Compost Land Management and Soil Carbon Sequestration

An Honors Program Project Presented to

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Abstract

Extensive fossil fuel burning has released carbon dioxide into the atmosphere. Under proper ecological conditions plants convert atmospheric carbon dioxide into stable soil organic matter, a natural and efficient means of mitigating climate change. In the symbiotic relationship between mycorrhizae and plants, mycorrhizae provide plants with essential nutrients in exchange for carbon sugars leaked from the plants. Mycorrhizae convert carbon sugars to an exudate called glomalin, a protein that assists in developing soil aggregates composed of sand, silt, and clay. These aggregates, called humus, store carbon for hundreds of years under healthy ecological conditions. Compost prompts soil microbes to aerobically transform organic matter into nutrients readily available to plants. Compost fosters the relationship between plants, mycorrhizae, and soil organisms to enrich the humification process. The Marin Carbon Project is an effort to augment this soil carbon sequestration process through compost application onto California rangelands. This project is modeled on the East Campus Hillside to determine if compost boosts carbon storage within soils. The Hillside area has 1.5 acres of a tallgrass prairie. Eight 10 x 10 meter prairie plots were treated with compost, another eight prairie area plots served as controls, and the remaining 6 plots were located in the lawn area for comparison. Soil samples were gathered from each plot by the ISAT 320 class and analyzed by the Waypoint Laboratory. Additional samples were collected and then burned in an on-campus muffle furnace to calculate the total carbon from each sample. The data assembled from the muffle furnace was analyzed spatially and statistically to investigate correlations between the soil treatment and percentage of stable soil carbon. Across the replications executed, soil treated with compost had the highest carbon percentage. Results from this experiment will be integrated into the ongoing study of the health of the East Campus Hillside.

Chapter 1 Introduction

1.1 Existing Dilemma

In 2013, scientists calculated that the concentration of carbon dioxide present within the atmosphere had risen to a level of 400 parts per million (ppm) for the first time in over five million years [1, p. XVIII]. It is estimated that to maintain a stable atmosphere suitable for human life, the concentration of carbon dioxide within the atmosphere must remain below 350 ppm [2, p. 16]. Although carbon dioxide occurs naturally in the atmosphere and is essential for keeping the Earth at a suitable temperature for human life, the sources of rising carbon dioxide emissions are largely anthropogenic. In 2014, the Environmental Protection Agency (EPA) calculated that 80.9% of the entire U.S. greenhouse gas emissions emitted by human actions were carbon dioxide [3]. Human activities have effectively altered the carbon cycle by pumping more emissions into the atmosphere and by altering stable reservoirs, such as the atmosphere, oceans, soils, and forests [3]. Fossil fuel usage has surged the amount of carbon dioxide emissions pumped into the atmosphere. Fossil fuels such as coal, natural gas, and oil are used for electricity, transportation, and industry. In 2014, combustion of fossil fuels to produce electricity accounted for 37% of all U.S. carbon dioxide emissions, and 30% of all the greenhouse gas emissions within the United States [3]. Usage of fossil fuels such as gasoline and diesel for transportation generated 31% of all the U.S. carbon dioxide emissions and 25% of the total U.S. greenhouse gas emissions in 2014 [3]. Industry accounts for the third largest source of carbon dioxide emissions in the United States. Industries utilize fossil fuel combustion for energy and emit carbon dioxide through chemical manufacturing processes. In the United States, carbon dioxide emissions from industries accounted for 15% of all U.S. carbon dioxide emissions and 12% of all U.S. greenhouse gas emissions in 2014 [3]. Aside from a direct increase in carbon dioxide emissions, land use and land cover change have also accounted for an alteration in the carbon cycle. As land is cleared during deforestation, densely packed plants and trees are cleared. These living organisms possess the capability to cycle large amounts of carbon through photosynthesis, and thus harbor a large quantity of carbon. Removing vegetation eradicates an effective means of naturally offsetting carbon dioxide emissions and thus an essential step in the carbon cycle. Greenhouse gas emissions such as carbon dioxide absorb energy and can either decelerate or half the loss of heat to space [4], which effectively warms the Earth. This destructive altering of the carbon cycle and boosting emissions has threatened the capability to sustain future generations due to the treat of climate change.

1.2 Counterbalancing the Carbon

The Earth has previously cycled this carbon effectively by absorbing it into its natural sinks – the atmosphere, oceans, forests, and soils. Because carbon dioxide is pumped into the atmosphere from anthropogenic sources at unsustainable rates, some of these reservoirs do not currently possess the capacity to effectively store carbon dioxide. As many of these emissions are released directly into the atmosphere, scientists have deemed the atmosphere "full" of carbon dioxide and thus unable to continue storing these emissions [1, p. 6]. Scientists have also warned that the oceans are slowly "filling up" and in a few decades will be saturated with carbon to an extent at which it can no longer store these emissions [1, p. 6]. Forests, which can stably store carbon when managed properly, are currently being stripped from the Earth or improperly managed. As forests are burned and trees die, the carbon dioxide is immediately released directly into the atmosphere again [1, p. 6]. The last carbon sink, soil, has been depleted of its carbon stocks and thus can serve as an effective means of harboring carbon. Due to ongoing cultivation occurring for millennia, up to 80% of soil carbon has been depleted [2, p. 15]. Poor soil management has released the carbon stored within the soil, accounting for a loss of up to 80 billion tons of carbon from the soil [2, p. 15]. Examples of poor soil management include tillage, chemical fertilizer application, overgrazing, monoculture farms, and poor perennial crop management. Because soils are depleted of their carbon, they are available to soak up the excess carbon dioxide currently lodged in the atmosphere. Through effective land management techniques the organic matter content of soils can be boosted, creating a reservoir of atmospheric carbon dioxide. Research has predicted that a 2% increase in the organic content of the planet's soils could absorb all the excess atmospheric carbon dioxide within a decade [1, p. 7].

1.3 Sequestration Procedure

The process of extracting atmospheric carbon dioxide naturally and storing it in the soil stably for an extended period is termed sequestration. Carbon sequestration is composed largely of four main steps that include photosynthesis, resynthesis, exudation, and humification [1, p. 19]. In the photosynthesis step, plants utilize sunlight energy as a means to break apart water molecules into their hydrogen and oxygen components. The oxygen is released directly back into the atmosphere and during the second stage of photosynthesis the hydrogen atoms are bound to carbon dioxide molecules from the atmosphere. When the hydrogen molecules combine with the carbon dioxide molecules, a simple carbohydrate called glucose is created. In the second, resynthesis, the glucose previously formulated is resynthesized into numerous carbon compounds by means of a sequence of complex chemical reactions. In the third step of sequestration, 30 to 40 percent [1, p. 19] of the carbon synthesized during photosynthesis is directly released into the soil through the plant roots. This leaked carbon is called liquid carbon or root exudates. When the carbon is expelled into the soil, it nurtures the soil microbes that assist in building topsoil. As these microbes such as bacteria and fungi consume the leaked carbon, they provide the plant with nutrients in exchange. These nutrients, such as phosphorus and nitrogen, were not otherwise available to these plants and thus an essential symbiotic relationship between the soil microbes and plants forms. As this relationship expands, mycorrhizal fungi begin to colonize the roots of their host plant to assist in connecting the plant to the surrounding environment through hyphae [1, p. 19]. Enabling this fungal colonization of plant roots enhances the plants ability to uptake water and mineral nutrients. The final step of sequestration involves the humification process. Humus is a chemically stable form of organic matter [1, p. 19]. Carbon storage as humus is highly resistant against decomposition and is capable of remaining within the soil for hundreds of years [1, p. 19] under proper land management practices. After the mycorrhizal fungi utilize the expelled carbon, they expel a protein called glomalin. This glycoprotein binds soil aggregates together that consist of sand, silt, and clay particles. The formation of these soil aggregates enhances the amount of stable soil carbon called humus.

1.4 Organic Matter Amendment Proposal

Organic matter amendments to the soil are recommended as a means to increase carbon storage within soils [5]. The implications of this organic matter amendment are both direct and indirect. An organic matter amendment directly inputs carbon into the soil from the amendment itself, and an increase in carbon storage within soils also occurs indirectly from boosted plant production [6]. An effective land management technique proposed is a compost application. Soil microbes are capable of effectively converting the organic matter present within compost into nutrients readily available for plants. The boost in organic matter thus fosters the relationship between actively growing plants and the soil microbes that assist in building the topsoil. Because composted materials are already partially decomposed, the organic matter incorporated into the soil through compost application tends to be more resilient with a higher carbon ratio than an application of fresh plant litter or animal manures [7]. While some of the added organic matter from compost is rapidly decomposed by soil microbes, a portion of the organic matter is merged into soil aggregates, which physically and biochemically shields the organic matter from decomposition [7]. Because the organic matter is protected from decomposition, these carbon pools will remain within the soil for decades before turning over [7]. Compost can thus serve as a slow release natural fertilizer for plants and soils, enhancing the carbon sequestration process and plant production. With enhanced plant production occurring in soils, more liquid carbon leaks into the soil, leading to a boosted humification process and amount of carbon stored.

1.5 Marin Carbon Project Model

This research is modeled after the Marin Carbon Project, which is currently an ongoing experiment that is taking place in Nicasio, Marin County, California. John Wick and Peggy Rathmann initiated this project in 2008, and are currently maintaining its continuation and dispersion to other testing sites. Peggy and John are working with lead scientist Whendee Silver, a biogeochemist and professor of ecosystem ecology at the University of California-Berkeley. In this collaborative study, the effects of an organic matter amendment consisting of composted green waste are studied. The researchers hypothesized that the addition of compost would boost the aboveground and belowground net primary productivity for at least one year [8]. The hypothesis was tested using replicated field experiments over a period of three years in two dominant annual grassland types present in California. This particular experiment under the Marin Carbon Project occurred over three growing seasons starting in October of 2008 [8]. This study involved untreated control plots and plots with a single ½ inch composted organic matter amendment. To prevent unintended negative impacts on forage growth, compost depth was consistently kept at a depth of ½ inch [9]. A buffer strip of 5 meters separated each 25 x 60 m plot in this study, and these plots were arranged into three randomized blocks to reduce bias [8]. After three years, researchers found that the single compost amendment increased the forage production by 50% and the soil carbon sequestration by 1 ton/hectare [10]. Research indicated that the compost application also boosted the net ecosystem carbon storage by 25-70% in the grasslands [10]. Researchers found that their results agreed with their stated hypothesis that the net primary productivity would increase; as they found that the production of grass on the composted plots was doubled [10]. From this study, it was concluded that if 1 Mg C ha^{-1} y^{-1} was sequestered on half of the available rangeland area in California, then 42 million metric tons of carbon dioxide emissions could be offset, which is corresponds to the yearly greenhouse gas emissions of commercial and residential energy resources in California [10].

1.6 Experimental Design

This research experiment was conducted on a 1.5-acre prairie on the ISAT Hillside at the James Madison University campus in Harrisonburg, Virginia. The study site was originally planted with grasses foreign to the landscape but as part of the ISAT Hillside Naturalization Project, the hillside is now composed of native grasses and wildflower species. The purpose of the ISAT Hillside Naturalization Project is to successfully convert a monoculture lawn into a polyculture, carbon sequestering natural prairie. Within the 1.5-acre prairie, 22 10 x 10 meter plots were randomly chosen using a scheme identified by Dr. Robert Brent. A tape-measuring device was utilized to locate the corner of each plot, and a GPS unit was subsequently used to record the location of each site. Students in the ISAT 320 Fall class remarked each location by using GPS units. Eight of the 10 x 10 meter plots located in the prairie were semi-randomly selected by Dr. Wayne Teel to receive a single half-inch compost amendment in March of 2015. Eight separate 10 x 10 meter plots in the prairie did not receive an organic matter amendment, and thus these plots served as the control in this study. There were six 10 x 10 meter plots located outside of the prairie in the lawn area that were also incorporated into this study to serve as a comparison for the prairie plots. About six months following the single compost application, soil samples were collected from each of the 22 study locations. Students within the Fall 2015 ISAT 320 Lab also collected their own individual samples for testing at a separate facility. The samples collected for the purpose of this experiment were tested on-campus within the JMU Environmental Lab by using a muffle-furnace and scale. By using a muffle furnace to dry and burn each soil sample, the weights before and after burning were compared to determine the percentage or organic matter burned from each sample (Eq. 1). Because carbon composes about 45% of organic matter, this percentage was used to then find the estimated amount of carbon burned from each sample (Eq. 2). This procedure was executed for a total of four replications to account for variability within the soil samples and uncertainty introduced within measurements.

1.7 Research Implications

By following the procedure utilized for the Marin Carbon Project, the purpose of this experiment was to determine if a singular amendment of composted green waste could assist in boosting the sequestration of carbon within the soil. Findings of boosted carbon sequestration within the study site would indicate that carbon dioxide atmospheric emissions were successfully offset through a natural and cost-effective procedure. With a successfully augmented carbon sequestration process implemented into the ISAT Hillside, a portion of carbon dioxide emissions present in the atmosphere from energy expenditures can be offset. This enhanced addition of carbon into the soil through plant roots not only would reduce emissions lingering in the atmosphere, but it would also boost overall soil and vegetation health. Widespread usage of this procedure would thus possess the power to effectively diminish the negative implications of amplified greenhouse gas emissions, such as climate change. Rather than relying on expensive technologies to remove carbon dioxide emissions from the atmosphere, an effective compost land management technique would serve as a real-world solution that can be applied globally at a fast rate but low cost.

Chapter 2 Literature Review

2.1 Marin Carbon Project

The primary study evaluated for this study was the ongoing Marin Carbon Project experiment. The main objective of the Marin Carbon Project was to "explore the value of local soil carbon sequestration in rangelands" [1, p. 10] in an attempt to benefit rural communities both ecologically and agriculturally. To facilitate the uptake of carbon dioxide, researcher Whendee Silver spread ½ inch of compost onto pastureland plots [1, p. 11]. The compost used within the Marin Carbon Project was a mixture of plant clippings and animal manure [1, p. 11], a common compost solution. Silver clarified that the compost intensifies plant growth while also lowers the soil temperature to a degree that doesn't stimulate heavy microbial activity, which would subsequently result in active microbes exhaling carbon dioxide back into the atmosphere [1, p. 11]. Visibly, Silver has found that the composted plots produced taller grass, meaning that the grass has a greater amount of carbon stowed within it [1, p. 11]. Silver has also calculated that the composted plots within the study successfully seize 50 percent more carbon from the atmosphere than the grass in the control plots [1, p. 11]. Silver estimated that the compost land management technique of offsetting carbon dioxide emissions within the atmosphere could be continued for 30 years before the soil carbon reached equilibrium. [1, p. 11].

As part of the Marin Carbon Project, Whendee Silver and Rebecca Ryals conducted a field experiment on valley grasslands at the Sierra Foothill Research and Extension Center in Browns Valley, California [8]. The research project was repeated on coastal grasslands in Nicasio, California [8]. The experiment began in October of 2008 and was performed until August of 2011[8]. The plot sizes were 25 x 60 m, and treatments consisted of composted organic matter and untreated control plots[8]. The organic amendment consisted of commercially available composted green waste, and the soil amendment was applied in December of 2008 [8]. The sites in this study have historically been grazed by cattle since 1900, and thus all plots in the study were grazed using a rotational system[8]. Calculation of the soil carbon content was executed prior to and following the organic matter amendment, which would have served as a useful step in the procedure of this hillside experiment. Soil samples in this study were collected using a 7 cm corer, and the sample depth was approximately 10 cm [8]. This study also collected nine separate samples from each plot to analyze spatial differences. To condition the soil sample, identifiable root and compost pieces were manually removed from the soil samples. To calculate the carbon content, a Carlo Erba Elantech elemental analyzer was used with an atropine being utilized as a standard that was altered to content using bulk density values for each plot [8]. To analyze the data statistically, a one-way analyses of variance (ANOVA) was implemented to find statistical significance in the soil carbon content between treatments [8].

From Silver and Ryal's comprehensive study, it was concluded that the organic matter amendment applied to both the valley and coastal grassland boosted the plant growth [8]. The aboveground net primary production (ANPP) in the composted plots was augmented by 70% at the valley grassland site, and 44% at the coastal grassland [8]. The level of enhancement in the aboveground plant growth was observed again during the second and third year of the study. Across all three years, the ANPP was amplified a total of (436 +/- 68) g C/m^2 in the valley grassland and (161 +/- 78) g C/m^2 at the coastal grassland [8]. Root biomass was also observed to significantly increase at the 0-10 cm depth for both the valley grassland and coastal grassland[8]. The p-value calculated in this study for the significance in the increased ANPP over the three-year period was 0.01 [8]. This p-value is less than 0.05, and thus indicates that this difference in aboveground plant growth between the compost amended plots and control plots was statistically significant.

In regards to net carbon storage within the ecosystem, Silver and Ryals found that following the organic matter amendment, the amended plots had an increase in their net ecosystem carbon storage of (17.7 +/- 1.4) Mg C/ha in the valley grassland and (13.8 +/- 1.8) Mg C/ha in the coastal grassland [8]. The p-value calculated to evaluate the statistical significant of this measurement was 0.0001, a value indicating that the difference between treatments in highly statistically significant [8]. Due to increased soil microbe activity, researchers also found that carbon dioxide emissions from soil respiration were also amplified by 18-19% [8]. The sequestration of carbon into the soils offset this release of carbon dioxide from soil microbes, and researchers concluded that the organic matter amendment minimized the rate at which carbon was abandoning the soil due to the enhanced net primary productivity observed [8]. When it was assumed that 50% of the soil respiration occurred from heterotrophic respiration, it was calculated that the rate of carbon sequestration was increased by 25 to 70 percent [8] due to an organic matter amendment. Without considering the carbon directly added to the soil from the composted material, carbon was sequestered into the soil at a rate of (51 +/- 77) g C/m^2 to (333 +/- 52) g C/m^2 [8].

The results of this study indicated that a single compost amendment holds the capacity to boost and sustain NPP for at least three years, without indication that the effect was shrinking [8]. The amplified plant activity thus offset the increased soil respiration from microbial activity following the compost amendment. The compost-amended plots in both the valley and coastal grassland exhibited elevated levels of carbon sequestration. The results from the Marin Carbon Project indicated that an organic compost amendment could naturally and effectively offset atmospheric carbon dioxide emissions while simultaneously boosting the soil health and fertility. By diverting green waste that would normally go into a landfill, the emission of methane gas was evaded. The composted green waste thus offset greenhouse gas emissions and stimulated the soil to withhold a higher concentration of carbon.

2.2 Jeffrey Creque Olive Farm

Jeffrey Creque, a co-founder of the Marin Carbon Project, is an agroecologist who employs a holistic land management approach and aims to encourage growth by working in harmony with nature and its processes. Creque warns against suppressing life by working against nature, and the detrimental effects it will have on the entire system of land management [1, p. 2-3]. Creque was a member of the research and management team at a 500-acre organic olive farm, and sought to evaluate the carbon content of the soil on the farm [1, p. 2]. Creque's strategy to amplifying the carbon storage of the soil consisted of four primary land management techniques. Creque encouraged land management practices to avoid tillage of the land by instead employing permanent cover crops underneath the olive trees on the farm [1, p. 7]. Creque also performed seasonal rotational grazing of sheep on the olive farm and reinstated riparian areas as a means of diminishing gullies formed on the property from widespread erosion [1, p. 7]. The principal land management technique integrated into the management of the olive farm was to apply heavy amounts of compost to the soil, produced on-site from olive mill waste, livestock manure, and landscaping debris taken from the farm [1, p. 7]. With this enhanced land management approach, Creque aspired to boost the organic matter content and fertility of the soil.

From his study, Creque found that he was able to double the carbon content of the soil from 2% to 4% in under ten years using his land management techniques [1, p. 7]. Creque annually collected dozens of soil samples from multiple sites on the farm and sent them to a laboratory for consistent, independent analysis. Creque established that his newly revived management practices on the olive farm were capturing a greater amount of carbon from the atmosphere than was being emitted into the atmosphere from soil microbe respiration and energy emissions [1, p. 8]. After his ten-year study on the olive farm, Creque was able to conclude that the carbon content of the soil increased to about 4% [1, p. 8]. Creque now encourages the diversion of organic waste from landfills, where it will boost heavy greenhouse gas emissions. By composting organic waste, Creque found that greenhouse gas emissions from landfills is curtailed while soil carbon content is amplified.

2.3 Marin and Sonoma Studies

Fields located on commercial dairy rangelands were utilized in this study to determine the degree to which augmented ecosystem carbon sequestration can offset greenhouse gas emissions and thus climate change. This study hypothesized that manure additions to the soil would amplify soil carbon content, but that the greenhouse gas emissions would potentially offset some or all of the carbon gained in the soil over a long-term period[11]. The soil samples in this study were gathered from ten dairy rangelands located in Marin and Sonoma counties in California [11]. Samples were collected between November of 2011 and March of 2012 [11]. A total of 26 fields were used as soil sample sites, all of which are grazed fields [11]. Eleven of the fields in this study received a solid manure amendment, two received solely a liquid manure amendment, four fields received both, and nine fields had no amendment added [11].

A 6.5-cm-diameter corer was used to collect samples from 0 to 20 cm, and a 5.5-cm-diameter corer was used to obtain samples from a depth of 20 to 50 cm [11]. Soil samples were passed through a 2-mm sieve in this study while visible root and plant fragments were manually removed [11]. The rocks separated from the soil samples were weighed to determine the rock concentration of the samples being analyzed. Prior to analyzing the soil samples, they were ground to a find powder after being dried [11]. A Carlo Erba Elantech elemental analyzer with an atropine standard calculated the carbon content of the samples analyzed [11]. To analyze statistical significance in this study, means were compared with analysis of means (ANOM) and a statistically significant difference was defined as having a p-value less than 0.10 [11].

From the field measurements, researchers found that there was variation in the soil carbon concentration within and between the dairies in this study. Overall, researchers found that the organic matter amendment consisting of manure increased the average soil carbon concentration by (1.07 +/- 0.81) percent carbon within the 5 to 10 cm soil depth [11]. At an increased depth of 10 to 20 cm, the average carbon content of the soil increased by (0.88 +/- 0.68) percent carbon in the sites that received an organic matter amendment [11]. At a soil depth of 0 to 5 cm, the difference in carbon content between the sites that received an organic matter amendment and the sites that served as controls was not statistically significant [11]. Researchers concluded that in the top 20 cm of the soil profile, fields with an organic matter amendment had higher soil carbon content average by (19.0 +/- 7.3) Mg C ha^{-1} [11].

Researchers from this study predicted that given a longer period of time following the organic matter amendment to the soil, the soil carbon content would increase at all soil depths analyzed in this study [11]. Due to high variation within the data collected, the differences in average carbon content of the soil across treatments could not be concluded to be statistically significant [11]. It was still concluded from this study that organic matter amendments to rangelands offer the potential to mitigate climate change by offsetting the concentration of carbon dioxide within the atmosphere. Long-term impacts of an organic matter amendment suggest that carbon concentration of the soil will continually rise as time elapses. Researchers predicted that the carbon pools in the soil analyzed would stabilize over time and factors such as quality, quantity, and time of the organic matter amendment must be optimized such that the amount of carbon sequestration can be maximized [11].

2.4 Marshwind Farm Study

A field study was conducted on Marshwind Farm, Masstown from 1998 to 2001 to determine the benefits that composted material can have on a pasture in terms of its soil physical properties and soil organic matter [12]. Treatments in this study consisted of compost derived from crop residue, dairy manure, sewage sludge, or liquid dairy manure [12]. An unfertilized control was also included in this study as a means of comparison for the amended plots. The mineral fertilizer treatments in this study were applied on an annual basis, but the organic matter amendments were solely applied in 1998 and 1999 [12]. Soil samples were collected in October of 2000 and 2001 using a split core sampler [12]. Ten samples were collected from each plot, and the samples collected included the top 15 cm of the soil profile [12]. A sieve was used to remove gravel, crowns, and large root pieces while any remaining visible root pieces were removed from the soil samples by hand [12]. To analyze the carbon content of the collected soil, the Dumas method of direct combustion was implemented the procedure [12]. Analyzing the statistical significance of differences between treatments used the General Linear Model of SAS software [12].

The composted plots in this study significantly boosted the soil carbon sequestration and mass per volume [12]. This trend of boosted soil carbon sequestration was observed two years following the final application of composted materials [12]. The compost amendment enhanced soil organic carbon from 29.3 g C kg^{-1} in the unfertilized control to 41.5-53.2 g C kg^{-1} in the amended soil plots [12]. Researchers reported that compost alone altered the soil organic carbon and mass by 5.2 to 9.7 Mg C ha^{-1} [12]. The amendments with lower carbon inputs, such as manure, reflected a lower gain in soil organic carbon in comparison to the composted material [12]. Because these treatments were applied to two different crop types, the crop types were found to respond differently to the soil amendments. This was an element excluded in the hillside experiment, but it is recommended that future work include specific plant types and densities.

This experiment demonstrated the overall trend that composted amendments applied to landscapes can boost the total carbon storage in the soil more efficiently than non-composted materials, yet both enhanced the soil quality by directly providing the soil with organic matter. Increased carbon storage was observed across all treatments, although the composted materials augmented carbon sequestration most dramatically. Researchers determined that composts can be matched to specific crops to provide the greatest results in increased soil fertility and organic matter content [12]. While this study focused on targeting specific crops with particular types of organic matter amendments, the conclusion confirmed that composted organic materials promote soil carbon improvements [12].

Chapter 3 Objectives

The main objective of this experiment was to determine if a single composted organic matter amendment could effectively increase the amount of carbon sequestered within the soil. The goal of this project was to replicate the Marin Carbon Project as closely as possible on the ISAT Hillside to investigate if the same results would be obtained. Because this was the first year this experiment was conducted on the ISAT Hillside, a sub-objective of this project was to develop an operational protocol to foster the successful continuation of this project. This experiment also aligns with the ISAT Hillside Naturalization Project, which is an ongoing project with the goal of nurturing the growth of a polyculture wildflower prairie. An area of study within this experiment was thus to determine if a monoculture grassland could be converted into a polyculture carbon-sequestering prairie. Lawn plots incorporated into this study serve as a comparison between the prairie plots and the grassland area. Integration of the grassland into this study will serve to further the research of the ISAT Hillside Naturalization Project to confirm if the health of the prairie surpasses that of the lawn in regards to carbon content.

While this project aligned with the Marin Carbon Project, it was still investigative in nature. The project was not built upon a previous project conducted on the ISAT Hillside and was instead the initiation of an ongoing study that will continue. The hypothesis of this study was that the plots that received a single compost amendment would have a higher content of soil carbon compared to the control prairie plots and the lawn plots. This project established a baseline for understanding the carbon content of the ISAT Hillside soil, as this data was not recorded prior to the study. By completing this study, it was expected that differences in soil carbon content would be observable between composted prairie plots, control prairie plots, and lawn plots. This was also the first composted organic matter amendment added onto the ISAT Hillside for an experimental study, meaning the project was entirely investigative. Due to climate and soil differences between this experimental study site and the location of the Marin Carbon Project, it was predicted that while the same trend in carbon content could be observed, the differences between treatments would vary between the two study sites.

Chapter 4 Methodology

4.1 Study Site Background

The study was conducted on a 1.5-acre grass prairie on the ISAT Hillside on the James Madison University campus in Harrisonburg, Virginia (-78.935, 38.4553) [13]. Harrisonburg is a city within the Shenandoah Valley region of Virginia that has an average annual high temperature of 63.6°F [13], as well as an average annual low temperature of 40.6°F [13]. Temperatures in this region can vary, as January has an average temperature range of 20-40°F [13], while the month of July has temperature averages ranging from 62-85 F[13]. Harrisonburg experiences an average temperature of 52.1°F [13], as well as an annual average precipitation of 36.41 inches [13]. The study site is part of the ISAT Hillside Naturalization Project, which started in the summer of 2011. The goal of the Naturalization Project is to foster the growth of a wildflower prairie as a means of reducing runoff, erosion, and the frequency of mowing. While the hillside was originally planted with grasses foreign to the landscape, the hillside now flourishes with native grasses and wildflower species.

4.2 Individual Study Plots

Within the 1.5-acre grass prairie, 22 plots were chosen for the purpose of the ISAT 320 lab, as well as this research project. Students in the ISAT 320 Fall 2015 class measured out the 10 x 10 meter plots and marked the corners of the plots with flags. Students also recorded the GPS coordinates from the center of their 10 x 10 meter plots using a handheld GPS. 16 of the designated plots were located within the ISAT Hillside prairie, while the remaining 6 plots were placed in the lawn.

Table 1 Soil Sample Location and Characteristic Data

Sample ID	Treatment	Latitude (°N)	Longitude (°W)	Sample Date	Sample Time
S1G1	No Amendment	38.43392309	-78.86427907	10/16/15	11:20 AM
S1G2	No Amendment	38.43408268	-78.86455648	10/16/15	11:30 AM
S1G3	Compost Amendment	38.43423693	-78.86437370	10/16/15	11:37 AM
S1G4	Compost Amendment	38.43440214	-78.86446446	10/16/15	11:51 AM
S1G5	Lawn	38.43368374	-78.86510613	10/29/15	2:17 PM
S2G1	No Amendment	38.43398150	-78.86461267	10/16/15	11:58 AM
S2G2	Compost Amendment	38.43404015	-78.86468196	10/16/15	12:06 PM
S2G3	Compost Amendment	38.43431917	-78.86476397	10/16/15	12:25 PM
S2G4	No Amendment	38.43443672	-78.86501815	10/29/15	2:09 PM
S2G5	Lawn	38.43415511	-78.86491214	10/29/15	2:05 PM
S3G1	Compost Amendment	38.43376151	-78.86470863	10/16/15	12:49 PM
S3G2	Compost Amendment	38.43406424	-78.86494924	10/16/15	12:55 PM
S3G3	No Amendment	38.43419078	-78.86508095	10/16/15	1:02 PM
S3G4	No Amendment	38.43424107	-78.86519343	10/16/15	1:18 PM
S3G5	Lawn	38.43388961	-78.86537521	10/14/15	3:26 PM
S3G6	Lawn	38.43462242	-78.86546072	10/16/15	1:30 PM
S4G1	Compost Amendment	38.43384265	-78.86494714	10/16/15	1:48 PM
S4G2	No Amendment	38.43384006	-78.86510831	10/14/15	3:43 PM
S4G3	No Amendment	38.43415571	-78.86531439	10/14/15	3:10 PM
S4G4	Compost Amendment	38.43446534	-78.86547287	10/16/15	1:41 PM
S4G5	Lawn	38.43449052	-78.86601356	10/29/15	2:23 PM
S4G6	Lawn	38.43413603	-78.86550191	10/14/15	3:19 PM

4.3 Implemented Treatments

Eight of the plots positioned on the hillside prairie received treatment of a single half-inch organic matter amendment. A random number generator was utilized to determine which plots received the organic matter amendment to reduce bias. Eight different plots on the prairie hillside did not receive an organic matter amendment served as controls. The remaining six plots in this study were located on the lawn and served as a comparison. The plots located in the lawn were subjected to higher rates of mowing and pedestrian traffic, as they were not sheltered like the plots within the prairie.

4.4 Composted Plots

The organic matter amendment was compost produced by Black Bear Composting, a company located in Crozet, Virginia. The compost was produced from local food scraps, leaves, and green waste. Food waste from the James Madison University campus was also incorporated into the compost. A half-inch of compost was spread on the eight selected plots by the ISAT/GEOG 429 Spring 2015 class in the third week of March.

4.5 Soil Sample Collection

Soil samples were collected from the plots between October 14, 2015 and October 29, 2015. Samples were taken from the designated 10 x 10 meter plots marked by students using the GPS coordinates in Table 1. While students took their own samples, separate soil samples were taken for the purposes of this experiment. An auger was used to dig a hole into the soil about 6 inches in depth. To maintain a consistent depth across soil samples, a ruler was used to ensure each sample was being taken from a depth of at least 6 inches. Due to the rocky nature of the soil in many of the plots, samples were difficult to collect at a consistent depth. A garden trowel was then used to scoop soil from the site and place it into a plastic bag. Care was taken to scrape the sides of the sample hole when collecting soil to ensure a full 6-inch profile was collected. Following sample collection, the handheld GPS unit was held next to the sample location for a minimum of 60 seconds while the unit collected coordinates. The GPS coordinate data was later downloaded and the average GPS coordinate from the 60-second data collection period was recorded for each sample.

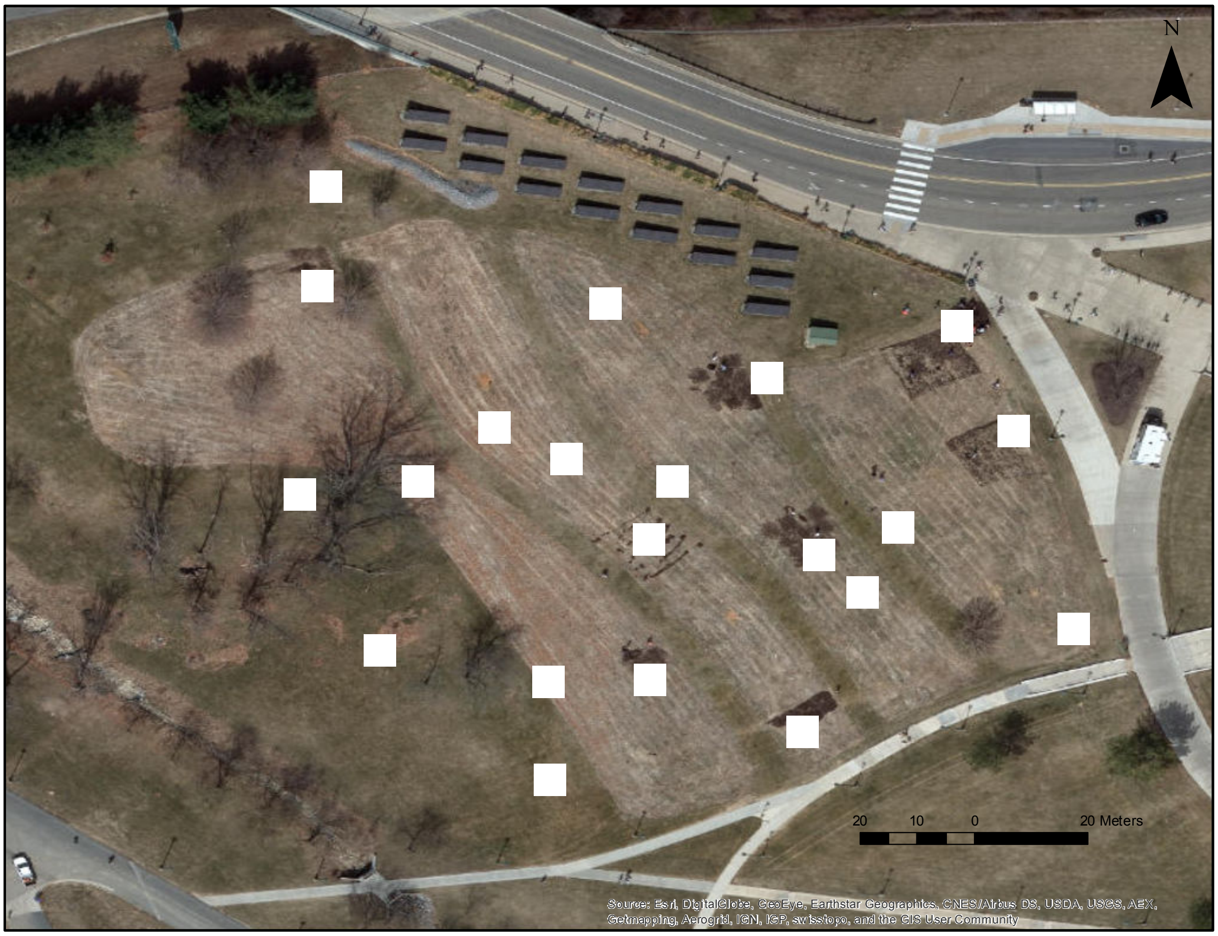

Figure 1: Geographic Coordinate Location of Soil Samples in ArcGIS

4.6 Sample Storage

The purchased muffle furnace for this experiment was not available until January 2016. The soil samples taken were thus stored in a refrigerator in the ISAT Environment Lab until all the necessary equipment was available. All samples were stored in individual plastic bags with their accompanying soil sample ID, sample date, and time of sample.

4.7 Sample Conditioning

Before the samples could be dried using the muffle furnace, they were mixed because they had remained stagnant in the refrigerator for two months. In the first two replications of this experiment, the soil samples were manually mixed by hand. The plastic bags containing the soil samples were shaken and mixed by hand. To improve the mixing procedure and determine if different mixing protocols yielded differing results, the soil samples were mechanically mixed in replications three and four. For these replications, the CSC Scientific Sieve Shaker catalog number 18480 (Figure 2) was utilized with solely sieve number 10. The specifications for this sieve indicate that the sieve filters particles above 2.00 millimeters, or 0.0787 inches. The nominal wire diameter for the sieve was 0.900 millimeters, or 0.0354 inches. The speed of shaking was adjusted for soil samples of different compositions. Soil samples composed primarily of heavy clay were shaken at a higher speed to try and break apart soil particles. Wet samples with primarily a clay composition were heated overnight in an oven at 35°C to dry and break apart the soil particles. While the sieve shaker assisted in removing rocks from the samples, roots were still capable of passing through the sieve and these remained in the soil sample. In all samples, roots and any identifiable compost litter were not removed through hand sorting.

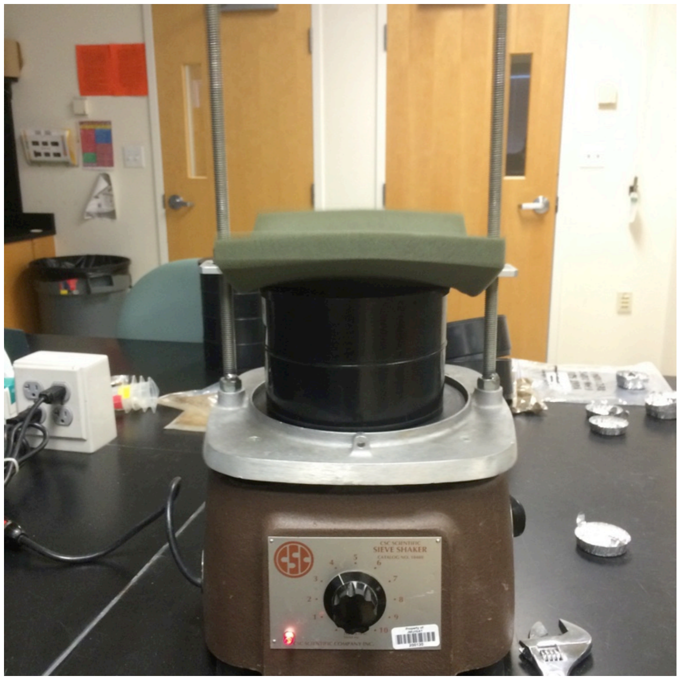

Figure 2: CSC Scientific Sieve Shaker

4.8 Muffle Furnace Preparation

Empty crucibles were labeled with a number and were assigned to a specific soil sample for testing. The empty crucibles were weighed before adding the soil sample. Before each weight measurement was taken, the scale was recalibrated to reduce systematic uncertainty in weight values. Subsequent to weighing the crucible, the crucible was handled using either gloves or tongs to avoiding adding weight to the crucibles from oils or dirt. After the weight of each crucible was recorded, about 5 grams of the soil samples were added to individual crucibles. Foil weigh boats were used when transferring soil from the plastic sample bags to the crucibles. Crucibles with added soil were again weighed to obtain the wet soil weight. When crucibles with the soil samples were not being handled, they were stored in a desiccator (Figure 3) to prevent the samples from coming into contact with water.

Figure 3: Desiccant Chamber Storing Sample Crucibles

4.9 Soil Drying and Burning

Crucibles with soil samples were placed in the muffle furnace (Figure 4) using gloves. The muffle furnace could fit a total of nine crucibles at a time. Samples were first heated at a temperature of 90°C for one hour (Figure 6). Dried samples were then cooled in the desiccation chamber while the remaining samples were heated. Dry sample weights were then taken and recorded using the scale. Samples were again placed in the muffle furnace and burned at 700°C for a period of 15 minutes (Figure 7). After burning samples at 700°C, samples were left to cool in the muffle furnace before transferring them to the desiccation chamber due to the extremely hot temperature of the crucibles. After cooling, samples were weighed a final time and the soil was then disposed of. In between replications, crucibles were rinsed thoroughly. Due to the small size of the muffle furnace, crucible tipping and spilling occurred sparingly. In the case of a spilled sample, the sample was disposed of and the soil burning process was restarted to ensure consistency. When recording the weight of each sample, the weights were not averaged and instead every number displayed on the scale was recorded.

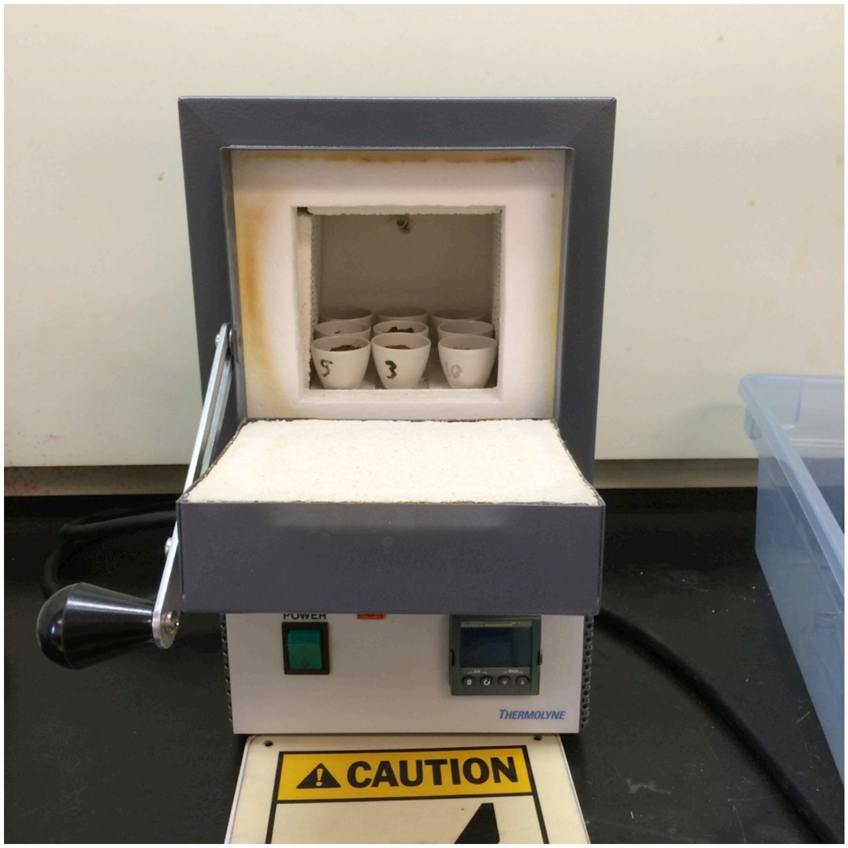

Figure 4: Muffle Furnace Used for Drying and Burning

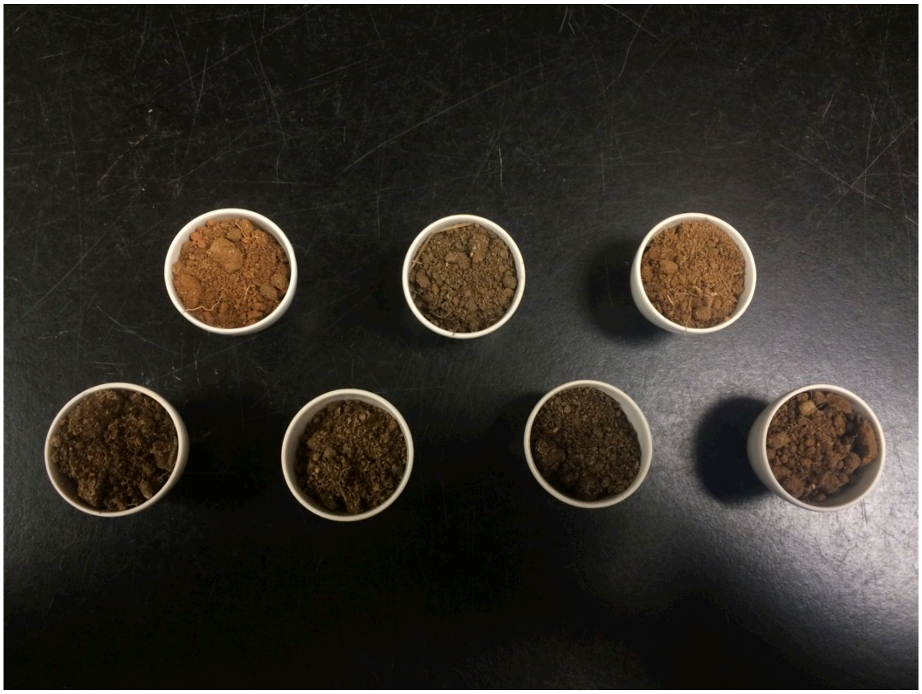

Figure 5: Soil samples before drying

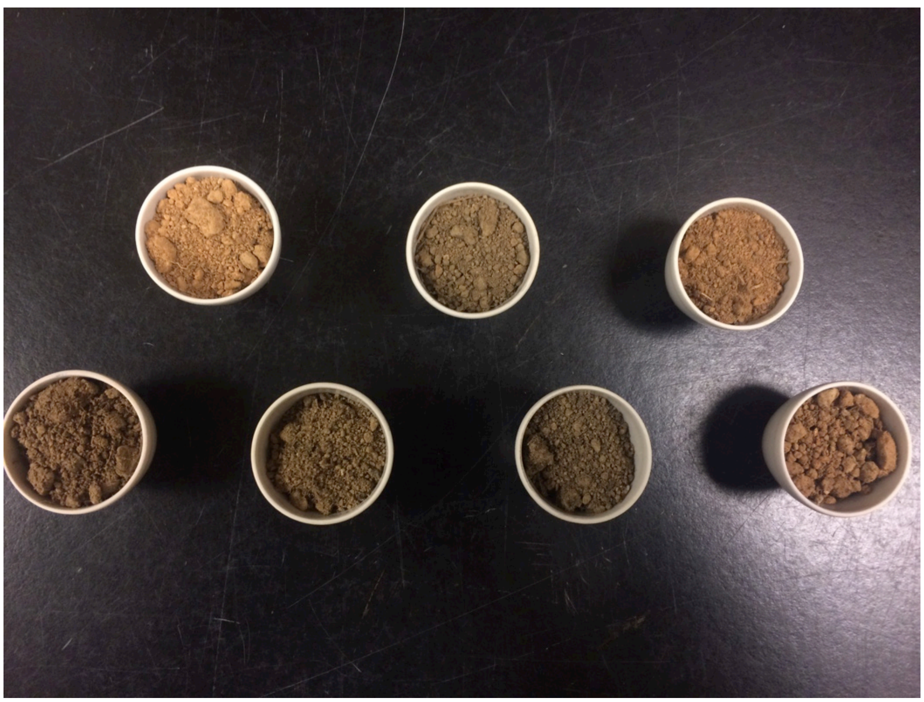

Figure 6: Soil Samples Following Drying Procedure

Figure 7: Soil Samples Following Burning Procedure

4.10 Soil Carbon Calculation

To find the dry weight of the soil, the weight of the crucible was subtracted from the sample weight following the initial 90°C burn. To calculate the percent of organic matter that was present in each sample, the following equation was used:

$$Eq. 1. Organic\ matter\ \% = \left(\frac{dry\ weight - organic\ matter\ burnoff\ weight}{dry\ weight}\right) * 100$$

Following the calculation of the percent organic matter in each sample, the following equation was used to find the total percent of carbon within each sample:

$$Eq. 2. Carbon\ \% = (organic\ matter\ \%) * (0.45)$$

Because carbon comprises about 45% of organic matter, the organic matter percentages were multiplied by 0.45 to find the percentage of carbon that was burned off within each sample. These calculations were used across all replications when finding the carbon content of the soil samples.

4.11 Carbon Content Visualization

To visually present the results calculated for each replication, ArcGIS was used. An image was produced for each replication, and the three treatments were assigned a color in the images to make it clear which treatment each data point correlated to. Before creating the images, each soil sample site was arranged into different classes depending on their carbon content percentage. The classifications were separated by 1% carbon content. Classification for each soil sample site varied between replications. To visually represent the different carbon content classifications, a different circle sized was used to each class. Classes associated with larger carbon percentages were given larger circle circumferences. For each replication, 22 circles were graphed in ArcGIS to make the difference in carbon content visually identifiable. Difference was also observable across treatments due to the different colors used for each treatment.

4.12 Statistical Analysis

To execute a statistical analysis of the data collected, GraphPad Scientific Software was used. The software performed an unpaired two-tailed t-test to compare two means. This was executed three times for each replication so that each treatment could be compared to each other. The average carbon content was compared for compost amendment vs. no compost amendment, compost amendment vs. lawn, and no compost amendment vs. lawn. Each run on the statistical software produced the two-tailed p value, the 95% confidence interval of the difference, the t value used, the degrees of freedom, and the standard error of difference in the data. This permitted determination of whether or not the difference between treatments was statistically significant or not. To validate the software-generated values, the equation below was used to manually calculate t-values:

$$Eq. 3.\, t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{(N_1 - 1)S_1^2 + (N_2 - 1)S_2^2}{N_1 + N_2 - 2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

where $\bar{x}_1$ is the mean of the first set of values, $\bar{x}_2$ is the mean of the second set of values, S_1 is the standard deviation of the first set of values, S_2 is the standard deviation of the second set of values, N_1 is the sample size of the first set of values, and N_2 is the sample size of the second set of values. After finding the t-values, a t-table was used to find the accompanying p-value.

Chapter 5 Results

5.1 Soil Carbon Content

To calculate the amount of carbon present within each sample analyzed, equations 1 and 2 were utilized. Four replications were executed in this study to determine if there was variation within the data. The plots that were composted in this experiment included S1G3, S1G4, S2G2, S2G3, S3G1, S3G2, S4G1, and S4G4. The plots located in the prairie that did not receive a compost amendment were S1G1, S1G2, S2G1, S2G4, S3G3, S3G4, S4G2, and S4G3. Finally, the plots located in the lawn are S1G5, S2G5, S3G5, S3G6, S4G5, and S4G6. The ISAT 320 Fall 2015 class established the group identifications. A sample of the calculation used to find the carbon content of the analyzed soil samples is provided below:

$$Organic\ matter\ \% = \left(\frac{dry\ weight - organic\ matter\ burnoff\ weight}{dry\ weight}\right) * 100$$

$$Replication\ 3\ S2G2\ Organic\ matter\ \% = \left(\frac{4.9037 - 4.2464}{4.9037}\right) * 100 = 13.4042\%$$

$$Carbon\ \% = (organic\ matter\ \%) * (0.45)$$

$$Replication\ 3\ S2G2\ Carbon\ \% = (13.4042) * (0.45) = 6.0319$$

Using these formulas, the percentage of carbon within each soil sample was calculated across each replication (Table 2, Table 3, Table 4, Table 5). The two formulas above were implemented in an identical fashion when calculating the carbon percentage of the soil samples for each replication. The three treatments – organic matter compost amendment, control without compost amendment, and lawn – were averaged across each replication (Table 6) and again averaged to obtain the overall treatment averages across all replications. The standard deviation was also calculated for each treatment across every replication and again to find the overall standard deviation across all replications (Table 6).

Table 2 Replication One Soil Carbon Content

Replication 1			
Sample ID	**Crucible**	**Organic Matter %**	**Carbon %**
S1G1	1	5.79	2.61
S1G2	3	6.31	2.84
S1G3	5	9.75	4.39
S1G4	6	6.78	3.05
S1G5	7	5.44	2.45
S2G1	8	6.59	2.97
S2G2	9	7.61	3.42
S2G3	10	8.11	3.65
S2G4	11	4.58	2.06
S2G5	12	11.83	5.32
S3G1	13	10.95	4.93
S3G2	14	17.31	7.79
S3G3	15	11.79	5.30
S3G4	16	8.37	3.77
S3G5	17	15.26	6.87
S3G6	18	10.99	4.95
S4G1	19	14.21	6.40
S4G2	20	12.22	5.50
S4G3	21	11.50	5.17
S4G4	22	13.38	6.02
S4G5	24	11.75	5.29
S4G6	25	6.69	3.01

Table 3 Replication Two Soil Carbon Content

Replication 2			
Sample ID	**Crucible**	**Organic Matter %**	**Carbon %**
S1G1	1	11.28	5.07
S1G2	3	16.43	7.39
S1G3	5	16.60	7.47
S1G4	6	15.67	7.05
S1G5	7	14.14	6.37
S2G1	8	12.91	5.81
S2G2	9	7.65	3.44
S2G3	10	18.47	8.31
S2G4	11	14.75	6.64
S2G5	12	14.96	6.73
S3G1	13	14.65	6.59
S3G2	14	18.04	8.12
S3G3	15	15.14	6.81
S3G4	16	12.40	5.58
S3G5	17	16.80	7.56
S3G6	18	13.36	6.01
S4G1	19	15.21	6.84
S4G2	20	11.45	5.15
S4G3	21	15.10	6.80
S4G4	22	15.55	7.00
S4G5	24	10.72	4.83
S4G6	25	8.79	3.95

Table 4 Replication Three Soil Carbon Content

Replication 3			
Sample ID	**Crucible**	**Organic Matter %**	**Carbon %**
S1G1	1	0.82	0.37
S1G2	3	13.21	5.95
S1G3	5	18.65	8.39
S1G4	6	10.56	4.75
S1G5	7	12.45	5.60
S2G1	8	10.05	4.52
S2G2	9	13.40	6.03
S2G3	10	18.41	8.29
S2G4	11	5.08	2.28
S2G5	12	15.52	6.98
S3G1	13	8.98	4.04
S3G2	14	15.11	6.80
S3G3	15	11.07	4.98
S3G4	16	7.17	3.23
S3G5	17	14.84	6.68
S3G6	18	10.17	4.58
S4G1	19	16.84	7.58
S4G2	20	16.69	7.51
S4G3	21	13.48	6.06
S4G4	22	9.12	4.11
S4G5	24	11.68	5.26
S4G6	25	8.78	3.95

Table 5 Replication Four Soil Carbon Content

Replication 4			
Sample ID	**Crucible**	**Organic Matter %**	**Carbon %**
S1G1	1	6.93	3.12
S1G2	3	7.80	3.51
S1G3	5	14.38	6.47
S1G4	6	9.31	4.19
S1G5	7	7.14	3.21
S2G1	8	8.89	4.00
S2G2	9	9.48	4.26
S2G3	10	13.29	5.98
S2G4	11	5.81	2.61
S2G5	12	10.03	4.52
S3G1	13	9.75	4.39
S3G2	14	13.39	6.02
S3G3	15	10.14	4.56
S3G4	16	6.09	2.74
S3G5	17	11.62	5.23
S3G6	18	10.35	4.66
S4G1	19	9.71	4.37
S4G2	20	10.43	4.69
S4G3	21	8.94	4.02
S4G4	22	x	x
S4G5	24	8.35	3.76
S4G6	25	6.01	2.70

Table 6 Average Soil Carbon Content Across All Replications

	Compost Amendment % Average	Compost Amendment Standard Deviation	No Compost Amendment % Average	No Compost Amendment Standard Deviation	Lawn % Average	Lawn Standard Deviation
Replication 1	4.96	1.66	3.78	1.37	4.65	1.64
Replication 2	6.85	1.51	6.16	0.86	5.91	1.31
Replication 3	6.25	1.80	4.93	1.78	5.51	1.18
Replication 4	5.10	1.01	3.66	0.79	4.01	0.96
All Replications	5.81	1.67	4.49	1.72	5.02	1.42

5.2 ArcGIS Visualization of Soil Carbon Content

To show the spatial distribution of carbon content within the soil samples, ArcGIS was used in visualizing the difference in soil carbon between treatments. Each replication was represented visually using ArcGIS (Figure 8, Figure 9, Figure 10, Figure 11) separately. In the ArcGIS program, a circle was used to represent each sample site. The size of the circle circumference for each sample correlated to the amount of carbon calculated from the soil samples. For samples with a higher carbon content percentage, a circle with a larger circumference was utilized. Either a blue, red, or black colored box surrounded each circle on the figure describing which treatment was associated with each sample site. Plot locations boxed in blue correlated to sites that were controls without a compost amendment, those boxed in red related to composted plots, and sites surrounded by a black box were those that were located in the lawn for comparison.

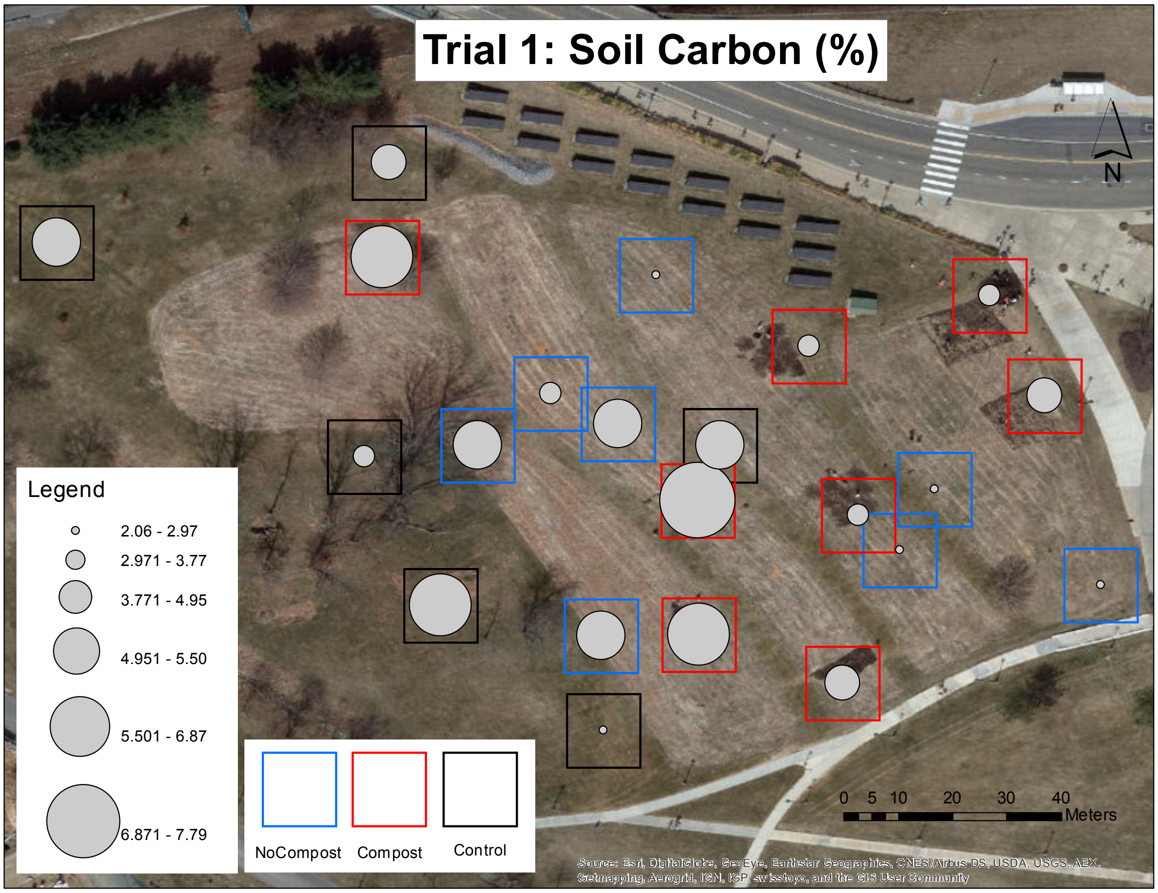

Figure 8: Replication One Soil Carbon Content Visualization in ArcGIS

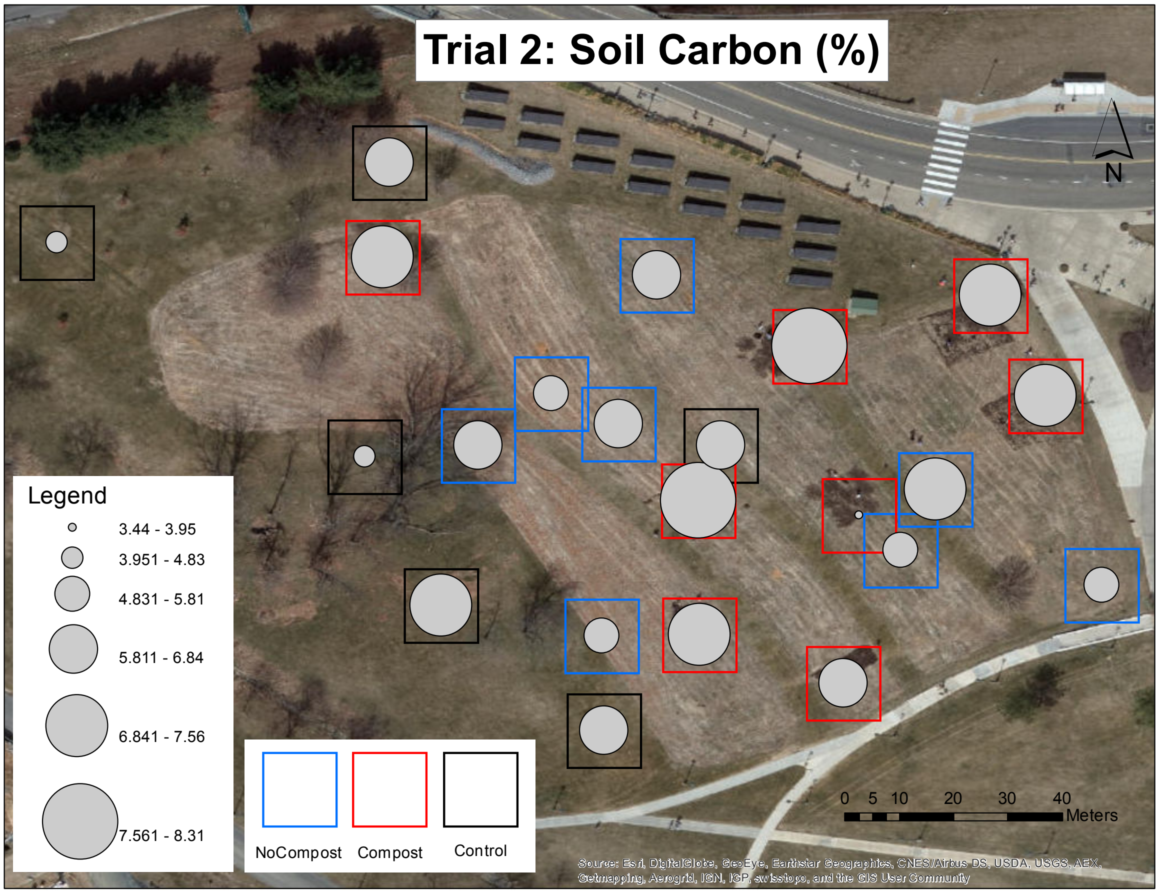

Figure 9: Replication Two Soil Carbon Content Visualization in ArcGIS

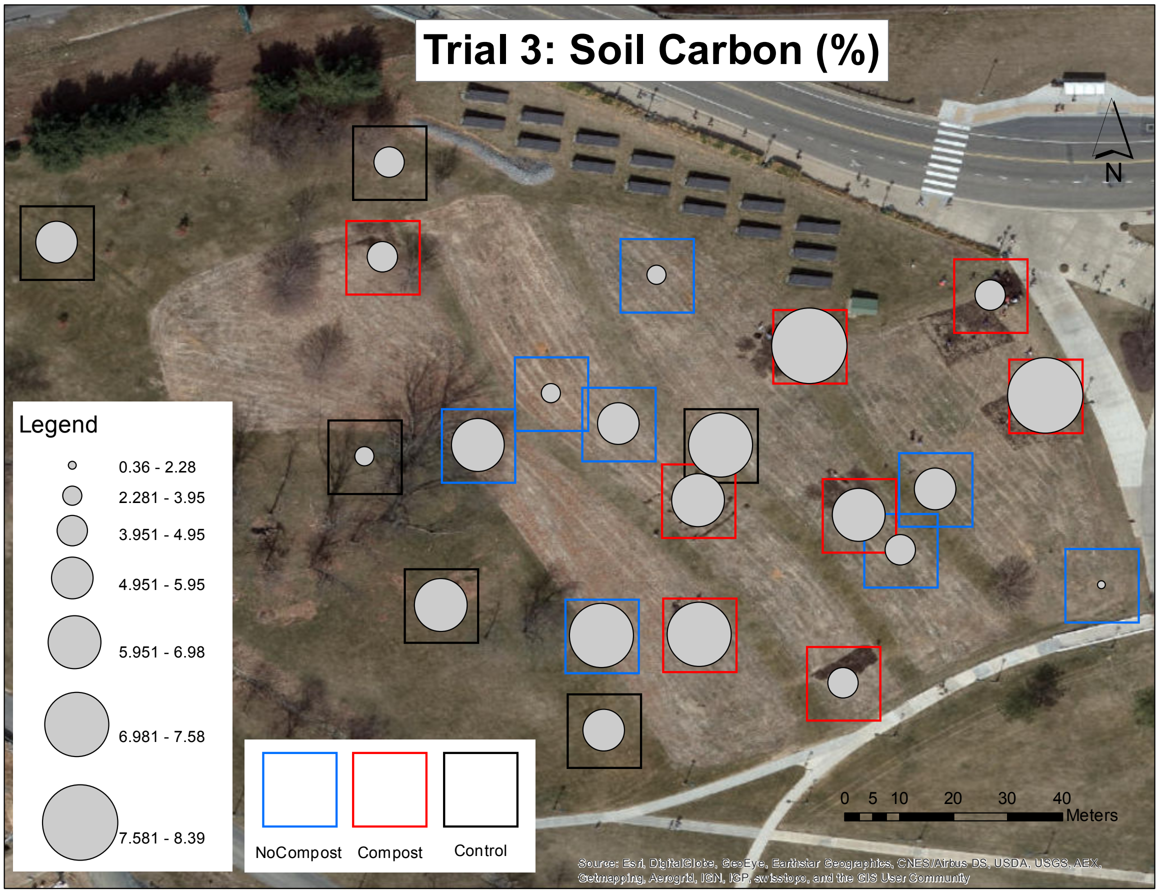

Figure 10: Replication Three Soil Carbon Content Visualization in ArcGIS

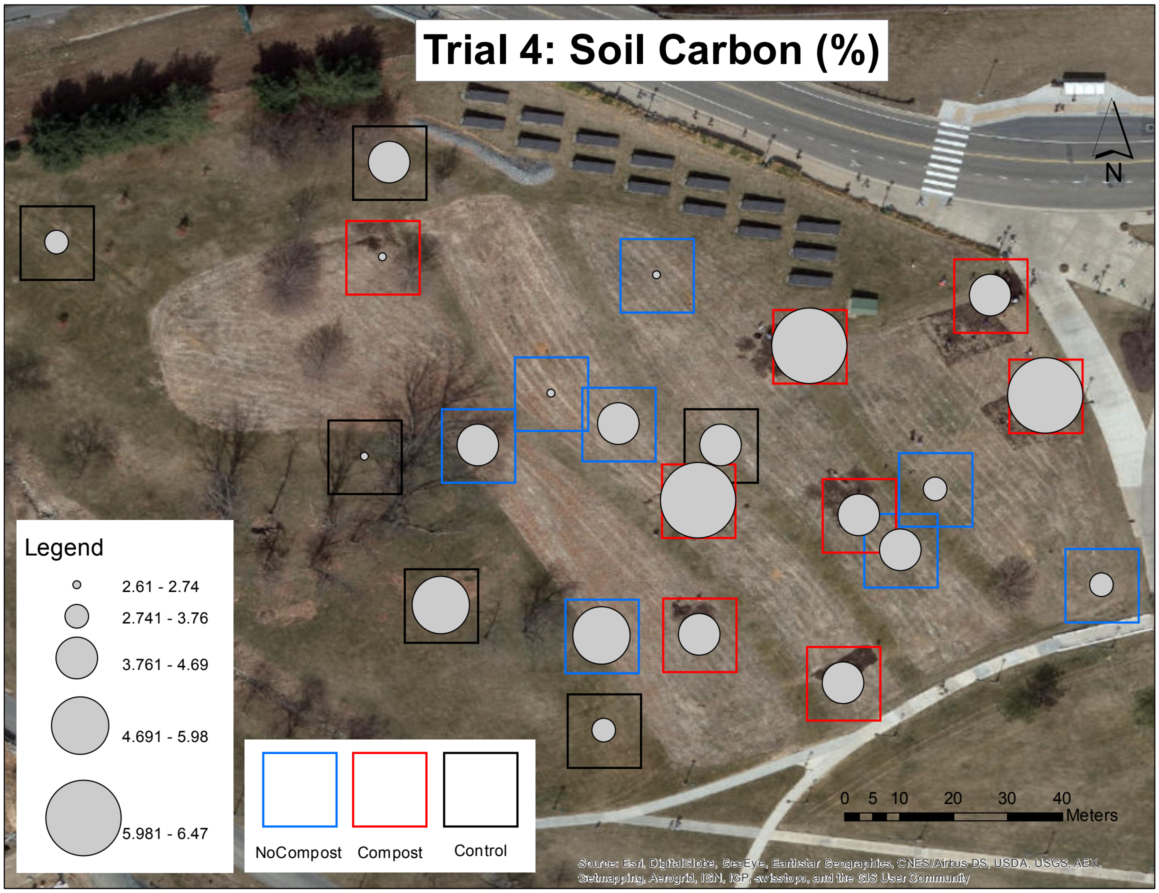

Figure 11: Replication Four Soil Carbon Content Visualization in ArcGIS

5.3 Statistical Analysis Between Treatments

To determine if the differences between treatments in this experiment were considered statistically significant, an unpaired two-tailed t-test was executed to compare the three treatments for each replication. GraphPad Scientific Software was used to calculate the p-values, and the values were manually verified. To validate the software-generated values, equation three was used to manually calculate the t-values. An example of the t-value calculation is provided below.

$$Eq. 3. t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{(N_1 - 1)S_1^2 + (N_2 - 1)S_2^2}{N_1 + N_2 - 2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

$$t = \frac{4.9556 - 3.7773}{\sqrt{\left(\frac{(8 - 1)1.6578^2 + (8 - 1)1.3685^2}{8 + 8 - 2}\right)\left(\frac{1}{8} + \frac{1}{8}\right)}} = 1.5505$$

The two-tailed p-values were calculated to compare compost amendment vs. no compost amendment, compost amendment vs. lawn, and no compost amendment vs. lawn. This procedure was completed for each replication (Table 7, Table 8, Table 9, Table 10) rather than combining each replication into a single statistical analysis. The replications were kept separate during statistical analysis due to variation observed between replications. The statistical analysis software also reported the 95% confidence interval of difference and the standard error of difference, both of which were recorded.

Table 7 Replication One Statistical Analysis Between Treatments

Replication 1					
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.1433	1.5505	-0.45 to 2.81	14	0.760
Compost Amendment vs. Lawn	0.735	0.3465	-1.63 to 2.25	12	0.891
No Compost Amendment vs. Lawn	0.2998	1.0836	-2.62 to 0.88	12	0.803

Table 8 Replication Two Statistical Analysis Between Treatments

Replication 2					
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.2757	1.1344	-0.62 to 2.01	14	0.614
Compost Amendment vs. Lawn	0.2439	1.2256	-0.74 to 2.63	12	0.771
No Compost Amendment vs. Lawn	0.6751	0.4296	-1.01 to 1.51	12	0.580

Table 9 Replication Three Statistical Analysis Between Treatments

Replication 3					
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.1791	1.4203	-0.69 to 3.32	13	0.926
Compost Amendment vs. Lawn	0.3984	0.8757	-1.10 to 2.59	12	0.846
No Compost Amendment vs. Lawn	0.5157	0.6715	-2.45 to 1.31	11	0.855

Table 10 Replication Four Statistical Analysis Between Treatments

	Replication 4				
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.0084	3.1049	0.44 to 2.44	13	0.464
Compost Amendment vs. Lawn	0.0727	1.9844	-0.12 to 2.29	11	0.547
No Compost Amendment vs. Lawn	0.461	0.7616	-1.37 to 0.66	12	0.467

5.4 Waypoint Analytical Soil Characteristics

Aside from the on-campus analysis of collected soil samples, the soil samples collected from students in the Fall 2015 ISAT 320 Lab had their samples sent to Waypoint Analytical in Richmond, Virginia. This laboratory separately analyzed each soil sample to calculate the soil characteristics. The soil attributes pertaining to this lab include the organic matter percentage (Table 11), the cation exchange capacity (Table 12), and the phosphorous content within the soil (Table 13).

Table 11 Soil Sample Organic Matter Percentages Obtained from Waypoint Laboratory

Waypoint Data	
Sample ID	**Organic Matter %**
S1G1	4.6
S1G2	5.8
S1G3	7.4
S1G4	7.8
S1G5	6.1
S2G1	3.9
S2G2	8.6
S2G3	8.6
S2G4	5.0
S2G5	6.3
S3G1	6.9
S3G2	4.8
S3G3	6.0
S3G4	2.7
S3G5	4.5
S3G6	6.6
S4G1	6.6
S4G2	5.9
S4G3	6.4
S4G4	3.7
S4G5	5.3
S4G6	6.2

Table 12 Soil Sample Cation Exchange Capacity Obtained from Waypoint Laboratory

Waypoint Data	
Sample ID	**Cation Exchange Capacity (meg/100g)**
S1G1	7.3
S1G2	10.2
S1G3	11.8
S1G4	13.8
S1G5	24.2
S2G1	6.8
S2G2	15.3
S2G3	18.7
S2G4	7.2
S2G5	12.0
S3G1	13.3
S3G2	11.4
S3G3	12.7
S3G4	8.6
S3G5	8.8
S3G6	10.1
S4G1	12.2
S4G2	27.1
S4G3	25.8
S4G4	11.3
S4G5	13.2
S4G6	18.7

Table 13 Soil Sample Phosphorous Content Obtained from Waypoint Laboratory

Waypoint Data	
Sample ID	**Phosphorus (ppm)**
S1G1	86
S1G2	85
S1G3	165
S1G4	99
S1G5	85
S2G1	47
S2G2	154
S2G3	177
S2G4	29
S2G5	88
S3G1	165
S3G2	120
S3G3	23
S3G4	12
S3G5	95
S3G6	43
S4G1	88
S4G2	30
S4G3	58
S4G4	36
S4G5	57
S4G6	55

Table 14 Average Soil Characteristic Data Obtained from Waypoint Laboratory

	Compost Amendment Average	Compost Amendment Standard Deviation	No Compost Amendment Average	No Compost Amendment Standard Deviation	Lawn Average	Lawn Standard Deviation
Organic Matter Percentage	6.800	1.753	5.038	1.257	5.833	0.784
Phosphorous (ppm)	125.50	48.893	46.250	28.019	70.500	21.427
Cation Exchange Capacity (meg/100g)	13.475	2.5138	13.213	8.4019	14.500	5.8570

5.5 Statistical Analysis of Waypoint Data Between Treatments

To again ascertain whether the differences between treatments after the compost application were statistically significant, GraphPad Scientific Software was used to analyze the Waypoint Analytical data. The same procedure was executed for the statistical analysis such that a two-tailed unpaired t-test was utilized to compare treatments. The software calculated the t-values and associated p-values, a 95% confidence interval of difference, and the standard error of difference. The software was used to compare treatments within the organic matter results (Table 14), the cation exchange capacity (Table 15), and the phosphorous content (Table 16). Again, to verify the software-calculated values the t-values were manually calculated using equation three. The same t-table was used when finding the p-values for the specific t-values. The same comparisons were made again consisting of compost amendment vs. no compost amendment, compost amendment vs. lawn, and no compost amendment vs. lawn.

Table 15 Statistical Analysis between Treatments of Carbon Percentages from Waypoint Laboratory

Waypoint Organic Matter %					
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.0365	2.3115	0.13 to 3.40	14	0.763
Compost Amendment vs. Lawn	0.2348	1.2508	-0.72 to 2.65	12	0.773
No Compost Amendment vs. Lawn	0.1995	1.3579	-2.03 to 0.48	12	0.586

Table 16 Statistical Analysis between Treatments of Cation Exchange Capacity from Waypoint Laboratory

Waypoint Cation Exchange Capacity (meg/100g)					
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.9337	0.0847	-6.39 to 6.91	14	3.101
Compost Amendment vs. Lawn	0.6624	0.4476	-6.01 to 3.96	12	2.29
No Compost Amendment vs. Lawn	0.7544	0.3201	-10.05 to 7.48	12	4.022

Table 17 Statistical Analysis between Treatments of Phosphorous Content from Waypoint Laboratory

Waypoint Phosphorus (ppm)					
	Two-tailed p-value	**t-value**	**95% C.I. of difference**	**df**	**Standard error of difference**
Compost Amendment vs. No Compost Amendment	0.0014	3.9777	36.52 to 121.98	14	19.924
Compost Amendment vs. Lawn	0.0251	2.5574	8.14 to 101.86	12	21.506
No Compost Amendment vs. Lawn	0.1035	1.7622	-54.23 to 5.73	12	13.761

Chapter 6 Discussion

6.1 Soil Carbon Content Interpretation

After executing four replications of analyzing carbon content from the soil samples, it was found that on average, the plots that received a compost amendment had the highest soil carbon content. There was variation observed between replications (Table 2, Table 3, Table 4, Table 5), which was likely due to a number of factors that introduced uncertainty into the measurements. The average carbon content of the composted plots varied from 4.96 percent to 6.85 percent (Table 6). In the prairie plots that didn't receive a compost amendment, the percentage of carbon found within the soil ranged from 3.66 percent to 6.16 percent (Table 6). For the lawn plots, the soil carbon content varies from 4.01 percent to 5.91 percent (Table 6). Although there was variation across replications, the trend observed was that the composted plots had, on average, the highest percentage of soil carbon with a total average of 5.81 +/- 1.67 percent (Table 6). The lawn plots had the second highest carbon content with an average percentage of 5.02 +/- 1.42 (Table 6). The lawn plots had a higher carbon content than the non-composted prairie plots due to high root abundance found within the lawn soil samples. Finally, the prairie plots without the compost amendment had the lowest carbon percentage average of 4.49 +/- 1.72 (Table 6). This aligns with the initially stated hypothesis that plots receiving a single compost amendment would have the highest percent of carbon within the soil.

The calculated percentage of carbon within each soil sample follows the trend observed in the Marin Carbon Project in which plots have a higher amount of carbon sequestration after receiving an organic matter amendment. Aside from the presentation of quantitative results, these values were analyzed spatially in ArcGIS (Figure 8, Figure 9, Figure 10, Figure 11). Looking at the images produced from the software, the trends are again observable. In replication one (Figure 8), the composted plots on average are associated with the largest circle size – meaning their carbon content is higher. The plots boxed in blue, which were located on the prairie but were not composted, have a much smaller percentage of soil carbon than the composted and lawn plots. There is variation within the data such that plots within each treatment exhibited diversity in results for soil carbon content. In replication two (Figure 9), the composted plots again exhibit the highest percentage of soil carbon. The values obtained in replication two vary from replication one because the calculated values for each treatment were on average higher in replication two than they were in replication one. In replication three (Figure 10), the results are similar to those found in replication two (Figure 9). The same overall trend is observable that the plots with the compost amendment have the highest percentage of soil carbon sequestered. The plots located in the lawn again have a higher soil carbon content than the non-composted prairie plots, which is explained by the high abundance of roots within the lawn. In replication four (Figure 11), the results are similar to those found in replication one (Figure 8). While there is again variation amongst treatments, the same trend is observable in the average soil carbon content between treatments.

While the averages of the soil carbon percentages indicate that the compost amendment successfully boosted the carbon sequestration in comparison to the control and lawn plots, uncertainty within this study created variation in results between replications and amongst treatments. Although the same trends were observable when analyzing the overall averages of each replication, there is variation in the data. The composted plots have the highest percentage of soil carbon, but the value calculated for a particular sample often varied between replications. For example, the percentage of carbon measured in sample S1G3 was 4.39 percent in replication one (Table 2), but when this same sample was analyzed again in replication two, the percentage found was 7.47 (Table 3). The percentage found varied again in replications three in four, as a value of 8.39 percent was calculated in replication three (Table 4), and a percentage of 6.47 was found in replication four (Table 5). Because the data varied between replications, it was difficult to compare the ArcGIS images to each other because each replication yielded different results. Aside from variation between replications, there was also disparity within the same treatments. In replication one (Table 2), for example, the percentage of soil carbon found in sample S3G2 was 7.79, while the percentage for S1G4 was 3.05. Although both of these plots received an identical organic matter amendment, there is a significant difference between the soil carbon content found. This could arise from natural spatial variation or uncertainty within measurements.

There are multiple notions pertaining to why there was such high variation in results within this study. The first is that the procedure for mixing the soil prior to placing it within the crucible was altered after executing the first two replications. In replications one and two, the sample was manually mixed by shaking the plastic bag with the soil sample. Because there was variation observed between replications one and two, it was predicted that the mixing method was not efficiently mixing the soil samples – which may have caused disparity in the results. The mixing procedure was consequently altered to acquire soil samples with thorough mixing. For replications three and four, the samples were mixed using a scientific sieve shaker. Only the sieve #10 was used in this procedure, which filters particles at 2.00 millimeters, or 0.0787 inches. Although this sample mixing procedure was enhanced, there was still variation within the results obtained from replication three (Table 4) and replication four (Table 5). Because the soil shaking technique was not an identical procedure utilized for all four replications, difference between the results across replications is in part due to this.

Another area of introduced uncertainty within this study is the high root abundance found within soil samples. This factor is likely the main contributor to the variation found across replications and within treatments. A high amount of roots within the soil samples offsets the weight output of the scales, thus influencing the total organic matter and carbon percentage calculated from the weight measurements. Even when executing the sieve shaker soil mixing procedure, the roots still passed through the sieve. These root particles were not manually removed from the soil samples and were thus included in the dried and burned weights. There was spatial difference in the carbon content, which is likely due to the diverse plant species altering the results differently. Because plant diversity and density was not considered by this study, it was not possible to determine how these plant roots were specifically altering the measured carbon content of each soil sample. Although the degree to which plant roots affected the results cannot be quantified, the source of uncertainty was identified and shape future studies so that the extent of plant root influence can be quantified and thoroughly analyzed.

Another route for uncertainty within these measurements is the rocky and shallow conditions of the soil where the samples were collected. Due to these conditions, it was difficult to penetrate the soil and collect a sample at consistent depths. To maintain uniformity within soil sample collection, every sample was collected to a depth of six inches. At some sample locations, obtaining a sample depth of even six inches was extremely difficult due to a high abundance of rocks within the soil. An ideal sample depth in this particular type of study is 20 centimeters, or roughly eight inches. This depth was not obtainable in this experiment due to rocky conditions. With an enhanced procedure or sampling equipment, soil samples could be collected at a depth of eight inches to thus boost the accuracy of the carbon content results. In replications one and two, the sieve shaker was not used and there was a higher abundance of rocks included in the sample as it was dried and burned. The presence of rocks offsets the weights and ultimately altered the soil carbon content calculation. Because some plots had a greater wealth of rocks, the calculated soil carbon content was more greatly affected. Overcoming this requires sieve shaking in all replications and confirming that the sieve size was appropriate for removing most to all of the rock fragments. The abundance of rocks was not entirely overcome in this experiment, indicating that the rocky and shallow properties of the study site contributed to the uncertainty and variation within results.

The study site in this experiment previously had a dirt road that ran directly through it. When James Madison University purchased the land, maintenance facilities planted directly over the dirt road. Although the road was covered in vegetation, the previous usage of the road compacted the soil underneath it. As a result, vegetation has more difficulty extending its roots into the soil. Overcoming soil compaction takes a significant amount of time for the roots to loosen the soil and make it more fertile. Plots located in the close proximity to where the road previously ran likely have more difficulty flourishing due to stunted root growth in the compacted soil. While this was not considered by this study, it is probable that the soil compaction from the dirt road affected the ability for some plots to boost their organic matter percentage. Plants need to extend their roots deep into the soil to come into contact with the beneficial soil microbes that assist in building topsoil. If plant growth is stunted due to compacted soil, it becomes more difficult for plant development to be heightened following a composted organic matter amendment. If this occurs, plants are less likely to have extensive relationships with soil microbes, meaning less of the root exudates from soil will be transformed into stable humus. The extensive impacts of compacted soil consequently contributed to spatial differences in soil carbon content found amongst soil samples.

Manually working within the laboratory also generates opportunities for human error to occur. Caution was always taken to strictly follow the same procedure, but it is impossible to completely avoid human error within experiments. If faults were observed during the experiment, such as tipping of crucibles, the procedure would be repeated to ensure correct weight values were obtained. Uncertainty and offset in the data was still instituted from human error during the soil sample collection and analyzing procedures.

6.2 Statistical Analysis Interpretation

6.2.1 Replication One

To determine if the difference between treatments in this study was statistically significant, an unpaired two-tailed t-test was executed for each replication. This was completed using GraphPad Scientific Software. The parameters evaluated by this software were the t-value, p-value, a 95% confidence interval of difference, the degrees of freedom, and the standard error of difference. In replication one, the p-value calculated for the compost amendment vs. no compost amendment was 0.1433 (Table 7). Because this p-value is less than 0.05, the difference between these two treatments in replication one cannot be considered statistically significant. A p-value of 0.1433 means that there is a 14.33% chance that the means of the two treatments overlap, but this still indicates that there is a 85.67% chance that these means are in fact different from each other. The standard error of difference value of 0.760 quantifies the uncertainty of the difference between the two means. The 95% confidence interval of the difference in between these treatments is interpreted as meaning that there is 95% assurance that the range between -0.45 and 2.81 contains the true population difference between the means of the two treatments.

The difference in means of the compost amendment and no compost amendment can thus not be considered statistically significant from these parameters.

When comparing the compost amendment mean to the lawn mean in replication one (Table 7), the p-value calculated was 0.735, which is not considered to be statistically significant. This is an extremely high p-value, which is interpreted to mean we are 73.5% sure the means of these two treatments overlap. The 95% confidence interval of difference was -1.63 to 2.25, meaning there is 95% confidence that the interval for the difference between the population mean compost amendment carbon content and mean lawn carbon content is within this range. This range includes the number zero, which is the null hypothesis that there is no difference between means, which is consistent of a p-value greater than 0.05. Ultimately, the difference between these two treatments cannot be considered statistically significant. The final comparison made in replication one, no compost amendment vs. lawn (Table 7) had a computed p-value of 0.2998. Again, because this value is greater than 0.05 it cannot by standard statistic procedures be considered a difference that is statistically significant. This p-value means that there is a 29.98% chance that the mean values for both treatments are the same. For each treatment in replication one, no difference between treatments could be considered statistically significant.

6.2.2 Replication Two

For replication two, the same parameters were again evaluated for the three different treatments to evaluate the statistical significance of the difference. For the compost amendment vs. no compost amendment (Table 8), a t-value of 1.1344 correlated to a p-value of 0.2757, a value too high to be considered to be statistically significant. The difference between the mean compost amendment carbon content and mean non-compost carbon content in replication two cannot be considered to be statistically significant.

When comparing the compost amendment carbon content average to the lawn carbon content average in replication two (Table 8), a p-value of 0.2439 was calculated. This being greater than 0.05, the difference in means of these two treatments cannot be considered to be statistically significant. There is a 24.39% chance that these means still overlap each other. From these values calculated by the statistical software, the difference between the average carbon content of the compost amended plots and lawn plots is not by statistics standards considered to be statistically significant. The final comparison in replication two was between the prairie plots that did not receive a compost amendment and the plots located in the lawn area (Table 8). For this statistical evaluation, a p-value of 0.6751 was calculated. This correlates to there being a 67.51% chance that the means carbon content values from the two treatments overlap each other, with only a 32.49% chance that the means are in fact different. As with replication one, the difference between treatments in replication two cannot be verified as being statistically significant.

6.2.3 Replication Three

For replication three, the same statistical analysis tool was used to compare the same three different treatments separately. In the comparison of compost amended plots and prairie plots without a compost amendment (Table 9), the p-value calculated was 0.1791. Although this p-value is lower than the p-values previously calculated, it is still not considered to be a statistically significant difference between treatments due to the p-value being higher than 0.05. Although the p-value was lower in this assessment, by statistical standards this difference is not considered to be statistically significant.

For the comparison of compost amended plots and lawn plots in replication three (Table 9), the t-value of 0.8757 correlated to a two-tailed p-value of 0.3984. Being higher than 0.05, this difference between treatments is not considered to be statistically significant as there is a 39.84% chance that these means overlap. Due to the p-value greater than 0.05, the difference between the means of these treatments is not statistically significant. The final comparison for replication three, prairie plots without a compost amendment and lawn plots (Table 9) had a p-value calculated to be 0.5157. With this p-value, there is a 51.57% chance that these means overlap, and a 48.43% chance that they do not. Because there is about half a chance that the means are the same and about the same probability that they are different, the difference between these treatments is not considered to be statistically significant. Ultimately, the difference between these two treatments is not great enough to be statistically significant.

6.2.4 Replication Four

In the final replication, the same procedure was followed to statistically analyze the difference between treatments. For the compost amended plots and prairie plots without a compost amendment (Table 10), the p-value calculated was 0.0084. This value is significantly lower than 0.05, and this difference is thus considered to be highly statistically significant. We could thus reject the null hypothesis that there was no difference between these two means. For the 95% confidence interval of the difference, the values ranged from 0.44 to 2.44. This range does not include the value zero, agreeing with the determination that the p-value is less than 0.05. This was the only difference between treatments in this experiment found to be statistically significant. This indicates that in replication four, the difference between the average carbon content of the plots in the prairie that received a compost amendment and the prairie plots without a compost amendment were statistically significant. This is the only statistically significant indication in this experiment that the compost amendment considerably boosted to carbon sequestration to an extent that created a difference between treatments to be statistically significant.

For the comparison of the compost amended plots and the lawn plots in replication four (Table 10), the calculated p-value was 0.0727. This value is extremely close to being under 0.05, but is just shy of being low enough to declare the difference between means to be statistically significant. This value still indicates that there is a 92.73% chance that there is a difference between the two means. While the p-value for these two treatments was still very low, by conventional statistical standards the difference between treatments is not statistically significant. The final statistical comparison made for this data consisting of comparing the mean carbon content of the prairie plots without a compost amendment to the mean carbon content of the lawn plots in replication four (Table 10). For this comparison, the p-value calculated was 0.461. This p-value is much larger than the necessary p-value of 0.05 to conclude that the difference is statistically significant.

6.2.5 Time Restraint

While the overall deduction of the statistical analysis is that the difference in carbon content of the soil between treatments is not statistically significant, it is predicted that this difference will become more apparent over time. The period between compost application and soil testing was approximately six months, as compared to the three-year period that elapsed in the Marin Carbon Project [10]. While a three-year period was not plausible for the purposes of this project, it would have more closely aligned with the procedure implemented in the Marin Carbon Project. It is predicted that as more time passes between the compost application to the hillside, the difference between treatments will become more apparent, potentially altering the statistical analysis such that these differences are considered statistically significant. It is possible that within this six-month period between compost application and soil sampling, weather conditions or outside factors impacted the ability of the soil to more significantly sequester carbon from the atmosphere. As more time elapses, the organic matter applied to the soil can stabilize and continually be boosted due to the enhanced plant growth from the compost amendment. A continuation of this experiment will determine whether a longer period of time will generate statistically significant differences in soil carbon content between the different treatments.

6.3 Waypoint Analytical Soil Characteristics Interpretation

The Waypoint Analytical laboratory solely analyzed the soil samples collected from the students in the ISAT 320 Fall 2015 class, as part of the laboratory procedure for this class. The separate samples collected to the intent of this experiment were only analyzed in the on-campus environmental lab rather than being sent to Waypoint Analytical. The samples collected by the students in the ISAT 320 lab were taken from each of the same 10 x 10 meter plots that were the soil site locations for this study as well. The soil depth at which students collected samples was not consistent, either being too shallow or too deep. As a result, separate holes for sample collection were dug for the intent of this experiment. There is thus uncertainty in the data provided from Waypoint Analytical because the sample depth was not consistent for each plot. Flawed sampling procedures also create instability when comparing the Waypoint Analytical results to the results obtained in this experiment. The prominent issue with inconsistent sampling depths from the student samples is that the soil characteristic data could be inaccurate because the soil sample did not incorporate an appropriate soil profile depth. Lastly, because the exact soil sample location within the 10 x 10 meter plots differed between student samples and personal samples, it is possible that differences between Waypoint data and data from this experiment contrast due to the spatial difference.

While Waypoint Analytical provided data for multiple soil characteristics, only the organic matter percentage (Table 11), phosphorous content (Table 13), and cation exchange capacity (Table 12) were incorporated into this study. For the organic matter content of the soil samples, Waypoint Analytical did not convert this into an estimation of the carbon content. Because it is likely that the laboratory also used an alternate procedure than the one used in this study, the results from Waypoint cannot be directly compared to those found in this experiment. Instead, the overall trends between treatments are compared to those found from this study. For the plots that received a compost amendment, the average organic matter percentage calculated from the Waypoint data was (6.8 +/- 1.753) percent (Table 14). The plots located in the prairie that did not receive a compost amendment had an average organic matter percentage of (5.038 +/- 1.257) percent (Table 14). Lastly, the lawn plots had an average organic matter content of (5.833 +/- 0.784) percent (Table 14). As observed from the data obtained through this study, the same trend is present that the composted plots have the highest average organic matter content. As also seen in previous results from this study, the lawn plots have the second highest average organic matter content and the prairie plots without a compost amendment have the lowest average percentage of organic matter.

The cation exchange capacity was next assessed (Table 12), which assesses the soil's capacity to retain cation nutrients important for plant growth. Following a composted organic matter amendment, it was predicted that the cation exchange capacity would increase to indicate that there was a higher availability of mineral nutrients available to the plant because the soil was capable of retaining these. This relates back to the previously mentioned mycorrhizae, which improves the plant uptake of water and mineral nutrients by the fungal hyphae. The average cation exchange capacity measurement of the plots that received a single compost amendment was (13.475 +/- 2.5138) meg/100g (Table 14). For the prairie plots without a compost amendment, the average cation exchange capacity was (13.213 +/- 8.4019) meg/100g (Table 14). While this value is close to that of the composted prairie plots, the prairie plots without a compost amendment have a much higher standard deviation of 8.4019, indicating that there is uncertainty within this measurement. Lastly, the lawn plots had the highest average cation exchange capacity measurement of (14.50 +/- 5.8570) meg/100g (Table 14). This indicates that the lawn plots have, on average, the greatest potential to store cations within the soil. The lawn plots are located at the bottom of the slope, which has a higher clay content and explains the difference in cation exchange capacity. Uncertainty within the cation exchange capacity of the soils can be attributed to the different composition of the soil samples in regard to their percent composition of sand, silt, and clay. Soil with higher proportions of clay and organic matter will have greater negative charge, meaning they will attract the positively charged particles, or cations [14]. Soil samples with high amounts of organic matter and clay will thus have a higher cation exchange capacity, and it is hard to decipher if this ability to retain nutrients is due to organic matter or clay. Incorporation of soil composition in future studies would assist in deciphering where exactly the large cation exchange capacity measurement is rooted.

The last aspect of the data provided by Waypoint Analytical was the phosphorous content of the soil. Because it was predicted that the composted organic matter amendment would enhance the activity of the fungi, bacteria, and soil microorganisms underground, it was also assumed that this amendment would thus enhance the availability of phosphorous within the soil. The soil microorganisms, specifically bacteria, are able to solubilize inorganic phosphorous to make it readily available for uptake by plants. As part of this holistic approach to analyzing the soil health after a compost amendment, the ability of the soil microorganisms to facilitate the availability of phosphorous for plants was incorporated into this study. The prairie plots that received a single compost amendment had an average phosphorous concentration of (125.50 +/- 48.893) ppm (Table 14). This measurement was associated with a high standard deviation of 48.893 ppm, indicating that there was variation between treatment plots and possible uncertainty introduced into measurements. The prairie plots that did not receive a compost amendment had an average phosphorous concentration of (46.25 +/- 28.019) ppm (Table 14). Again, there is high variation amongst the plots due to this elevated standard deviation measurement. Lastly, the lawn plots had an average phosphorous content of (70.50 +/- 21.427) ppm (Table 14). The standard deviation of this measurement is lower, but still relatively high in comparison to the average phosphorous measured. The compost amended prairie plots by far had the highest concentration of phosphorous in the soil, indicating that there were possibly more soil microorganisms present or there was a higher amount of phosphorous being solubilized due to the activated soil microbes following the organic matter amendment. The composted plots had the highest availability of phosphorous, meaning the plants in these plots had greater access to this macronutrient.

6.4 Waypoint Analytical Statistical Analysis Interpretation

6.4.1 Organic Matter Percentage

To assess the statistical significance between treatments from the Waypoint Analytical data, an unpaired two-tailed t-test was again used from the same GraphPad Statistical Software. The same parameters previously studied were again analyzed for the Waypoint data. The statistical analysis done for the Waypoint data was with the organic matter percent measured across the three different treatments. When comparing the compost amended plots to the prairie plots without a compost amendment (Table 15), a p-value of 0.0365 was calculated. This p-value is less than 0.05, meaning that the difference in organic matter content between composted and non-composted plots is statistically significant. The uncertainty in this difference measurement, calculated by the standard error of difference, was 0.763. The 95% confidence interval of the difference ranged from 0.13 to 3.40. Because this range does not contain the value zero, the null hypothesis that there was no difference in the mean organic matter content between these two treatments is thus rejected. As found in replication four from this experiment, the difference in organic matter content between the composted plots and prairie plots that did not receive a compost amendment is considered statistically significant. This agrees with the originally stated hypothesis that a single compost amendment would boost the carbon sequestration, and thus the organic matter content of the soil.

Next, the compost-amended prairie plots were compared to the lawn plots to analyze the difference in average organic matter content (Table 15). The calculated p-value for this comparison was 0.2348, meaning that there is a 23.48% chance that the organic matter averages between these two treatments overlap. Being that this percentage is greater than 5%, the difference in organic matter content between the compost amended plots and lawn plots was not statistically significant. The uncertainty in this measure of difference for this comparison of treatments was 0.773. Finally, the 95% confidence interval of difference ranged from -0.72 to 2.65, meaning that the actual population difference between these treatments is within this range. Because the p-value is greater than 0.05, we failed to reject the null hypothesis that there is no difference between means. The difference in organic matter content of the compost amended prairie plots and lawn plots is thus not statistically significant.

The average organic matter content of the prairie plots without a compost amendment was then compared to the average organic matter content of the lawn plots (Table 15). The p-value for this comparison was 0.1995, which is higher than 0.05 and is thus not a statistically significant difference. An 80.05% still exists that there is a difference in the average organic matter percentage between the non-composted amended prairie plots and the lawn plots. The uncertainty in this measurement of difference between treatments is 0.586. The 95% confidence interval of difference indicates that the actual average population difference between the non-composted prairie plots and lawn plots is between -2.03 and 0.48. Because this confidence interval of difference contains the number zero, we fail to reject the null hypothesis that there is no difference between the average organic matter content of these treatments.

6.4.2 Cation Exchange Capacity

The next statistical analysis was to compare the cation exchange capacity differences between treatments. The compost amended prairie plots were compared to the non-composted prairie plots (Table 16). The p-value for this comparison was 0.9337, an extremely high p-value that indicates that the difference between treatments is not statistically significant. There is only a 6.63% chance that the means for these two treatments do not overlap, which is extremely low. The uncertainty of the measurement of difference for this replication was 3.101. The 95% confidence interval of the difference was -6.39 to 6.91. This means that the true difference of the population means between these treatments is within this range. The difference in average cation exchange capacity between the compost amended plots and non-composted prairie plots was not statistically significant.

The cation exchange capacity differences between the compost amended plots and lawn plots was next compared (Table 16). The p-value from the two-tailed t-test between these two treatments was 0.6624, which is significantly greater than the necessary p-value of 0.05 to conclude that the difference is statistically significant. A p-value of 0.6624 indicates that there is only a 33.76% chance that the means of these two treatments no not overlap. The measurement of uncertainty in the difference between these two treatments was 2.29. The confidence interval of the difference indicated that there was 95% certainty that the true population mean differences between these two treatments was between -6.01 and 3.96. Because this confidence interval contains the number zero, we failed to reject the null hypothesis that there was no difference in the average cation exchange capacity between the compost amended plots and the plots located in the lawn area.

The final comparison made for the cation exchange capacity averages was between the non-composted prairie plots and the lawn plots (Table 16). For this statistical analysis, a p-value of 0.7544 was found. This p-value signifies that there is a 75.44% chance that the mean cation exchange capacity of the non-composted prairie plots and lawn plots overlap, which greatly exceeds the necessary 5% value to conclude that the difference in mean values is statistically significant. The uncertainty measurement of this difference calculation is 4.022. The 95% confidence interval calculated by the statistical software produced a range of values from -10.05 to 7.48. This means that the true difference in population cation exchange capacity averages is within this range, which is a range with a relatively large span of values. The difference between average cation exchange capacity between non-composted prairie plots and lawn plots is not statistically significant, and there is a considerable amount of uncertainty within this statistical calculation.

6.4.3 Phosphorous Concentration

The final statistical analysis executed for the Waypoint data was with the phosphorous content found from the soil samples. The phosphorous content between the compost amended plots and non-composted prairie plots (Table 17) was the first comparison executed in this section. The p-value calculated in this statistical analysis was 0.0014, which is an extremely low value indicating the difference in phosphorous content between these two treatments was very highly statistically significant. There is only a 0.14% chance that the averages in phosphorous content of these different treatments overlap. This indicates the compost amended plots have a notably higher content of phosphorous available for plants. The uncertainty of the difference, the standard error of difference, was calculated to be 19.924. The 95% confidence interval of the difference in phosphorous content ranged from 36.52 to 121.98. This means that there is 95% certainty that the actual population difference between average phosphorous content between compost amended and control plots is within this range. From this statistical analysis, the null hypothesis that there is no difference between the means is rejected, as this difference in phosphorous concentration between treatments is considered statistically significant.

Next, the phosphorous concentration of the compost amended plots and lawn plots was compared (Table 17) with the statistical software. The two-tailed p-value produced was 0.0251, a value lower than the value of 0.05 needed to verify that the difference is statistically significant. Again, because the composted plots had such a drastically high concentration of phosphorous in comparison to the other treatments, the null hypothesis that there is no difference between the mean phosphorous concentration of composted plots and lawn plots is rejected. The measurement of uncertainty in the differences between treatments was 21.506. The 95%

confidence interval of the difference ranged from 8.14 to 101.86, which is a range with an extremely wide span. This wide span originates from the high standard deviation values that were found when evaluating the phosphorous content within specific treatments (Table 14). The diversity in plant density and species across the hillside likely affected the phosphorous concentration spatially, which explains why there is such high uncertainty in the measurement of phosphorous. Although there is uncertainty within the statistical analysis, it is still concluded that the difference in phosphorous concentration between composted plots and lawn plots is statistically significant.

The final statistical analysis evaluated the difference in phosphorous concentration between prairie plots without a compost amendment and the lawn plots (Table 17). The statistical software calculated a p-value of 0.1035, a value slightly too high for the differences between these treatments to be considered statistically significant. The p-value still indicates that there is a 89.65% chance that the two means being evaluated do not overlap each other, but a 95% chance is necessary to conclude that the difference is statistically significant. The measurement of uncertainty calculated in this statistical analysis of difference between means is 13.761. The 95% confidence interval was interpreted such that the actual difference in population means is between -54.23 and 5.73. Again the span of this range is extremely wide, which is a result in the large standard deviations found for the phosphorous concentrations. Ultimately, the difference in average phosphorous concentration between non-composted prairie plots and lawn plots is not considered statistically significant, and thus we fail to reject the null hypothesis that there is no difference between means.

6.5 Data Omitted

Aside from the predominant areas of uncertainty in the study that affected the carbon content and soil characteristics measured, there were additional areas to be addressed that introduced uncertainty during the investigation of results. The first being an outlier encountered in replication three of the soil carbon content investigation (Table 4). The carbon percentage measured from soil sample S1G1 was found to be 0.37, which is an extremely low value that would indicate there is organic matter content of the soil is nearly absent. This outlier was not included in the average soil carbon content calculations made in this study (Table 6) because it would offset the interpretation of soil carbon content. The value was also omitted in the statistical analysis executed for replication three results (Table 9). Although this value was rejected from the mathematical calculations completed in this study, the value was still displayed on the ArcGIS visualization of the results (Figure 10). This outlier is likely due to human error during the laboratory procedure for finding the carbon content of soil samples. A low carbon percentage calculation denotes that there was an extremely small difference in the weight of the sample before and after the sample was burned at 700°C. Because this small of a value was not observed in any other soil samples, this specific result was considered an outlier in this study that did not accurately represent the soil carbon content of the particular sample.

Another issue addressed in this experiment was an insignificant amount of soil for the carbon content evaluation. Because the soil samples were collected in the Fall 2015 semester and weren't analyzed until the muffle furnace was available in the Spring 2016 semester, the soil analyzed was limited to the amount originally collected. Due to the variation observed in carbon content between replications and within treatments, more replications were completed than originally intended. By replication four, the amount of soil left was extremely limited, and soil sample S4G4 was unable to be analyzed (Table 5) because it had been expended after the third replication. As a result, the mathematical calculations performed during this experiment had to exclude this sample (Table 6). The statistical analysis executed for each replication also excluded this soil sample (Table 10), and fewer samples in a statistical analysis generate a weaker statistical analysis. This particular sample was also absent in the ArcGIS visualization of results (Figure 11). To avoid this issue in future work, it is recommended that researchers collect samples with a greater volume of soil to thwart a deficiency of soil to be analyzed.

Chapter 7 Conclusion

The results of this study indicate that based on average soil carbon content, the plots that received a singular composted organic matter amendment had the highest percentage of carbon stored within the soil. The composted plots had an average carbon percentage of (5.81 +/- 1.67) (Table 6), compared to an average carbon percentage of (4.49 +/- 1.72) (Table 6) within the control prairie plots that did receive a compost amendment. The composted plots had in increase in carbon sequestration that resulted in over a 1% difference in carbon content than the non-composted control plots located in the prairie. After only six months between compost application and soil sampling, this difference in carbon content between the composted and non-composted plots is significant and indicates that the compost amendment successfully boosted the capture and sequestration process of carbon by the plants on the hillside.

The average carbon percentage of the plots located in the lawn was (5.02 +/- 1.42) (Table 6), which was higher than the average carbon content of the non-composted prairie plots due to the high root abundance of the grass area. The composted plots had slightly less than a 1% increase in carbon content compared to the plots in the grass area. Because the grass area plots served as a comparison between the naturalization hillside and managed grass area, the results indicate that a composted, natural prairie can enhance the carbon sequestration within the soil. To heighten soil carbon sequestration, optimal conditions consist of a naturalized prairie with the addition of a single compost amendment consisting of organic matter. Implementing this land amendment assists in offsetting carbon dioxide emissions being emitted into the atmosphere from fossil fuel burning for energy resources. Composted organic matter amendments to the soil provide a natural, efficient, cost-effective, and immediate contribution to the resolution of a carbon dioxide saturated atmosphere.

While differences between treatments were observed in terms of average carbon content percentage, these distinctions were not considered statistically significant according to conventional statistical criteria. The only statistically significant difference in average carbon content was found in replication four between the compost amended plots and non-composted prairie plots (Table 10). Aside from this single indication of statistical significance in the difference between treatments, statistical analysis did not indicate that the carbon content of soils was significantly altered following a compost amendment. It is predicted that as more time elapses after the single compost amendment, this difference between treatments will become more apparent and statistically significant. To align with the timeline of the Marin Carbon Project, the carbon content of the soils should be evaluated for at least a three-year period to observe the development of carbon content within the soil. While the difference in carbon percentage of the hillside between treatments is not currently statistically significant, it is probable that as more time elapses, these differences will become statistically significant and align more closely with the results obtained by the Marin Carbon Project.

There was a high level of variation within the data obtained throughout this study, both between replications and within the different treatment results. While uncertainty is unavoidable, these avenues were identified so that in future work the influence it has on the results can be minimized. Possible areas of uncertainty in this study included the soil mixing procedure that was altered after replication two, the high root abundance present within the soil samples, the shallow and rocky conditions of the soil, compaction from the previous dirt road, faulty measurement devices, and human error. By identifying the channels by which uncertainty affects the data, more variables can be included in future work that attempt to quantify this uncertainty and address the degree to which it offsets the data. Being that this was the first year of this study on the hillside, the project was investigative and nature and the sources of uncertainty were not previously identified. Completion of this experiment and data assessment will potentially alleviate some areas of uncertainty and error in the data with future experiments concerning carbon sequestration on the hillside.

Aside from the boost in average carbon content of the soil, Waypoint Analytical data indicated that there were additional benefits acquired by the soil following the organic matter amendment. The average phosphorous content of the compost-amended plots was (125.50 +/- 48.893) ppm (Table 14), in comparison to the control prairie plots that had an average phosphorous concentration of (46.250 +/- 28.019) ppm (Table 14). The phosphorous average of the lawn plots, (70.50 +/- 21.427) ppm (Table 14), was also higher than that of the control plots. The average phosphorous content of the compost-amended plots was statistically significantly different from the non-composted control plots and lawn plots (Table 17), indicating that there was a higher availability of phosphorous for uptake by plants following the organic matter amendment. Because phosphorous is a macronutrient, a greater amount of phosphorous present in the soil reduces the need for fertilizer application to sustain plant growth. Although there was high variability in phosphorous measurements made between the designated plots, the application of organic matter significantly increased the availability of phosphorous to plants such that plant growth could be boosted and carbon sequestration facilitated.

Statistical analysis of the cation exchange capacity data from Waypoint Analytical indicated that the difference between treatments was not statistically significant (Table 16). The cation exchange capacity remained relatively uniform across treatments (Table 14), which agrees with the data from this experiment that there wasn’t a statistically significant difference between average carbon content of treatments. An increase in organic matter content or clay content would boost the cation exchange capacity of soil due to their negative charge. Without a statistically significant difference in carbon content of composted and non-composted plots, the cation exchange capacity of the soil would not be considerably altered. As with the average carbon content of the soil on the hillside, it is predicted that as a greater amount of time passes after the compost amendment, the difference in cation exchange capacity of the soil will become more prominent. It is projected that the cation exchange capacity of the composted plots will increase more than that of the control prairie plots. By introducing a study of the soil composition into future work, differences in cation exchange capacity can be attributed more clearly to either a high content of clay or organic matter.

The organic matter content calculated by Waypoint Analytical (Table 14) indicated that the average organic matter content percentage the composted plots, (6.80 +/- 1.753), was more than 2% higher than the non-composted control plots. This average organic matter percentage was also nearly 1% higher than the average organic matter percentage of the lawn plots. The difference between the composted plots and non-composted prairie plots (Table 15) was by conventional standards, considered statistically significant. This is in unison with the statistical analysis conducted for replication four (Table 10), meaning that there is indication from both this experiment and Waypoint Analytical that the compost amendment to the hillside enhanced the soil carbon sequestration. While there is variation amongst the data obtained from this experiment and Waypoint Analytical, as time elapses and sources of uncertainty are addressed, the difference between treatments is predicted to become more apparent as it did in the Marin Carbon Project following a three-year analysis [10].

The definitive conclusion reached in this study is that a singular compost amendment can serve as an effective land management technique for boosting the average carbon percentage of the soil. To determine the long-term effects of a compost amendment, studies must be maintained that analyze the trend of carbon content within the soil. The results of this experiment indicate that there is upward trend of carbon sequestration on the composted plots, but the difference in soil carbon content between various treatments is not yet statistically significant. With a greater lapse of time between compost amendment and soil sampling, it is predicted that the difference in soil carbon percentage between treatments will become more evident and thus statistically significant. Multiple areas of uncertainty were identified in this study, and it is recommended that future studies incorporate an analysis of these sources of uncertainty so the degree to which they affect the output data are minimized.

Chapter 8 Future Work

This experiment was modeled after the ongoing Marin Carbon Project, which is currently an ongoing study being executed that was initiated in 2008. The research experiment conduced on the ISAT Hillside was started in March of 2015. The time elapsed between the compost application and soil testing under the Marin Carbon Project was roughly three years [10]. Because the period between the compost amendment application and the soil sampling was about six months, this experiment requires greater time and research to follow the timeline of the Marin Carbon Project. This experiment will be continued under the direction of Dr. Wayne Teel to determine if the difference between treatments on the Hillside becomes more apparent over time. Continuation of this experiment will allow researchers to conclude if a greater portion of time between the organic matter amendment application and soil testing accounts for a more observable difference between the composted and non-composted plots. The difference in average carbon content between treatments in this experiment could not be concluded to be statistically significant. With a greater time lapse, it is possible that the difference in soil carbon content between treatments will intensify and thus be considered statistically significant.

In this experiment, there were a number of factors that introduced uncertainty within the data. Soil samples collected often harbored a high root abundance, which alters the weight differences during the burning process. During future work, a more accurate depiction of the soil carbon content can be obtained if a procedure is implemented to remove the root biomass found in soil samples. The sieve shaker mixing technique must be used before each sample is heated in the muffle furnace to ensure the same procedure is implemented across all replications. An identical procedure for each replication allows the results from each replication to be more appropriately compared. Using a smaller sieve size could potentially remove the abundance of roots within soil samples, and if this method fails it is recommended that root fragments be manually removed from the soil sample. Research into procedures for removing root biomass from soil samples would provide researchers with background data and past procedures that were implemented to account for this issue.

Another area of ambiguity within this study is the complication that occurred when collecting soil samples. Due to the shallow and rocky properties of the sample locations, a depth of approximately six inches was utilized when collecting samples. This was the maximum depth obtainable with the equipment used, and soil samples were frequently littered with a large portion of rocks. An ideal sample depth for this study type is eight inches, which is greater than the soil depth obtained in this experiment. A new sampling technique or equipment could overcome the soil sampling difficulties and allow future studies to analyze soil from a depth of eight inches. Enhancing this sampling technique will allow this study to more closely follow previous studies investigating soil organic matter. A sample depth of at least eight inches will additionally grant the researchers with a more accurate depiction of the soil characteristics.

The study site in this experiment previously had a dirt road running parallel to the prairie strips. When the land was purchased by James Madison University, the maintenance department simply planted over the old dirt road. The presence of this road is likely to have highly compacted the soil that it ran over, making it more difficult for vegetation roots to penetrate into the ground. While soil compaction is analyzed in the ISAT 320 Lab, this soil characteristic was not incorporated into this study. This study could be enhanced by delving into the soil compaction on the sample site from the past road, and this factor could possibly contribute to the analysis of carbon content found within the soil. When the carbon content of the plots was spatially analyzed in ArcGIS, having the soil compaction background knowledge would provide the researcher with a greater understanding of any discrepancies within the results.

Aside from recommendations for resolving uncertainties within this experiment, additional study factors can be incorporated into the study to greater understand the effects of an organic compost amendment. For future work concerning this experiment, it is recommended that more samples be collected from each 10 x 10 meter plot to determine if there are spatial differences in the soil carbon content amongst each plot. With more soil samples in total, more samples can be analyzed in the lab to produce a greater volume of results. With more samples analyzed and more replications, the statistical analysis becomes more representative of the results. A greater amount of data reduces the occurrence of random error and generates results that are more representative of the actual soil carbon content. To acquire more soil samples to be analyzed, it is also recommended that a larger team be formed for future studies to make the soil sampling and analyzing procedure simpler and more efficient.

To broaden the scope of this experiment, additional factors can be incorporated into the study. The ISAT Hillside hosts a plethora of vegetation species, and it is possible that the different plant species release carbon at different rates. Previous studies have indicated that compost successfully boosts plant growth, and the extent of this outcome can be analyzed in this study. By documenting the plant diversity and density on the Hillside, this can be compared against the varying soil carbon content values calculated. The incorporation of plant studies into this experiment introduces several variables and reactions to be analyzed. With boosted plant diversity on the Hillside, it is likely that the soil would become more resilient due to enhanced microbial life. With heightened microbial activity, the abundance of nutrients such as phosphorous and nitrogen would likely increase surrounding the plants. A new procedure for analyzing soil microbe activity would have to be researched and incorporated into this study to allow future work to address the effect of plant species and microbial diversity. In addition, a procedure would have to be implemented to allow researchers to calculate the amount of different nutrients within the soil to connect these different variables. Again, a larger team would make this process more feasible.

The cation exchange capacity of the soil was analyzed using the data provided by Waypoint Analytical. Because organic matter and clay are both negatively charged, they attract these cations, or positively charged particles. The organic amendment was predicted to boost the cation exchange capacity of soil, meaning that the soil would be capable of retaining these mineral nutrients as a source of nutrients for the plants growing. The soil composition was not evaluated in this study, but would provide deeper insight into whether the differences in cation exchange capacity between treatments was due to an abundance of clay particles or organic matter. The cation exchange capacity affects the soil fertility, and thus is necessary to have a high cation exchange capacity due to the presence of organic matter. Without a suitable cation exchange capacity, the soil would be limited in nutrient availability, and would also be inefficient storing these nutrients. Although an appropriate amount of clay is necessary to attract and retain cations, an extremely high clay level would induce anaerobic conditions due to the compaction of the soil and inability for air to exist. Soil can thus have a high cation exchange capacity due to clay, but also have inhabitable conditions for soil microbes and plant growth. There were soil samples collected in this study that were primarily composed of clay, but because soil composition was not evaluated in this study, it wasn't possible to draw connections between clay content and cation exchange capacity. In future studies, soil composition should be determined so that any outliers of cation exchange capacity can be analyzed and determined to either arise from organic matter or clay content.

A final area of study that could be integrated into this experiment would be observing an increase in water holding capacity of the soil. Past studies analyzing the effects of an organic matter amendment have incorporated an analysis of the difference in the water holding capacity of the soil. To boost the extent of this research a procedure to measure the water holding capacity could be replicated from past studies. It was concluded in this experiment that the compost amendment increased the amount of soil aggregates within the soil, thus correlating to a higher proportion of humus and carbon sequestered in the soil. It can then be predicted that with an increase of soil aggregates, the soil becomes more “spongy” and capable of retaining water to enhance soil health and reduce any runoff. Research is necessary to determine efficient means of determining the water holding capacity of soil. Adding this aspect into the study would extend the scope of the project beyond means by which the compost amendment reduces carbon dioxide emissions in the atmosphere. It would thus serve to incorporate a greater understanding of the boost in soil health that is correlated to an organic matter amendment.

Chapter 9 Unintended Consequences

A possible unintended yet positive consequence of this study involves the potential for this project to impact the landscape management techniques of JMU facilities. Boosted plant growth and water holding capacity of the hillside following the compost application likely enhances the hillside's resistance to erosion and runoff. The decreased runoff in turn reduces the amount of pollution entering the stream at the bottom of the hillside. The overall health of both the hillside and stream are thus boosted following the compost amendment. While this was not an area of study within this project, this lessens the burden for the university facilities management to maintain the health of the hillside and stream because the water is naturally filtered as retained in the soil as it travels down the hillside. Aside from the positive environmental outcomes, this would also assist in reducing the cost of landscape and water quality management.

Finally, an additional positive unintended consequence would be the reduction of food waste stemming from James Madison University. Because the compost in this study was partly composed of food waste from the university, the amount of food being deposited in landfills was decreased. Gases emitted from landfills are about 50 percent methane and 50 percent carbon dioxide and water vapor, as well as minute amounts of nitrogen, oxygen, hydrogen, other organic compounds, and trace amounts of inorganic compounds [15]. By decreasing the proportion of food going to landfills, this project has the unintended consequence of decreasing the amount of landfill gases being emitted into the atmosphere. Not only is the organic compost amendment directly offsetting atmospheric carbon dioxide emissions, but by decreasing the volume of food being stored in landfills this project also has indirectly diminished atmospheric pollution from landfills.

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