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Assessing Impacts of Biochar on Microbial Community Structure, Fermentation Intermediates, and Nutrient Degradation in Manure Lagoons

A Thesis Presented by Courteney Hales
to the Faculty of the College of Engineering and Mathematical Sciences
of the University of Vermont

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Abstract

Manure management practices have the capacity to impact greenhouse gas emissions and promote climate resilient food systems. Previous studies have found that using biochar as a manure lagoon cap is effective for reducing odor and ammonia emissions, while simultaneously increasing methane emissions. However, it is unknown what other impacts biochar addition could have on manure lagoon sludge. This study assessed the impact of biochar enrichment on microbial community structure, nutrient degradation, and fermentation intermediates in manure lagoons. Additionally, analysis of these variables may provide insights into how biochar-enriched manure might enhance crop rhizosphere health, when land applied as a fertilizer. Six bioreactors were constructed; three were filled with only raw manure and the remaining three contained raw manure enriched with biochar. Headspace gas and manure samples were collected every week, for a total of twelve weeks. Gas chromatography, chemical analyses, and microbial metagenomic sequencing was performed on a range of samples. The results generated from this study suggest that fermentation intermediate concentrations, organic matter degradation, and methanogenic microbial populations are higher in biochar enriched manure samples. These results corroborate with the observed increase in methane emissions after week six of the experiment. One-way ANOVA statistical analyses were performed, where the methanogenic microbial community analysis results were of statistical significance. In the future, this study could be extended to investigate how biochar-enriched manure lagoon sludge impacts crop rhizosphere health and crop growth, when applied as a fertilizer.

Table of Contents

Acknowledgements	2
Abstract	3
Introduction	6
Literature Review and Background	9
Problem Statement	14
Methodology	15
Experimental Overview.....	15
Reactor Set-up.....	16
Manure Collection and Experimental Set-up	17
Sample Collection and Preparation	18
Chemical Analyses	18
Microbial Community Analysis	21
Results and Discussion	22
Headspace Gas Emissions.....	22
Chemical Oxygen Demand (COD)	26
Ammonia ($NH_3 - N$)	28
Volatile Fatty Acids (VFAs)	30
Summary of Headspace Gas, Nutrient, and VFA Transformations	36
Microbial Community Analysis	36
Conclusion	42
Error Discussion	46
Future Work	47
References	48
Appendices	51
Appendix 1: Supporting Images.....	51
Appendix 2: Unprocessed Data.....	54
Appendix 3: Processed Data	63
Appendix 4: Microbial Community Analysis Codes	68

List of Figures

Figure 1: Average Headspace Carbon Dioxide Concentration Experimental Variation between Treatments.....	23
Figure 2: Average Headspace Methane Concentration Experimental Variation between Treatments.....	25
Figure 3: Variation in Treatment Bioreactor’s Average sCOD Concentrations (mg/L) from Week 1 and 12	27
Figure 4: Variation in Treatment Bioreactor’s Average Ammonia Concentrations (mg/L) from Week 1 and 12	29
Figure 5: Variation in Treatment Bioreactor’s Average Acetate Concentrations (mg/L) from Weeks 1, 6, and 11	31
Figure 6: Variation in Treatment Bioreactor’s Average Propionate Concentrations (mg/L) from Weeks 1, 6, and 11	33
Figure 7: Variation in Treatment Bioreactor’s Average Butyrate Concentrations (mg/L) from Weeks 1, 6, and 11	34
Figure 8: Heatmap of Variation in Treatment Bioreactor’s Abundant Phylum from Week 12 ..	37
Figure 9: Heatmap of Variation in Treatment Bioreactor’s Abundant Genera from Week 12 ...	38
Figure 10: Heatmap of Variation in Treatment Bioreactor’s <i>Methanocorpusculum</i> Genus for Week 12	41

Introduction

The global climate crisis due to anthropogenic activities is occurring at an unprecedented rate and poses a major challenge to environmental health and human security. The major driver of the climate crisis is the emission of greenhouse gases (GHGs) from human activities. The five key GHGs are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), chlorofluorocarbons, and water vapor – and their atmospheric concentrations are increasing (NASA, 2023). The planet is already experiencing the severe effects of global climate change – such as melting glaciers, intense heat waves, wide-scale droughts, wildfires, and extreme flooding (NASA, 2023). Combating climate change requires not only addressing all major GHG-producing activities, but also understanding how to engineer more sustainable and resilient human systems. In order to engineer these systems, we must consider the interconnected relationship between the water-energy-food nexus and the climate crisis. There is a reciprocal relationship between climate change impacts and society's food systems: food systems emit GHGs and climate change impacts humankind's ability to produce food.

Rapidly growing populations and changing diets are increasing the demand for food. Production is struggling to keep up as crop yields level off, natural resource availability is stretched thin, and soil health is diminished. A 2020 study found that 8.9% of the global population lacks access to adequate quantities of food (FAO, 2020). This food security challenge is intensified by the agricultural system's extreme vulnerability to climate change. Climate change impacts are already being felt in the form of increasing temperatures, shifting agroecosystem boundaries, invasive crops and pests, and more frequent extreme weather events (World Bank, 2021). On farms, there has been an overall reduction in crop yields, quality of major cereals, and livestock productivity. It is recommended that substantial investments in adaptation will be required to

achieve production and food quality increases to meet future demand (World Bank, 2021). However, the problem also works in reverse as global agricultural systems are known to be responsible for 19- 29% of total GHG emissions (World Bank, 2021). Feeding a growing planet while fighting climate change will require rethinking how food and its byproducts are managed. Climate-smart Agriculture (CSA) is an integrated approach for managing landscapes that addresses the interlinked challenges of food security and accelerating climate change (World Bank, 2021, “Achieving the Triple Win of CSA” section). The CSA aims to achieve three goals: increase food productivity, enhance resilience, and reduce GHG emissions (World Bank, 2021). However, strategies for reducing GHG emissions from food systems are underdeveloped and underutilized.

The United States has a robust agricultural system that produces nearly \$330 billion per year in agricultural goods. This sector ensures a reliable food supply and supports job growth and economic development, therefore playing a critical role in the U.S. and global economies (U.S. EPA, 2021). However, the United States agricultural sector accounted for approximately 11% of 2020 greenhouse gas emissions, according to the United States Environmental Protection Agency (EPA) (EPA, 2020, “Overview” section). Of this, manure management practices account for 12% of the total GHG emissions from the agricultural sector in the United States (EPA, 2020, “Agriculture Sector Emissions” section). Using manure as a soil amendment is a common manure management practice. Some studies suggest that this could result in increased microbial biomass, which subsequently heightens soil microbial diversity and improves soil health (Velis, 2021). Microbial activity in the rhizosphere is essential for nutrient cycling – which is essential for crop growth. Therefore, developing sustainable manure management techniques is essential for reducing GHG emissions and creating more climate-resilient food systems across the United States and world.

The EPA suggests two ways to reduce CH_4 and N_2O emissions from manure storage: capturing methane from manure decomposition to produce renewable energy through anaerobic digestion or controlling the way manure decomposes within manure storage lagoons (EPA, 2020, “Agriculture Sector Emissions” section). Anaerobic digestion involves the construction of reactors that can convert the manure into biogas through an anaerobic microbial process and then combust the biogas to generate heat and electricity. Anaerobic digestion captures methane rather than releasing it to the atmosphere, but it requires large investments that often prohibit small farms from taking advantage of the technology. On the other hand, developing sustainable techniques for controlling the way manure decomposes within storage lagoons, could prove to be a more economical solution for small farms.

Biochar has emerged as a potential solution to a multitude of agricultural challenges, including improving soil health and reducing emissions from manure lagoons. Further, biochar has been used to increase methane production and subsequent energy recovery in anaerobic digestion. Biochar is similar to charcoal, and it is produced by the burning of biomass in the absence of oxygen. A few studies have been conducted on the use of biochar as a cap in manure lagoons with some evidence supporting odor reduction, increased GHG emissions, and reduction in ammonia (NH_3) emissions (Maurer et al., 2017; Dougherty et al., 2016). While this is not promising for the purpose of GHG emission reduction, it is useful for reducing ammonia emissions which are responsible for negatively impacting human and animal health, while also damaging ecosystems (Teagasc, 2020).

In regard to the creation of more climate-resilient food systems with improved nutrient cycling and microbial soil health, the land application of manure can be an appropriate management technique. It is known that managing organic matter, crop rotation, and other

activities is fundamental to promoting a healthy ratio of organic matter consumption. The application of manure helps stimulate microbial activity – improving soil aggregation – which results in increased water infiltration and resistance to erosion (Wortmann et al., 2020). Although biochar is generally used as a soil amendment for carbon storage, it is unknown how biochar impacts nutrient cycling and microbial communities in manure.

This research will build on existing biochar research by assessing the impacts of biochar enrichment on microbial community structure, nutrient degradation, and fermentation intermediates within manure lagoon sludge, prior to land application. The ultimate goal of this research is to gain a better understanding of how biochar impacts manure lagoon sludge. With improved understanding of biochar's impacts on manure, we can more readily assess its potential impacts on GHG emission, land application, crop rhizosphere health, and subsequent food system resilience.

Literature Review and Background

Biochar has a structure with lots of empty pore space; although the exterior surface appears small, the interior surface area is vast. One study suggests that the exterior surface area of biochar can vary from 23 to 46 $m^2 g^{-1}$, the interior surface area can vary from 0.6 to 1.4 $m^2 g^{-1}$, and the micropore volume can vary from 0.008 to 0.009 $cm^3 g^{-1}$, depending on the type of biochar (Sari et al., 2014). In addition to the storage capacity, the surface of biochar also contains surface functional groups, creating a lot of probable binding and reaction sites. This gives biochar the ability to attract and absorb a variety of chemicals, including nitrogen, phosphorous, and potassium ions. Studies have also revealed that biochar produced at different temperatures and with varying methods can have distinctive properties (Zhang et al., 2018). However, research is still being done

to fully understand the chemical and physical properties of biochar and mechanisms of its remediation properties remain unknown.

There have been a multitude of studies investigating biochar for manure management (Graves et al., 2022). Biochar has also been used in manure lagoons as a cover/cap to reduce odor production and gas emissions. One study tested non-activated biochar for swine manure treatment and mitigation of ammonia, hydrogen peroxide, odorous Volatile Organic Compounds (VOCs), and greenhouse gasses (Maurer et al., 2017). This pilot-scale experiment tested the effects of time and dosage of biochar application on gaseous emissions from deep-pit swine manure storage. The results suggested that biochar application as a cover resulted in significant reductions in ammonia (NH_3) emissions (12.7 – 22.6% reduction as compared to the control). Conversely, the methane (CH_4) emissions significantly increased by 22.1 to 24.5% (Maurer et al., 2017). Another study focused on the use of biochar to reduce gas emissions and odor from a dairy manure lagoon (Dougherty et al., 2016). This experiment tested two different types of biochar; one made from Douglas-fir chips pyrolyzed at 650° F (FC650) and the other from Douglas-fir hog fuel pyrolyzed at 600° F (HF600). The results suggested that the HF600 biochar cover reduced the mean headspace ammonia concentrations by 72 to 80%. No significant reduction in ammonia concentrations were found using the FC650 bio-cover. Additionally, nutrient uptake ranged from 0.21 to 4.88 mg N g^{-1} biochar and the odor reduction study produced positive results (Dougherty et al., 2016). This study also emphasized that the methods for applying and removing the biochar covers should be further investigated.

Biochar has also been tested in anaerobic digestors with a multitude of waste sources. One study analyzed the effect of biochar addition on microbial community and methane production during anaerobic digestion (AD) of food wastes (Sugiarto et al., 2020). The study was conducted

using pine sawdust biochar, and daily biogas samples were measured using the volume displacement method which allowed for analysis of methane concentrations. The results revealed that compared to the control without biochar addition, the addition of raw biochar significantly increased the methane production yield by 46.9% (Sugiarto et al., 2020). Volatile Fatty Acid (VFA) and microbial analysis was also done, and the results suggested that the iron (Fe) containing minerals in biochar enhanced VFA degradation – improving methane production by anaerobically digested food waste (Sugiarto et al., 2020). Another study investigated the impacts of different biochar types on the anaerobic digestion of sewage sludge (Zhang et al., 2019). In this study 9 types of biochar generated from three different feedstocks were tested on AD sewage sludge. The results found that all types of biochar used in the test significantly increased methane production, with the maximum cumulative methane yield being 218.45 L per kg VS – obtained from the corn straw biochar pyrolyzed at 600° F which exhibited the largest specific surface area (Zhang et al., 2019). It was also suggested that biochar addition enhanced AD stability by increasing buffering capacity, releasing VFA accumulation and alleviating ammonia inhibition. Simultaneously, microbial community analysis revealed that biochar treatment facilitated acetoclastic methanogens compared to the hydrogenotrophic methanogens (Zhang et al., 2019). Overall, the use of biochar in AD systems showed a significant increase in methane emissions and subsequent increase in possible energy production.

Lastly, biochar has also been used as a soil and compost amendment. One study investigated the long-term effect of biochar on yield-scaled greenhouse gas emissions (YSGE) in a rice paddy cropping system in south China (Qin et al., 2016). The experiment was a four-year field study using the static chamber gas chromatography method. The results indicated that long-term application of biochar significantly decreased methane (CH_4) and gross greenhouse gas

emissions (Qin et al., 2016). Another study focused on the optimization of food waste compost with the use of biochar (Waqas et al., 2017). The experiment was done with two types of biochar produced from lawn waste at different temperatures and applied at rates of 10 and 15% (w/w) of the total waste to an in-vessel compost bioreactor. The study specifically looked at acceleration of the degradation and mineralization rates of food waste compost. The results highlighted that biochar significantly improved the composting process and physiochemical properties of the final compost (Waqas et al., 2017). Lastly, a review paper provides insights into the impact of biochar on chemical characteristics and microbial community structure during composting of organic waste (Wu et al., 2017). The article review highlighted that composting is an environmentally friendly technology for treating and recycling a wide range of organic wastes, but it still has some problems to solve – biochar has been demonstrated as an efficient amendment for composting due to its positive role in reducing GHG emissions and nitrogen losses, accelerating the decomposition and humification of organic matter, and promoting microbial activity (Wu et al., 2017). Overall, the use of biochar in composting tends to decrease GHG emissions and improve the properties of the final compost. Additionally, the use of biochar as a soil amendment tends to reduce methane emissions.

Based on the above literature review it has become evident that, while previous studies have shown positive results for biochar reducing methane emissions within soil and compost systems (both of which are aerobic), this is not the case for manure lagoon or anaerobic digestion systems. It is also evident that biochar addition as a cap in manure lagoon systems, generally decreases ammonia emissions and increases nutrient uptake by the biochar. The studies performed on anaerobic digestion systems generally concluded that the presence of biochar enhanced VFA

degradation, which ultimately increased methane emissions. They also concluded from microbial analysis that biochar enhancements facilitated acetoclastic methanogens.

It may be known that the use of a biochar cap/cover over manure lagoons increases methane emissions and nutrient uptake, as well as decreases ammonia emissions and odor. However, it is not fully understood how mixing/incorporating biochar into manure lagoon sludge impacts the nutrient cycling, VFA presence, and microbial community structure over time. The analysis of these three elements can be integrated to assess how biochar might impact methane emissions, ammonia concentrations, and odors. Additionally, it could aid in the understanding of what microbial communities and nutrients are present in the biochar-enriched manure, that could be useful later for crop rhizosphere health.

Within a manure lagoon, an anaerobic environment is created that can result in the production of CH_4 . Methane production relies on a 4-step process. In the first step, known as hydrolysis, complex organics are broken down into their constituent units. Secondly, during acidogenesis, hydrolysis products undergo fermentation and are converted to a variety of acids and alcohols along with H_2 and CO_2 . Third, during acetogenesis, fermentation intermediates are converted to acetate. Lastly, a process known as methanogenesis occurs whereby H_2 , CO_2 , and acetate are converted to CH_4 . For further clarification refer to the diagram of an anaerobic digestion food web (Appendix 1, Figure 1). Volatile Fatty Acids (VFAs) – such as acetate, propionate, and butyrate – are key fermentation intermediates and identifying these can be helpful with determining how biochar might be affecting the manure lagoon fermentation processes and subsequent gas emissions.

As described above, the analysis of nutrient availability and microbial community structure can be integrated to assess how biochar-enriched manure could be useful for crop rhizosphere

health. In general, the carbon content of manure helps increase microbial biomass and soil respiration rates by acting as a feed source for native soil microorganisms (Velis et al., 2021). By adding manure as a fertilizer, the diversity of bacterial populations in the soil increases (Graham et al., 2020). These organisms play an essential role in nutrient cycling, making nutrients more readily available for plants, and improving the physical properties of soil. The presence of microorganisms expedites the breakdown of organic substances and mineralizes organic nitrogen and phosphorus within manure, transforming it into a plant-available form of nutrients (Velis et al., 2021). Therefore, by analyzing the microbial communities present and nutrient degradation, it would aid in assessing the impact biochar-enriched manure could have on future crop rhizosphere health.

Problem Statement

The major aim of this research is to determine if biochar addition in manure lagoon sludge changes the microbial community structure compared to that of manure lagoon sludge without biochar. The sub hypotheses for this research are as follows:

- (1) Volatile fatty acid (i.e., fermentation intermediate) concentrations are higher in liquid manure samples after addition of biochar.
- (2) Organic matter degradation is higher in liquid manure samples after addition of biochar and releases higher amounts of nitrogen than samples without biochar.
- (3) Methanogenic microbial populations are higher in enrichments with biochar than without biochar.

Methodology

Experimental Overview

The experiment used six custom-built bioreactors: three containing manure sludge with biochar (10% w/w) and the other three containing only manure sludge (control). Each bioreactor contained a liquid sampling, headspace (gas) sampling, and venting tube (See Appendix 1.2 for photos of reactors). The bioreactors were placed on a shaker table for the entirety of a 12-week long experiment to ensure homogeneity and avoid settling. Every week a manure sample (2 ml) was collected from each bioreactor using the liquid sampling tube and a syringe. The samples were then mechanically separated into their supernatant and biomass constituents using a centrifuge. Once centrifuged, the supernatant was pipetted into a separate tube (~ 2 ml) and stored in a -20°C freezer until samples are analyzed. The biomass samples left over were stored in a -80° C freezer until samples are analyzed. Additionally, every week a gas sample was taken from each reactor using the headspace sampling tube and a syringe and immediately analyzed using gas chromatography with a thermal conductivity detector (GC-TCD; Shimadzu GC2030). The gas concentrations in the control and biochar-treated samples were compared using one-way ANOVA analysis.

After the 12-week period, the stored biomass samples were analyzed using metagenomic DNA sequencing. The microbial communities were compared based on metagenomic analysis with Nanopore generated sequences. Heatmaps of abundant genera and phyla were compared to identify differences in microbial communities between the treatments. The stored supernatant samples undergo VFA concentration determination using gas chromatography – mass spectrometry (GC-MS; Shimadzu GC2030 + TQ8000), Chemical Oxygen Demand (COD; Hach Colorimetric High

Range Test Kit) testing and Ammonia ($NH_3 - N$; Hach Cyanurate and Salicylate Test Kit) testing. The data were statistically analyzed to test the hypotheses. The COD and $NH_3 - N$ concentrations in the control and biochar-treated samples were compared using one-way ANOVA analysis. Using these results, the impacts of biochar on VFA production, ammonia release, and the microbial community populations during manure storage were assessed.

Reactor Set-up

The bioreactors that were used in this experiment were constructed by hand, but their constituent parts were purchased online. The bioreactor containers used were 2L wide mouth, round, LDPE, lab quality Nalgene bottles with PP closure caps. In order to install the sampling tubes, the reactor lid was drilled-out with approximately four 8 mm holes – specifically for a vent tube, gas tube, and two liquid tubes. The sampling tubes installed within the reactors were borosilicate glass tubing with an outer diameter of 8 mm and an inner diameter of 5 mm. The glass tubing was cut into the correct dimensions using a metal tube cutter and the rough edges were sanded down for safety. The vent and gas sampling tubes were installed with epoxy to about $\frac{1}{4}$ of the bottle length below the lid. The liquid sampling tubes were installed with epoxy to about $\frac{3}{4}$ of the bottle length below the lid. The specific positioning of the sampling tubes within the bioreactor were decided upon because the manure mixture would be fill up to about half the volume of the bottle (~1 L). This would allow the liquid sampling tubes to be submerged halfway within the manure mixture for the entirety of the experiment and allow the gas sampling tube to be constantly within the reactor headspace.

Once the glass sampling tubes were installed, the plastic sampling tube extensions were connected and fastened, with hose clamps, to all 4 sampling tubes on each reactor. The plastic

sampling tubes used were Cole Palmer C-Flex tubing with an outer diameter of 11 mm and an inner diameter of 8 mm. The plastic sampling tube extensions were used to allow for syringe connections on the liquid and gas sampling ports, as well as for the ability to vent gasses to a fume hood. Once the bioreactor's construction was completed for the experiment, they were all placed in a large plastic tray, which was fastened to a shaker table. See Appendix 1 for photos of reactor setup.

Manure Collection and Experimental Set-up

The manure used in the experiment was collected from the University of Vermont CREAM program dairy farm (the UVM Miller Farm). The manure collected was fresh from the barn on the day of collection and was collected in a 5-gallon bucket using a shovel from their manure pit. Once the manure was collected, the 5-gallon sample bucket was immediately brought to the laboratory.

On the same day as manure collection, the raw manure was transferred from the 5-gallon bucket into the six bioreactors. Approximately 500 g of raw manure was scooped into each bioreactor and an additional 500 ml of water was added. The three control bioreactors were sealed and labeled. Approximately 10% w/w (~100 g) biochar was added and thoroughly incorporated into the raw manure within the other three bioreactors. These biochar-enriched manure bioreactors were then sealed and labeled. All bioreactors were positioned in a large tray atop a shaker table. To initiate the experiment, the shaker table was set to 90 rpm, and the vent tubes were placed inside a nearby fume hood. Some of the remaining raw manure that was collected was transferred into two 500 ml Nalgene bottles and placed in the -20° C freezer. The rest of the raw manure was safely disposed of, and the 5-gallon bucket was cleaned out appropriately.

Sample Collection and Preparation

Manure Sampling

Manure sampling took place every week for a total of 12 weeks. The manure sampling was done through the use of a syringe and the liquid sampling port. Once manure was extracted into the syringe through vacuum action, it was deposited into and filled two 1 ml sample tubes. This was done for each of the six reactors. Once all the samples were collected, they were run in a centrifuge at 10,000 rpm for 10 minutes. This was done to separate the biosolids from the supernatant liquid.

Sample Preparation

Once the biosolids had settled to the bottom of the 1 ml sample vials, the liquid supernatant from the two 1 ml sample vials was pipetted out and into a separate 2 ml vial. These 2 ml vials were then stored in the -20° C freezer until chemical analyses were performed. Once the supernatant liquid was removed from the 1 ml vials, the biosolids remaining in the vials were stored in the -80° C freezer until microbial community analyses were performed.

Chemical Analyses

Headspace Gas Emissions

Gas headspace sampling occurred on the same day, every week of the 12-week experiment. The gasses from each reactor were extracted from the headspace using the headspace port and the GC-TCD compatible gas syringe. The syringe was screwed onto the headspace port and 10 ml of gas was vacuumed out into the syringe. The syringe was then locked

with the gas inside and then transported to the GC-TCD. Finally, the syringe was screwed into the apparatus, unlocked, and the gas was then pushed out into the GC-TCD - the 'run' button was pressed to initiate the gas analysis. After approximately 15 minutes, the run was complete, and the next sample was ready to be analyzed. This procedure was completed for all 6 bioreactors every week. The results of this analysis generated relative gas concentrations recorded in percent of total gas in the sample. The gasses of interest were methane (CH_4) and carbon dioxide (CO_2). The processed results from week 12 were also statistically analyzed using One-way ANOVA analysis, generated through R. This was done to determine if there was a statistically significant difference between the control and biochar-enriched bioreactor samples during the last week of the experiment.

Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is the amount of dissolved oxygen that must be present to oxidize chemical organic materials (HACH). High levels of COD indicate that, if the substrate was discharged into water, it can easily deplete dissolved oxygen in the water – leading to negative environmental and regulatory consequences (HACH). In the context of this research, “total COD” represents organic material in the dairy farm manure. However, the “soluble COD” is what is actually measured within the supernatant samples. The COD concentrations of week 1 and week 12 samples were measured in triplicate using a High Range (HR) HACH Test ‘N Tube (TNT) kit. The range of the test kit is 2 to 1500 mg/L COD. The standard testing procedure relies on USEPA approved methods for wastewater analyses – Methods 410.4 and 8000.

In general, this procedure calls for the preparation of Potassium Hydrogen Phthalate (KHP) calibration standards. These standards have known COD concentrations and are used to create a calibration curve, by plotting absorbance against concentration. The standards and

samples are analyzed using a spectrophotometer at 620 nm. The calibration curve then allows for regression analysis of the samples with unknown COD concentrations. The processed results from week 12 were also statistically analyzed using One-way ANOVA analysis, generated through an R code. This was done to determine if there was a statistically significant difference between the control and biochar-enriched bioreactor samples during last week of the experiment.

Ammonia ($NH_3 - N$)

Ammonia is a colorless, pungent gaseous compound of hydrogen and nitrogen, that is highly soluble in water. Ammonia is formed naturally as a product of the microbiological decay of nitrogenous organic matter, such as animal and plant protein (HACH). Ammonia in groundwater is normal due to microbial processes, however its presence in surface water usually indicates domestic pollution. Excess ammonia can damage vegetation and is highly toxic to aquatic life (HACH).

Ammonia is important in agricultural food systems because plants cannot fix nitrogen directly from the atmosphere, and rather they rely on nitrogen-fixing bacteria to convert nitrogen into ammonia. Ammonia can then be used by plants to create other essential organic molecules needed by complex organisms (HACH). To enhance this natural process, ammonia is often added to fertilizers – hence the use of manure lagoon sludge for its high concentration of ammonia.

The ammonia concentrations of week 1 and week 12 supernatant samples were measured in triplicate using an ammonia/ammonium HACH test kit, which includes a combination of “powder pillows” (containing all reagents) and TNT vials. The range of the test kit is 0.01 to 0.5 mg/L $NH_3 - N$. The standard testing procedure relies on USEPA approved methods for

wastewater analyses – Methods 350.1 and 8000. This test method relies on the Salicylate method.

In general, this procedure calls for the preparation of ammonium chloride (NH_4Cl) calibration standards. These standards have known ammonia concentrations and are used to create a calibration curve, by plotting absorbance against concentration. The standards and samples are analyzed using a spectrophotometer at 655 nm. The calibration curve then allows for regression analysis of the samples with unknown ammonia concentrations. The processed results from week 12 were also statistically analyzed using One-way ANOVA analysis, generated through an R code. This was done to determine if there was a statistically significant difference between the control and biochar-enriched bioreactor samples during last week of the experiment.

Volatile Fatty Acids (VFAs)

VFAs such as acetate, propionate, and butyrate are key fermentation intermediates in anaerobic environments. The VFA concentrations of week 1, 6, and 12 samples were measured in duplicate using the GC-MS. The standard testing procedure relies on USEPA approved methods for anaerobic digester analyses – Methods 5560D. The VFAs of interest were acetic acid, propionic acid, and butyric acid.

Microbial Community Analysis

For microbial community analysis, the stored week 12 and raw manure biomass samples were analyzed using metagenomic DNA sequencing. DNA was extracted from the samples with a Qiagen Power Soil DNA Extraction kit and DNA quality and quantity were analyzed using Qubit and Nanodrop. Metagenomic analysis was conducted with Nanopore generated sequences,

through the use of the SqueezeMeta package on the Vermont Advanced Computing Core (Tamames & Puente-Sánchez, 2018). SqueezeMeta (SQM Tools) is a fully automatic pipeline for metagenomics covering all the steps of analysis. The SQM package and Nanopore-generated data sequence was then run on R to generate heatmaps of abundant genera and phyla. These heatmaps were compared to identify how the microbial communities present compare between the treatments. The specific microbial community of interest was methanogens, who are responsible for the process of methanogenesis and subsequent methane production.

Results and Discussion

Headspace Gas Emissions

Carbon dioxide (CO₂) is a greenhouse gas and also indicates if biological activity is occurring in the manure. The current atmospheric concentration of CO₂ is about 0.05%. In Figure 1 below, the variation in average headspace carbon dioxide gas concentration percentages throughout the experimental period are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 1.

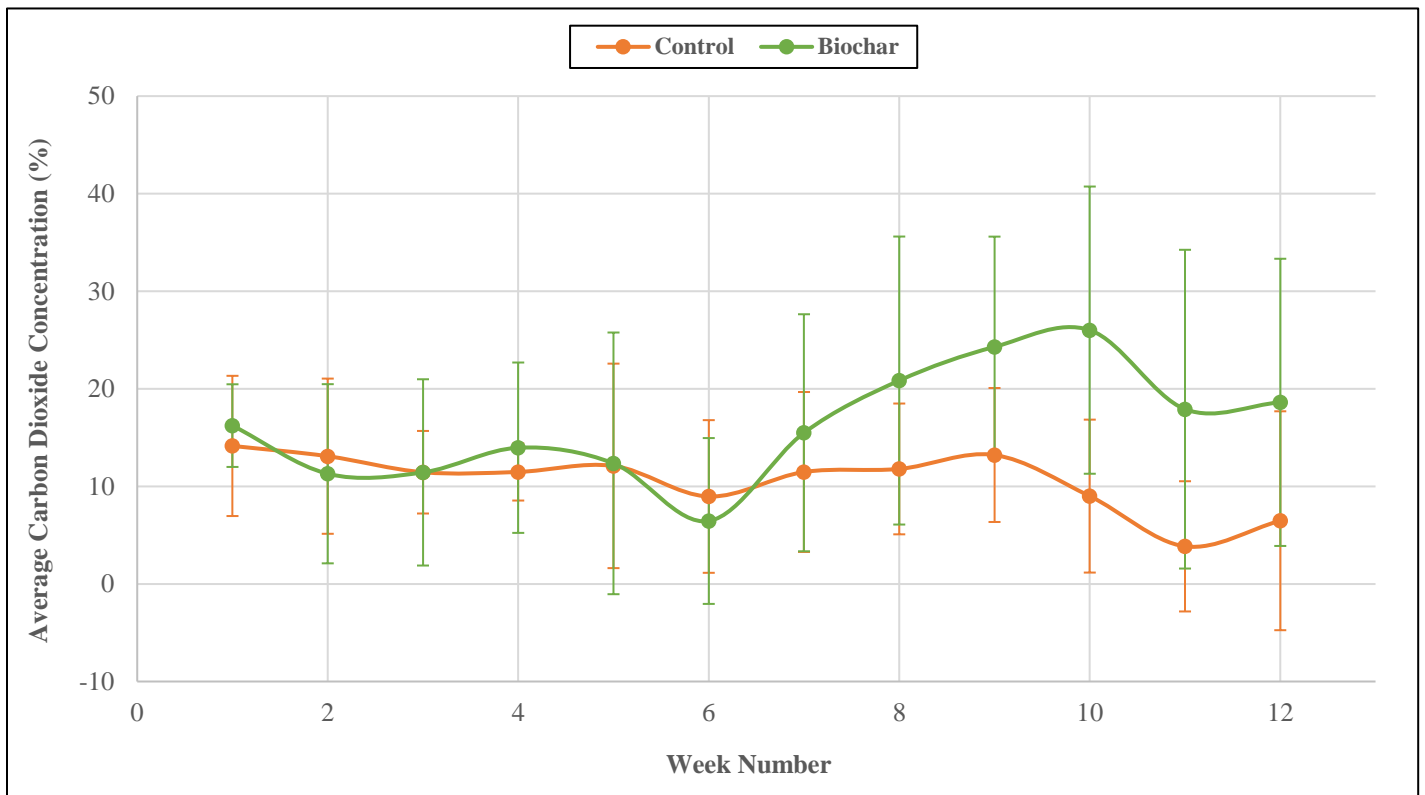


Figure 1: Average Headspace Carbon Dioxide Concentration Experimental Variation between Treatments

When evaluating the CO₂ emissions (Figure 1), it can be seen that there is little variation in carbon dioxide emissions between the biochar-enriched and control bioreactors for the first 6 weeks of the experiment. Concentrations ranged from 6.5% to 25% and were all much higher than atmospheric concentrations, suggesting that there is biological activity occurring in the manure and that organic material is being oxidized. Starting at around the 7th week, the biochar-enriched and control bioreactors CO₂ emissions diverge, and the biochar-enriched bioreactors display higher headspace CO₂ concentrations. During week 10, the treatments show the largest difference in carbon emissions of approximately 17.01%. Although the treatments differ in carbon dioxide concentrations, they seem to show similar general trends. The first 6 weeks show fluctuations in emissions from a maximum of ~16% to a minimum of ~6%. At around week 7,

both treatments show an increasing trend until about week 9 or 10. Then there is a general sharp decrease in CO₂ concentrations until week 11. From week 11 to week 12, the CO₂ concentrations start to increase again. The experiment ended after 12 weeks; therefore, it is unknown how the carbon dioxide emissions would have behaved after week 12.

Based on these results, it is possible that the biochar enrichment influenced the increase in headspace CO₂ gas concentrations from the manure bioreactors. However, it is also important to consider that the different biochar-treated bottles had very different results (see Appendix 2 for unprocessed data). The error bars on most of the data points are quite large, and this is because there was large variation in the gas concentrations between the 3 bioreactors (with the same treatment) and those 3 values were averaged to create the data points displayed. The One-way ANOVA statistical analysis performed on the week 12 data generated a p-value of 0.32, which is greater than 0.05. Therefore, the difference between the biochar-treated and untreated manure is not statistically significant.

Methane (CH_4), being a potent GHG, was also analyzed. Atmospheric concentrations of CH_4 are very small (0.00019%). In Figure 2 below, the variation in average headspace CH_4 gas concentration percentages throughout the experimental period are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 2.

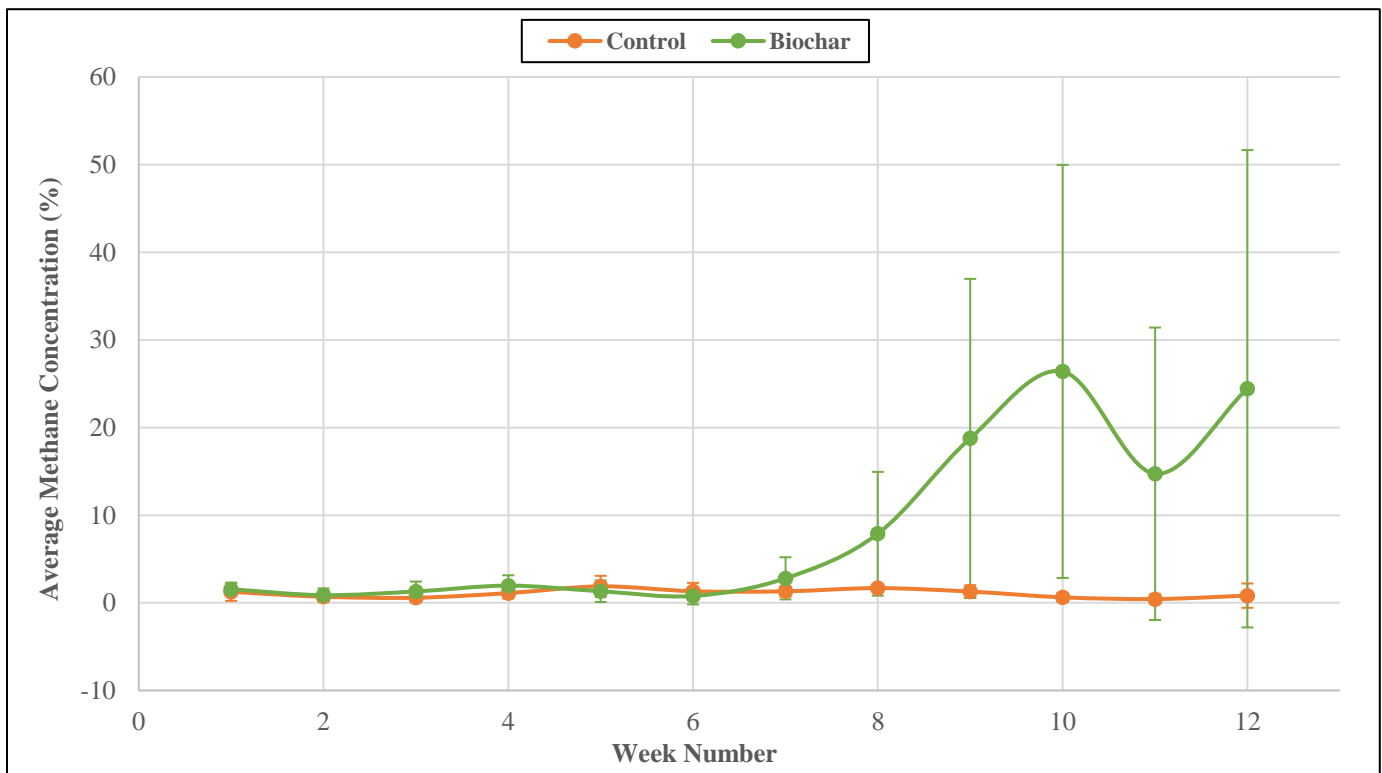


Figure 2: Average Headspace Methane Concentration Experimental Variation between Treatments

When evaluating the CH_4 emissions (Figure 2), it can once again be seen that there is little variation in CH_4 emissions between the biochar-enriched and control bioreactors for the first 6 weeks of the experiment. Starting at around the 7th week, the biochar-enriched and control bioreactors CH_4 emissions diverge, and the biochar-enriched bioreactors display higher headspace CH_4 concentrations. During week 10, the treatments show the largest difference in CH_4 of approximately 25%. Over the first 6 weeks both treatments show fluctuations in

emissions from a maximum of ~ 2% to a minimum of ~ 0.7%. At around week 7, the CH₄ concentrations within the biochar-enriched bioreactor headspaces increases rapidly until it reaches a maximum at week 10 of approximately 26%. From week 10 to 11, there is a sharp decrease to approximately 15%. Lastly, from week 11 to 12, there is a sharp increase again back up to approximately 24%. On the other hand, the control bioreactor continues to fluctuate between ~1.7 % and ~ 0.6% from week 7 to 12. The experiment ended after 12 weeks; therefore, it is unknown how the CH₄ emissions would have behaved after week 12.

Based on these results, it is possible that the biochar enrichment influenced the increase in headspace CH₄ gas concentrations from the manure bioreactors. There was large variation between the bottles with the same treatment, however, indicated by the large error bars from week 7 to 12 are quite large (Figure 2). The One-way ANOVA statistical analysis performed on the week 12 data generated a p-value of 0.208, which is greater than 0.05 suggesting that the impacts of biochar on CH₄ emissions are not statistically significant.

Chemical Oxygen Demand (COD)

Chemical oxygen demand is a measure of bulk organic material. For this analysis, samples were filtered prior to COD measurement so that the data represent the soluble fraction of COD (sCOD). The sCOD can increase as particulate organics are solubilized and decrease as sCOD is converted to gaseous products, such as methane, that leave the reactor. The sCOD data was processed to create bar charts illustrating the difference in sCOD concentrations between the control and biochar-enriched bioreactors during the first and last weeks of the study.

In Figure 3 below, the variation between average sCOD concentrations (mg/L) during weeks 1 and 12 are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 3.

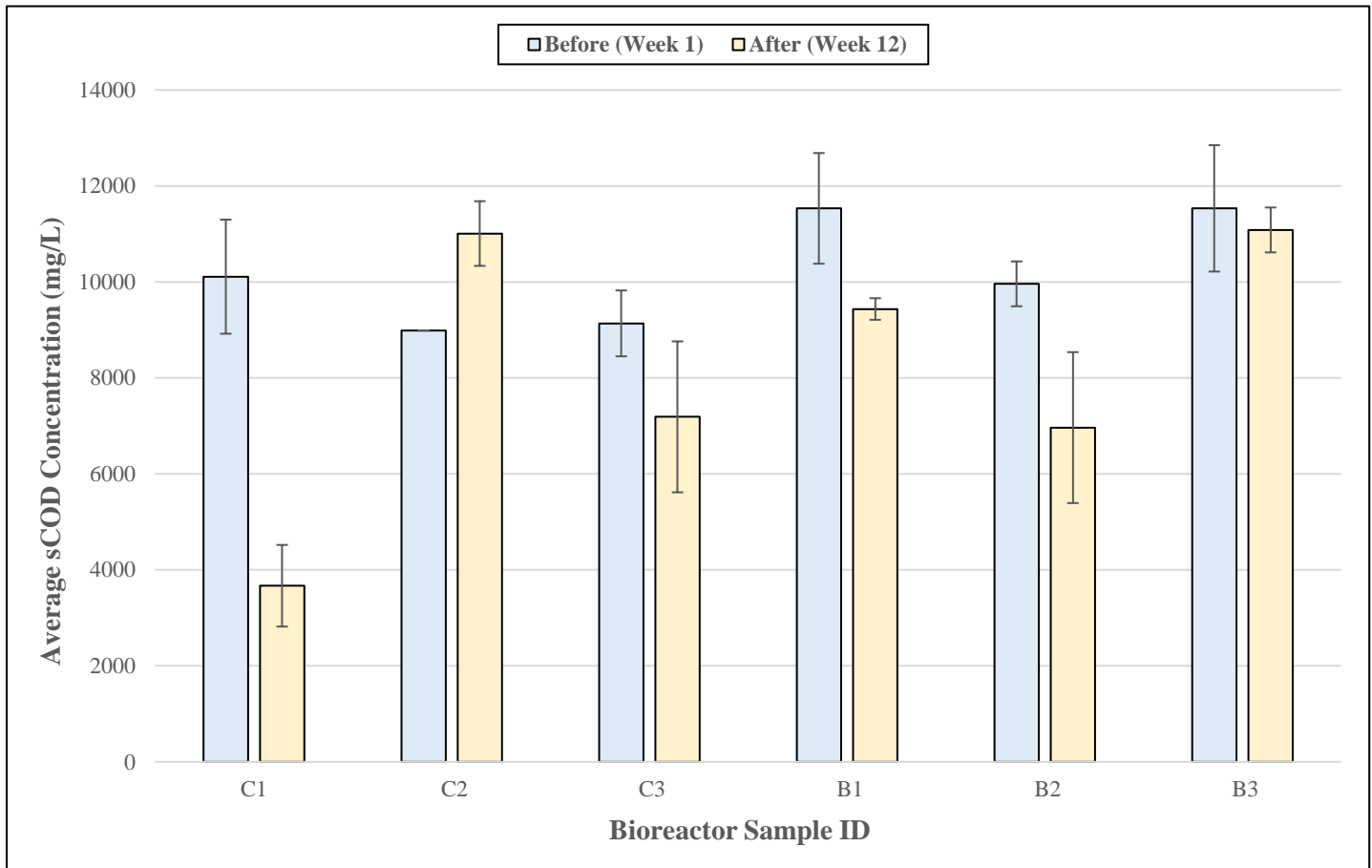


Figure 3: Variation in Treatment Bioreactor’s Average sCOD Concentrations (mg/L) from Week 1 and 12

When evaluating Figure 3 above, it can be seen that there are slight variations in the sCOD concentration between all six bioreactors during Week 1, with slightly higher concentrations in the biochar-enriched bioreactors. When analyzing the change in sCOD concentrations between week 1 and 12, it becomes evident that for almost all 6 bioreactors there is an overall decrease. It is important to highlight that control bioreactor 2 (C2) shows an increase in sCOD concentration, however the week 1 data from this reactor is not reliable. This is

because there was not enough week 1 sample collected from this bioreactor to complete triplicate sCOD tests, therefore only one sample was tested. Considering this and discounting reactor C2 in the analysis, all reactors showed a decrease in sCOD concentrations from week 1 to 12. This general decrease in sCOD concentrations indicates that the sCOD is being converted to gaseous products, such as methane, in both the control and biochar-enriched bioreactors.

The One-way ANOVA statistical analysis performed on the week 12 data generated a p-value of 0.165, which is greater than 0.05. Therefore, the results are not statistically significant and there is no significant difference in the change in sCOD concentrations between the biochar-enriched and control bioreactor samples.

Ammonia ($NH_3 - N$)

Ammonia concentrations can increase as organic matter is degraded and organic nitrogen is transformed to ammonia/ammonium. The ammonia data was processed to create bar charts illustrating the difference in ammonia concentrations between the control and biochar-enriched bioreactors during the first and last weeks of the study. In Figure 4 below, the variation between average ammonia concentrations (mg/L) during weeks 1 and 12 are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 4.

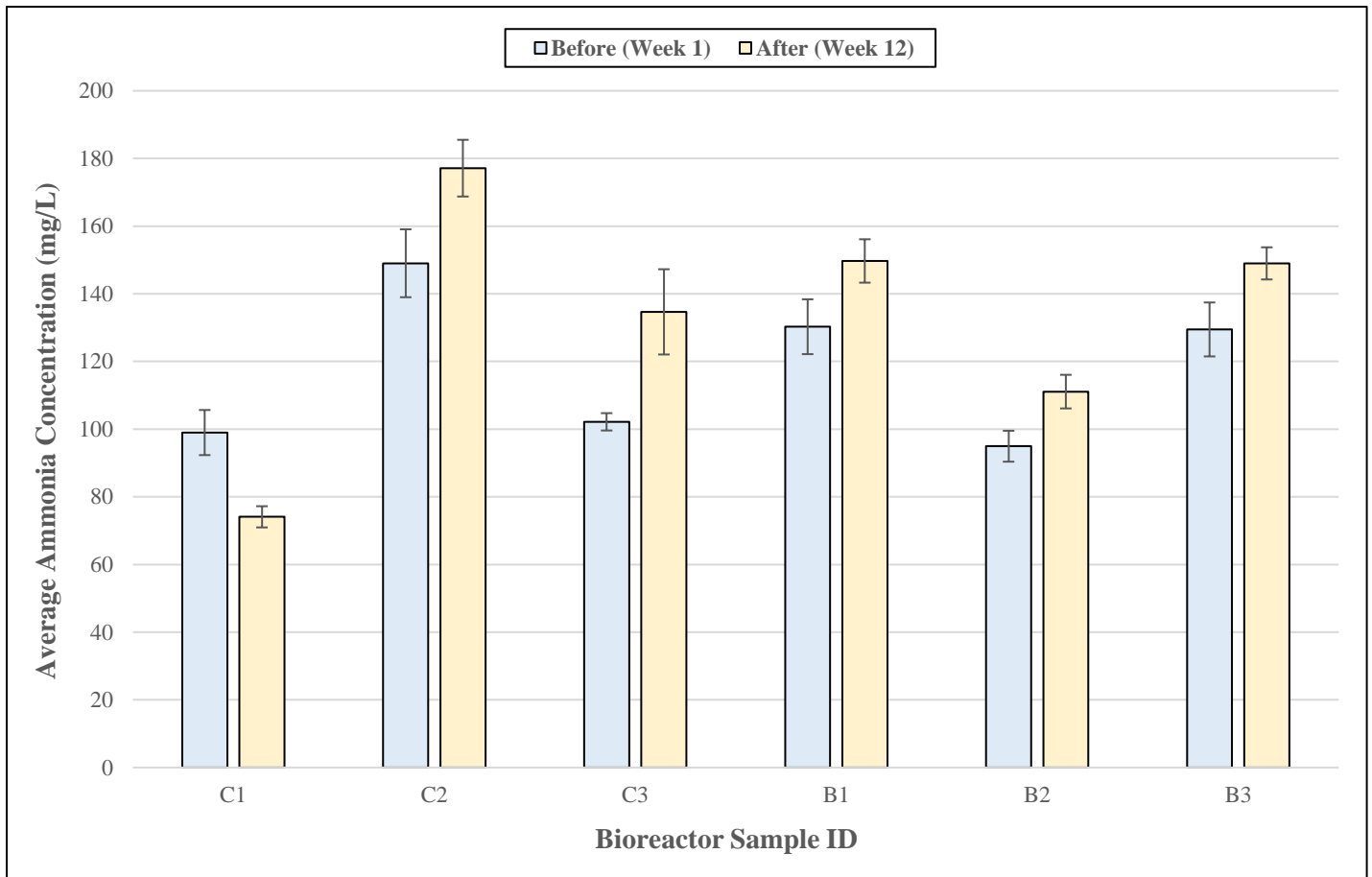


Figure 4: Variation in Treatment Bioreactor's Average Ammonia Concentrations (mg/L) from Week 1 and 12

When evaluating Figure 4 above, it can be seen that there are some variations in the ammonia concentration between all six bioreactors during Week 1. There seems to be no significant difference between the biochar-enriched and control bioreactors during the first week. When analyzing the change in ammonia concentrations between Week 1 and 12, it becomes evident that for five of the bioreactors there is an overall increase. The only exception to this observation is the control bioreactor 1 (C1), where there is an observed decrease in the ammonia concentration. This could be due to ammonia volatilization, but the exact reason for the decrease in ammonia in this single reactor is unknown.

Based on these results, there seems to be some difference in the change in ammonia concentrations between the biochar-enriched and control bioreactor samples. The control samples seem to have slightly higher increases in ammonia concentrations between week 1 and 12, than the biochar-enriched samples. Based on this observation, it is possible that the biochar enrichment produced lower increases in ammonia concentrations between the first and last week of the study. The One-way ANOVA statistical analysis performed on the week 12 data generated a p-value of 0.801, which is greater than 0.05. Therefore, the results are not statistically significant and there is no significant difference in the change in ammonia concentrations between the biochar-enriched and control bioreactor samples.

Volatile Fatty Acids (VFAs)

VFAs are common fermentation products and intermediates of anaerobic digestion. VFAs are expected to increase as degradation of organic matter occurs but decrease as methane is produced. The VFA data were analyzed to assess acetate, propionate, and butyrate concentrations between the control and biochar-enriched bioreactors from weeks 1, 6, and 11 of the experiment. Samples from week 11 were used because there was not enough sample liquid left from week 12 to complete COD, ammonia *and* VFA analysis.

In Figure 5 below, the variation between average acetate concentrations (mg/L) during weeks 1, 6 and 11 are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 5.

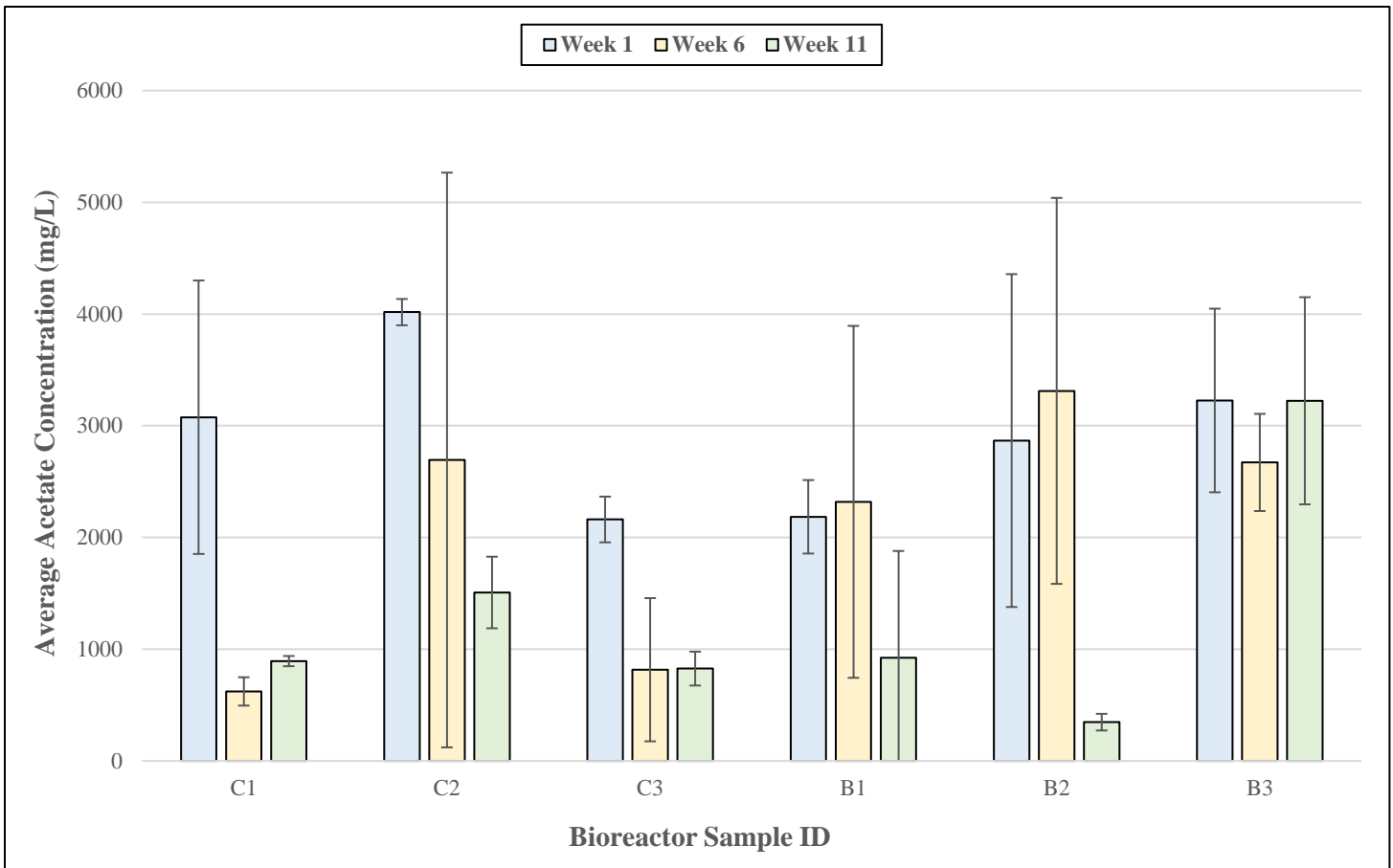


Figure 5: Variation in Treatment Bioreactor’s Average Acetate Concentrations (mg/L) from Weeks 1, 6, and 11

When evaluating Figure 5 above, it can be seen that there is some variation in the acetate concentration between all six bioreactors during Week 1. The control bioreactor 2 (C2) has an abnormally high acetate concentration when compared to the other two control bioreactors. However, the rest of the bioreactors all have relatively similar acetate concentrations in week 1. There seems to be no significant difference between the biochar-enriched and control bioreactors during the first week. Halfway through the experiment (during Week 6) all control bioreactors seem to have experienced a decrease in acetate concentrations, whereas two of the biochar-enriched bioreactors experienced the inverse – a slight increase in acetate concentrations. During the second last week of the experiment (week 11) two the control bioreactors experienced a

further decrease in acetate concentrations, whereas two of the biochar-enriched bioreactors experienced a dramatic decrease in acetate concentrations. Ultimately, both the control bioreactor 1 and biochar-enriched bioreactor 3 did not show similar trends to the other bioreactors in its treatment group.

Based on the results from the 2 remaining bioreactor treatment pairs, it becomes evident that the biochar-enriched bioreactors experienced an increase in acetate concentrations from week 1 to 6, and a subsequent dramatic decrease from week 6 to 11. On the other hand, the control bioreactors experienced a general decline in acetate concentrations from week 1 to 11. This could possibly illustrate that biochar enrichments influence the increase in production of acetate during the first 6 weeks of addition.

In Figure 6 below, the variation between average propionate concentrations (mg/L) during weeks 1, 6 and 11 are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 6.

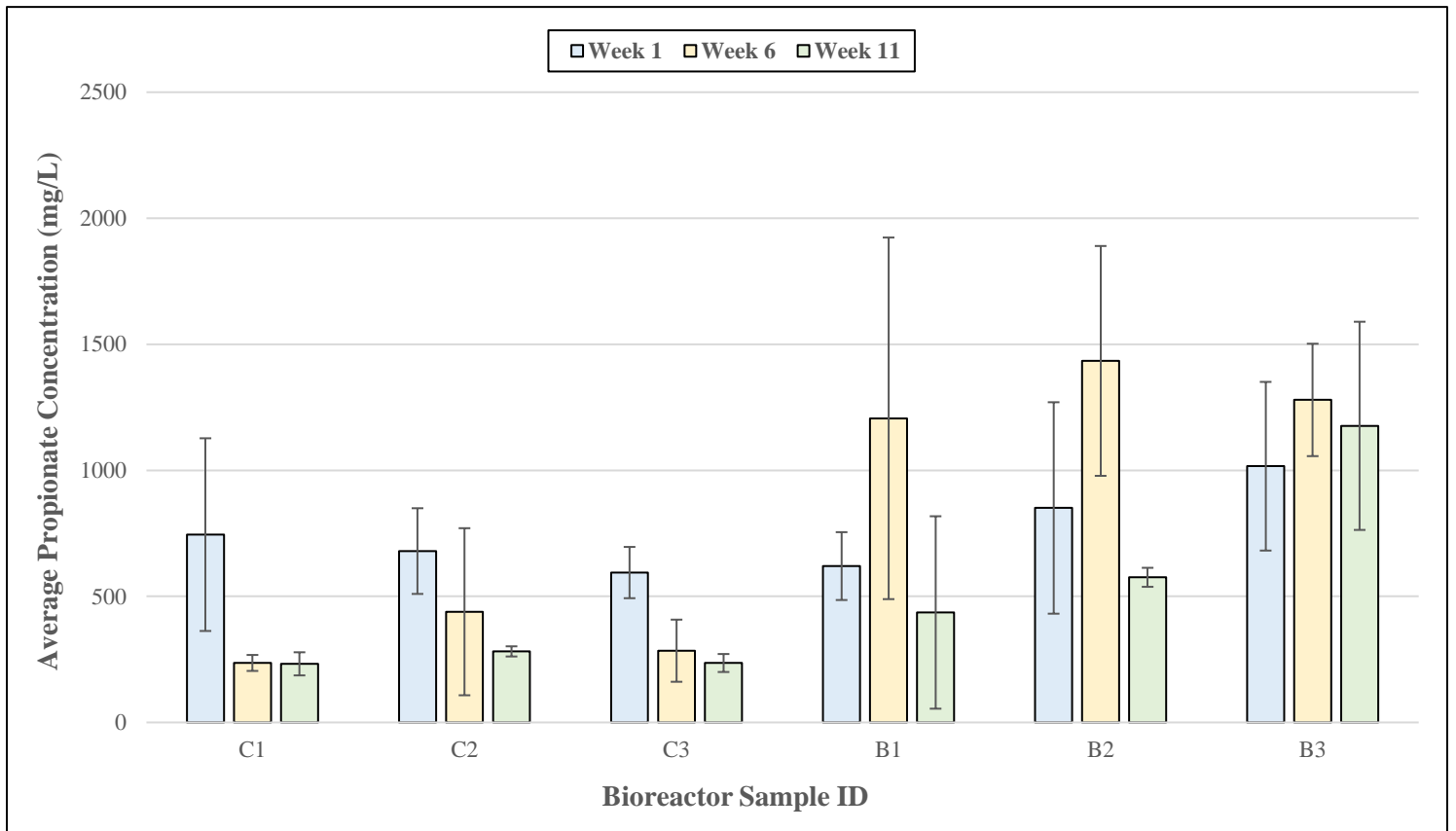


Figure 6: Variation in Treatment Bioreactor’s Average Propionate Concentrations (mg/L) from Weeks 1, 6, and 11

When evaluating Figure 6 above, it can be seen that there is some variation in the propionate concentration between all 6 bioreactors during week 1. There seems to be no significant difference between the biochar-enriched and control bioreactors during the first week. Halfway through the experiment (during week 6) all control bioreactors seem to have experienced a decrease in propionate concentrations, whereas all the biochar-enriched bioreactors experienced the inverse – a dramatic increase in propionate concentrations. During the second last week of the experiment (week 11) all the control bioreactors experienced either no change or a further decrease in propionate concentrations, whereas all the biochar-enriched bioreactors experienced a decrease in propionate concentrations.

Based on these results, it becomes evident that the biochar-enriched bioreactors experienced a drastic increase in propionate concentrations from week 1 to 6, and a subsequent decrease from week 6 to 11. On the other hand, the control bioreactors experienced a general decline in propionate concentrations from week 6 to 11. This could possibly illustrate that biochar enrichments influence the increase in production of propionate during the first 6 weeks of addition.

In Figure 7 below, the variation between average butyrate concentrations (mg/L) during weeks 1, 6 and 11 are shown – comparing the biochar-enriched bioreactors to the control bioreactors. The processed data tables can be found in Appendix 3, Table 7.

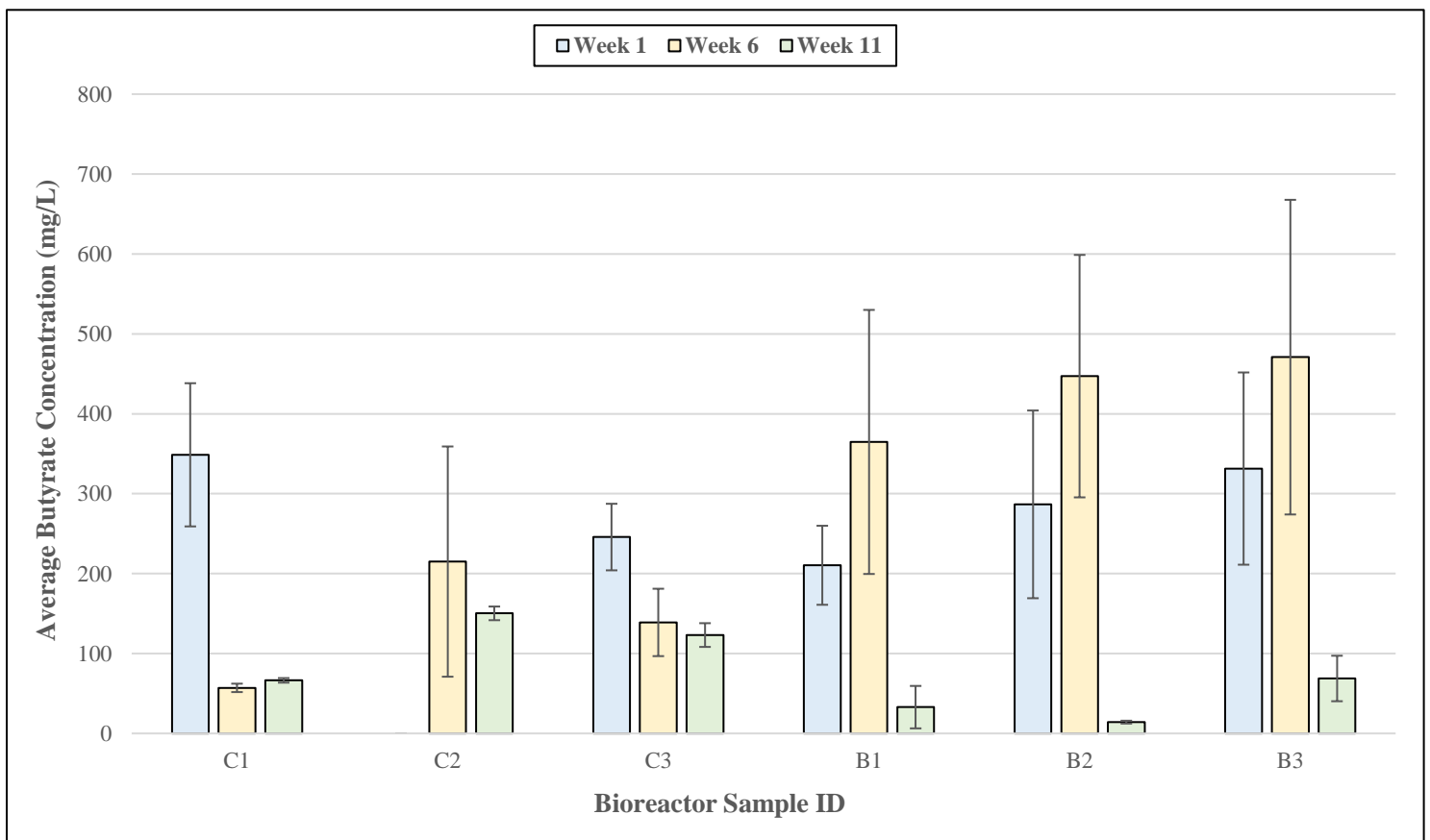


Figure 7: Variation in Treatment Bioreactor’s Average Butyrate Concentrations (mg/L) from Weeks 1, 6, and 11

When evaluating Figure 7 above, it can be seen that there is some variation in the propionate concentration between all 6 bioreactors during week 1. There seems to be no significant difference between the biochar-enriched and control bioreactors during the first week. It is important to note that control bioreactor 2 (C2) does not have data to show for week 1 – this is because there was very little sample liquid left to work with from week 1 and the sample was therefore heavily diluted. Due to the sample from C2 being heavily diluted, no butyrate concentration was detected. Since this is the case, control bioreactor 2 can be labeled as an outlier and should be excluded from the analysis. Halfway through the experiment (during week 6) all remaining control bioreactors seem to have experienced a decrease in butyrate concentrations, whereas all the biochar-enriched bioreactors experienced the inverse – a dramatic increase in butyrate concentrations. During the second last week of the experiment (week 11), control bioreactor 1 (C1) experienced a minuscule increase in butyrate concentration and control bioreactor 3 (C3) experienced a further decrease in butyrate concentration. On the other hand, all the biochar-enriched bioreactors experienced a drastic decrease in butyrate concentrations.

Based on these results, it becomes evident that the biochar-enriched bioreactors experienced a drastic increase in butyrate concentrations from week 1 to 6, and a subsequent dramatic decrease from week 6 to 11. On the other hand, the two control bioreactors experienced a general decline in butyrate concentrations from week 1 to 11. This could possibly illustrate that biochar enrichments influence the increase in production of butyrate during the first 6 weeks of addition.

Summary of Headspace Gas, Nutrient, and VFA Transformations

It appears that the biochar enrichment influenced the increase in headspace methane and carbon dioxide gas concentrations from the manure bioreactors. Further, there is no significant difference in the change in sCOD concentrations between the biochar-enriched and control bioreactor samples. However, the general decrease in sCOD concentrations indicates that the sCOD is being converted to gaseous products, such as methane, in both the control and biochar-enriched bioreactors. It is also likely that the biochar enrichment produced lower increases in ammonia concentrations between the first and last week of the study. This could indicate that less organic matter is being degraded in the biochar-enriched bioreactors, or that ammonia is being sequestered, as has been observed in previous studies with biochar (add citation). It appears that biochar enrichment could be influencing the accumulation of all three volatile fatty acids analyzed (acetate, propionate, and butyrate) during the first six weeks of application. It also seems that the decrease in VFA concentrations (all three) within the biochar-enriched bioreactors occurs over a similar time period to the increase in methane emissions. These results and evaluations are promising, however the error present in the measurements must be highlighted and considered when formulating a final conclusion.

Microbial Community Analysis

Week 12 and raw manure biomass samples were analyzed using metagenomic DNA sequencing. Metagenomic analysis was conducted with Nanopore generated sequences, through the use of SqueezeMeta. The SQM package and Nanopore-generated data sequence was then run on R to generate heatmaps of abundant genera and phyla. In Figure 8 below, the heatmap of

abundant phylum for the biochar-enriched bioreactors, control bioreactors, and raw manure is presented. The R script used to generate the heatmap can be found in Appendix 4, Figure 1.

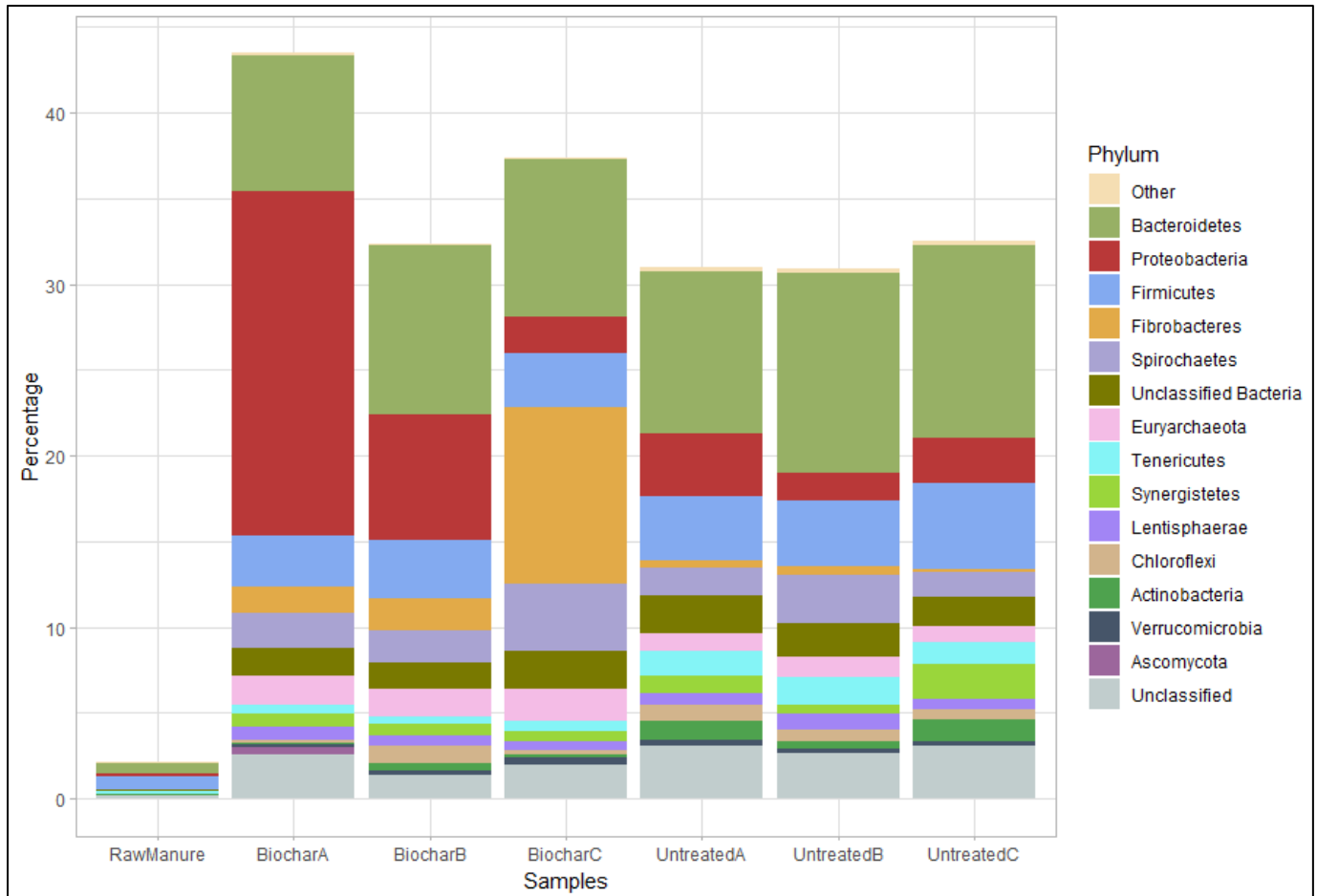


Figure 8: Heatmap of Variation in Treatment Bioreactor's Abundant Phylum from Week 12

When evaluating Figure 8 above, it can be seen that there are total of 14 identified phyla in the bioreactor samples after the duration of the experiment. Firstly, in the biochar-enriched bioreactor 1 (Biochar A) there is a notably large abundance of *Proteobacteria* and in the biochar-enriched bioreactor 3 (Biochar C) there is a notably large abundance of *Fibrobacteres*. Both other biochar-enriched bioreactors also have larger *Fibrobacteres* abundances than the control

bioreactors. Secondly, in control bioreactors 1 and 3 (Untreated A and C) there is a larger abundance of *Actinobacteria*, as compared to the biochar-enriched bioreactors. Thirdly, in all control bioreactors there is a larger abundance of *Tenericutes*, as compared to the biochar-enriched reactors. Lastly, all three biochar-enriched bioreactors have larger abundances of *Euryarchaeota*, as compared to the control bioreactors. All other phyla have relatively similar abundances in both the control and biochar-enriched bioreactors.

In Figure 9 below, the heatmap of abundant genera for the biochar-enriched bioreactors, control bioreactors, and raw manure is presented. The R script used to generate the heatmap can be found in Appendix 4, Figure 2.

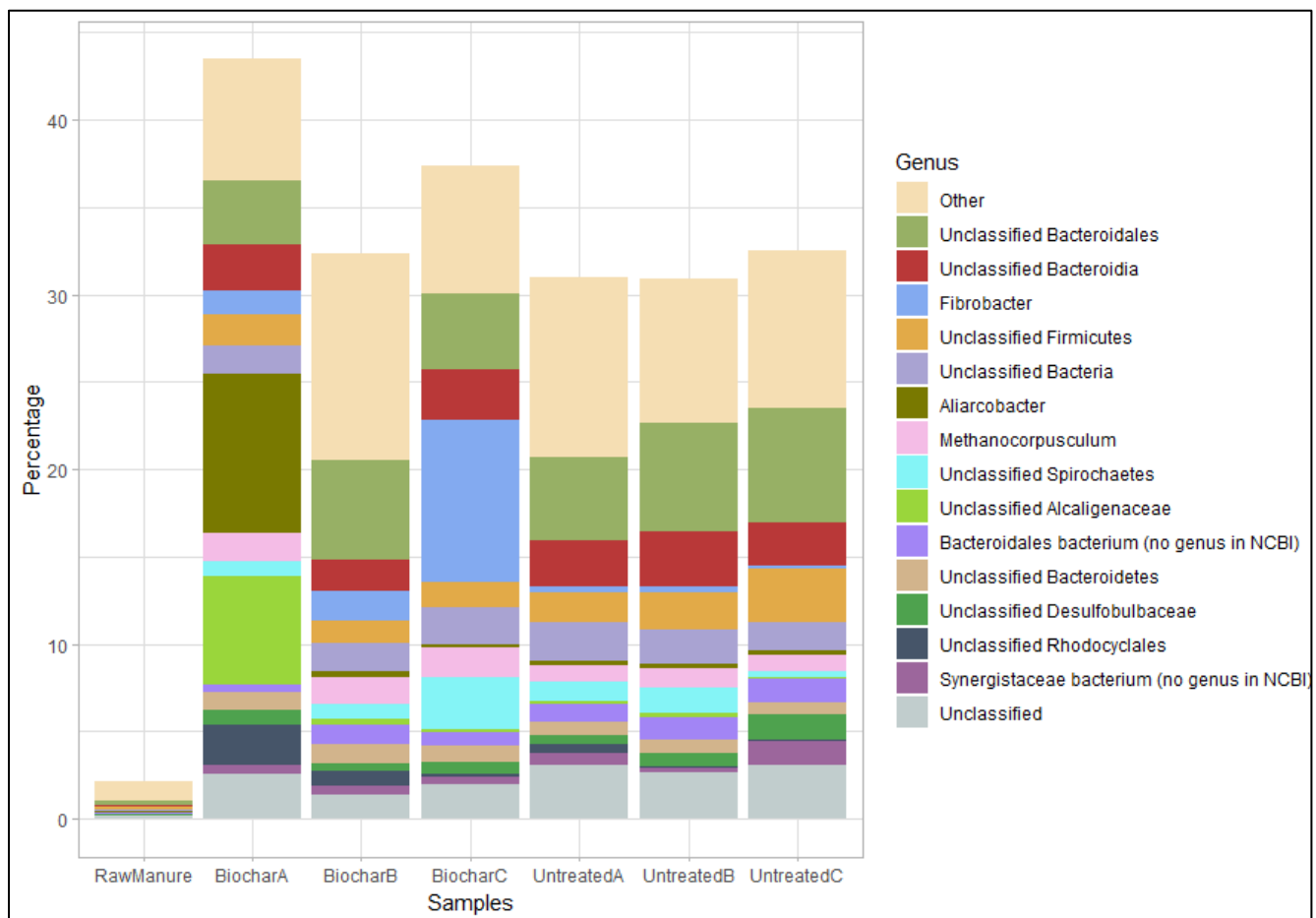


Figure 9: Heatmap of Variation in Treatment Bioreactor's Abundant Genera from Week 12

When evaluating Figure 9, it can be seen that in the biochar-enriched bioreactor 1 (Biochar A) there is a notable large abundance of *Aliarcobacter*, Unclassified *Alcaligenaceae*, and Unclassified *Rhodocyclales* – which is unlike the other biochar-enriched bioreactors. Secondly, in the biochar-enriched bioreactor 3 (Biochar C), there is a notably large abundance of *Fibrobacter* and Unclassified *Spirochaetes*. There are also larger abundances of *Fibrobacter* in the other two biochar-enriched bioreactors, as compared to the control bioreactors. Lastly and most interestingly, in all biochar-enriched bioreactors there is a larger abundance of *Methanocorpusculum*, as compared to the control bioreactors.

In taxonomy, *Methanocorpusculum* is a genus of microbes within the family *Methanocorpusculaceae*. The name *Methanocorpusculum* has Latin roots and means “*bodies that produce methane*” (LPSN). The species within *Methanocorpusculum* were first isolated from biodigester wastewater and activated sludge from anaerobic digestors (LPSN). In nature, they live in freshwater environments. This genera typically reduces carbon dioxide to methane using hydrogen or secondary alcohols (LPSN). The presence of these microbes indicate that methanogenesis is likely taking place within the manure bioreactors.

The genera *Fibrobacter* and related species are recognized as major bacterial degraders of lignocellulosic material in the herbivore gut (Ransom-Jones et al., 2012). Therefore, their presence in cow excrement is justified. Historically, members of the genus *Fibrobacter* were thought to only occupy mammalian intestinal tracts. However, additional 16S rRNA gene-targeted molecular approaches have shown that novel variations within the genus are present in landfill sites and freshwater lakes (Ransom-Jones et al., 2012).

Additionally, *Aliarcobacter* is a globally emerging foodborne and zoonotic pathogen (Muller et al., 2020). In one particular study, 27 *Aliarcobacter cryaerophilus* strains were

investigated from water poultry in Thuringia, Germany (Muller et al., 2020). Little is known about the species' genomic features and diversity, antibiotic resistance, and virulence (Muller et al., 2020). The *Alcaligenaceae* are a family of bacteria, within the order *Burkholderiales*. The members of this family are usually found in water, soil, humans, and other animals (Garrity et al., 2005). The specific genera within this family of bacteria was unclassified, however it is interesting to note that some species, like *Bordetella* are pathogenic for humans and some animals (Garrity et al., 2005). *Rhodocyclales* are an order of the class *Betaproteobacteria* in the phylum “*Pseudomonadota*” (Garrity et al., 2005). *Rhodocyclales* is an abundant bacterial order in wastewater treatment systems – however its phylogenomics, prevalence of denitrifying genes in sub-lineages and distribution in wastewater treatment plants (WWTPs) worldwide have not been well characterized (Wang et al., 2020). Lastly, *Spirochaete* are a group of spiral-shaped bacteria, some of which are serious pathogens for humans (Britannica, 2020). *Spirochaetes* are free-living nonpathogenic inhabitants of mud and water, typically thriving in anaerobic environments (Britannica, 2020).

In Figure 10 below, the heatmap of abundant *Methanocorpusculum* for the biochar-enriched bioreactors, control bioreactors, and raw manure is presented. The R script used to generate the heatmap can be found in Appendix 4, Figure 3.

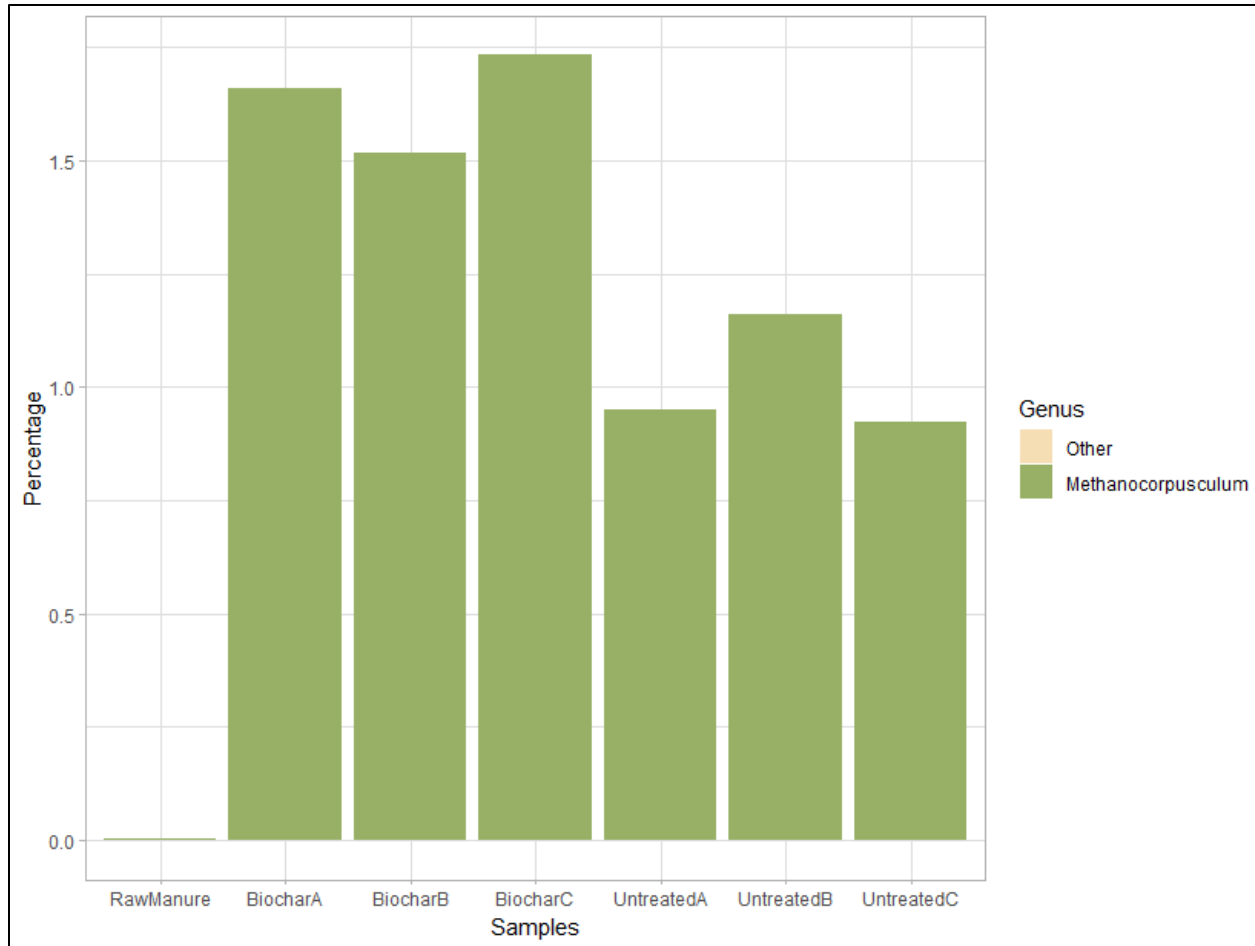


Figure 10: Heatmap of Variation in Treatment Bioreactor’s *Methanocorpusculum* Genus for Week 12

When evaluating Figure 10, it becomes more evident that there is a noticeable difference in abundance of the *Methanocorpusculum* genera between the control and biochar-enriched bioreactors in week 12. The abundance in the biochar-enriched bioreactors ranges from approximately just above 1.5% to just below 1.75%. On the other hand, the abundance in the control bioreactors ranges from approximately 0.85% to 1.15%. The One-way ANOVA statistical analysis performed on the week 12 data generated a p-value of 0.0048, which is less than 0.05. Therefore, the results are statistically significant, and it confirms that there is a

significant difference in the abundance of the *Methanocorpusculum* genera between the biochar-enriched and control bioreactor samples.

Based on these results, it is likely that the biochar enrichment in the manure bioreactors influenced the higher abundance of *Methanocorpusculum* microbes, which ultimately leads to higher rates of methanogenesis and methane production. Additionally, it is also likely that the biochar enrichment in the manure bioreactors influenced the higher abundance of *Fibrobacter* microbes, which are typically responsible for degrading lignocellulosic material in herbivore guts. It is also important to note that there was a higher abundance of *Aliarcobacter*, *Alcaligenaceae*, *Rhodocyclales*, and *Spirochaete* in some of the biochar-enriched bioreactors. Their presence in the manure bioreactors makes sense because as mentioned above, these genera are commonly present in either natural environments (e.g., soil, mud, or freshwater), animals, or anaerobic environments (e.g., wastewater sludge) - all of which are characteristic of manure lagoon sludge. Ultimately, the biochar enrichment in the manure lagoons potentially promoted their growth.

Conclusion

In summary, the results from the study produced the following conclusions. The biochar-enriched bioreactors showed increased carbon dioxide and methane headspace concentrations/emissions starting around week 7, as compared to the control bioreactors. However, the one-way ANOVA analysis deemed the difference between the control and biochar-enriched bioreactors, not statistically significant. Overall, this means that the biochar enrichment could possibly have influenced the increased methane emissions, but the correlation is not certain.

The chemical oxygen demand (COD) concentration results showed that generally the COD concentration decreased from week 1 to 12. Soluble COD concentrations tend to decrease when methane is being produced. However, there was no noticeable difference between the biochar-enriched and control bioreactors. The one-way ANOVA analysis confirmed this evaluation, as no statistically significant difference was found.

The ammonia concentration results showed that generally the ammonia concentration increased from week 1 to 12. However, there seemed to be a difference in the magnitude of the increase – the biochar-enriched bioreactors experienced a smaller increase in concentration than the control bioreactors. Generally, when ammonia concentrations increase it means that organic material is being degraded. On the other hand, when ammonia concentrations decrease the organic material is being volatilized. However, the one-way ANOVA analysis deemed the difference between the control and biochar-enriched bioreactors, not statistically significant. This means that the biochar enrichment could possibly have influenced the inhibition of the ammonia concentration increase over the 12-week study period.

The volatile fatty acid (VFA) concentration results show a fairly similar pattern between all three VFAs of concern – acetate, propionate, and butyrate. The biochar-enriched bioreactor showed a rather dramatic increase in VFA concentrations between week 1 and 6, whereas the control bioreactors showed a general decrease in VFA concentrations. From week 6 to 12, the biochar-enriched bioreactors showed a general decrease in concentration, whereas the control bioreactors showed a further decrease or no change. One-way ANOVA analysis was deemed unnecessary for these data sets; however, it is important to consider that some of these VFA measurements have large associated error bars. Overall, this means that the biochar enrichment

could possibly have influenced the drastic increase in acetate, propionate, and butyrate concentrations from week 1 to 6, but the correlation is not certain.

The microbial community analysis results from week 12 generally show that the biochar-enriched bioreactors contained a higher abundance and diversity of microbial phyla and genera. More specifically, the biochar-enriched bioreactors had a higher abundance of the *Methanocorpusculum* microbes, as compared to the control bioreactors. One-way ANOVA analysis was performed on the *Methanocorpusculum* genera data, and the results were deemed statistically significant. This is an important finding as *Methanocorpusculum* microbes are responsible for facilitating the methanogenesis process that produces methane emissions. Overall, this means that biochar enrichment could possibly have influenced the increase in the abundance of the methane-producing microbial community within the manure bioreactors.

Ultimately, without considering the error involved within the results, majority of the results from this study corroborate with one another. Firstly, lower final ammonia concentrations in the biochar-enriched bioreactors indicates that more organic material was volatilized - converted into volatile organic compounds and methane gas - as compared to the control bioreactors. Secondly, the VFA results indicated that the biochar enrichment could possibly have influenced the drastic increase in acetate, propionate, and butyrate concentrations from week 1 to 6. Thirdly, the microbial community analysis results showed that biochar enrichment could possibly have influenced the increase in abundance of the methane-producing microbial community within the manure bioreactors. Lastly, the headspace methane concentration results showed that the biochar-enriched bioreactors produced higher methane concentrations from week 7 to 12.

Considering these results, it has become evident that biochar enrichment in bioreactors, that are representative of manure lagoons, influences the increase in organic material decomposition/transformation to volatile fatty acids over the first 6 weeks of addition. The availability of organic material (e.g., COD and NH_3) and increased fermentation intermediate (e.g., VFAs) concentrations drives the growth of methanogenic microbial communities. The increased abundance of methanogenic microbes and fermentation intermediates drives the increased production of methane gas emissions from week 7 to 12. Thus, the microbial communities present in the manure lagoons are highly interconnected with the organic material and fermentation intermediate concentrations. Based on this evaluation, it is plausible that biochar enrichment in manure lagoons facilitates higher methane emissions.

Therefore, this research study proves that sub hypotheses (1), (2), and (3) to be not incorrect. However, the time scale of sub hypothesis (1) is important to note – as the increase in VFA concentrations occurs only from week 1 to 6, after biochar addition. In addition to satisfying these hypotheses, the results also establish that biochar enrichment in manure lagoons could possibly increase the diversity and abundance of microbial communities. When considering the cropland application of manure, increased microbial diversity and activity has shown to improve water infiltration and reduce soil erosion. Therefore, the use of biochar-enriched manure as a crop fertilizer could likely improve crop rhizosphere health and subsequently enhance climate-resistant food systems. Future research investigating the impact of using biochar-enriched manure, as a fertilizer, on crop yield and growth is necessary.

Error Discussion

While these findings are promising, it is incredibly important to discuss the error associated with the results. All results, except for the microbial community analysis results, were deemed not statistically significant. This was due to both human and instrumental error. There is some error associated with the measurements made by the instruments that were used to conduct the COD, ammonia and VFA testing. There is also human error associated with the experimental setup, sampling, and testing. Within the experimental setup error, there were challenges with keeping the sampling tubes fixed to the lids, as the weight of the plastic tubing would often pull the adhesive epoxy from the lid – loosening the sampling tube, allowing headspace gas to escape, and making the bioreactors no longer air-tight. This could have possibly impacted the accuracy and reliability of the headspace gas concentration data. There was also significant sampling error, as there was not enough sample from week 1 to run the COD, ammonia, and VFA tests. A few week 1 samples had to be heavily diluted to achieve the required liquid volumes for the tests - much of the sample was lost through filtration. These dilutions caused problems in the results when the concentration was not high enough to be detected. There was also not enough week 12 sample to perform the VFA tests, and that is why week 11 samples were used instead. To avoid this problem in the future, one must carefully plan out in advance how much sample is required to run all the tests needed. Lastly, human error when performing analytical tests is a concern. There is bound to be error when measuring out or transferring substances using pipettes or other methodology. It is incredibly challenging to prevent human error, but one can compensate for this error by repeating tests and incorporating multiple replicates. Lastly, this study made use of biological triplicates to analyze the impacts of biochar enrichment. However, there is also known to be huge variation in biological replicates due to

stochastic variations in microbial community assembly. Given the complexity of the microbial communities present, the biological replicates could have diverged during incubation. Since the results show large differences among bioreactors with the same treatment, this study highlights the importance of performing biological replicates and suggests that the impacts of biochar may depend on the microbial community present in manure storage and treatment.

Future Work

Firstly, to gain a better understanding of how biochar enrichment impacts microbial communities, nutrient degradation, and fermentation intermediates in manure lagoon sludge, the experiment should be conducted for a longer period of time – one that accurately simulates how long manure would typically be stored in a manure lagoon before being applied as a fertilizer. Secondly, a greater volume of sample should have been taken during week 1, 6 and 12. The volume of sample should be decided prior to the start of the experiment and enough to complete all tests/analyses, with at least a 10 to 15% safety factor. Lastly, considering the expansion of the experimental timeline, samples should probably only be taken every six weeks. In the future, this study could also be extended to test how biochar-enriched manure lagoon sludge impacts crop rhizosphere health and crop growth, when applied as a fertilizer. Furthermore, this could also broadly investigate if the land application of biochar-enriched manure improves food system climate-resilience.

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Appendices

Appendix 1: Supporting Images

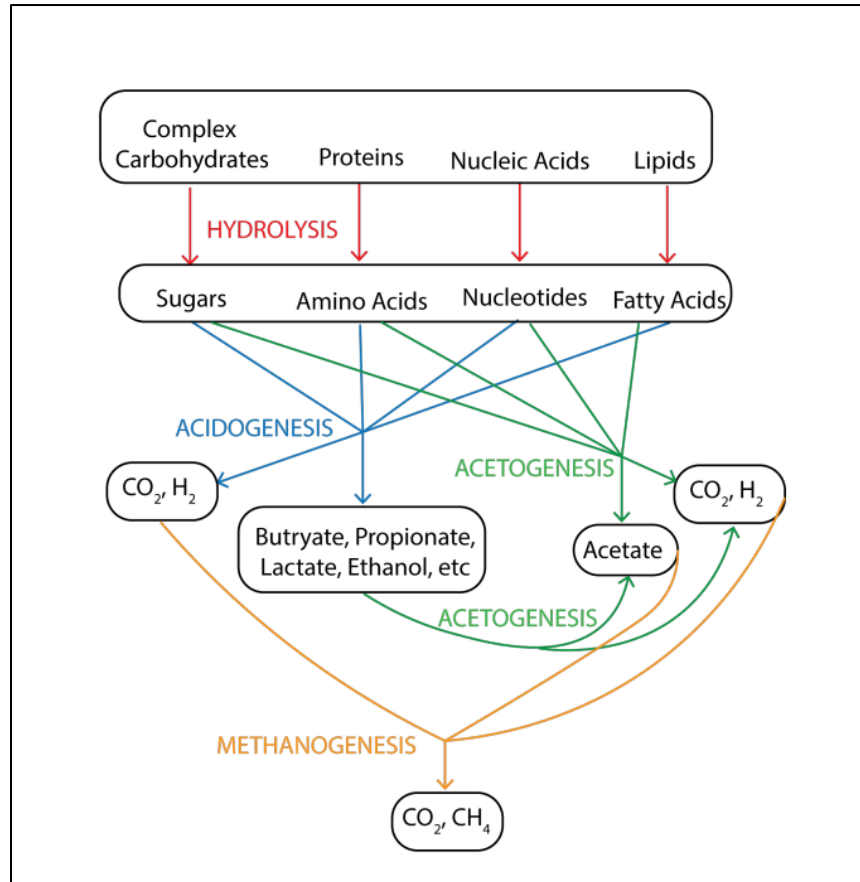


Figure 1: Anaerobic Digestion Food Web (Created by: Dr. Matthew Scarborough)



Figure 2: Constructed Bioreactor Containers (Taken by: Courtney Hales)

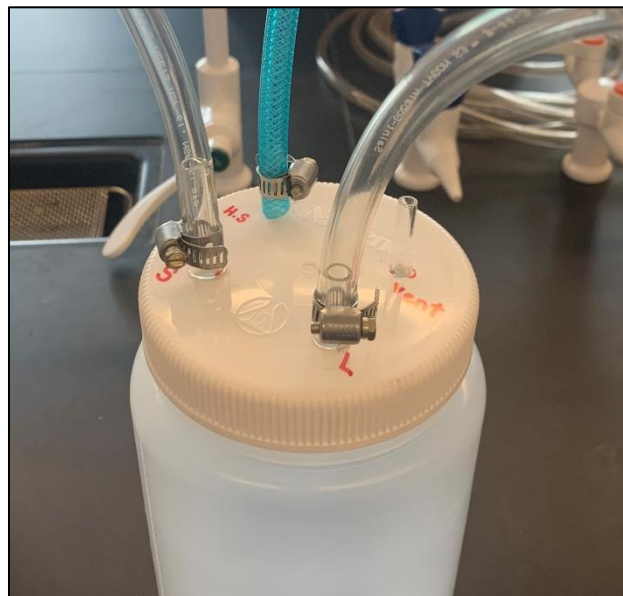


Figure 3: Headspace Sampling, Liquid Sampling and Venting Tubes (Taken by: Courtney Hales)



Figure 4: Bioreactors Filled with Manure Lagoon Sludge on Shaker Table (Taken by: Courtney Hales)

Appendix 2: Unprocessed Data

Headspace Gas Sampling

Table 1: Week 1 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	6.827	18.925	84.374	0.21	13.342	68.311	0.212
C2	14.415	12.73	82.265	1.288	8.923	66.625	1.212
C3	21.19	8.719	78.635	2.268	5.988	63.89	2.088
Control Average	14.144	13.458	81.758	1.255	9.418	66.275	1.171
Control Std. Dev.	7.185	5.142	2.903	1.029	3.702	2.231	0.939
B1	20.758	8.513	79.209	2.406	5.82	64.304	2.207
B2	15.556	11.157	82.628	1.127	7.756	66.918	1.061
B3	12.364	15.441	81.396	1.1	10.904	66.068	1.038
Biochar Average	16.226	11.704	81.078	1.544	8.160	65.763	1.435
Biochar Std. Dev.	4.237	3.496	1.732	0.746	2.566	1.333	0.668

Table 2: Week 2 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	5.341	19.684	84.839	0.445	13.927	68.812	0.432
C2	12.698	14.152	82.883	0.582	9.971	67.263	0.552
C3	21.235	11.451	76.564	1.058	8.073	62.450	0.981
Control Average	13.091	15.096	81.429	0.695	10.657	66.175	0.655
Control Std. Dev.	7.954	4.197	4.325	0.322	2.987	3.318	0.289
B1	13.081	16.752	79.081	1.245	11.916	64.449	1.161
B2	19.448	15.989	73.371	1.388	11.438	60.198	1.278
B3	1.347	23.518	85.307	0.000	16.577	69.171	0.000
Biochar Average	11.292	18.753	79.253	0.878	13.310	64.606	0.813
Biochar Std. Dev.	9.182	4.144	5.970	0.763	2.839	4.489	0.707

Table 3: Week 3 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	6.857	17.356	86.528	0.282	12.274	70.054	0.280
C2	12.297	13.101	84.759	0.774	9.200	68.666	0.734
C3	15.191	12.231	82.975	0.696	8.581	67.337	0.657
Control Average	11.448	14.229	84.754	0.584	10.018	68.686	0.557
Control Std. Dev.	4.231	2.742	1.777	0.264	1.978	1.359	0.243
B1	11.874	16.254	81.609	1.249	11.534	66.394	1.169
B2	20.749	11.247	76.767	2.454	7.927	62.685	2.237
B3	1.671	23.029	86.064	0.202	16.256	69.829	0.000
Biochar Average	11.431	16.843	81.480	1.302	11.906	66.303	1.135
Biochar Std. Dev.	9.547	5.913	4.650	1.127	4.177	3.573	1.119

Table 4: Week 4 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	10.860	14.298	84.777	0.791	10.111	68.731	0.751
C2	14.672	11.554	82.315	1.822	8.108	66.866	1.683
C3	8.906	15.930	84.946	0.716	11.271	68.848	0.677
Control Average	11.479	13.927	84.013	1.110	9.830	68.148	1.037
Control Std. Dev.	2.932	2.211	1.473	0.618	1.600	1.112	0.561
B1	11.229	15.534	81.634	1.870	11.038	66.452	1.725
B2	23.732	10.513	73.391	3.187	7.436	60.171	2.866
B3	6.926	18.675	83.667	0.836	13.256	67.975	0.786
Biochar Average	13.962	14.907	79.564	1.964	10.577	64.866	1.792
Biochar Std. Dev.	8.730	4.117	5.442	1.178	2.937	4.137	1.042

Table 5: Week 5 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	0.000	20.279	85.153	0.727	14.404	69.160	0.688
C2	18.352	12.292	77.021	3.134	8.733	62.962	2.822
C3	17.946	7.247	84.015	1.775	4.845	68.125	1.613
Control Average	12.099	13.273	82.063	1.879	9.327	66.749	1.708
Control Std. Dev.	10.480	6.571	4.403	1.207	4.807	3.320	1.070
B1	8.765	16.807	83.667	1.387	11.942	68.028	1.280
B2	27.200	6.816	75.069	2.516	4.622	61.418	2.255
B3	1.109	23.314	86.428	0.068	16.505	70.181	0.000
Biochar Average	12.358	15.646	81.721	1.324	11.023	66.542	1.178
Biochar Std. Dev.	13.411	8.310	5.924	1.225	5.995	4.567	1.131

Table 6: Week 6 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	0.000	19.285	86.422	0.251	13.761	70.250	0.247
C2	12.464	16.011	80.511	2.086	11.452	65.817	1.905
C3	14.427	9.326	85.921	1.634	6.434	69.722	1.490
Control Average	8.964	14.874	84.285	1.324	10.549	68.596	1.214
Control Std. Dev.	7.825	5.076	3.278	0.956	3.746	2.421	0.863
B1	3.271	21.361	86.028	0.490	15.213	69.981	0.462
B2	16.082	13.134	80.068	1.855	9.348	65.437	1.681
B3	0.000	24.264	86.724	0.000	17.213	70.568	0.000
Biochar Average	6.451	19.586	84.273	0.782	13.925	68.662	0.714
Biochar Std. Dev.	8.500	5.773	3.659	0.961	4.088	2.808	0.868

Table 7: Week 7 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	3.633	20.822	84.283	0.809	14.897	68.653	0.760
C2	10.800	14.548	83.198	1.151	10.363	67.749	1.063
C3	19.987	4.093	84.509	1.989	2.422	68.509	1.795
Control Average	11.473	13.154	83.997	1.316	9.227	68.304	1.206
Control Std. Dev.	8.198	8.451	0.701	0.607	6.315	0.486	0.532
B1	6.177	18.227	84.777	1.033	12.993	68.936	0.956
B2	29.233	3.326	73.192	5.540	1.925	60.033	4.822
B3	11.068	12.081	85.224	1.826	8.507	69.186	1.660
Biochar Average	15.493	11.211	81.064	2.800	7.808	66.052	2.479
Biochar Std. Dev.	12.148	7.488	6.821	2.406	5.567	5.214	2.059

Table 8: Week 8 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	4.066	20.312	83.796	1.210	14.564	68.257	1.118
C2	15.161	12.176	80.238	2.135	8.647	65.485	1.926
C3	16.131	6.284	85.829	1.691	4.116	69.515	1.528
Control Average	11.786	12.924	83.288	1.679	9.109	67.752	1.524
Control Std. Dev.	6.703	7.044	2.830	0.463	5.239	2.062	0.404
B1	4.184	19.879	84.363	0.887	14.215	68.677	0.816
B2	32.276	2.538	61.187	15.007	1.426	50.817	12.454
B3	26.079	1.204	75.496	7.763	0.217	61.719	6.652
Biochar Average	20.846	7.874	73.682	7.886	5.286	60.404	6.641
Biochar Std. Dev.	14.759	10.418	11.694	7.061	7.756	9.002	5.819

Table 9: Week 9 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	5.344	19.045	85.950	0.464	13.644	69.927	0.000
C2	17.972	7.636	84.201	1.849	5.188	68.346	1.673
C3	16.331	6.686	86.747	1.540	4.422	70.300	1.398
Control Average	13.216	11.122	85.633	1.284	7.751	69.524	1.024
Control Std. Dev.	6.866	6.878	1.302	0.727	5.118	1.037	0.897
B1	11.253	14.055	81.839	3.787	10.053	66.751	3.376
B2	31.085	1.070	42.242	39.019	0.452	35.955	30.719
B3	30.552	4.550	63.618	13.508	3.006	52.791	11.318
Biochar Average	24.297	6.558	62.566	18.771	4.504	51.832	15.138
Biochar Std. Dev.	11.299	6.721	19.819	18.196	4.973	15.420	14.066

Table 10: Week 10 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	0.000	22.178	86.462	0.086	15.848	70.210	0.000
C2	12.688	10.123	86.547	0.981	7.045	70.135	0.904
C3	14.314	8.396	87.910	0.824	5.743	71.110	0.761
Control Average	9.001	13.566	86.973	0.630	9.545	70.485	0.555
Control Std. Dev.	7.837	7.508	0.813	0.478	5.497	0.543	0.486
B1	10.486	14.225	81.652	4.187	10.199	66.582	3.758
B2	27.784	1.059	33.054	51.118	0.520	28.455	0.000
B3	39.763	1.115	48.420	23.906	0.409	40.871	19.391
Biochar Average	26.011	5.466	54.375	26.404	3.709	45.303	7.716
Biochar Std. Dev.	14.719	7.585	24.840	23.565	5.620	19.446	10.284

Table 11: Week 11 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	0.000	22.981	86.130	0.133	16.420	69.968	0.000
C2	11.559	10.773	87.328	1.072	7.535	70.673	0.999
C3	0.000	21.604	87.960	0.098	15.417	71.274	0.000
Control Average	3.853	18.453	87.139	0.434	13.124	70.638	0.333
Control Std. Dev.	6.674	6.686	0.929	0.553	4.866	0.654	0.577
B1	10.441	14.485	76.917	8.056	10.407	62.921	7.053
B2	6.650	16.642	84.216	2.417	11.895	68.388	2.228
B3	36.647	2.851	39.620	33.712	1.893	33.767	26.775
Biochar Average	17.913	11.326	66.918	14.728	8.065	55.025	12.019
Biochar Std. Dev.	16.335	7.418	23.921	16.680	5.397	18.612	13.005

Table 12: Week 12 Headspace Gas Sampling Unprocessed Data

Sample ID	Line 1 Conc. (%)				Line 2 Conc. (%)		
	Carbon Dioxide	Oxygen/Argon	Nitrogen	Methane	Oxygen	Nitrogen	Methane
C1	0.000	22.435	86.018	0.046	16.042	69.840	0.000
C2	19.430	4.058	84.871	2.425	2.393	68.746	2.186
C3	0.000	23.389	85.943	0.000	16.701	69.862	0.000
Control Average	6.477	16.627	85.611	0.824	11.712	69.483	0.729
Control Std. Dev.	11.218	10.896	0.642	1.387	8.077	0.638	1.262
B1	13.086	12.422	66.363	18.285	8.988	54.841	15.137
B2	7.453	16.551	85.550	0.790	11.822	69.403	0.000
B3	35.291	0.511	23.680	54.213	0.166	20.695	0.000
Biochar Average	18.610	9.828	58.531	24.429	6.992	48.313	5.046
Biochar Std. Dev.	14.718	8.329	31.670	27.236	6.079	25.002	8.739

Chemical Oxygen Demand (COD)

Table 13: COD Calibration Standards

COD Concentration (mg/L)	Spectrophotometer Measurement
0	0
93.75	0.045
187.5	0.089
375	0.186
750	0.339
1125	0.531
1500	0.633

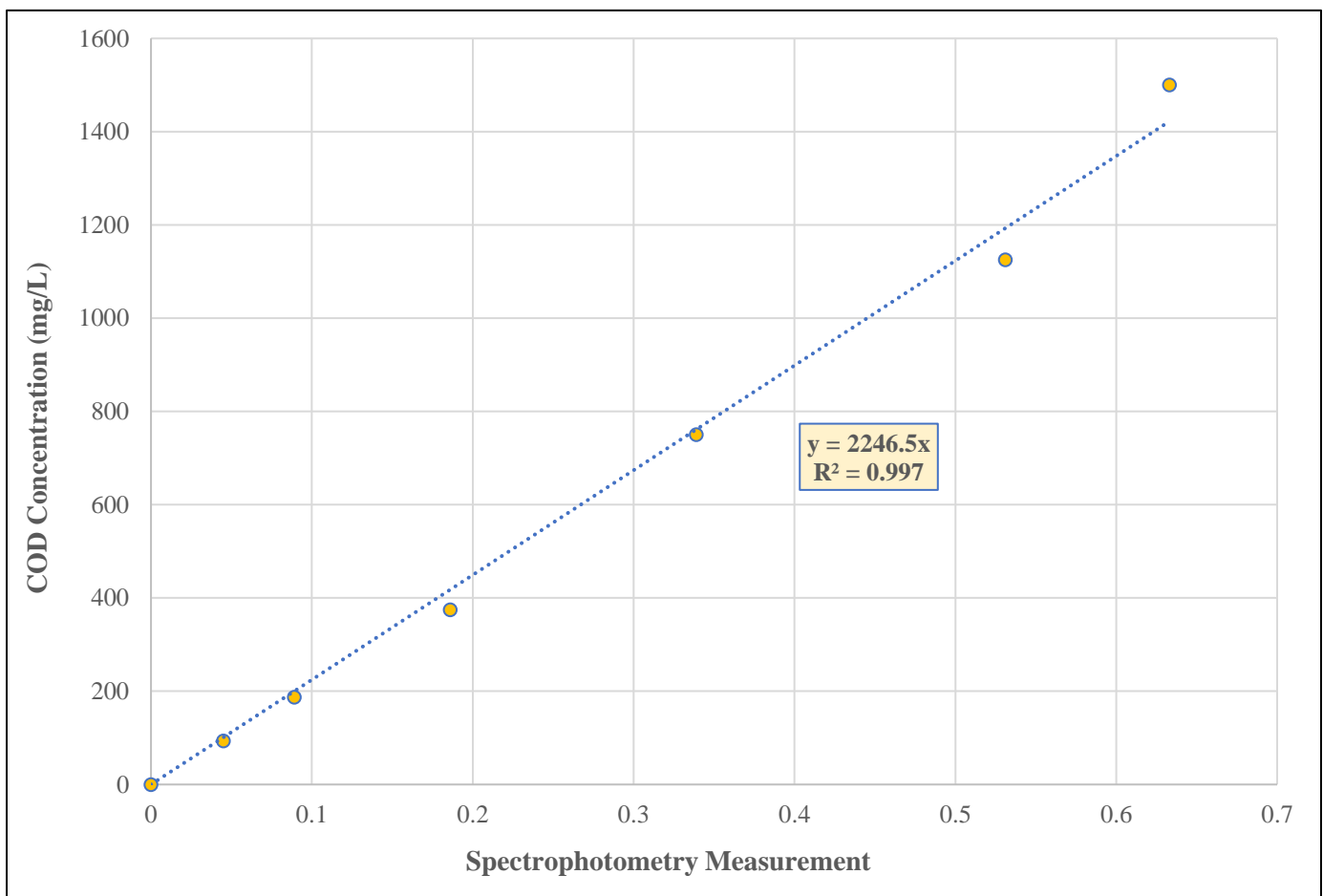


Figure 1: COD Calibration Curve

Table 14: COD Testing Unprocessed Data

Sample ID	Spectrophotometry Measurement				
	Trial 1	Trial 2	Trial 3	Average	Std Deviation
C1.1	0.051	0.041	0.043	0.0450	0.0052915
C1.12	0.019	0.012	0.018	0.0163	0.0037859
C2.1	0.040	-	-	0.0400	-
C2.12	0.052	0.049	0.046	0.0490	0.0030000
C3.1	0.040	0.044	0.038	0.0407	0.0030551
C3.12	0.024	0.037	0.035	0.0320	0.0070000
B1.1	0.047	0.050	0.057	0.0513	0.0051316
B1.12	0.041	0.043	0.042	0.0420	0.0010000
B2.1	0.042	0.045	0.046	0.0443	0.0020817
B2.12	0.026	0.028	0.039	0.0310	0.0070000
B3.1	0.049	0.047	0.058	0.0513	0.0058595
B3.12	0.051	0.047	0.050	0.0493	0.0020817

Ammonia ($NH_3 - N$)**Table 15: Ammonia Calibration Standards**

Ammonia Concentration (mg/L)	Spectrophotometer Measurement
0	0
0.1255	0.137
0.25	0.264
0.375	0.397
0.5	0.541

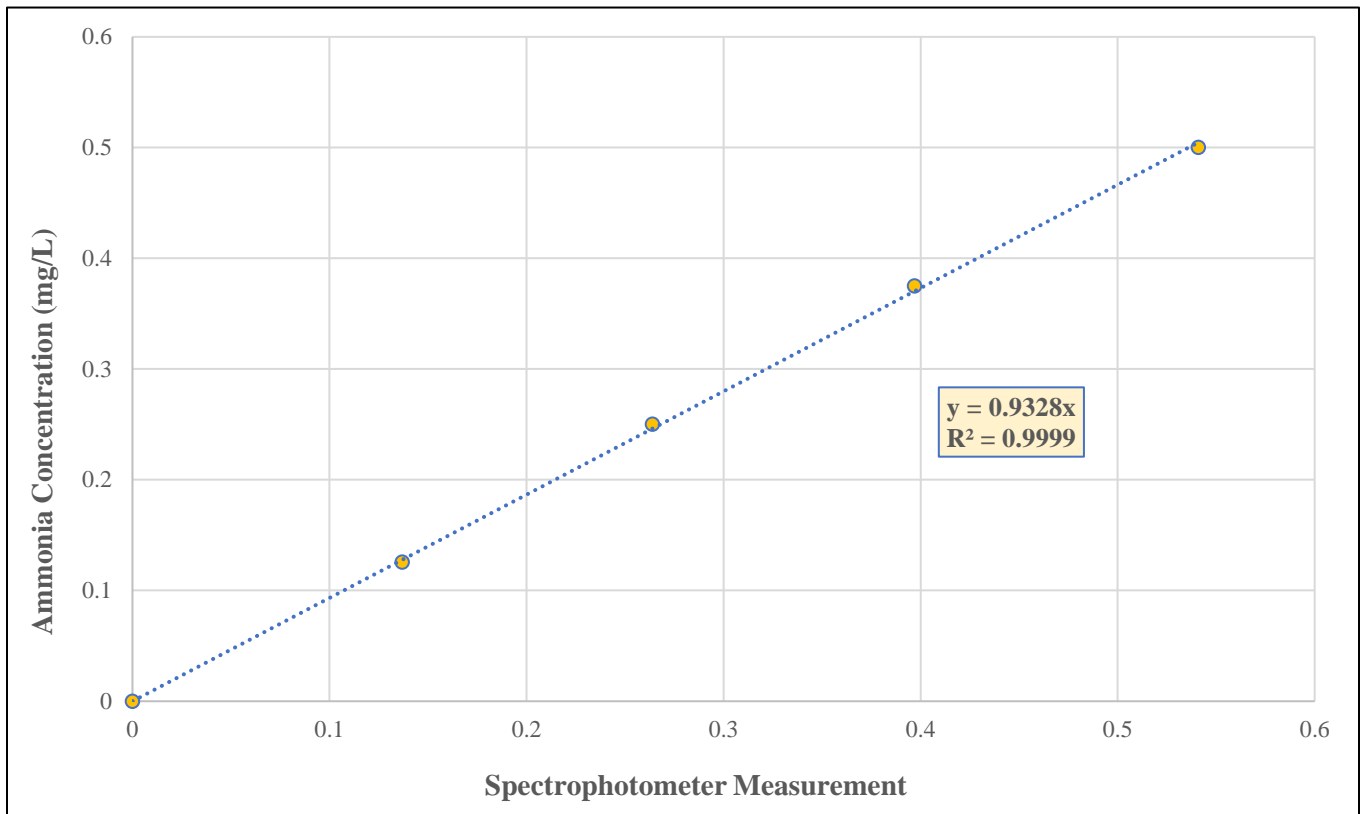


Figure 2: Ammonia Calibration Curve

Table 16: Ammonia Testing Unprocessed Data

Sample ID	Spectrophotometry Measurement				
	Trial 1	Trial 2	Trial 3	Average	Std. Deviation
C1.1	1.003	1.040	1.141	1.0613	0.0714306
C1.12	0.757	0.804	0.822	0.7943	0.0335609
C2.1	1.487	1.604	1.702	1.5977	0.1076398
C2.12	1.805	1.908	1.984	1.8990	0.0898387
C3.1	1.068	1.095	1.123	1.0953	0.0275015
C3.12	1.311	1.439	1.581	1.4437	0.1350605
B1.1	1.315	1.387	1.488	1.3967	0.0869042
B1.12	1.533	1.612	1.670	1.6050	0.0687677
B2.1	0.969	1.018	1.067	1.0180	0.0490000
B2.12	1.252	1.154	1.167	1.1910	0.0532259
B3.1	1.295	1.406	1.463	1.3880	0.0854342
B3.12	1.620	1.539	1.633	1.5973	0.0509346

Appendix 3: Processed Data

Headspace Gas Sampling

Table 1: Carbon Dioxide Concentration Processed Data

Week	Control Carbon Dioxide Concentration		Biochar Carbon Dioxide Concentration	
	Average (%)	Standard Deviation (%)	Average (%)	Standard Deviation (%)
1	14.144	7.185	16.226	4.237
2	13.091	7.954	11.292	9.182
3	11.448	4.231	11.431	9.547
4	11.479	2.932	13.962	8.730
5	12.099	10.480	12.358	13.411
6	8.964	7.825	6.451	8.500
7	11.473	8.198	15.493	12.148
8	11.786	6.703	20.846	14.759
9	13.216	6.866	24.297	11.299
10	9.001	7.837	26.011	14.719
11	3.853	6.674	17.913	16.335
12	6.477	11.218	18.610	14.718

Table 2: Methane Concentration Processed Data

Week	Control Methane Concentration		Biochar Methane Concentration	
	Average (%)	Standard Deviation (%)	Average (%)	Standard Deviation (%)
1	1.255	1.029	1.544	0.746
2	0.695	0.322	0.878	0.763
3	0.584	0.264	1.302	1.127
4	1.110	0.618	1.964	1.178
5	1.879	1.207	1.324	1.225
6	1.324	0.956	0.782	0.961
7	1.316	0.607	2.800	2.406
8	1.679	0.463	7.886	7.061
9	1.284	0.727	18.771	18.196
10	0.630	0.478	26.404	23.565
11	0.434	0.553	14.728	16.680
12	0.824	1.387	24.429	27.236

Chemical Oxygen Demand (COD)

Table 3: COD Concentration Processed Data

Sample ID	Average Spectrophotometer Value	Average COD Concentration 1:100 Diluted (mg/L)	Average COD Concentration Undiluted (mg/L)	Std Deviation Undiluted (mg/L)
C1.1	0.0450	101.09	10109.25	1188.74
C1.12	0.0163	36.69	3669.28	850.51
C2.1	0.0400	89.86	8986.00	0.00
C2.12	0.0490	110.08	11007.85	673.95
C3.1	0.0407	91.36	9135.77	686.32
C3.12	0.0320	71.89	7188.80	1572.55
B1.1	0.0513	115.32	11532.03	1152.81
B1.12	0.0420	94.35	9435.30	224.65
B2.1	0.0443	99.59	9959.48	467.65
B2.12	0.0310	69.64	6964.15	1572.55
B3.1	0.0513	115.32	11532.03	1316.33
B3.12	0.0493	110.83	11082.73	467.65

Ammonia ($NH_3 - N$)

Table 4: Ammonia Concentration Processed Data

Sample ID	Average Spectrophotometer Value	Average Ammonia Conc. 1:100 Diluted (mg/L)	Average Ammonia Conc. Undiluted (mg/L)	Std. Deviation Undiluted (mg/L)
C1.1	1.061	0.9900	99.0012	6.6630
C1.12	0.794	0.7410	74.0954	3.1306
C2.1	1.598	1.4903	149.0303	10.0406
C2.12	1.899	1.7714	177.1387	8.3802
C3.1	1.095	1.0217	102.1727	2.5653
C3.12	1.444	1.3467	134.6652	12.5984
B1.1	1.397	1.3028	130.2811	8.1064
B1.12	1.605	1.4971	149.7144	6.4147
B2.1	1.018	0.9496	94.9590	4.5707
B2.12	1.191	1.1110	111.0965	4.9649
B3.1	1.388	1.2947	129.4726	7.9693
B3.12	1.597	1.4900	148.9993	4.7512

Volatile Fatty Acids (VFAs)

Table 5: Acetate Concentration Processed Data

Week 1 (mg/L)				
Sample ID	Acetate (1)	Acetate (2)	Average Acetate	Acetate Standard Deviation
C1	2210.70	3941.40	3076.05	1223.79
C2	4100.00	3933.60	4016.80	117.66
C3	2016.20	2305.60	2160.90	204.64
B1	1952.50	2417.60	2185.05	328.88
B2	1813.90	3920.60	2867.25	1489.66
B3	3807.30	2645.00	3226.15	821.87
Week 6 (mg/L)				
Sample ID	Acetate (1)	Acetate (2)	Average Acetate	Acetate Standard Deviation
C1	532.41	711.71	622.06	126.78
C2	4512.21	874.64	2693.43	2572.15
C3	1269.44	362.91	816.18	641.01
B1	3432.67	1205.18	2318.93	1575.07
B2	4533.46	2090.66	3312.06	1727.32
B3	2363.95	2978.85	2671.40	434.80
Week 11 (mg/L)				
Sample ID	Acetate (1)	Acetate (2)	Average Acetate	Acetate Standard Deviation
C1	861.05	925.78	893.42	45.77
C2	1281.23	1733.99	1507.61	320.15
C3	933.49	719.12	826.31	151.58
B1	1599.00	246.03	922.52	956.69
B2	399.86	294.19	347.03	74.72
B3	2567.97	3878.83	3223.40	926.92

Table 6: Propionate Concentration Processed Data

Week 1 (mg/L)				
Sample ID	Propionate (1)	Propionate (2)	Average Propionate	Propionate Standard Deviation
C1	474.80	1015.30	745.05	382.19
C2	800.00	559.60	679.80	169.99
C3	522.70	666.50	594.60	101.68
B1	525.10	715.40	620.25	134.56
B2	554.30	1147.40	850.85	419.39
B3	1252.90	779.80	1016.35	334.53
Week 6 (mg/L)				
Sample ID	Propionate (1)	Propionate (2)	Average Propionate	Propionate Standard Deviation
C1	213.68	258.50	236.09	31.69
C2	673.49	204.82	439.16	331.40
C3	371.77	197.45	284.61	123.26
B1	1713.46	699.24	1206.35	717.16
B2	1756.42	1111.81	1434.12	455.81
B3	1121.73	1437.07	1279.40	222.98
Week 11 (mg/L)				
Sample ID	Propionate (1)	Propionate (2)	Average Propionate	Propionate Standard Deviation
C1	200.12	264.93	232.53	45.83
C2	267.59	296.06	281.83	20.13
C3	261.01	210.62	235.82	35.63
B1	706.15	166.39	436.27	381.67
B2	602.34	549.11	575.73	37.64
B3	884.64	1468.56	1176.60	412.89

Table 7: Butyrate Concentration Processed Data

Week 1 (mg/L)				
Sample ID	Butyrate (1)	Butyrate (2)	Average Butyrate	Butyrate Standard Deviation
C1	285.30	412.00	348.65	89.59
C2	0.00	0.00	0.00	0.00
C3	216.30	275.20	245.75	41.65
B1	175.50	245.40	210.45	49.43
B2	203.60	369.70	286.65	117.45
B3	416.50	246.40	331.45	120.28
Week 6 (mg/L)				
Sample ID	Butyrate (1)	Butyrate (2)	Average Butyrate	Butyrate Standard Deviation
C1	53.33	60.75	57.04	5.25
C2	316.90	113.20	215.05	144.04
C3	168.73	109.12	138.93	42.15
B1	481.59	247.94	364.77	165.22
B2	554.36	339.84	447.10	151.69
B3	331.67	610.09	470.88	196.87
Week 11 (mg/L)				
Sample ID	Butyrate (1)	Butyrate (2)	Average Butyrate	Butyrate Standard Deviation
C1	64.42	68.44	66.43	2.84
C2	144.23	156.31	150.27	8.54
C3	133.62	112.63	123.13	14.84
B1	51.70	14.15	32.93	26.55
B2	15.42	12.92	14.17	1.77
B3	48.65	88.92	68.79	28.48

Appendix 4: Microbial Community Analysis Codes

```
library(data.table)
library(reshape2)
library(pathview)
library(ggplot2)
library(SQMtools)

setwd("C:/Users/court/OneDrive/Desktop/HCol Thesis/Data and Results/Biosolids Testing (DNA)")
load("Courteney_Manure.RData")

plotTaxonomy(project, rank = 'phylum', count = 'percent', N=15, ignore_unmapped =T)
```

Figure 1: Phylum Heatmap R Code

```
library(data.table)
library(reshape2)
library(pathview)
library(ggplot2)
library(SQMtools)

setwd("C:/Users/court/OneDrive/Desktop/HCol Thesis/Data and Results/Biosolids Testing (DNA)")
load("Courteney_Manure.RData")

plotTaxonomy(project, rank = 'genus', count = 'percent', N=15, ignore_unmapped =T)
```

Figure 2: Genus Heatmap R Code

```
library(data.table)
library(reshape2)
library(pathview)
library(ggplot2)
library(SQMtools)

setwd("C:/Users/court/OneDrive/Desktop/HCol Thesis/Data and Results/Biosolids Testing (DNA)")
load("Courteney_Manure.RData")

plotTaxonomy(project, rank = 'genus', count = 'percent', N=15, ignore_unmapped =T)
plotTaxonomy(project, rank = 'genus', count = 'percent', tax = c("Methanocorpusculum"))
Meth <- subsetTax(project, "genus", "Methanocorpusculum")
plotTaxonomy(Meth, rank = 'genus', count = 'percent', ignore_unmapped =T)
```

Figure 3: Methanocorpusculum Genera Heatmap R Code