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Fielding Sustainability: The Potential for Compost Extract as an Amendment on Athletic Turfgrass

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Fielding Sustainability: The Potential for Compost Extract as an Amendment on Athletic Turfgrass

by

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Abstract

Sustainability and green initiatives are being pushed across the country and the globe, but athletics are not seeing the same pressures as intensely. Golf courses are largely the only athletic arena that have received pressure and are beginning to implement more sustainable management practices. Agriculture is also making strides to use less chemicals through organic farming and improve soil health with cover crops. Meanwhile, the rest of the athletic world continues to contribute to soil compaction, chemical maintenance, and runoff all of which affect the quality of the local environment. The strides to improve athletics' sustainability record can be seen in the energy efficient building, recycling programs, and reduced water use. However, outside on track, football, baseball, softball, lacrosse, soccer, and other fields, soil is dug up, replaced with a sandy soil, leveled, compacted, and planted with non-native turfgrass. The turfgrass receives massive amounts of chemical fertilizers, herbicides, and pesticides. When rain or irrigation falls on the fields the water quickly drains through the sandy soil under the field leaving little water for the grass roots.

As an alternative to these traditional maintenance strategies, this experiment evaluated compost extract on University of South Carolina's track infield. Compost was used in place of chemical fertilizers so that the soil and turfgrass could be compared. Viewing soil as more than a medium for turfgrass to grow, this study was interested in not

only the aboveground grass, but also the soil health and diversity of the below-ground microbial community. Remote sensing, specifically the Trimble® GreenSeeker® was used in analyzing the turfgrass throughout the three months of treatment. Additionally, soil samples were taken to determine soil chemistry as well as soil biology. Soil cores were taken to determine the bulk density, which is a critical component to athletics for athlete safety and field playability. The experiment suggests that with more research, compost extract could be used in place of chemical fertilizers or as part of a more environmentally friendly field maintenance regime for turfgrass when the environment is appropriate for growth.

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List of Abbreviations

ANOVA .... Analysis of Variance

AOI .... Area of Interest

CEC .... Cation Exchange Capacity

J-S .... Johnson-Su Compost

NDVI.. Normalized Differential Vegetative Index

NTEP...National Turfgrass Evaluation Program

OM .... Organic Matter

PLFA...Phospholipid Fatty Acid

Total%BS... Total Percent Base Saturation

Chapter One: Introduction and Literature Review

1.1 Introduction

Sports have been a part of human history for thousands of years. From early Olympic foot races to modern day events, athletics has grown and organized dramatically over time. With this organization and increasing popularity of nearly fifty internationally regulated outdoor sports, athletic fields have also become common (Roland, 2017). Many of these sports require fields covered in grass and subsequently maintained with fertilizers, pesticides, and irrigation. Newer than the prevalence of athletics is the field of sustainability. Sustainability focuses on the interrelatedness of environmental protection, social communities, and economic viability (Johnson, 2014). Sustainability is a prevalent topic in current events and sustainability initiatives appear at a variety of levels from global down to local, including specific communities like colleges. Many field maintenance practices work against the environmental component of sustainability (Johnson, 2014). However, little research addresses the sustainable or unsustainable nature of most sports. The literature does address the largely unsustainable nature of golf; scientists are investigating the reasons as to why golf is viewed as unsustainable. Since the rest of athletics have not had the same pressure to go green, their efforts are focused on buildings, particularly energy usage, not the maintenance practices done on outside (Henly, 2013). The buildings do have environmental impacts and energy efficient buildings can save costs,

but the management of turfgrass fields would have a major environmental impact on both the soil and water quality in the surrounding areas.

Coming from a background of collegiate athletics puts National Collegiate Athletic Association (NCAA) fields at the forefront of my mind and project. As a softball player, I have spent the last thirteen years of my life on fields. In college, the pellets of bright yellow fertilizer were easily visible all over the field and surrounding areas. Now in a larger conference with a bigger budget, even more chemicals are used to ensure that the fields are green and playable year-round. Tying my athletic life to my environmental interests, I am in a unique position to bridge these fields of study in a way that the athletic department is more open to since I am not an outsider. In initial investigations before starting this project, I was found that many chemicals used in field maintenance are skin or eye irritants. This made sense after fighting allergic reactions to the grass on softball fields most of my career. Moving athletic fields away from such heavy chemical maintenance would not only help the environment but also be better for the student athletes while cutting costs for the university.

A unique program that has moved to a more sustainable framework of turfgrass management is the University of Colorado at Boulder (CU). In an informative trip to Colorado (June 17 – 20, 2018), I learned that CU uses only organic products as part of their campus sustainable initiatives. They have developed unique compost tea system including an injector into their campus-wide sprinkler system. After meeting with directors (athletic director Rick George, director of athletic grounds Ryan Newman, and Environmental Center director Dave Newport) and implementers of the program on campus (outdoor services turfgrass manager Ryan Heiland), it was very apparent that the university not only

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wanted to support green grass and healthier, lively soils, but they also wanted to so in a way that had far less negative environmental impacts. Through this program, they are able to use only compost tea and organic products paired with management practices such as moving, aerating, and seeding. While implementing this throughout all of campus had substantial costs associated with it, the transition within athletics was more streamlined since they had most of the management practices and personnel in place already. For athletics, this can actually cut costs over time. The organic fertilizers are less expensive, and they have found over time that they need substantially less fertilizer and have actually been able to cut usage by more than half while using them with the compost tea. Additionally, in comparison to implementing the program campus wide, there were not extra labor costs within athletics maintenance. This unique program has helped CU improve soil health, maintain green fields, and majorly cut their environmental impacts. The program immediately caught my interest and directly showed that there are other methods of field maintenance than the common chemical narrative.

1.2 Goals

One goal of this project is to determine if compost extract can be a successful method (or supplement) for field maintenance. The second goal is to encourage the University to consider alternative maintenance practices like compost extract to reduce their environmental impact.

1.3 Hypotheses

We proposed the following hypotheses for this research:

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Null Hypothesis 1: Plots treated with compost extract will show no significant difference in plant color or vigor compared to conventionally treated plots.

Alternate Hypothesis 1: Plots treated with compost extract will show significantly improved plant color or vigor compared to conventionally treated plots.

Null Hypothesis 2: Plots treated with compost extract will show no measurable changes in belowground microbiodiversity, conventional soil test values, bulk density, or infiltration rates compared to conventionally treated plots.

Alternate Hypothesis 2: Plots treated with compost extract will show measurable improvements in belowground microbiodiversity, conventional soil test values, bulk density, or infiltration rates compared to conventionally treated plots.

1.4 Literature Review

Compost by definition is decaying organic matter and can be added to soil as fertilizer for plants. According to Dr. David Johnson of the Johnson Su compost method to be utilized in this project,

> "The Johnson-Su composting method creates compost teeming with microorganisms that improve soil health and plant growth and increase the soil's potential to sequester carbon. This simple composting method produces a biologically enhanced compost by creating an environment where beneficial soil microorganisms and thrive and multiply. When this biologically alive compost is applied to the soil the microorganisms inoculate the soil and work in harmony with growing plants to improve soil health and increase the amount of carbon drawn out of the atmosphere and into the soil" (Johnson, 2018).

Compost of this nature aims to improve soil health and therefore positively affect the plants growing in it.

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Ingham (2014) extensively describes the soil food web. This food web is made up of the microorganisms that live in the soil that are fed by plants that in turn gain energy from photosynthesis, ultimately the sun's energy. A diverse soil food web full of beneficial microbes create a healthier soil that then can better support the plants while also helping to provide more available nutrients. The diverse community of microbes, including bacteria, fungi, protozoa, microscopic arthropods, and nematodes, build a food web within the soil. Ingham explains that this food web is integral to the soil health and anything growing in it. These microbes can work with the plant roots, break down organics into nutrients making them more available to plants, outcompete unwanted pathogens, and help create additional small pore space in the soils. All together, these factors make healthier soils that then can directly impact the plants growing in the soil. (Ingham, 2014)

For turfgrass fields in particular, the color and growth rate are critical components. The field needs to look green to be aesthetically pleasing for play, and the grass needs to grow at a rate fast enough to rebound from frequent wear. Garling and Boehm (2001) published research addressing the effectiveness of compost for these two components (aesthetics and resilience) looking at golf courses. Through their research, they found that the color of the grass was enhanced through compost application for a duration of five to eight weeks on frequently low-cut fairways. They attribute this increase in foliar nitrogen to the high nitrogen compost applied in the study (Garling & Boehm, 2001). With the increased nitrogen, the turfgrass is also capable of handling more wear from frequent use. This wear is commonly a reason why additional chemical nitrogen fertilizers are used in athletics, so the increased nitrogen levels are a crucial finding.

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In addition to nitrogen application, turfgrass fields utilize other fertilizers and pesticides to keep a healthy stand of only the desired turfgrass. Joyce (1998) addresses the wide-spread misuse and overuse of fertilizers and pesticides in her article. The heavy and unregulated chemical use of fertilizers is noted as an environmental issue since they spread beyond the boundaries of the golf course and also potentially pose a human health issue as they seep into groundwater and move into surface water. The chemicals in fertilizers used are required to have Data Safety Sheets (formally Material Safety Data Sheets – MSDS), however, the amount and frequency of using chemical treatments is not particularly regulated. Pesticides on the other hand are regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Even with FIFRA regulating pesticides, Joyce notes that there are still considerable concerns regarding both non-target organisms as well as the potential for human health concerns. (Joyce, 1998)

In turfgrass just like any other monoculture, disease is always a concern. In a forum where the grass being green and healthy is vital, fungi like *Rhizoctonia solani* that cause brown spots across the grass, are not tolerated. Multiple studies from Boulter, Boland, and Trevors (2000, 2002) found that compost can be a successful treatment in suppressing turfgrass diseases. Their research claims that the disease suppression is attributed to physical, chemical, and biological aspects of compost. The biological communities of microorganisms that compost supports assist in outcompeting the fungal diseases while supporting the turfgrass (Boulter, Boland, Trevors, 2002). Additionally, the compost's physiochemical components that improve the soil structure and water retention help to support and strengthen the turfgrass to outcompete the disease (Boulter, Boland, Trevors, 2000). These studies directly support Ingham's (2014) claims that a healthier soil with a

diverse belowground microbial population (2014) will be able to better support plant growth and outcompete pests. Boulter, Boland, and Trevors (2000) suggest that compost is an effective way to create healthier soils.

Before an athletic field is ever cut or lined, the footprint is typically dug up completely. This is an area that athletic fields tend to differ from other forums like agriculture and golf courses. The native soil is replaced with a very sandy soil. The new soil profile allows water to more quickly infiltrate, so the field is playable quicker after a rain event. Since most athletic fields are built on sandy soils, the research that compost can improve that soil type is critical. In a study conducted by Glab et al. (2018), compost with a variety of different additives were tested on a sandy loam soil. The study found that the addition of biochar to compost led to the most improvement over a three-month testing period, but all of the compost mixtures (even plant material) showed at least some improvement (reduction) in soil bulk density and total porosity, meaning the soil showed less overall compaction and had more pore space which would improve water infiltration and drainage. On top of improvements in bulk density and porosity, the study also found that the available water capacity in the soil was improved by using the compost mixtures (Glab et al, 2018).

When compost is incorporated in playing fields, it is often as a topsoil mixture (Fuller, 1998). The article reviews the use of topdressing made with 20% solid compost mixed with sand on both recreational fields in New York and on the Buffalo Bills practice field. According to Farrell (1998) the compost topdressing adds nutrients to the nutrient poor sandy soils and holds water. For this to be successful, it is noted that the field needs a good drainage system and the compost mixture needs to be fine, a "three-eighths inch-

minus particle" (Farrell, 1998). Additionally, the importance of testing and tailoring amendments to the compost is heavily stressed. Even though this compost topdressing method was found to be successful, it is generally cost-prohibitive to secondary schools and recreational fields.

Application rate and timing are particularly important for compost use. Muir, Butler, Helton, and McFarland researched (2010) the use of dairy manure compost on coastal bermudagrass, *Cynodon dactylon*. They analyzed both the seasonal timing of application as well as the rate with and without the addition of supplemental inorganic nitrogen. The applications were in November, January, and March on the plots in Texas. Three different rates of dairy manure compost were applied to the surface of the grass with either no added nitrogen or 112 kilograms of nitrogen as ammonium nitrate (Muir, Butler, Helton, & McFarland, 2010). Additionally, there was a nitrogen fertilizer only treatment and a check plot. A positive yield response was found with the dairy compost manure both with and without the supplemental nitrogen after the first year. A positive response to the November and March applications of the compost compared to the January application was also found after year one (Muir, Butler, Helton, & McFarland, 2010). It is suggested in the article that this might be because the roots still had some activity and November and starting activity in March allowing them to store the nitrogen for the spring and summer growing season.

From the literature, compost appears to be a valuable soil amendment in multiple different forms including extract. Some of the potential benefits include limiting the use of synthetic fertilizers, suppressing disease, improving the belowground microbiodiversity,

and supporting color and growth. Building from this literature, this experiment will evaluate the effectiveness of compost extract on a turfgrass field.

Chapter Two: Methodology

2.1 Area of Interest (AOI)

The project took place on the infield of University of South Carolina's Track and Field Facility, Weems Baskins Track (Figure 2.1) located on the corner of Rosewood and Marion streets in Columbia, South Carolina (33.983910, -81.020545) and covered with Bermuda turfgrass, *Cynodon dactylon*. The track field is used because it is Bermuda grass year-round and is not over seeded with Rye grass for the winter. This means that the turfgrass is not chemically killed off to promote the cool weather species and allows for natural dormancy to end the trials. The average annual temperature is 63.8 degrees Fahrenheit with summer temperatures into the 90s. The average annual rainfall is 44.29 inches, but the field also has irrigation (U.S. climate data, 2018).

2.2 Experimental Design

To compare the efficacy of compost extract to synthetic fertilizer four treatments were used namely: 1. Control (also referred to as a check), 2. young compost: aged 4 months before first use, 3. mature compost: aged 14 months before first use, and 4. conventional maintenance. There were four replicates for each treatment, making up sixteen plots and the experiment was repeated, i.e. two parallel experiments were conducted (Figure 2.2). The plots measured two meters by two meters making each

experiment a block of eight meters by eight meters. Within each replicate, the treatments

were randomized using an online random number generator to limit any potential bias.
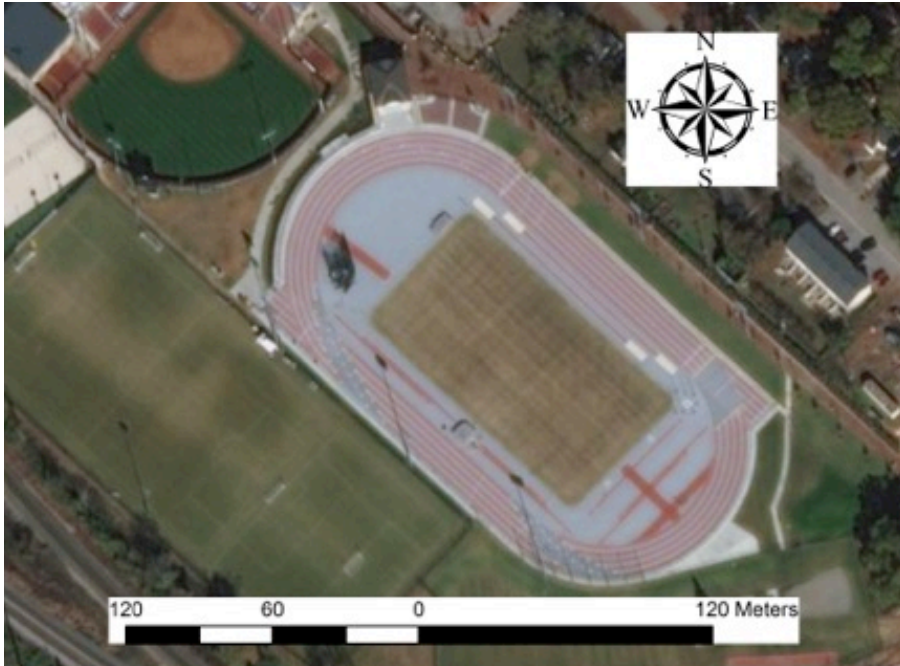
Figure 2.1: AOI: Weems Baskins Track at University of South Carolina

Experiment 1

1	2
3	4

3	4
2	1

2	3
1	4

3	2
1	4

Experiment 2

3	2
1	4

2	1
3	4

3	2
4	1

4	1
2	3
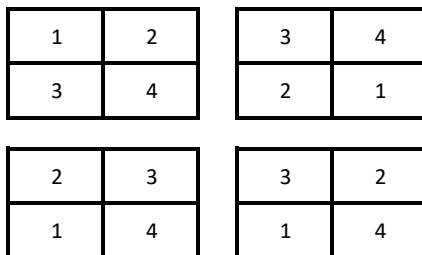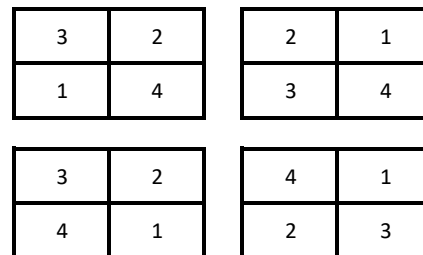
Figure 2.2: Schematic Representation of Plot Experimental Design

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2.3 Pre-Trial Preparations

2.3.1 Site Preparation

On the track field, the experimental plots were within one irrigation zone. Each experimental plot was selected based on uniformity of the turfgrass by visual density and color.

To establish the plots, all measurements were conducted with a tape measure, first the borders and then the diagonals to ensure the size and shape. The 2 meter by 2 meter plots were marked out after the original square was set. The plots of each experiment were marked with stakes that laid flat in the ground for athlete safety and also with spray paint for easy visibility. All samples for the project were taken from the central 1 square meter region of the two meters by two meters plots – creating a buffer around the measurement zone and limiting crossover of fertilizer and/or compost extract between treatments.

2.3.2 Johnson-Su Compost

The Johnson-Su method was chosen for this project for numerous reasons; it is static and aerobic, so it favors fungal development, and it is aged to allow for increased microbial diversity. The lack of turning is important as to not disturb or break-up the fungi and nematodes (Johnson, 2018).

2.3.3 Compost Preparation

We applied the Johnson-Su method as follows (Johnson Su Best Management Practices, 2017):

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To ensure the pile promotes beneficial soil microbes, the pile cannot go anaerobic. On April 20, 2018, a bioreactor was built with simple materials including a recycled pallet, chicken wire, sediment netting, and PVC pipes (Figure 2.3). The reactor was a round vessel three feet in diameter. The reactor was placed on top of a pallet while four large PVC tubes were stood in the pile when it was built to ensure adequate oxygenation of the compost. After the first 24 hours, the tubes were removed, but the space remained empty to make sure oxygen was available to the entire pile. No point in the reactor was more than eight inches from either a hole or the edge of the pile to provide oxygen. Figures 2.3 and 2.4 show visuals of the compost pile in the early stages.

The compost included wood chips, green leafy material, and dried cow manure. All of the ingredients were put through a wood chipper to ensure there were no large pieces and then immersed in water before being manually added into the bioreactor with 5-gallon buckets. The ratio of ingredients was approximately five parts woody material to two parts green material to one part manure. In addition, a small amount coffee grounds and soaked pea seeds that had been through the wood chipper were added to increase the nitrogen content.

The carbon to nitrogen (C:N) ratio is an important figure for compost (USDA, 2011). The nitrogen is an early food source for bacteria that help to begin the decomposition process and help the pile to go thermophilic. To determine the C:N ratios of the compost and its amendments, samples of the compost were collected throughout the pile and sent to Clemson Regulatory Services May 2, 2018.

Figure 2.3: Compost Pile Day 1 with PVC Pipes to be removed after 24 hours

Figure 2.4: Top View of Compost Pile with PVC Pipes Removed, illustrating that no part of the pile is more than 30 centimeters from air

The compost method and ingredients also supported a thermophilic period. In the first 2 weeks of establishing the pile, the temperature should get over 131 degrees Fahrenheit to kill off any pathogens (Ingham, 2014). This is especially important since this compost was used on turfgrass that athletes will be in contact with and cow manure was included in the production. The pile however, should not go above 160 degrees Fahrenheit because that then kills off many of the beneficial microbes.

2.4 Timeline

A detailed timeline for the application and sampling to be done throughout the project is shown in Table 2.1.

2.5 Plot Treatments

Every other Monday, prior to application, the compost extract and fertilizer had to be prepared. All four treatments were prepared in the laboratory on the afternoon that they were applied to ensure that the compost did not go anaerobic in the bottles.

2.5.1 Compost Extract

There were two sources of compost; the young had been aged in the bioreactor as discussed for four months and a fourteen-month-old compost acquired from Braeburn Farms in North Carolina. To prepare the compost extract, compost was taken from each pile (the young and mature) that day. Both of the extracts were prepared separately but following the same procedures. 270 milliliters of compost were measured out in a graduated cylinder.

Table 2.1: Timeline of Project Application and Sampling

	Week of>	7/30/2018	8/6/2018	8/13/2018	8/20/2018	8/27/2018	9/3/2018	9/10/2018	9/17/2018	9/24/2018	10/1/2018	10/8/2018	10/15/2018	10/22/2018	10/29/2018	11/5/2018	11/12/2018	11/19/2018	11/26/2018	12/3/2018	12/10/2018
Laboratory/Method	Event Type[1]	1	1	2,3	3	2,3	3	2,3	3	2,3	3	2,3	3	2,3	3	2,3	3	4	4	4	4
Clemson ST minerals with %OM	All Plots (n=32)		32																	32	
Ward: Haney & PLFA	Composite Each TRT x 2 Exp (n=8)		8									8								8	
Elemental (C, N, P, K) Compost Analysis	Composites of Compost Pile (n=2)	2																			
Infiltration	All Plots (n=32)		32																	8	
Bulk Density	All Plots (n=32)		32															32			
Soil Microscopy Examination	Composite Each TRT x 2 Exp (n=8)		8										8								8
Compost Microscope Exam	Composites of Compost Pile (n=2)	2																			
GreenSeeker® - NDVI	All Plots (n=32)		32	32	32	32	32	32	32	32	32	32	32	32	32	32					
NTEP Visual Assessment	All Plots (n=32)		32	32	32	32	32	32	32	32	32	32	32	32	32	32					

1. **Sample Event Type: 1=Baseline. 2=Extract Application, 3= Routine NDVI/NTEP, 4=Final Sampling**

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The cylinder was tapped to allow the solid compost to settle down for an accurate measurement. The compost was then poured into an approximately 400-micron paint screening net over a large beaker filled with about 1,500 milliliters of aerated water. The compost in the netting was massaged in the water for 60 seconds per Ingham's protocol (2009). The netting was then removed and wrung of excess water. This compost filled water was poured into a two-liter bottle and topped with aerated water up to 2250 mL for spraying.

2.5.2 Fertilizer Preparation

Preparing the fertilizer followed the recommendations that the University of South Carolina athletics department uses made by Harrell's field maintenance company, just scaled to the plots. The nitrogen, P_2O_5, and K_2O values of the fertilizer mixture are 48-0-36 (Harrell's, 2016). 27 grams of each of the four liquid chemicals was weighed out in beakers. The two-liter bottle was filled with 1,000 milliliters of aerated water and then the chemicals were added one at a time. The bottle was filled the rest of the way to 2,250 milliliters with more aerated water. The bottle was capped and swirled to mix the chemicals throughout it.

2.5.3 Application

A backpack sprayer without filters on the sprayer nozzle was used to apply the compost extract, fertilizer, and water. A tank of pressurized air was attached with a pressure gauge and a hose to the two-liter bottle of each given solution. The solution was then pushed from the bottle through a hose to the sprayer and out onto the grass. The sprayer

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sprays a width of approximately one-meter when held at about one-third of a meter above the ground, so each plot needs two passes to cover the two-meters. The compost extract solutions were always mixed in their bottles and applied first. After one extract was applied on the eight plots the second extract was used on the associated eight plots. The fertilizer was the applied and finally the water only treatment. Between each different treatment, the spray system was thoroughly cleaned to remove any residual product. Again, after the final treatment of water only, an entire bottle of water was sprayed through the sprayer to clean it.

2.6 Sampling and Measurements

2.6.1 Sampling Methods

The direct measurement methods included soil sampling for minerals and percent organic matter run through Clemson Regulatory Services. Additionally, soil samples were collected for Haney and Phospholipid Fatty Acid (PLFA) analysis by Ward laboratories to determine the microbial biomass in the soil (Ward Laboratories, Inc., 2018).

For the soil samples sent to Clemson, six-inch soil samples were taken with a soil sampler, bagged, and mailed. The guidelines for soil samples given by the extension were followed. When composite samples were sent, each plot was still sampled, and the soil was mixed in each bag before shipping. (Clemson Regulatory Service, 2018)

The samples sent to Ward for PLFA and Haney analysis were all three-inch soil cores. These samples were taken with an apple corer to ensure that only the top three inches were collected. The samples sent to Ward were sent as composites of each treatment, thus

several cores from each treatment were taken and combined from each experiment. These cores were then mixed and bagged. The sample bags of samples were allowed to stay ventilated to allow the soil microbes adequate oxygen for respiration. These samples were bagged, put on ice, and sent by overnight carrier.

2.6.2 Observation Methods

Remote sensing from both visual and mechanical techniques was used as a practical method of allowing more frequent measurements to be taken without increasing the project budget or damaging the turf.

The National Turfgrass Evaluation Program (NTEP) visual protocol was used to evaluate color, density, and uniformity of the plot surfaces weekly. Per this protocol, the observations were performed with the sun to the observer's back. The center of each plot was observed only after first setting minimally adequate (6) standards and rating one section of grass not in the experiments. A perfect score (9) could be given in the experiment but only if the plot was deemed ideal in all categories (Table 2.1).

Table 2.2: Components of Turfgrass Quality for 1-9 Ranking

Quality Component	1 Score	9 Score
Uniformity	Poor uniformity	Good uniformity
Shoot density	Low shoot density	High shoot density
Leaf texture	Course leaf texture	Fine lead texture
Leaf orientation	Random orientation	Upright orientation
Smoothness	Poor smoothness	Good smoothness
Color	Light or yellow green	Dark green

A Trimble® GreenSeeker® (Trimble Inc., Sunnyvale CA) was used to sense the Normalized Difference Vegetation Index (NDVI) of the turfgrass. The GreenSeeker® was steadied with a stand 61 centimeters above the turf to get readings from center 25

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centimeters of each plot. Three readings were taken from each plot in the center and the mode was be used for the NDVI of the plot. The readings were also taken with the sun to the observer's back; care was taken to avoid interference from the observer's shadow. The GreenSeeker® observations therefore matched the orientation of the NTEP observations for a more valid comparison.

The NTEP and NDVI readings were taken at the same time period each week; each Wednesday between 10 AM and 3PM (weather permitting). The plots also were not be observed in the same order each time to limit sampling bias; NTEP observations were made first as a means to limit the bias of the observer, then the NDVI was recorded with the GreenSeeker®. For the majority of the experiment, the NTEP and NDVI were recorded by a trained undergraduate to limit personal bias.

In the final stage of sampling, Mr. Clark Cox, University of South Carolina Assistant Athletic Director for sports turf and landscaping will analyze the plots and rank the treatments within each repetition. Cox's position gives him the predominant opinion about the turfgrass at the university and is an expert opinion within the field regarding the practical outcome of the experiments. In this ranking, his observations were blind, i.e., he did not know which treatment was applied to each plot. He simply ranked the plots based on what he looks for in athletic field turfgrass.

2.6.3 Infiltration and Bulk Density Methods

Infiltration tests were conducted within an infiltration ring and bulk density will be tested from soil cores. They were tested at the beginning and end of the experiment. Both of these tests were performed on campus either on site or in the lab. For both of these tests,

the middle section of the plot was not be used to avoid altering what was used for sampling in other tests.

The infiltration test used a 15.8-centimeter diameter infiltration ring (USDA, 2018) which was hammered three inches into the ground with a rubber mallet. Then, the ring was covered with plastic wrap. Once 498 milliliters (25.4 mm or one inch) of water was measured in a graduated cylinder and the timer was ready, the water was poured into the ring on top of the saran wrap. The plastic wrap was removed, and the timer is started. Once the first inch of water was completely gone from the surface of the grass, the timer was stopped; then this was repeated with a second inch of water. After, the time for the first inch and second inch of water was converted into inches per hour.

For bulk density, a slide hammer (AMS Inc. American Falls, ID) was used to collect tubes of soil. The tubes were then capped on each end to prevent any soil or moisture from escaping. Each tube collects 146.2 cubic centimeters of soil. Once in the laboratory, each tube was emptied individually into an aluminum tin and weighed. The tins of soil were heated in an oven for twenty-four hours at 105 degrees Celsius (USDA, 2018). After being heated, the soil was weighed again. From this, the percent moisture and bulk density is calculated using Equation 2.1.

Equation 2.1: Bulk Density in g/cm^3

Bulk Density = Weight/Volume

Where:

Weight = Weight of dry sample in grams

Volume = Volume of sample in cm^3

2.6.4 Direct Microscopy

Direct microscopy was used to evaluate the compost to ensure that there was not a substantial presence of harmful microbes in the compost pile. The microscopy was also performed on composite soil samples from the plots (one composite of each treatment from each experiment) to track microbial diversity throughout the study. Ingham and Rollins' Soil Food Web methodology and worksheet were used for the examination (Ingham and Rollins, 2009). For this, each sample was mixed, then one milliliter was diluted to five milliliters of sample with dechlorinated water. After thirty seconds of gentle mixing, one drop was mounted and observed under a microscope. First a scan of the entire slide at 40 times magnification was conducted for nematodes and then eight random fields at 400 times magnification were observed to count protozoans and fungi. The solution was diluted further to 1 in 500 and again mounted on a slide for a bacterial count of eight random field of views at 400 times magnification. The data were then entered into worksheets to calculate microbial biomasses for bacteria, fungi, protozoans, and nematodes and then to calculate fungal-to-bacterial ratios.

2.7 Statistical Analysis

For analysis of results, t-tests and analysis of variance (ANOVA) were used. Alongside ANOVA, the Fisher Multiple Comparison Correction method was also used for groupings. Letters in Fisher Grouping Column that are equal indicate no significant difference. All results were calculated at a 95% significance level or $\alpha=0.05$.

Chapter Three: Results

3.1 Context of the Experiments

3.1.1 Compost compared to soils

The temperatures of the compost pile from April 21, 2018 to May 27, 2018 are displayed in Figure 3.1. The pile went thermophilic on April 29, 2018. Over time, the pile cooled back to ambient temperature.
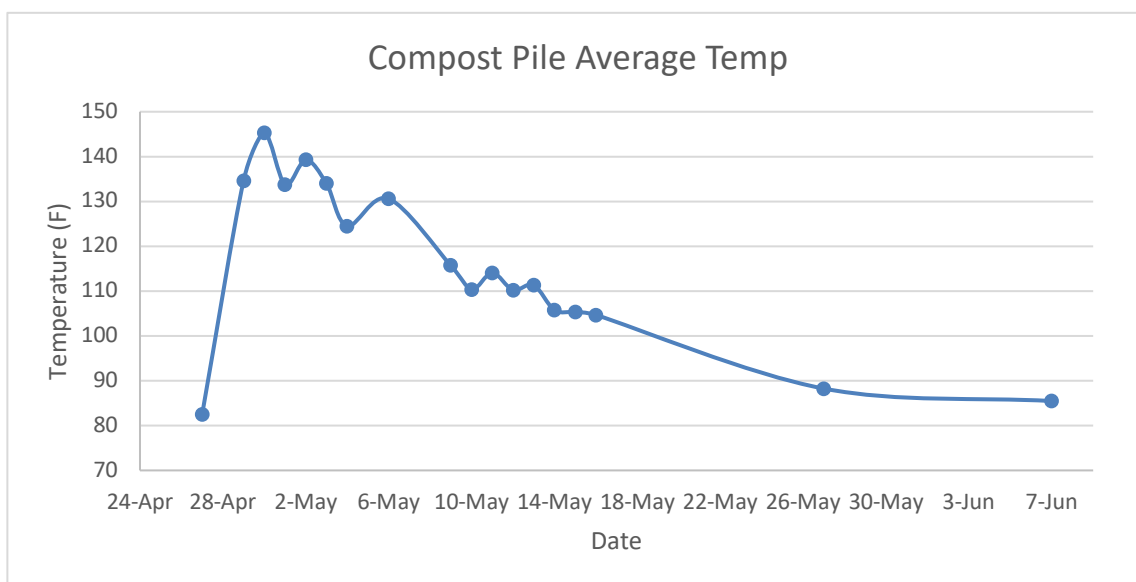
Figure 3.1: Compost Pile Temperatures

The average C:N ratio was 39:1 as found through compost analysis results from Clemson Regulatory Services, and the pile did indeed reach a thermophilic phase.

According to the United States Department of Agriculture (USDA), the ideal C:N ratio for compost is 30:1, so the pile is still a little more carbon heavy than the ideal, but it is close to the ideal ratio (USDA, 2011).

From the Ward Laboratories compost analysis test, the young Johnson-Su (J-S) at four months of aging can be compared to the mature J-S that had aged for about one year in terms of the traditional fertilizer components of nitrogen, phosphorus, and potassium. The total percent nitrogen in the young J-S was 1.93% and 1.22% in the mature J-S. The percent phosphorus in terms of P_2O_5 was 0.74% in the young and 0.61% in the mature. The percent potassium in terms of K_2O was 0.60% in the young and 0.78% in the mature.

PLFA tests of the compost and soils from Experiment A and B were done prior to the start of the experiments also by Ward Laboratories. Those results and also the PLFA results from a South Carolina hayfield for a reference point are displayed in Figure 3.2. The graph displays the total biomass broken down by type. It is apparent that the compost has significantly more microbial life than the soils, which is why it is being used as a soil inoculant. The athletic field soil in Experiments A and B have much more microbial biomass than the hayfield, which was not as expected.

The young J-S showed much more microbial biomass than the older J-S (Figure 3.2). The increased amount of microbial biomass in the young compost compared to the older compost is not surprising because Johnson (2018) explains that in the older compost the microbes have used most of the food sources and for spores and cysts until a new food source becomes available in the soils. Additionally, he specifies that compost at 22 weeks has 424 species and compost at 60 weeks has 453 species present (Johnson, 2018). The top

80% of biomass has 57 different species in 22-week-old compost but in the 60-week-old compost that top 80% of biomass has 99 different species.
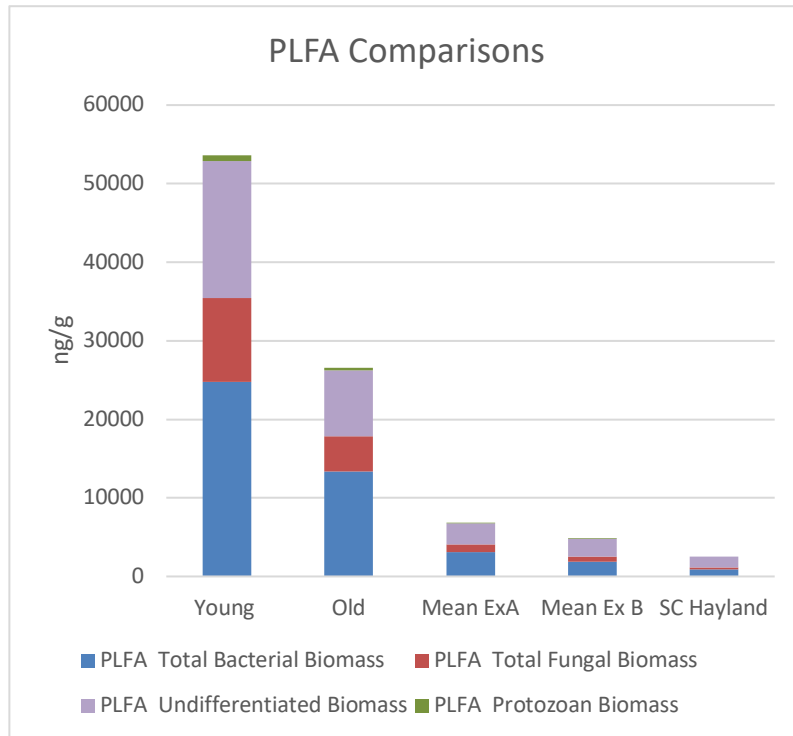
Figure 3.2: PLFA comparison of compost and soils in ng/g

3.1.2 Temperature and Precipitation

The temperatures and precipitation through the course of the experiment at shown in Figures 3.3 and 3.4 respectively (NOAA). The temperatures throughout the experiment were warmer than average in August, September, October, and December. In November, the average temperature was 2 degrees Fahrenheit cooler than the average. The rainfall was less than the average in August by 2.01 inches. For the remainder of the experiment, the rainfall was more than the recorded monthly averages. In September, there was 2.55 more

inches, in October there was 3.22 more inches, in November there was 3.81 more inches, and in December there was 4.03 more inches of rainfall.

Daily Maxium Temperatures
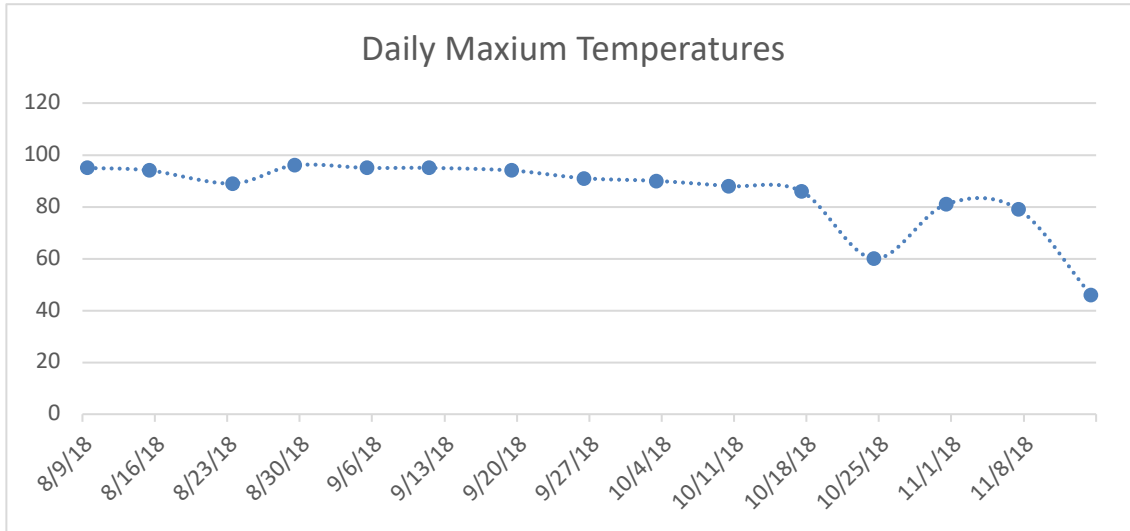
Figure 3.3: Daily Maximum Temperature in Fahrenheit
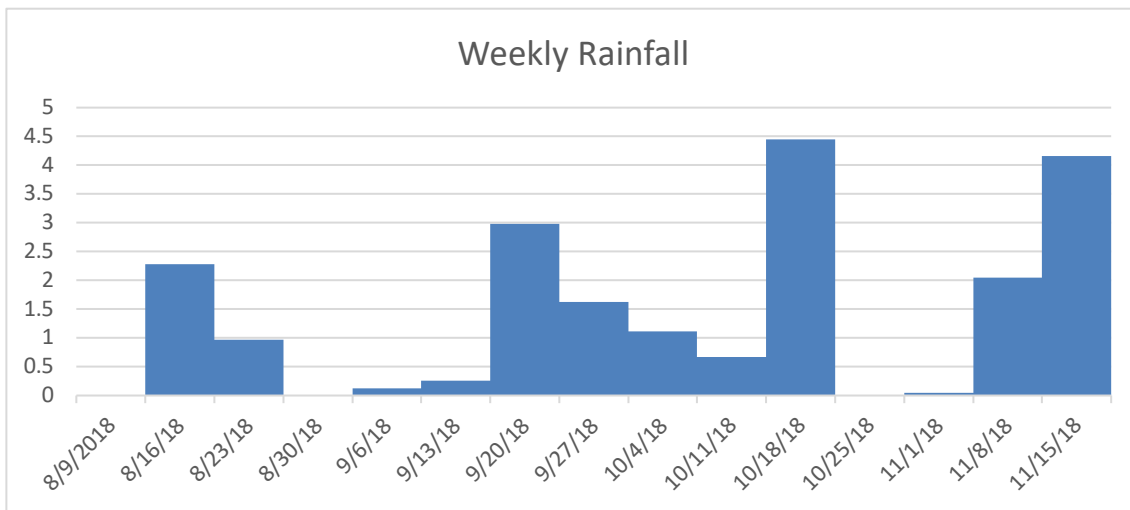
Weekly Rainfall

Figure 3.4: Weekly Rainfall in Inches

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3.1.3 Baseline differences

The baseline NDVI and NTEP are shown in Table 3.1 and Table 3.2. The experiments were not significantly different to begin the experiments. At the midpoint in October, there were will no significant differences between the experiments in NDVI or NTEP. However, at the last week of the experiments, Experiment A had significantly higher NDVI scores than Experiment B. Based on NTEP, Experiment B was significantly better than Experiment A in the final week.

Table 3.1: Comparison of Mean NDVI Readings in Experiments A and B (n=16 for each Experiment).

Date	EXA	EXB	Significant Difference?
8/9/2018	0.68	0.68	N
10/10/2018	0.63	0.64	N
11/7/2018	0.51	0.52	N
11/14/2019	0.54	0.50	Y

Table 3.2: Comparison of Mean NTEP Readings in Experiments A and B (n=16 for each Experiment).

Date	EXA	EXB	Significant Difference?
8/9/2018	7.287	7.281	N
10/10/2018	7.125	7.125	N
11/14/2018	6.75	7.0938	Y

Even though experiment A and B were selected by choosing visually uniform areas and the borders of each experiment are less than about 2 yards apart, the baseline soil data showed substantial differences between Experiment A and Experiment B. Based on results from Clemson Regulatory Services chemical soil tests, Experiment B has what is considered to be heavier soil. The soil in Experiment B has more organic matter (OM),

higher potassium levels (K), and greater cation exchange capacity (CEC). The corresponding average values found for each experimental plot are shown in the Table 3.3 (For each experiment n=16. Soil test values for P, K, Ca, Mg, Zn, Mn and B are in ppm, %OM is in percentage based on Loss of Ignition (LOI), CEC units are in milliequivalents per 100 grams (meq/100 g) of soil. Total Percent base saturation (Total %BS) is the percentage of CE sites occupied by base cations (K^+, Ca^{2+}, Mg^{2+} and Na^+) and pH is dimensionless.)

Table 3.3: Mean Chemical Soil Test Analysis (Mehlich 1) from Clemson Regulatory Services Laboratory comparing Experiment A and Experiment B at baseline (8/6/2018).

Variable	EXA	EXB	Significant Difference?
SoilpH	7.26	7.37	Y
P	10	7	N
K	64	80	Y
Ca	726	749	N
Mg	91	120	Y
Zn	2.0	1.4	N
Mn	13.7	14.2	N
B	0.1	0.1	N
OM	1.0	1.1	Y
CEC	2.8	2.9	N
Total%BS	83.6	85.8	N

3.2 Hypothesis 1

3.2.1 NDVI

In the baseline sampling for NDVI in Experiment A, the treatments did not all start equivalent to one another (Table 3.4). Treatment 4 had a significantly lower NDVI than Treatments 1 and 3. In Experiment B, all of the treatments began statistically similar.

At the halfway point of application and data collection (Oct 10, 2018), each of the 4 treatments on both experimental plots were not significantly different based on NDVI (Table 3.4 and 3.5). In response to Hypothesis 1 that the four different treatments will not show a difference in color or density, based on the NDVI at the midpoint of both Experiment A and Experiment B the null cannot be rejected.

The variation throughout the experiment can be seen in in Figure 3.5. The nitrogen fertilizer plots (TRT 4) did not significantly outperform the 2 compost plots (TRT 2 - young compost & TRT 3 - mature compost). Likewise, the compost plots did not significantly outperform the check plots (TRT 1). With cooler temperatures in the second half of the experiment, the NDVI values declined (Figure 3.5). During the same timeframe, Experiment B's values increased above those of A after lagging for most of the experiment. By the end of the trial, in both Experiments A and B, the fertilizer treatment had the highest average NDVI (Tables 3.4 and Table 3.5). In Experiment A, Treatment 4 NDVI readings were significantly higher than the other 3 treatments based on groupings from Fisher LSD Method (Table 3.4). In experiment B, the NDVI was not significantly different between the treatments based on ANOVA or Fisher comparisons (Table 3.5).

Table 3.4: Analysis of Variance (ANOVA) Comparison of Mean NDVI Readings in Experiment A by Treatment (TRT) using the Fisher Multiple Comparison Method.

Date	TRT	Mean NDVI EXP A	Grouping
8/9/2018	1	0.69	A
	2	0.68	A B
	3	0.69	A
	4	0.67	B
10/10/2018	1	0.64	A
	2	0.64	A
	3	0.61	A
	4	0.65	A
11/7/2018	1	0.50	A
	2	0.51	A
	3	0.50	A
	4	0.52	A
11/14/2018	1	0.53	B
	2	0.53	B
	3	0.51	B
	4	0.59	A

Table 3.5: Analysis of Variance (ANOVA) Comparison of Mean NDVI Readings in Experiment B by Treatment (TRT) using the Fisher Multiple Comparison Method.

Date	TRT	Mean NDVI EX B	Grouping
8/9/2018	1	0.68	A
	2	0.68	A
	3	0.68	A
	4	0.68	A
10/10/2018	1	0.62	A
	2	0.64	A
	3	0.63	A
	4	0.66	A
11/14/2018	1	0.50	A
	2	0.50	A
	3	0.50	A
	4	0.52	A
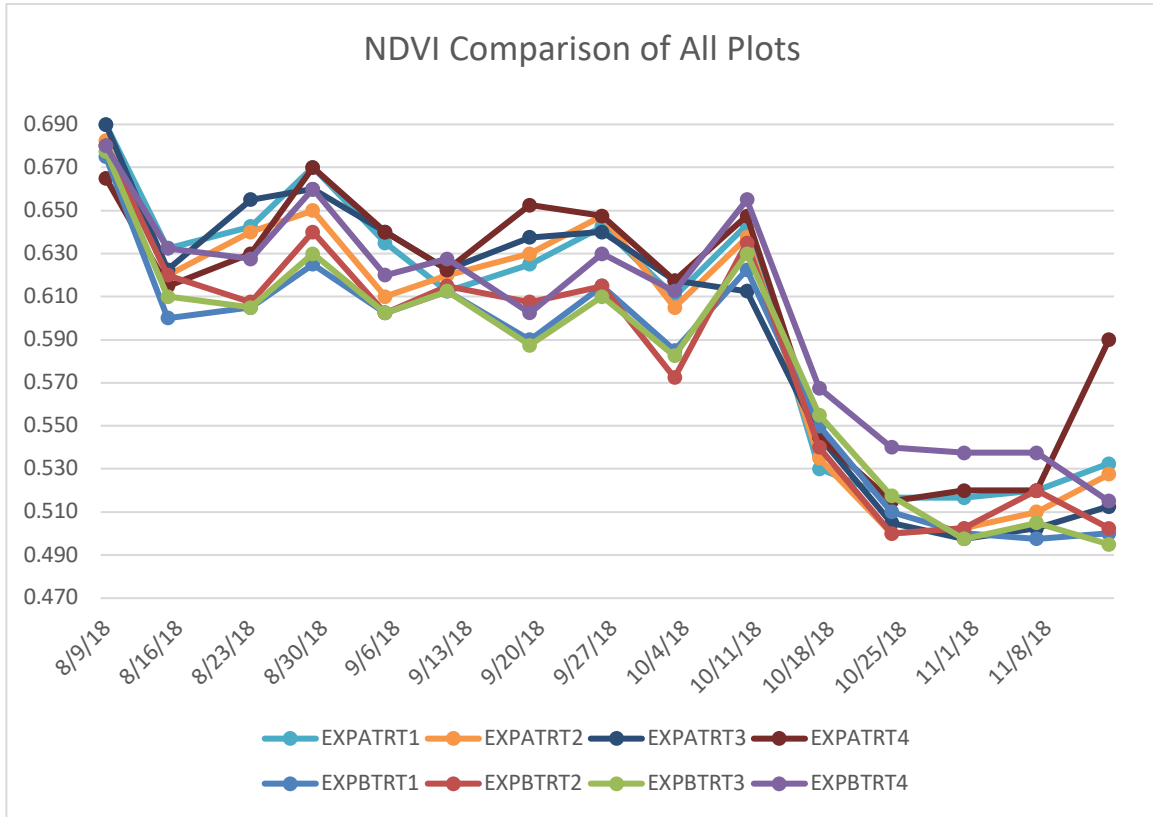
Figure 3.5: NDVI of All Plots

3.2.2 NTEP

At the baseline sampling, the NTEP for each experiment showed no significant difference between the treatments (Table 3.6 and Table 3.7). Likewise, by the midpoint of the experiments in October, the NTEP still showed no significant variation between the treatments in either Experiment A or B. Similar to the results from NDVI, at the final sampling, there were significant differences. By the final sampling for the trials, the grass treated with nitrogen fertilizer was visibly greener in patches than those treated with compost extract or water as indicated by the NTEP scores. In Experiment A, the final

sampling still showed no significant difference (Table 3.6), but in Experiment B, Treatment 4 was significantly better than the others (Table 3.7).

Figure 3.6 shows the variation in NTEP throughout the experiments. Like with the NDVI, the scores decreased overall, with a more dramatic decrease in the fall when temperatures cooled.

For Clark Cox's expert evaluation of the plots, he noted that the density of the grass was uniform throughout the two experiments. He did observe increased greenness on the nitrogen treated plots. In his evaluation, he would did not deem the nitrogen plots any more playable but did comment on the aesthetic aspect in a cooler season.

Table 3.6: Analysis of Variance (ANOVA) Comparison of Mean NTEP Readings in Experiment A by Treatment (TRT) using the Fisher Multiple Comparison Method.

Date	TRT	Mean	Grouping
8/9/2018	1	7.1	A
	2	7.4	A
	3	7.3	A
	4	7.4	A
10/10/2018	1	7	A
	2	7.3	A
	3	7	A
	4	7.3	A
11/14/2018	1	6.6	A
	2	6.6	A
	3	6.6	A
	4	7.1	A

Table 3.7: Analysis of Variance (ANOVA) Comparison of Mean NTEP Readings in Experiment B by Treatment (TRT) using the Fisher Multiple Comparison Method

Date	TRT	Mean	Grouping
8/9/2018	2	7.1	A
	3	7.5	A
	4	7.1	A
10/10/2018	1	7	A
	2	7	A
	3	7.3	A
	4	7.3	A
11/14/2018	1	7	B
	2	6.9	B
	3	7	B
	4	7.5	A
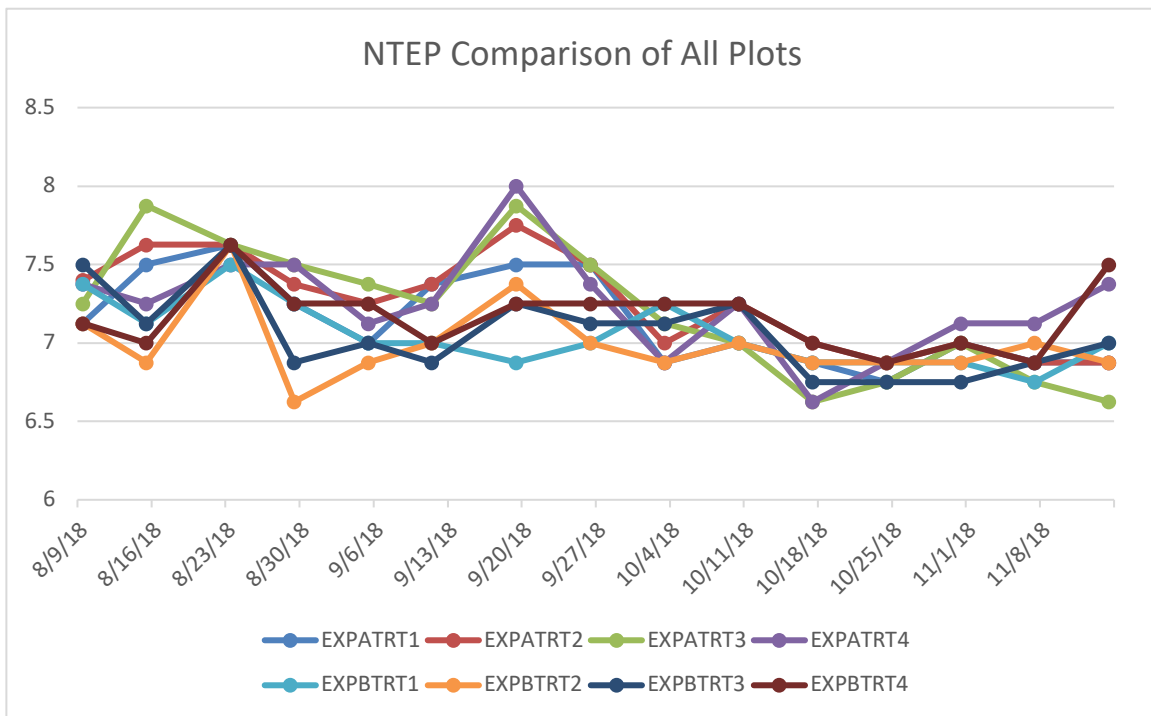
Figure 3.6: NTEP of All Plots

3.3 Hypothesis 2

3.3.1 Conventional Soil Tests

In Experiment A, the Clemson Regulatory Services chemical soil analysis results indicated no statistical variation between treatments at the baseline sampling (Table 3.8). Soil test values for P, K, Ca, Mg, Zn, Mn and B are in ppm, %OM is in percentage based on Loss of Ignition (LOI), CEC units are in milliequivalents per 100 grams (meq/100 g) of soil. Total Percent Base Saturation (Total %BS) is the percentage of cation exchange sites occupied by base cations (K^+, Ca^{2+}, Mg^{2+} and Na^+) and pH is dimensionless. By the final sampling in December, the soil test values only showed statistical variation in the pH, CEC, and total percent base saturation (the statistically different values are shaded in Table 3.8). In the changes shown from baseline to final sampling, there are no significant difference.

In Experiment B, the baseline samples did show significant differences on the chemical soil test values. Treatment 4 was significantly different than the other treatments for phosphorus, potassium, boron, and total percent base saturation as shown by the shaded areas in Table 3.9 For the final sample results, pH and total percent base saturation were the only significant differences between the treatments. Treatment 3 had the highest pH, while treatment 1 had the lowest percent base saturation. Again, the changes from baseline to final sampling are shown, but they do not have any significant differences.

Table 3.8: Mean Chemical Soil Test Analysis (Mehlich 1) from Clemson Regulatory Services Laboratory for Experiment A.

Sample Event	TRT	pH	P	K	Ca	Mg	Zn	Mn	B	OM	CEC	Total%BS
Baseline	1	7.2	5.6	32	374	50	0.9	6.5	0.1	1.1	3.0	80.0
	2	7.3	3.6	31	338	42	0.6	6.5	0.1	0.9	2.5	84.8
	3	7.3	4.3	33	354	45	0.7	7.5	0.1	1.0	2.7	84.3
	4	7.3	6.6	33	386	46	0.7	6.9	0.1	1.0	2.8	85.3
Final	1	7.3	10.5	33	363	56	0.6	8.1	0.2	1.7	3.2	**74.8**
	2	7.3	9.6	32	331	49	0.5	8.5	0.1	1.0	**2.6**	85.5
	3	7.4	10.3	31	343	50	0.5	8.6	0.1	1.0	2.9	**78.0**
	4	**7.2**	10.9	35	352	53	0.8	10.0	0.2	1.1	**2.7**	85.8
Change	1	0.1	4.9	1	-11	6	-0.3	1.6	0.1	0.6	0.2	-5.3
	2	0.1	6.0	1	-7	7	-0.1	2.0	0.1	0.0	0.1	0.8
	3	0.1	6.0	-2	-11	5	-0.2	1.1	0.1	0.1	0.2	-6.3
	4	0.0	4.3	2	-34	7	0.1	3.1	0.1	0.2	-0.1	0.5

Table 3.9: Mean Chemical Soil Test Analysis (Mehlich 1) from Clemson Regulatory Services Laboratory for Experiment B.

Sample Event		pH	P	K	Ca	Mg	Zn	Mn	B	OM	CEC	Total%BS
Baseline	1	7.4	**5.0**	**73**	704	108	1.2	14.0	0.13	1.1	2.7	85.0
	2	7.3	8.3	85	770	116	1.6	13.3	0.13	1.1	3.0	86.3
	3	7.4	6.8	76	719	121	1.2	14.0	0.13	1.1	2.9	84.8
	4	7.4	**9.8**	**86**	805	137	1.7	15.5	**0.20**	1.2	3.1	**87.0**
Final	1	7.3	15.8	59	643	110.8	1.225	15.75	0.25	1.1	2.9	**76.5**
	2	7.3	17.8	70	689.8	124.3	1.275	14	0.275	1.1	2.8	85.0
	3	**7.4**	19.0	72	665	125.8	1.25	17.5	0.3	1.2	2.9	80.0
	4	7.3	19.0	63	673	123.8	1.25	15	0.3	1.1	2.7	84.8
Change	1	-0.1	10.8	-14	-60.5	3.3	0.0	1.8	0.1	0.1	0.2	-8.5
	2	0.0	9.5	-14	-80.2	8.3	-0.3	0.8	0.2	0.0	-0.2	-1.3
	3	0.0	12.3	-5	-53.5	4.8	0.1	3.5	0.2	0.1	0.1	-4.8
	4	-0.1	9.3	-23	-131.8	-13.0	-0.4	-0.5	0.1	0.0	-0.4	-2.3

3.4 Additional Indicators

3.4.1 Infiltration Rates

The baseline infiltration varied between experiments. Based on an analysis of variance at α=0.05 Experiment A had an overall faster rate of infiltration for the second inch of water than Experiment B. In Experiment A at the baseline, Treatment 3 had significantly worse infiltration than Treatment 4. In Experiment B, all of the treatments were statistically similar.

Infiltration throughout the experiment was not improved. Based on the final infiltration tests, the infiltration on the field decreased significantly from August to December as shown in Table 3.10 and Table 3.11. The infiltration rate decreased 71% on average. Compared to the check plots in both experiments, none of the treatments made substantial improvements in the infiltration by December.

Table 3.10: Infiltration rates in inches per hour for Experiment A

Inches/hr	Baseline	Final	Difference	% Change
A TRT 1	11.9	3.2	8.8	-74%
A TRT 2	11.4	2.9	8.6	-75%
A TRT 3	9.7	2.7	7.0	-72%
A TRT 4	14.3	3.8	10.5	-74%

Table 3.11: Infiltration rates in inches per hour for Experiment B

Inches/hr	Baseline	Final	Difference	% Change
B TRT 1	6.7	3.0	3.7	-55%
B TRT2	10.1	2.6	7.4	-74%
B TRT 3	12.1	3.1	9.0	-74%
B TRT 4	8.2	2.4	5.7	-70%

3.4.2 Bulk Density

The baseline bulk density results showed no significant difference between the experiments (Table 3.12). There was also no significant difference between treatments within Experiment A or Experiment B. Bulk density showed little variation from the baseline samples through the end of the experiment. By the final sampling, there was no significant change in the bulk density in either experiment (Table 3.13). Null Hypothesis 2 cannot be rejected because there was not a significant improvement in infiltration or bulk density between any of the treatments.

Table 3.12: Bulk Density baseline sampling comparison between Experiment A and B in g/cm^3

Sampling	TRT	EX A	EX B
Baseline	1	1.58	1.58
	2	1.58	1.56
	3	1.54	1.60
	4	1.55	1.57

Table 3.13: Bulk Density final sampling comparison between Experiment A and B in g/cm^3

Sampling	TRT	EX A	EX B
Final	1	1.59	1.56
	2	1.58	1.49
	3	1.60	1.57
	4	1.58	1.57

3.4.3 PLFA

From Ward Laboratories, the PLFA results (Table 3.14) for the baseline of the experiment did not show a significant difference between the two experiments for fungal

to bacterial ratio. In Experiment A, the ratio was on average slightly lower than in experiment B. The total biomass was the highest in Treatment 1 for both experiments (Table 3.14). In Experiment A, the fungal and protozoan biomasses increased from the baseline to the midpoint of the experiment for Treatments 2, 3, and 4. From the midpoint to the end of the trial, the biomasses overall declined. The changes from the baseline to the final sampling are shown in Table 3.14. The fungal to bacterial ratios in the compost treated plots did not decrease but none of the changes observed in the fungal to bacterial ratios differed significantly. Additionally, for the changes from baseline to the final sample event, Treatment 3 with the mature compost was the only treatment not to have a decline in fungi, arbuscular mycorrhizal, and protozoan biomasses.

For Experiment B, from the baseline sampling to the midpoint, the same trend of increases in fungal and protozoan biomasses for Treatments 2, 3, and 4 were not seen (Table 3.15). Also, in Experiment B, Treatment 2 with the young compost was the treatment to have increases from the baseline to final sampling for bacteria, fungi, arbuscular mycorrhizal, and protozoan biomasses. In both experiments, there is not an increase in the diversity index from the baseline to the final sampling for the trials so the null of hypothesis 2 cannot be rejected stating that the microbial diversity had been increased.

3.4.4 Direct Microscopy

The direct microscopy showed improved fungal to bacterial ratios in all of the treatments except for Treatment 4 in both experiments from baseline analysis to the midpoint (Table 3.16 – Where bacteria and fungi are in μg organisms/g of soil and

flagellates, amoebae, ciliates, and nematodes are numbers/mL solution). Thereafter, the fungal to bacterial ratio remained roughly the same in Experiment A from the midpoint to the final sampling and overall declined from midway to the final sampling in Experiment B. During all three of the microscopy examinations, the only treatments that had any nematodes in the samples were Treatments 1, 2, and 3. All of the nematodes observed were bacterial feeders. The large variation in bacterial biomass indicates the difficulty to accurately count the bacteria in the samples with this method. This discrepancy in bacteria numbers then plays a large role in the fungi to bacteria ratio.

Table 3.14: PLFA Results for Experiment A.

	EX A TRT	Total Bacteria Biomass (ng/g)	Total Fungi Biomass (ng/g)	Arbuscular Mycorrhizal Biomass (ng/g)	Protozoa Biomass (ng/g)	Undifferentiated Biomass (ng/g)	Fungi: Bacteria	Predator: Prey	Diversity Index
Baseline	1	4167	1324	522	135	4738	0.318	0.032	1.645
	2	2817	750	313	84	2219	0.266	0.030	1.599
	3	2286	604	202	66	1891	0.264	0.029	1.543
	4	3270	931	425	102	2022	0.285	0.031	1.648
Midpoint	1	2544	544	263	67	2004	0.214	0.026	1.476
	2	4627	1435	532	144	3235	0.310	0.031	1.588
	3	3266	686	336	77	2538	0.210	0.024	1.525
	4	3948	1092	465	151	2805	0.277	0.038	1.573
Final	1	2298	597	263	82	1638	0.260	0.036	1.578
	2	1501	423	165	59	1100	0.282	0.039	1.576
	3	2155	624	269	91	1582	0.290	0.042	1.626
	4	2026	527	229	62	1323	0.260	0.030	1.568
Change	1	-1869	-726	-259	-53	-3101	-0.06	0.00	-0.07
	2	-1317	-327	-148	-25	-1120	0.02	0.01	-0.02
	3	-130	20	67	25	-309	0.03	0.01	0.08
	4	-1244	-404	-196	-40	-700	-0.02	0.00	-0.08

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Table 3.15: PLFA Results for Experiment B.

	EX B TRT	Total Bacteria Biomass (ng/g)	Total Fungi Biomass (ng/g)	Arbuscular Mycorrhizal Biomass (ng/g)	Protozoa Biomass (ng/g)	Undifferentiated Biomass (ng/g)	Fungi: Bacteria	Predator: Prey	Diversity Index
Baseline	1	1675	725	285	121	3063	0.433	0.072	1.736
	2	1013	491	135	40	2489	0.485	0.039	1.646
	3	2536	674	260	69	2100	0.266	0.027	1.566
	4	2286	663	262	82	1503	0.290	0.036	1.604
Midpoint	1	3954	878	399	100	3343	0.222	0.025	1.505
	2	2595	474	224	34	2121	0.183	0.013	1.416
	3	2324	456	206	50	2618	0.196	0.022	1.439
	4	3450	704	346	74	2596	0.204	0.021	1.483
Final	1	2482	654	284	71	1706	0.263	0.029	1.589
	2	2353	522	254	48	1695	0.222	0.021	1.469
	3	1628	321	150	30	1202	0.198	0.018	1.439
	4	1492	264	139	0	1374	0.177	0.000	1.391
Change	1	807	-71	-2	-50	-1357	-0.17	-0.04	-0.15
	2	1339	31	119	8	-794	-0.26	-0.02	-0.18
	3	-908	-353	-110	-39	-897	-0.07	-0.01	-0.13
	4	-794	-399	-123	-82	-130	-0.11	-0.04	-0.21

Table 3.16: Direct Microscopy Results for Experiment A and B at Baseline, Midpoint, and Final Samplings.

	EX	TRT	Bacteria	Fungi	Flagellates	Amoebae	Ciliates	Nematodes	Fungal:Bacterial Ratio
Baseline	A	1	4064	279	0	0	114,150	0	0.07
		2	2218	170	114,150	0	894,175	0	0.08
		3	1712	5	0	0	38,050	0	0.00
		4	807	94	38,050	19,025	57,075	0	0.12
	B	1	12480	18	19,025	19,025	171,225	50	0.001
		2	1594	36	19,025	0	19,025	0	0.02
		3	13698	16	38,050	0	95,125	50	0.001
		4	2306	111	0	0	95,125	0	0.05
Midpoint	A	1	5030	358	0	38,050	0	0	0.07
		2	5175	723	0	38,050	0	0	0.14
		3	4947	433	76,100	152,200	0	0	0.09
		4	5631	299	0	38,050	0	0	0.05
	B	1	7230	937	76,100	38,050	38,050	0	0.130
		2	5517	1239	38,050	114,150	0	50	0.22
		3	6697	898	38,050	76,100	0	0	0.134
		4	7306	50	76,100	0	0	0	0.01
Final	A	1	4071	358	0	38,050	0	0	0.09
		2	4870	723	0	190,250	0	50	0.15
		3	4186	433	38,050	152,200	38,050	0	0.10
		4	5517	299	76,100	0	0	0	0.05
	B	1	4947	181	0	76,100	76,100	0	0.037
		2	4528	156	38,050	38,050	38,050	0	0.03
		3	4642	293	38,050	114,150	114,150	0	0.063
		4	5631	112	0	114,150	114,150	0	0.02

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Chapter Four: Discussion and Conclusions

4.1 Experimental Design

Both experiments were selected to be uniform throughout and to one another, however as the baseline samples indicated the above ground turf was uniform but the soil below ground was not.

The check plot did not significantly lag behind the treated plots before the grass was approaching dormancy. This could be for a number of different reasons related to the experiment's design. First, even though sampling was focused on the middle region of the plots, the small size of the plots could allow for nutrients to be passed underground between the superficial markings. According to Richard Duble (N.D), a turfgrass specialist, Bermuda turfgrass rhizomes can extend laterally up to six feet. On the other hand, the application was foliar so the majority of applied liquid should not infiltrate deep into the root system. Late in the trial when the cooler temperatures prevailed, the nitrogen fertilizer only helped to green those plots; it was not evenly dispersed throughout. Another reason the check plots could have done as well as they did is because a pre-emergent herbicide carried on a slow release nitrogen and phosphorus fertilizer was applied in July 2018, a month before the start of the trials. The pre-emergent could be responsible for maintaining the check plot.

4.2 Baseline

From the baseline sampling, the differences in the field even in close proximity became apparent. The plots were visually similar, but the soil did not show the same uniformity (Table 3.3). This indicates Ingham's (2014) emphasis on the importance of soils as a dynamic and very localized environment not just a static, uniform anchor for roots. This diversity was prevalent even in soils that had been replaced with a specified sandy soil and covered with the same turfgrass. Additionally, the soils already contained much more life than we had initially anticipated (Figure 3.2). Almost sterile soils were expected, but in reality, the sandy soils had far more life than was found in a hayfield also in South Carolina. Even with efforts to have uniform plots, aspects both above and below ground still showed variation.

Since Experiments A and B were set up to be independent trials and differences were prevalent in the baseline test, it is especially appropriate to evaluate Experiment A and B separately as planned. Experiment B had more organic matter which according to Farrell (1998) increases water retention and slows infiltration rates which were also observed in the baseline tests (Table 3.10 and 3.11). In future testing, soil samples would be taken before the plots are marked to eliminate the variability of the soil.

4.3 Johnson-Su Compost

One of the major questions this experiment addressed is the timeframe in which J-S compost is usable. Johnson (2018) suggests aging the compost for close to one year before use to have higher microbial diversity. The mature compost used was aged about 14 months. However, the young J-S was only aged four months before use. It was surprising

that the mature J-S did not display more nematodes and a higher fungal to bacterial ratio (Figure 3.2). Neither the direct microscopy or the PLFA analysis of the compost showed a significant increase in fungal to bacterial ratio from the young to the mature. It is hypothesized that this might be because organisms sporulate and encyst in the mature compost once the early food supplies are gone (Johnson, 2018).

4.4 Application

4.4.1 Application Method

A sprayer was used to closely resemble the current application methods of fertilizer used by the university. This would allow for an easier transition utilizing the equipment that is already there as well as being cost-effective for the department. Furthermore, the University of Colorado runs their compost tea through their irrigation system. This would be an ideal set up for efficiency, so being effective through a sprayer would is a precursor. However, the sprayer adds a lot of force and pressure to the microbes that could be injuring or killing them and reducing the efficacy of the J-S application. In further studies, different application methods should be considered to investigate the potential impacts on the microbes.

On top of the physical pressure of the sprayer, the sprayer had to be cleaned out several times during the experiment even after flushing it with water after every use. No filter was used on the end of the nozzles, but when the pipes and hoses were cleaned, it was apparent that some compost remained in the spraying apparatus. This brings to question the potential of some microbes and nutrients getting trapped in the sprayer. In University of Colorado's trials, the sprinklers were not clogged, and they found up to eighty percent

of the microbes in the tea coming out of the sprinkler nozzle onto fields (Ryan Heiland, personal communication, June 18, 2018).

4.4.2 Application Rate

The application rate of compost was modeled after the research of Boulter and Boulter (2000) using a 1:5 to 1:10 ratio of solid compost to water. Then the amount of liquid applied to the grass (7.5 gal/1000 ft^2) mirrored that of the Harrell's program that the University of South Carolina uses for nitrogen fertilizer. The actual dosage of nitrogen could not be matched because that would require an unrealistic amount of compost since the compost is much less available nitrogen.

The application rate of compost should be further investigated and has been experimented with in potted plant experiments within the Kloot laboratory. Through preliminary, unpublished investigations, a higher dosage for a seed inoculated with young J-S compost appeared to result in a better outcome. Doses ranging from 0 to 22.5 milligrams (in increments of 2.5 mg) of J-S extract were added to corn seeds, and the resulting plants varied in size (Figure 4.1). Without the outliers from the trial, a significant relationship between the amount of J-S and the weight of the corn plant was found. For field application then, the required amount of J-S to be effective should be studied and then used to reach peak results without being wasteful.

4.4.3 Seasonality of Application

The applications for the trial started midway through the turfgrass growing season. Traditionally, small, frequent dose nitrogen treatments are used during the playing season

on athletic fields as prescribed by Harrel's (2016). The compost was applied following the same recommendation philosophy. However, based on the research done by Muir et al (2010) the applications were all during dormancy. Their findings showed the most success with applications in November and March. Applying to the dormant grass to prepare for the next growing season should be considered to benefit the roots and in turn overall plant health as indicated by Muir et al (2010).

In the laboratory setting, the compost extract is applied to the seed in the soil. This is giving significant results unlike those in the field trial. It is possible that the turfgrass could benefit from extract application at planting not just as a foliar application.
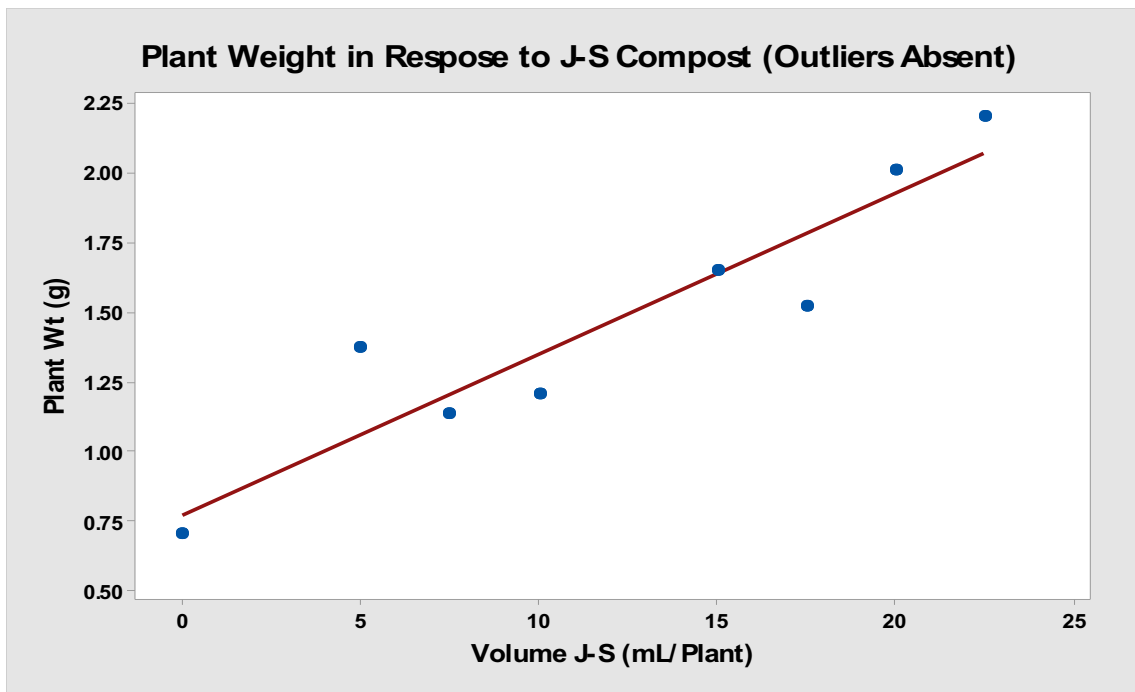

Figure 4.1: Plant Weight Based on Johnson-Su Extract Added

4.5 Sampling Analysis

4.5.1 Hypothesis 1: Aboveground – NDVI and NTEP

The biggest benefits of using the Greenseeker for NDVI data are that it is handheld and portable, that it provides a substantial amount of data without a lot of time, it limits human bias, and it is easy to use. The weekly readings provide continuous data throughout the course of the trial. The readings from the Greenseeker detect more change than NTEP and averaging the three readings helps ensure validity.

As anticipated, the NDVI values varied with the temperature because Bermuda grass is a warm season variety, as the temperatures declined so did the NDVI values (Figure 4.2).
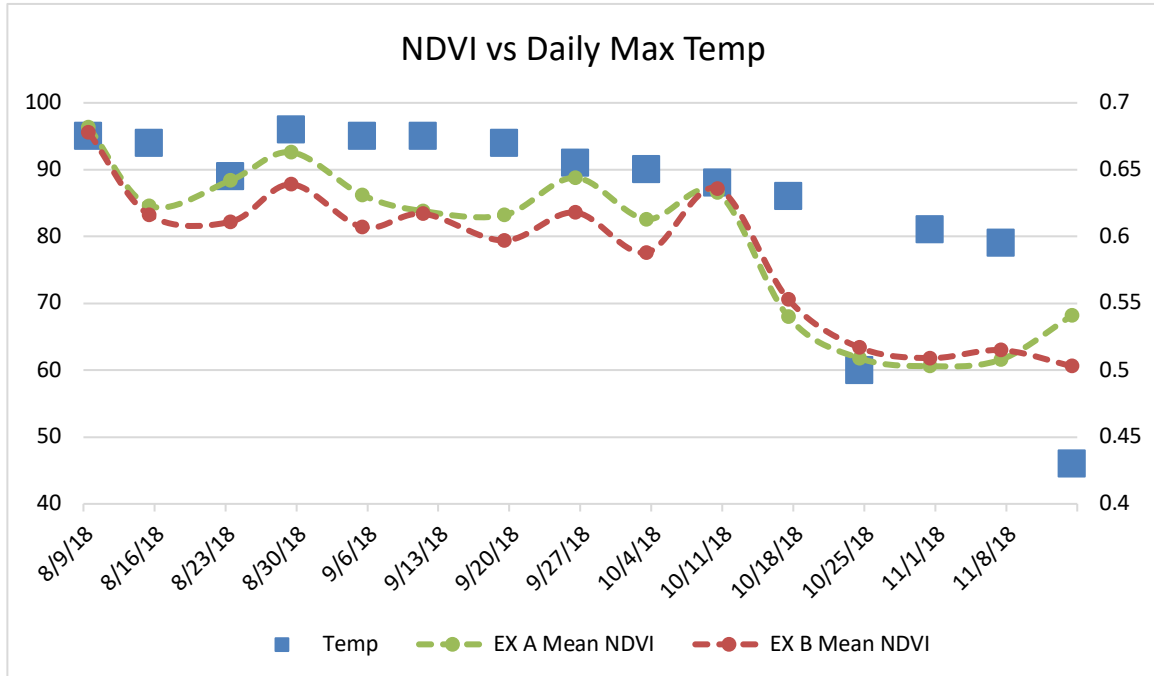

Figure 4.2: Plot of Temperature in Degrees Fahrenheit and NDVI Values

The decline in the NDVI values throughout the course of the experiments were expected because of the relationship to temperature. Until the final week, the null of Hypothesis 1 could not be rejected because none of the treatments were statistically different in either experiment (Tables 3.4 and 3.5). The final outcomes of Experiment A indicate that the NDVI readings for the nitrogen treatment were significantly higher, but that was not confirmed with Experiment B.

From before the experiment started, Mr. Clark Cox questioned the use of NTEP, out of concern that a biased sampler could look for what she wants to see and therefore skew the results. For this reason, an undergraduate volunteer was taught the protocol and did observations as a blind observer. The protocol is easy to follow, has no associated cost, and provides more data to the experiment. In conjunction with NDVI, the sampling bias is limited because of the use of the Greenseeker® instead of the human eye. Throughout the experiment, the NTEP and NDVI values followed one another and showed similar trends.

The downside of using NTEP is the limited variation. Since 6 is minimally adequate and 9 is perfect, there is not a large range breaking down to only half and integer. For this reason, NDVI was able to show more subtle variation in the turfgrass (Figure 3.5 compared to Figure 3.6).

For most of the trial, the Experiment B averaged a lower NTEP than Experiment A (Figure 3.6). Once the cooler weather set in, experiment B averaged higher scores. NTEP displaying these differences means they were noticeable to the human eye even with an unknown causation.

4.5.2 Hypothesis 2: Belowground – Clemson and Ward Laboratory Analyses

Before the experiments, Experiment B was "heavier" soil with higher CEC's, implying a higher clay content and also more organic matter, but in the final sampling Experiment B did not have significantly higher CEC, calcium, magnesium, and potassium (Table 3.3). According to Fuller (1998), compost when applied to topdressing does increase the OM. The compost could have potentially boosted the OM in Experiment A to level it with Experiment B. Most of the changes seen in Table 4.1 are not substantial differences and can likely be attributed sampling or testing error. However, the major changes seen in phosphorous and boron are significant. Without any addition of P_2O_5, all of the treatments saw major increases. Likewise, there were large increases in boron values without any inputs.

The fungal to bacterial ratio as well as the total fungi biomasses from the PLFA tests overall declined from the baseline to the final sampling in both experiments (Table 4.2). This was unexpected because the compost was intended to support increased fungal biomass especially since the J-S method was selected for its support of fungal hyphae (Johnson, 2018). This can be attributed to the seasonality of soil diversity. At the final sampling, temperatures were approaching freezing, which could have caused some thermophilic microbes to become dormant or die before the soil samples were taken for analysis.

Table 4.1: Percent Changes from Baseline to Final Sampling in Chemical Soil Test Values from Clemson Regulatory Services

EX	TRT	pH	P	K	Ca	Mg	Zn	Mn	B	OM	CEC	Total% BS
A	1	1%	87%	4%	-3%	12%	-33%	25%	200%	49%	8%	-7%
	2	1%	166%	3%	-2%	17%	-14%	31%	100%	5%	2%	1%
	3	1%	141%	-6%	-3%	11%	-23%	15%	100%	8%	8%	-7%
	4	-1%	64%	7%	-9%	16%	14%	45%	200%	18%	-4%	1%
B	1	-1%	215%	-19%	-9%	3%	0%	13%	100%	5%	6%	-10%
	2	0%	115%	-17%	-10%	7%	-19%	6%	120%	0%	-5%	-1%
	3	0%	181%	-6%	-7%	4%	4%	25%	140%	11%	3%	-6%
	4	-1%	95%	-27%	-16%	-10%	-24%	-3%	50%	-2%	-13%	-3%

Table 4.2: Percent Changes from Baseline to Final Sampling in PLFA

EX	TRT	Total Bacteria Biomass	Total Fungi Biomass	Protozoa Biomass	Undifferentiated Biomass	Fungi: Bacteria	Predator: Prey	Diversity Index
A	1	-45%	-55%	-39%	-65%	-18%	10%	-4%
	2	-47%	-44%	-30%	-50%	6%	32%	-1%
	3	-6%	3%	38%	-16%	10%	47%	5%
	4	-38%	-43%	-40%	-35%	-9%	-2%	-5%
B	1	48%	-10%	-41%	-44%	-39%	-60%	-8%
	2	132%	6%	21%	-32%	-54%	-48%	-11%
	3	-36%	-52%	-57%	-43%	-26%	-32%	-8%
	4	-35%	-60%	-100%	-9%	-39%	-100%	-13%

4.5.4 Infiltration

The infiltration did not improve from baseline to the end of the experiment (Table 3.10 and 3.11). By December, the infiltration rates were so slow that a representative sample of the four treatments from one replicate were taken due to time constraints. The decline in infiltration rates are likely related to the particularly wet fall experienced (Figure 3.4). During the trial, there were two hurricanes (Hurricane Florence in September and Hurricane Michael in October) that came through the state leaving upwards of 243 more millimeters of rain than average over the three months of the experiment (NOAA, 2018). The track infield does not have a full tile drainage system under the sand to help alleviate this extra moisture like many of the other fields on campus.

As the experiment was preformed, Null Hypothesis 2 cannot be rejected because there was not an improvement in the infiltration. Infiltration is a key component of athletic fields because universities need the fields to be playable as quickly as possible after rain events. For a fairer comparison of infiltration, the infiltration rates observed in August should be compared to the following August instead of dormancy in December to limit the seasonal variation factor.

4.5.5 Bulk Density

The bulk density is an important factor for soil health but especially for athletic fields. The lower the bulk density, the more pore space there is in the soil. In turn, this means that the soil is less compacted, which is beneficial for the health of athletes. The bulk density was uniform throughout the experiments and plots at baseline and showed

almost no change through the course of the experiment (Table 3.12 and 3.13). Boulter and Boulter's research saw improvements in bulk density through the use of compost, so it is possible that with a longer trial, the bulk density could be reduced.

4.6 Direct Microscopy

Direct microscopy was used in this experiment in addition to the PLFA tests run by Ward Laboratories. This allowed for the microscopy results to be cross-checked with those found through. Even though the PLFA and direct microscopy fungal to bacterial ratios did not match numerically, they do follow the same trends. For the fungal counts from direct microscopy compared to the fungi biomass in PLFA tests, Figure 4.3 indicates a relationship between the methods. The direct microscopy method takes time to complete but it has less direct monetary costs associated with it beyond the upfront cost of materials and time needed while PLFA testing has both testing and overnight shipping costs but provides more numeric data.

The microscopy indicates trends in the soil and show the diversity of life in the soil. According to Ingham (2009), large numbers of ciliates generally indicate poor soil or compost quality and can suggest anaerobic conditions. The substantial increase in ciliates observed in the soil samples under the microscope in Experiment B indicates poor soil conditions (Table 4.3). This could suggest that the soils were waterlogged after the particularly wet fall. Another benefit of the direct microscopy is the researcher's ability to directly observe the organisms instead of just receiving a report or reading about them in a book. The movement and size variation are much more impactful under a microscope.
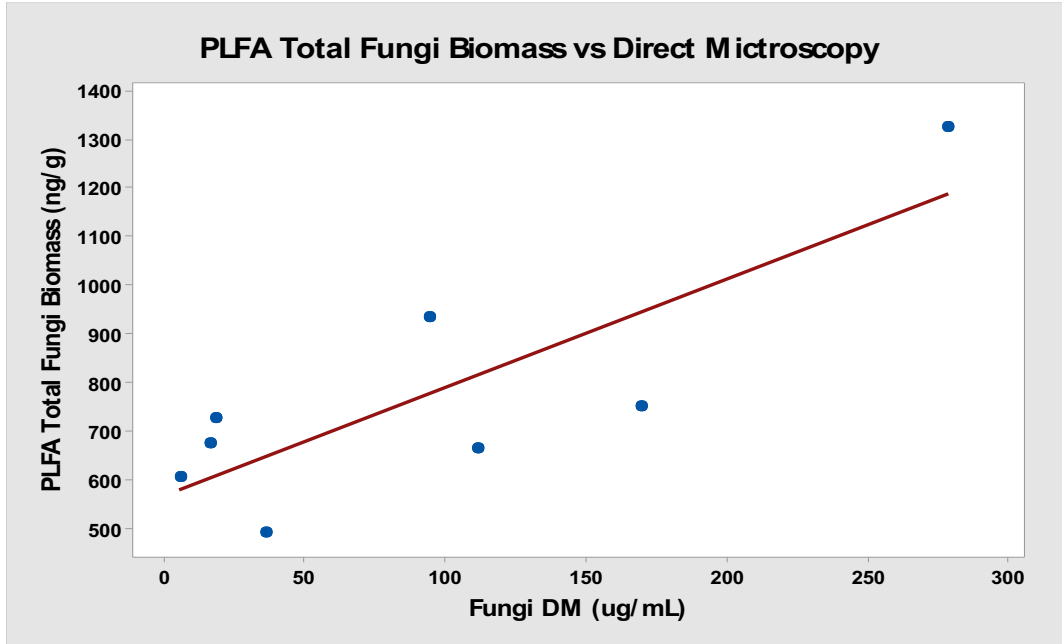
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Figure 4.3: PLFA Results Versus Direct Microscopy for Fungal Biomass

Table 4.3: Changes in Direct Microscopy from Baseline to Final Samples.

EX	TRT	Bacteria	Fungi	Flagellates	Amoebae	Ciliates	Nematodes
A	1	8	79	0	38050	-114150	0
	2	2652	553	-114150	190250	-894175	50
	3	2473	428	38050	152200	0	0
	4	4711	206	38050	-19025	-57075	0
B	1	-7534	163	-19025	57075	-95125	-50
	2	2934	120	19025	38050	19025	0
	3	-9056	277	0	114150	19025	-50
	4	3326	1	0	114150	19025	0

4.7 Conclusions

4.7.1 Experiment Conclusions

The original goals of this project were to investigate if compost extract could be

used for field maintenance and to encourage the university to consider more

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environmentally friendly maintenance practices. Overall, these goals were met through the course of the experiment. There were no significant differences in the turfgrass the majority of the trial and this project sparked conversation within the athletics department about alternative maintenance practices. In terms of the hypotheses, while the temperatures were warm and the grass was not approaching dormancy, Null Hypothesis 1 was retained as expected in that there were no significant differences in the turfgrass color, vigor, or biomass among any of the treatments. Likewise, for Hypothesis 2, the null hypothesis also could not be rejected because there were no significant improvements in microbiodiversity, soil test values, bulk density, or infiltration rates. However, the statistical similarities found in each of the plots indicates that any of the treatments could be part of a successful maintenance regimen.

Throughout the course of the experiment, we learned a great deal for future trials. It was continually confirmed just how dynamic soils are, and the complexities of field work presented themselves routinely. Additionally, through the process, we uncovered more variables to be considered.

4.7.2 Future Considerations

From this experiment, it is evident that more research is needed. The dosage, application method and timing, and experimental design should all be studied further. In addition to the extract as a fertilizer, the use of compost extract as a weed suppressant should also be considered in future trials. With additional time in future experiments, the roots should be studied in addition to the turfgrass and soil since the root stand is a major factor for athlete safety on playing fields. This should include root density as well as

investigating the implications of compost on arbuscular mycorrhizal fungi on turfgrass roots. Finally, the response to wear should be further studied since nitrogen fertility is often used on fields with significant wear from athletes.

4.7.3 Management Implications

This experiment highlighted the potential compost extract has on athletic fields in place of or to supplement synthetic fertilizer treatments. When the conditions are appropriate for the Bermuda grass, the nitrogen fertilizer did not outcompete the compost treatment. Compost extract is cost-effective way to replace some of the chemical additives currently used by the university's athletic department. The same spraying equipment could be used, and it could eventually be connected to the irrigation system. This would likely be in conjunction with some synthetic nitrogen when the turfgrass needs to be pushed, for example when the temperatures are cool. The use of the extract should lead to less runoff, less nitrogen leaching through the soil, saved costs, and no chemical skin irritation for athletes.

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Appendix A: Experiment A Visual

Figure A.1: Experiment A Plot on Site

Appendix B: Harrell's Fertilizer Program for University of South Carolina 2016

Annual Program

USC Athletic Department
1300 Heyward Street
Columbia, SC 29208

Program Name: Stone Foliar Program   **Square Feet:** 95832   **# of Acres:** 2.2
Tank Capacity (Gallons): 175   **App Rate Gallons/1000:** 1   **Total Coverage (Sq ft):** 175000
 # Tanks Needed/App: 0.75

Date: 8/1/16

Sprayable

Foliar

Product	Oz/1000	Oz/Tank	Gal/App	N	P	K	Ca	Mg	S	Fe	Mn	Si	B	Zn	Cu	Mo
200295 - Harrell's Max Nitrate Plus 2.5 Gal	2.44	320.25	2.5	0.013				0.004		0.004	0.011		0.001			
820442 - Harrell's Bio Max 12-0-6 w/UMAXX 2.5 Gal	2.44	320.25	2.5	0.024		0.012				0.002	0.002					
820816 - Harrell's Title Phyte 2.5 Gal	2.44	320.25	2.5			0.076										
822160 - Harrell's N-30+ w/Umaxx & SE 2.5gal	2.44	320.25	2.5	0.062												

Paint/Dye

Product	Oz/1000	Oz/Tank	Gal/App	N	P	K	Ca	Mg	S	Fe	Mn	Si	B	Zn	Cu	Mo
HSMPARSG - Harrell's Par SG 1 Gal Q-A	0.24	31.5	0.25													

10.25 Gallons per Tank

Totals: N 0.099   P 0.088   K 0.004   Mn 0.013   Fe 0.006   Zn 0.001

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Appendix C: Compost Extract and Fertilizer Prepared

Figure C.1: From left to right - Young J-S (TRT 2), Mature J-S (TRT 3), & Fertilizer (TRT 4)
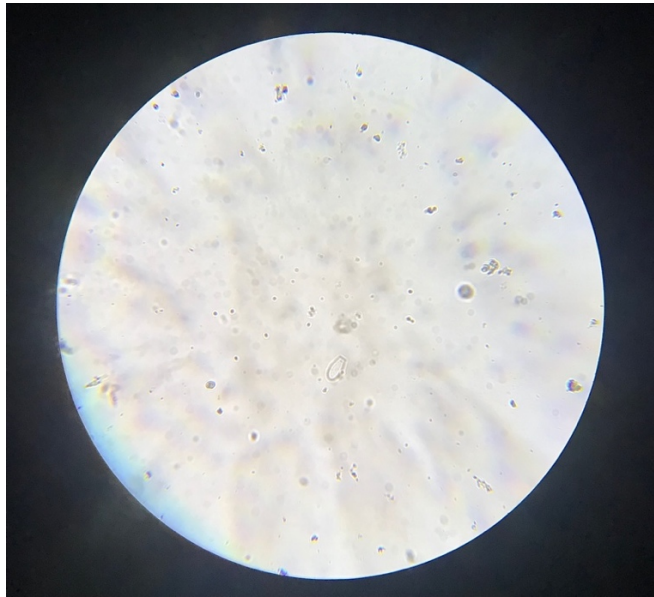
Appendix D: Direct Microscopy Images

Figure D.1: Bacteria Feeding Nematode

Figure D.2: Testate Amoeba