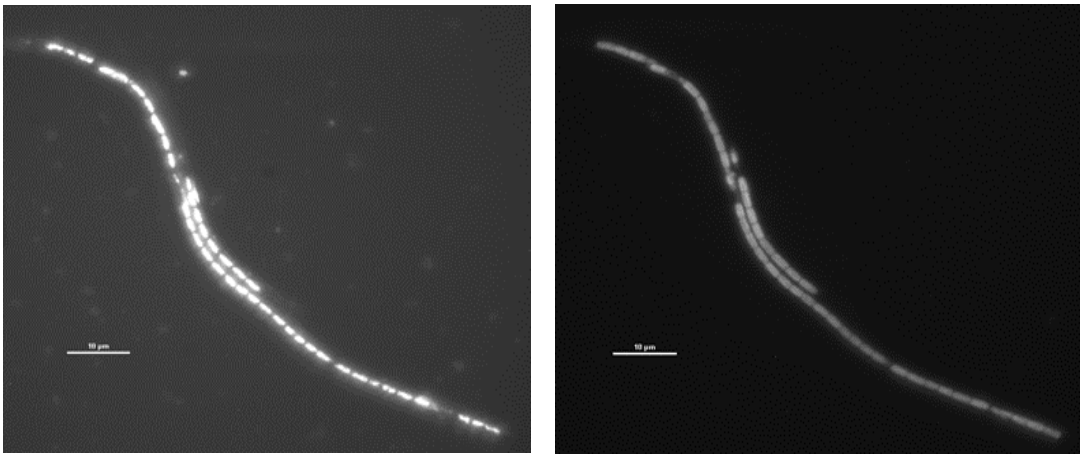

Figure 28. FISH images EUK (bacterial pellet). The images are Fluorescent in situ hybridization pictures of Eukarya present in the bacterial pellet treatment on day 0. Both pictures are images of Eukarya taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of bacterial pellet was prepared using both the DAPI stain and the EUK516 probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera.

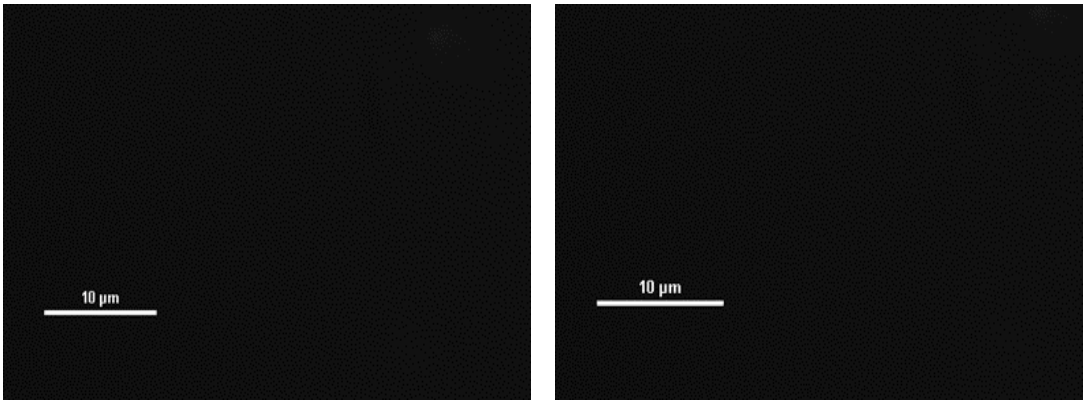

Figure 29. FISH images EUK (control). The images are Fluorescent in situ hybridization pictures of Eukarya present in the water control on day 0. Both pictures are images of Eukarya taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of water control was prepared using both the DAPI stain and the EUK516 probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. The water used for the control did not contribute many Eukarya.

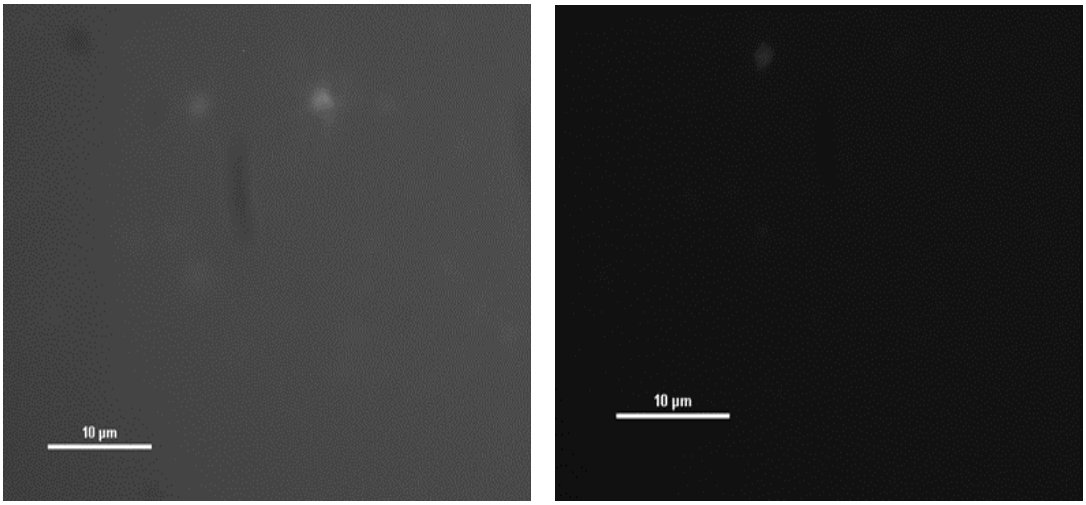

Figure 30. FISH images EUK (sand). The images are Fluorescent in situ hybridization pictures of Eukarya present in the sand used for the soil microcosms. Both pictures are images of Eukarya taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of sand was prepared using both the DAPI stain and the EUK516 probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. The soil used for the soil microcosm did not contribute many Eukarya.

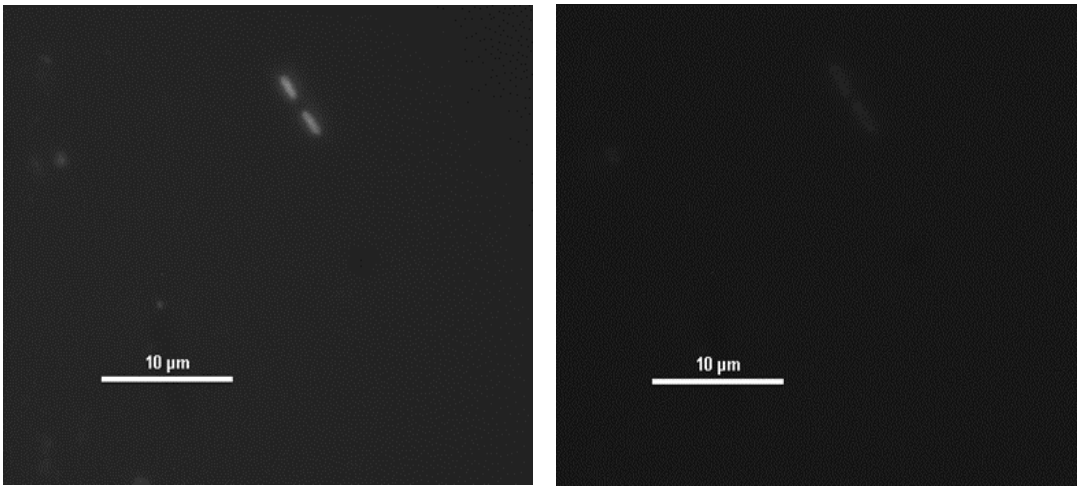

Figure 31. FISH images EUK (supernatant). The images are Fluorescent in situ hybridization pictures of Eukarya present in the supernatant treatment. Both pictures are images of Eukarya taken under two different lenses that fluoresce differently depending on the stain or probe. The sample of supernatant was prepared using both the DAPI stain and the EUK516 probe. The picture on the left was under the DAPI light filter while the picture on the right is under Cy3 light filter (probe light). Both images were taken with a Nikon Eclipse 80i-microscope camera. The supernatant was separated from the bacterial pellet.

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