COMPARATIVE ASSESSMENT OF THE EFFECTS BIOCHAR PARTICLE SIZE HAS ON MICROBIAL ACTIVITY

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CLAYTON POPE

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APPROVED BY:

Dr. Jeremy Ferrell, Ph.D.
Chairperson, Thesis Committee

Dr. Matthew Ogwu, Ph.D.
Member, Thesis Committee

Dr. Ok-Youn Yu, Ph.D.
Member, Thesis Committee

Dr. Ok-Youn Yu, Ph.D.
Chairperson, Department of Sustainable Technology and the Built Environment

Ashley Colquitt, Ph.D.
Associate Vice Provost and Dean, Cratis D. Williams School of Graduate Studies

Abstract

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Clayton Pope
B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Dr. Jeremy Ferrell

The detrimental consequences of industrial agriculture, exacerbated by climate change, have intensified the need for solutions to address the critical challenges it poses. Modern agricultural practices have led to widespread soil degradation and nutrient depletion, endangering future food production. Improving soil health has emerged as a potential remedy, not only for addressing industrial agriculture's issues but also for combating climate change by sequestering carbon, enhancing fertility, reducing fertilizer use, and protecting waterways. Biochar, a soil amendment, has garnered attention as a solution, but its viability remains uncertain. This study aimed to investigate how different biochar particle sizes influence microbial respiration when inoculated with effective microorganisms 1 (EM1). The primary goal is to determine which particle size of biochar elicits the most substantial microbial respiration, measured in terms of CO_2 flux. The hypothesis posits that the smallest particle size of biochar will yield the highest microbial response in terms of CO_2 flux. Two trials were conducted, one with biochar alone (Trial 1) and another with biochar incorporated into soil (Trial 2). Both trials assess the impact of different particle sizes on microbial activity by measuring CO_2 flux. The samples are inoculated

with EM1, and data is collected over a four-week period. Data is analyzed using LI-COR equipment. The results revealed a complex yet uncertain relationship between particle size and microbial activity. While the 2-4mm particle size consistently exhibited the highest CO_2 flux values in both trials, the smaller particle sizes, such as <2mm, showed increased inoculant retention, potentially leading to waterlogged conditions and decreased microbial activity. Biochar, regardless of particle size, outperformed inoculated soil in promoting microbial activity. Affirming biochar's potential as a soil amendment and microbial carrier. Biochar remains a promising soil amendment for enhancing soil health and crop productivity when used in combination with microbial inoculants. Overall, this study contributes to understanding the link between biochar particle size and microbial activity after inoculation. While this research does not directly assess the effects of inoculated biochar on plant growth, microbial activity is indicative of nutrient uptake and soil quality, vital for preserving soil health. The findings suggest that biochar, especially with a particle size of 2-4mm, has the potential to enhance soil quality, crop productivity, and the sustainability of future food systems. Further research should explore the mechanisms driving these observations and their long-term effects on soil and plant growth.

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Introduction

Statement of the Problem

In the face of the catastrophic impacts of industrial agriculture, which are further exacerbated by climate change, the need for solutions to address these consequences has become increasingly urgent. Degraded soils, caused by modern agricultural practices, have resulted in severe nutrient depletion in soils worldwide, posing a threat to future food production. Many have recognized that improving soil health could play a significant role in addressing not only industrial agriculture, but the converging crisis, climate change, by sequestering carbon, enhancing fertility, reducing fertilizer use, protecting waterways, and more. Biochar, a soil amendment, has emerged as a potential solution, but its viability remains uncertain.

As the foundation of the food web and terrestrial life, soil health is crucial, and the application of soil amendments, such as biochar, is imperative to preserve soil quality for future generations in the face of rapid soil degradation. While research has shown that biochar has numerous beneficial effects on soil health and plant growth, there is limited data on the optimal particle size of biochar for the most favorable response. For instance, there is a lack of clear understanding regarding which particle size of biochar (e.g., powdered, larger than 2mm, etc.) elicits the highest microbial response after inoculation and subsequently provides the most beneficial impact when added to the soil.

Purpose of the Study

The purpose of this study is to investigate the relationship between biochar particle size and microbial activity in biochar. By characterizing biochar by particle size, the study aims to determine how different particle sizes impact microbial activity in biochar. In doing so, the overall goal of this research is to determine what particle size of biochar leads to the most

microbial respiration after inoculation with a uniform compound (EM1). This study intends to reach a conclusion by quantifying the microbial activity in terms of CO_2 flux in the separate samples of inoculated biochar through a series of comparative tests, and analyzing the data by evaluating time frames for when microbial activity is at its peak and when it begins to decrease.

Research Hypothesis and Questions

The hypothesis for this study is that the smallest particle size sample of biochar will generate the greatest microbial response in terms of CO_2 flux. The larger particle size samples will likely have significant microbial activity, due to the uniform inoculation with EM1, but respiration rate and particle size will likely be negatively correlated. In other words, as the particle becomes smaller, the rate of CO_2 flux in the sample will likely increase. The research question(s) are as follows: How does the particle size of biochar affect microbial respiration in a liquid inoculation of EM1? Which particle size has the greatest microbial response?

Significance of the Study

The significance of this study lies in its ability to contribute to the understanding of the relationship between biochar particle size and microbial activity after inoculation. Although this study will not incorporate the inoculated biochar into a plant growth experiment, microbial activity is ultimately indicative of nutrient uptake by plants and overall soil quality. Therefore increased microbial activity lends to healthier soils. With industrial agriculture practices causing rapid degradation of soil, the preservation of soil health has become a crucial concern. Biochar, as a soil amendment, has been shown to have several benefits for soil health and plant growth, but the particle size that leads to the most favorable response remains unknown. By investigating the impact of different biochar particle sizes on microbial activity, this study aims to provide insight into which particle size of biochar is most effective for soil health preservation and

improvement. The results of this study could inform the application of biochar in agricultural practices, leading to improved soil health, increased crop productivity, and a more sustainable food system for future generations.

Limitations of the Study

Biochar is a soil amendment. Hence, in practice, biochar is commonly applied to the soil in the field in order to experience its beneficial results. This study is limited because biochar samples will be tested independent from the soil in a lab environment. The lab environment presents ambient conditions in the room, and samples are kept in containers limiting gas exchange to some degree. Through analyzing microbial activity across the different biochar samples, this study will be able to determine what sample induces the highest microbial response, but will not be able to determine whether the effects of that response are relayed to plant growth upon incorporation to the soil. A general, but often accurate assumption is made that more microbial activity will be better for the soil. Additionally, this study is limited through the choice of a specific inoculant, EM1. Biochar is commonly inoculated with several different things to induce microbial activity, like compost for example. Generally, EM1 is not a common inoculant for biochar. This study will utilize EM1 because its ingredients are well defined and widely studied. However, this study cannot determine whether other inoculants would have the same response to biochar particle size as EM1.

Review of Literature

Brief History of Agriculture

Approximately 12,000 years ago, agriculture began to emerge independently in various communities worldwide (Van der Crabben, 2021). Agriculture, the cultivation of crops, and the raising of livestock for food, marked a departure from the hunter-gatherer lifestyle that had sustained human populations for hundreds of thousands of years. Access to a constant and reliable food supply became a more favorable lifestyle. From agriculture grew permanent communities, and eventually, the modern cities seen across the globe today (Driver, 2018). The adoption of agriculture did not occur due to a single factor and is believed to have happened independently across the globe. Climate conditions and pressure on natural resources are among the numerous potential influences that may have played a role in this progression (Driver, 2018). Whatever the origins, it is evident that agriculture sowed the seeds for the development of modern civilization.

Agriculture evolved through three major periods - domestication, Pre-1800 to Green Revolution and Post-Green Revolution, beginning with domestication (Van der Crabben, 2021). Historical evidence suggests that the initial emergence of plant domestication can be traced back nearly 12,000 years to the regions of the Fertile Crescent, parts of China, and Central America, with the earliest domesticated species including wheat, barley, chickpeas, lentils, rice, and fruit trees (Van der Crabben, 2021). The initial domestication of plants and animals progressed into more sophisticated forms of agriculture over the thousands of years following. Innovations in irrigation, plowing, food storage techniques, and the establishment of a dedicated agricultural workforce in the form of “farmers” significantly increased agricultural production worldwide (Van der Crabben, 2021).

In roughly 1,000 AD, the advent of European monasteries brought about the next big agricultural progression, the creation of marginal landscapes (Mason, 2014). This innovation saw floodplains drained, woodlands converted to plains, and areas of low fertility altered to be fit for agricultural use (Mason, 2014). Throughout history, agricultural practices continued to evolve, with the mastery of crop rotation being achieved in 17th century England and the rise of various farming techniques, such as the French market gardeners' use of horse manure and raised beds, in the 18th century. By the 19th century, selective breeding was backed by science and the manufacturing of basic farm equipment became widespread, paving the way for the green revolution (Tauger, 2008).

Green Revolution

The 20th century, home to the industrial revolution, also housed its agricultural counterpart, the green revolution. Characterized by a surge in agricultural production coinciding with global population growth, the green revolution was equally as important as the Industrial one. Formally beginning in Mexico with the creation of a hybrid wheat boasting a much higher yield, the keynote techniques behind the green revolution quickly spread worldwide (Tauger, 2008). In addition to plant genetics and monocropping, the basis for the green revolution was a multitude of techniques designed to significantly enhance crop productivity. Techniques include enriching the soil by applying powerful synthetic fertilizers and combating plant pathogens and pests with chemical pesticides. Coupled with modern irrigation methods and farm equipment, the techniques doubled and tripled crop yields globally (Tauger, 2008).

The Green Revolution transformed rural economies into modern ones, and food poverty and hunger were no longer at the forefront of societal issues in much of the developed world (El Bilali et al., 2018). The global population nearly quadrupled from 1.6 billion in 1900 to 7 billion

over the years following the green revolution (Driver, 2018). Today, the techniques leveraged during the green revolution continue to be the hallmark of modern agriculture and the backbone of the commercial/industrial agricultural systems that feed most of the world's population.

Modern Agriculture

Commonly referred to as, modern, industrial, or commercial agriculture, the associated agricultural practices are largely characterized by the techniques established during the green revolution. This high-input, high-output model based on the implementation of advanced farming machinery and irrigation systems allows for the cultivation of fields of crops in excess of thousands of acres. Monoculture and genetically modified crops allow for favorable plant qualities and eliminate a large portion of plant diseases, meaning seamless and efficient management of large fields (Tauger, 2008). Consistent application of chemical pesticides ensures optimal productivity and efficiency, while synthetic fertilizers substitute the natural soil nutrients, negating the need for crop rotation and enabling the intensive utilization of the same land (Tauger, 2008). Through the widespread employment of these practices, the time for small community and subsistence farming is long past, massive commercial fields are representative of crop cultivation in many parts of the world (Dimitri et al., 2005).

As a result of these techniques, food prices remain low, and the populations of many countries are adequately fed. Over time, however, these techniques have been drawn into question as they can be related to several negative impacts seen in both the natural ecosystems and agricultural environments. The techniques which once fore fronted the societal shift away from poverty and towards prosperity, now pose a great ultimatum as they threaten the health of the environment and its matrices.

Agricultural Consequences

Soils are extremely diverse and dynamic. They play a fundamental role in supporting plant, microbe, insect and other communities, interacting with the atmosphere, regulating water cycles and more (Hunt et al., 2010). The Green Revolution, characterized by the implementation of techniques aimed at enhancing crop productivity, has had significant impacts on soils and the global agricultural landscape as a whole (Gomiero, 2016). The expansion of agricultural land, elevated water consumption, soil degradation, and chemical runoff have resulted in the contamination of soil, air, and water in both agricultural lands and surrounding natural ecosystems, including the world's oceans (Lindwall, 2019). Agricultural contaminants threaten nearly every part of the earth's ecosystems, as climate change puts additional pressure on the world's food systems, the vulnerabilities of the modern food system have become increasingly apparent.

Water is an essential input for agricultural production, agriculture accounts for nearly 70% of total global freshwater withdrawals (Lindwall, 2019). The tremendous allocation of freshwater to agriculture, however, also leads to significant pollution of these water sources. Agriculture is the largest contributor of non-point source pollution to surface water and groundwater (Lindwall, 2019). Agricultural runoff, which contains chemical fertilizers and pesticides applied to the fields, ends up in local waterways and groundwater resources, contaminating these essential sources of water. As noted by Lindwall (2019), the excessive use of fertilizers in agriculture can cause significant environmental harm, including the stimulation of algal blooms in waterways, which can have negative impacts on stream health.

Soil, as a complex ecosystem, holds immense value as a resource crucial for food production. Unfortunately, intensive modern agriculture has led to the rapid degradation of soils

on a global scale (Gomiero, 2016). Monocropping and intense tillage, in conjunction with the application of chemical pesticides and synthetic fertilizers, have contaminated the soil and hampered its health (Pimentel, 2006). Soil health and quality are important because the soil is the foundation of terrestrial life and thereby the food web. According to Bamdad et al. (2021), healthy soils harbor a diversity of microbial and fungal life, ranging from bacteria to nematodes to mycorrhizal fungi, which play a vital role in the cycle of nutrients and life by breaking down minerals and biomaterials to provide essential nutrients to growing plants. In addition, healthy soil acts as a sponge, supplying water and oxygen to both plant roots and microbial life (Bamdad et al., 2021).

Throughout history, soil health was maintained using novel techniques such as crop rotation, intercropping, cover cropping, and more. In commercial agriculture soil health isn't maintained, rather it is substituted. Mono cropping and intensive use of the same fields every season doesn't provide adequate time for the soil to naturally replace lost nutrients through nutrient cycling (Bamdad et al., 2021). As a result, the soil becomes depleted of nutrients, forcing crops in intensive agriculture systems to rely on constant inputs of synthetic chemical fertilizers, particularly nitrogen-based fertilizers. Poor quality and degraded soils lead to rapid soil erosion and intensified runoff, furthering water pollution and soil degradation (Pimentel, 2006). Soil erosion refers to topsoil particles wearing away through wind, water, and farming activities like tillage (Gomiero, 2016). The loss of topsoil, which is rich in organic matter and macronutrients vital for crop growth, only perpetuates the need for chemical fertilizers, creating a cycle that exacerbates soil degradation with each planting season.

Building Soil Health

The impacts of modern agriculture present a significant risk to future food security if left unmitigated (El Bilali et al., 2018). The increasing global population will require even more food to be produced, putting further stress on cultivated lands and necessitating the creation of new ones. To achieve food security for present and future generations, all components of food systems need to be resilient, efficient, and most importantly sustainable (El Bilali et al., 2018). In the realm of sustainable agriculture, food producers have the advantage of drawing from a rich history of innovative farming techniques that are far less environmentally detrimental and can help mitigate the negative impacts of modern agricultural practices. These techniques shed light on a food system that shifts away from the chemically intensive, monoculture approach of industrial agriculture and back towards the traditional methods that work with the ecosystem instead of against it (Yang, T. et al., 2020). Methods include: intercropping, crop rotation, no-till farming, cover cropping, integrated grazing, agroforestry, regenerative agriculture, and others seen throughout the history of agriculture (Yang, T. et al., 2020). By embracing these methods, food producers can support natural resource conservation, reduce pollution and soil erosion, enhance biodiversity among crops, and most importantly, promote soil health.

Building soil health is important because the quality of the soil directly relates to the quality of the crops grown within it, as well as the surrounding ecosystems. Building soil health pertains to soil structure, porosity, water retention, aeration, nutrient content, and biological activity, all of which are important factors to consider when amending soil (Yang, T. et al., 2020). Outside of the traditional production methods like cover cropping, crop rotation, and more that generally promote soil health and quality, there are several soil amendments that aim specifically to build soil health.

Soil Amendments

Although more common in practice, soil amendments are not limited to traditional farming methods and have the potential to be applied to modern commercial agriculture systems as well. Soil amendments are materials applied to or mixed into the topsoil to change soil properties and improve plant growth (Traunfeld, 2020). Typically, in the form of organic matter, soil amendments can be used to improve soil structure and drainage, reduce erosion, improve plant growth, alter ph, add nutrients, and encourage microbiological activity in the soil **(**Traunfeld, 2020). Presently, there are several different soil amendments gaining traction.

Biosolids, or composted sludge from livestock, is a form of soil amendment rich in organic matter, nutrients, and microbial life (Garbowski et al., 2023). Compost is a common soil amendment consisting of a mixture of organic materials such as food waste, grass clippings, leaves, etc. Compost serves to increase soil organic matter content and nutrients, as well as improve soil physical properties (Bamdad et al., 2021). Another form of composting, vermicompost, utilizes various species of worms to achieve the decomposition process and convert organic materials such as food leftovers and scrap paper into a mixture of decomposed organic matter and earthworm castings (Adhikary, 2012). Vermicompost is rich in both microbiological activity and plant-available nutrients and has been shown to improve the physical properties of soil (Adhikary, 2012). Biochar, a less widely studied soil amendment, is charred organic matter made by burning biomass such as wood waste in the absence of oxygen, known as pyrolysis. The end product is a carbon-rich charcoal that is stable, porous, and variable depending on the feedstock and the process used (Hunt et al., 2010).

Biochar

Biochar, a highly porous and carbon-rich product derived from the pyrolysis of biomass, is generated through thermal decomposition under oxygen-restricted conditions at temperatures above 250 degrees C (Garbowski et al., 2023). According to the International Biochar Initiative (IBI), biochar is defined as a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment. Although the research on biochar is still emerging, its application in soils has a long history. Evidence of extensive use of biochar can be seen in the highly fertile Terra Preta and Terra Mulata soils in the Amazon Basin, created by ancient indigenous cultures over 2000 years ago (Hunt et al., 2010). Despite heavy tropical rains for centuries, the abundant biochar in the soil has helped maintain the region's fertility (Hunt et al., 2010). Hunt et al. (2010) further explain that biochar can be found in soils worldwide due to natural occurrences such as wildfires in forests and grasslands. The North American Prairie region, located between the Mississippi River and Rocky Mountains, is known for its naturally high levels of biochar, making it one of the most fertile regions in the world. Laboratory studies estimate that the average lifetime of biochar in soil ranges from 1300 to 4000 years (Hunt et al., 2010).

Uses of Biochar

Biochar can be a simple yet powerful tool to amend degraded soils and potentially combat climate change. When organic materials decay, greenhouse gasses, such as carbon dioxide and methane, are released into the atmosphere. However, by subjecting organic material to pyrolysis, much of the carbon becomes "fixed" into a more stable form and can effectively be sequestered when applied elsewhere (Hunt et al., 2010). Bamdad et al. (2021) stated that biochar has various uses beyond soil amendment, such as acting as a catalyst, producing gas/liquid

adsorbents for eliminating pollutants, supplementing cement or building materials, and manufacturing biosensors. That said, biochar is an extremely promising soil amendment with the ability to contribute to the reduction of agricultural and urban solid waste, while also improving soil and plant quality. Numerous scientific publications report on the agronomic benefits of biochar applied to different types of soils (Bamdad et al., 2021). Biochar has diverse potential applications as a soil amendment as well, including pollution remediation, improvement of soil fertility, enrichment of volatile matter, enhancement of soil structure, and carbon sequestration (Tomczyk et al., 2020).

Unlike typical fertilizers, biochar does not directly provide nutrients to plants, but rather supports the uptake of water and nutrients by plants and enhances microbial life through its porosity and surface area. Therefore, biochar is often used in combination with other fertilizers, microorganisms, and nutrient-rich compounds such as compost to inject nutrients and microbiological life into the soil mix (Hunt et al., 2010). Additionally, most biochar is alkaline and can serve as an alternative to lime for ameliorating acidic soils, thus increasing nutrient availability (Bamdad et al., 2021). Similar to liming, biochar can adsorb cations and anions from the soil solution, reducing leaching of nutrients introduced with fertilizers (Garbowski et al., 2023). This capability makes biochar an effective sorbent for reducing chemical runoff, leaching, and pollution (Bamdad et al., 2021). Organic and inorganic compounds, such as heavy metals and pesticides, can be adsorbed to the surface of biochar through pore-filling mechanisms, and the complex structure of biochar traps pollutants and reduces them into less mobile species (Bamdad et al., 2021). Biochar has many potential applications, in and outside of the soil. Although, its benefits when applied to the soil are profound and widely studied.

Benefits and Drawbacks of Biochar

Biochar possesses several beneficial properties from an agricultural perspective, including a high surface area per unit volume, high porosity, high pH value, high CEC, high carbon content, low content of resins, and potential for nutrient and microorganism inoculation (Garbowski et al., 2023). These properties allow for the improvement of soil physical, chemical, and biological characteristics (Tomczyk et al., 2020). The large specific surface area and porosity of biochar enable it to adsorb and retain nutrients and water, as well as provide a conducive habitat for beneficial microorganisms to thrive (Hunt et al., 2010). The alkalinity and high CEC of biochar can also amend acidic and depleted soils by increasing nutrient availability and reducing nutrient leaching (Tomczyk et al., 2020). Additionally, biochar is inherently carbon-negative, contributing to long-term carbon sequestration in the soil for thousands of years (Hunt et al., 2010). In summary, the benefits of biochar include promoting plant productivity by enhancing nutrient availability, reducing erosion through soil structure improvement, mitigating nutrient leaching, mitigating losses of gaseous components, and introducing beneficial microbial life to the soil (Tomczyk et al., 2020).

While the benefits of biochar have been extensively studied, its potential negative effects are not as well understood. Some studies have highlighted biochar's cytotoxic effect, which is attributed to microscopic biochar particles attaching to living cells and causing oxidative stress, changes in cell morphology, and inhibiting nutrient transport across cell membranes (Garbowski et al., 2023). Moreover, microbial decomposition of biochar in soil may temporarily limit the availability of nitrogen and other nutrients to plants (Hunt et al., 2010).

Current State of Biochar Production

Biochar is produced by heating organic material under conditions of limited or no oxygen, a process known as pyrolysis (Hunt et al., 2010). Pyrolysis-based production of biochar offers several benefits, including reducing biomass waste volume, lowering the risk of pathogens, organic pollutants, and heavy metal availability, and most importantly, decreasing greenhouse gas emissions associated with biomass (Garbowski et al., 2023). There are various methods for producing biochar, ranging from low-cost to commercial-grade approaches. Historically biomass was piled and covered with soil in pits, and burned slowly with limited air, which eventually evolved into handmade reactors such as firebrick pits, clay burners, kilns, iron retorts, and large metal bins for low cost biochar production (Gabhane et al., 2020). Commercial production of biochar typically involves large steel ovens or expensive pyrolysis reactors, with costs ranging from $50,000 to over $1,000,000 (Gabhane et al., 2020). Over time, various approaches such as flash, vacuum, and microwave-pyrolysis, gasification, torrefaction, hydrothermal carbonization, and electro-modified techniques have been developed for biochar production (Gabhane et al., 2020). Regardless of the production method, biochar intended for agricultural use should meet the requirements specified by the European Biochar Certificate (EBC) or the International Biochar Initiative (IBI) (Garbowski et al., 2023).

Key Metrics in Biochar Quality

Biochar has the potential to enrich soil properties and interact with soil biota due to its unique physical and chemical characteristics, including a stable carbon-based structure, large surface area, accommodating pH, high porosity, large cation exchange capacity, and high water holding capacity (Hunt et al., 2010). However, these properties are subject to change and can vary significantly across different biochar. The composition of biochar is influenced by various

factors such as the type of source materials, and production parameters, which can impact its performance as a soil amendment (Bamdad et al., 2021). Research on biochar has yielded mixed results, indicating that biochar is not a single product but a wide range of chemically diverse products that can have different effects on different soils under different conditions. Moreover, soils are highly diverse and dynamic, and there is no one-size-fits-all solution for biochar. Therefore, selecting suitable production conditions to achieve desired biochar properties requires understanding the dependencies, influencing factors, and desired outcomes (Tomczyk et al., 2020). Production parameters, particularly pyrolysis temperature and feedstock type, have the most significant effect on biochar properties (Tomczyk et al., 2020).

Temperature

Temperature plays a crucial role in determining the characteristics of biochar produced (Ippolito et al., 2020). There are two predominant types of pyrolysis systems used in biochar production: fast and slow pyrolysis, which differ in terms of heating rate and duration (Kookana et al., 2011). Fast pyrolysis occurs at temperatures above 550 degrees Celsius, while slow pyrolysis typically ranges from 200 to 500 degrees Celsius (Kookana et al., 2011). Generally, slow pyrolysis produces higher biochar yields but with lower quality, while fast pyrolysis results in lower yields but higher quality biochar (Bamdad et al., 2021).

Research indicates that high pyrolysis temperature promotes the production of biochar with a well-developed specific surface area, high porosity, pH, ash, and carbon content, but with lower cation exchange capacity (CEC) and volatile matter content (Tomczyk et al., 2020). Surface area and porosity of biochar are crucial properties that influence water movement and storage within the biochar (Bamdad et al., 2021), and both properties tend to increase with temperature as they are interconnected (Kookana et al., 2011). Furthermore, biochar produced at

higher temperatures are known to be effective in increasing water retention in soil (Bamdad et al., 2021).

On the other hand, biochar produced at lower pyrolysis temperatures tends to have higher CEC and contains more volatile matter, which may result in higher adsorption characteristics for heavy metals (Bamdad et al., 2021). Additionally, nutrient availability changes significantly with increasing pyrolysis temperature, with higher temperatures generally resulting in increased content of carbon, phosphorus, potassium, calcium, and decreased content of nitrogen, hydrogen, and oxygen (Ippolito et al., 2020). In general, slow pyrolysis tends to produce biochar with higher nitrogen, sulfur, available phosphorus, calcium, and magnesium content (Ippolito et al., 2020).

Feedstock

The properties of biochar vary greatly with the type of feedstock used (Ippolito et al., 2020). The nature of the feedstock can result in different types of biochar with different chemical structures (Bamdad et al., 2021). Various organic materials, including crop and forestry waste, urban yard waste, industrial biomass by-products, animal manures, and municipal sewage sludge, can be used to produce biochar (Kookana et al., 2011). These materials are often classified as either woody or non-woody biomass, with woody biomass primarily comprising residues from forestry and trees (Tomczyk et al., 2020). The characteristics of woody biomass are low moisture, low ash, high stability, high calorific value, high bulk density, and less voidage, resulting in durable, coarse biochar with high carbon content of up to 80% (Bamdad et al., 2021). At any comparable temperature, the specific surface area of the wood biochar was up to two times greater than that of the grass biochar (Kookana et al., 2011).

In contrast, non-woody biomass consists of agricultural crops and residues, grasses, animal waste, urban and industrial solid waste, and generally has higher moisture and ash content, lower calorific value, higher CEC, low bulk density, and higher voidage (Tomczyk et al., 2020). Although, the feedstock type affects the chemical structure and properties of the resulting biochar (Bamdad et al., 2021). For instance, biochar derived from animal litter and solid waste feedstocks exhibit lower surface areas, carbon content, volatile matter, and high CEC compared to biochar produced from crop residue and wood biomass, even at higher pyrolysis temperatures (Tomczyk et al., 2020). On the other hand, biochar enriched in nitrogen and phosphorus can be derived from different types of manure and seaweeds (Bamdad et al., 2021). Ultimately, the chemical and structural makeup of the biomass feedstock plays a significant role in determining the composition, behavior, function, and fate of the resulting biochar in soils (Kookana et al., 2011).

Particle Size

Particle size, the variable in this study, is another key metric in biochar quality and plays a crucial role in the performance of biochar as a microbial vehicle (Bamdad et al., 2021). Although the physical features of biochar, such as particle size, are believed to significantly affect the soil-plant-microbe system, there are limited studies on this topic (De Jesus Duarte et al., 2019; Bamdad et al., 2021; Chen et al., 2017). Furthermore, little is known about the effect of biochar particle size on soil microbial community, structure, and function (Chen et al., 2017). That said, previous studies aim to shed light on particle size as a key metric in biochar. For instance, De Jesus Duarte et al. (2019) conducted an experiment with particle sizes ranging from (<0.15 mm; 0.15–2 mm and >2 mm) and found that smaller particle size (<0.15 mm) increased water retention, while biochar particles in the range of 0.15-2 mm increased porosity. Liao &

Thomas (2019) manipulated biochar particle size by sieving or grinding to generate particles in two size ranges (0.06–0.5 mm and 2–4 mm), determining that small particle size biochar had the largest liming effect and enhanced water retention capacity.

Literature focused specifically on biochar particle size in tandem with microbial communities is less commonly available, but generally conclusive. For example, Sarfraz et al. (2020) hypothesized that biochar particle size and incubation temperature can significantly influence the microbial community in soil. A laboratory incubation study was established having varying particle sizes ((≤0.5 mm (fine), 0.5–1.0 mm (medium) and 1.0–2.0 mm (large)) under different incubation temperatures. Results showed that fine particle biochar resulted in higher bacterial species richness. Sarfraz et al. (2020) suggested that fine particle biochar and high incubation temperature may provide better habitat for microorganisms compared to the other particle sizes. Zhao et al. (2020) divided biochar into two groups (< 1 mm and 2.5–5 mm) and discovered that biochar enhanced microbial biomass and activity but collectively the fine biochar had a stronger effect on soil microbial community than coarse biochar. The study ultimately emphasized that the relationship between particle size and soil microbial community needs to be considered when using biochar for soil amendment (Zhao et al., 2020).

Chen et al. (2017) ground biochar into three different particle sizes (fine, medium, and course) and determined that the fine-sized biochar induced significantly higher total microbial concentrations than the medium and coarse particles regardless of addition rate. Chen et al. (2017) ultimately suggested that the microbial community structures were largely dependent on particle size, and that the fine particle biochar may additionally produce a better habitat for microorganisms. This suggestion is one of the main operating assumptions of the hypothesis presented in this study. Bamdad et al. (2021) reviewed a study in which biochar was separated by

particle size and observed the better cell growth of bacteria in conjunction with the ground biochar's (1 mm), than that with the same crushed biochar (2 mm), due to increased surface area/particle contact. Conversely, another study found that coarser ground biochar (>150 μm) performed better with a bacterial partner while the same extremely fine biochar (< 150 μm) performed worse (Bamdad et al., 2021). Ultimately, Bamdad et al. (2021) recommends further research in this area to find the optimum particle size of biochar to be used as an effective microbial carrier. Overall, these studies suggest that particle size is a critical factor influencing the performance of biochar in soil, particularly in relation to water retention, porosity, soil ph, nutrient availability, and microbial community structure.

Charging and Inoculation

Biochar serves as a unique soil amendment that does not directly provide nutrients to plants, but instead supports the uptake of water and nutrients by enhancing porosity and surface area. To inject nutrients and microbiological life, biochar is typically mixed with compost or another microbe-rich compound, a process known as inoculation or charging (Hunt et al., 2010). The porosity and surface area of biochar create a favorable habitat for microorganisms, which in turn contribute to nutrient cycling and serve as food for beneficial soil biota such as protozoa, mites, and nematodes, ultimately benefiting the soil and plant rhizospheres. Due to its stable organic structure and architecture, biochar has been studied as an environmentally friendly and cost-effective carrier for inoculants (Bamdad et al., 2021). In some cases, biochar has been inoculated with specific microbes to target the needs of particular plants. Common inoculants for biochar include compost, compost tea, vermicompost, digester effluent, and commercial fertilizers (Garbowski et al., 2023).

EM1 as an Inoculant

This study will utilize EM1 to inoculate biochar. EM1 is not technically a common inoculant for biochar, but it is extensively used within agriculture as a soil amendment and inoculant. Known as *effective microorganisms 1*, EM1 is a ready to use, organic soil amendment packed full of beneficial microbes (Abd El-Mageed et al., 2020). These beneficial microbes help improve soil quality, fertility, pest and disease resistance and boost plant growth and increase yields. The host of beneficial microbes, including photosynthesizing bacteria, multiple strains of lactic acid bacteria, yeasts and more help break down organic matter, turning it into food for plants and releasing essential minerals (Abd El-Mageed et al., 2020).

Minimal literature exists regarding EM1s application to biochar, but some previous studies do cite this application. Yang, X. et al. (2019) combined biochar with effective microorganisms' treatments to explore the influences on the growth of tobacco. The results showed that the biochar and EM application had a significantly increased net photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration. Cui et al. (2021) conducted an experiment with biochar and EM1 treatments and showed that biochar addition in combination with EM significantly increased seed germination, plant height, stem diameter, total biomass and plant nutrient uptake of S. cannabina. Abd El-Mageed et al. (2020) combined biochar with effective microorganisms to study the effects on soil properties finding that biochar in combination with EMs significantly increased plant growth, and productivity, macro- and micro-nutrient concentration, as well as dehydration tolerance and irrigation use efficiency. While biochar and EM1 have not been previously studied in terms of microbial respiration rates, the literature shows that EM1 is an effective inoculant for biochar.

Microbes in Soil

When it comes to soil health, the microbiology surrounding soil microbes plays a crucial role in plant growth (Bell et al., 2021). Microbes are single-celled organisms, including bacteria, fungi, protozoa, and viruses. Coexisting plants and beneficial soil microorganisms form symbiotic relationships within the soil, which are vital for various soil reactions and functions such as organic matter decomposition, humus formation, aggregate formation and stabilization, and nutrient cycling. A diverse and active microbial community enhances soil suitability for plant growth as the combination of nutrients and microbial organisms is essential for healthy and productive plants (Bell et al., 2021). The rhizosphere of soil, where the microbial community is thousands of times richer than in the bulk soil, has yielded a large number of isolated and extensively studied microorganisms (Bamdad et al., 2021). Bamdad et al. (2021) states that the structure of the rhizosphere microbial community is the outcome of complex interactions and feedbacks among plant roots, the physical/chemical properties of the soil, and the microorganisms. Biochar is commonly applied directly within the rhizosphere and therefore should be examined as a microbial carrier.

Microbial Respiration Analysis in Biochar

This study aims to measure CO_2 flux from microbes using LI-COR technology, which has been previously employed in similar studies. For example, in a study focused on assessing the impact of different pyrolysis methods on biochar, researchers used a LICOR 8100 machine to determine CO_2 flux levels in separate samples of biochar (Bruun et al., 2012). Similarly, Sun et al. (2014) used a LI-COR 8100 to successfully take and analyze CO_2 flux measurements in soils where biochar had been applied. Another study used the LI-COR 8100 to determine the effect of biochar on CO_2 respiration in different soil types (Zhou et al., 2017). By comparing soil

respiration levels before and after the application of biochar, the LI-COR 8100 CO_2 flux system allowed them to calculate the difference. While LI-COR technology is commonly used in the literature, its application to biochar is relatively new. These studies were influential in devising a reliable method to measure respiration in biochar samples.

Methodology

Research Methodology

The purpose of this research is to gain a better understanding of the relationship between biochar particle size and microbial activity. Specifically, this study aims to investigate the impact of different particle sizes of Biochar on microbial activity and population, both the absence and presence of soil. The overall objective is to determine the particle size of biochar that results in the highest level of microbial activity after inoculation with a uniform microbial compound, effective microorganisms 1 (EM1). In order to accurately evaluate which particle size of biochar will result in the highest microbial activity and CO_2 flux, a series of steps must be followed to ensure consistency and limited variability. The study encompassed two distinct trials, denoted as Trial 1 and Trial 2, each featuring four different sample groups with variable particle sizes. Trial 1 involved biochar exclusively, whereas Trial 2 introduced a mixture of biochar and soil, with the inoculated material accounting for 10% of the total weight of the wet soil. In both trials, three replicants were maintained for each of the four sample groups, amounting to a total of 12 individual samples.

In any case, the first step in the process was the production of biochar in a single burn. Following production, the biochar was crushed using a hammermill, and then sieved in order to separate and differentiate samples by particle size. In Trial 1, four distinct particle sizes of biochar were examined, each being replicated three times, resulting in a total of 12 individual samples. Trial 1 particle sizes included 9.75mm-4mm, 2-4mm, < 2mm, and a mixed distribution of the previous three particle sizes denoted as the “Mixed mm” sample. For Trial 2, all particle sizes were the same except the 9.75-4mm biochar samples were replaced with inoculated soil in order to provide a reference as to what CO_2 flux values would look like without biochar.

Following the sieving process, the samples were dried and sterilized in a lab oven set at 65.5 degrees Celsius (150º F)for a duration of one day. The biochar samples were then separated into their respective buckets by weight and inoculated with EM1 through specific procedures that will remain consistent for all samples. Following a two-day incubation period with occasional mixing the samples underwent a one-day straining process before commencing respiration testing. Trial 1 assessed the inoculated biochar in isolation, whereas Trial 2 integrated the inoculated biochar into a uniform potting soil at a ratio of 10% by wet weight. The soil had a moisture content of roughly ~30%. Microbial activity was measured using a LI-COR 8200-01s smart chamber in conjunction with the LI-870 CO_2/H2O gas analyzer. This instrumentation allowed for the real-time monitoring of CO_2 fluxes (μmol m^2 s^1)) within the samples. The CO_2 flux values were employed to gauge the levels of microbial activity and respiration in the individual samples daily over a four-week period.

Biochar Properties

In order to begin with the experimental process, biochar must first be produced. The biochar used in this study was produced at the Appalachian State University NEXUS facility in Boone, North Carolina using an octagonal kiln. The temperature in the kiln reaches in excess of 500 degrees C**.** The feedstock used for biochar production was primarily comprised of mixed hardwood, with nearly 90% consisting of Hybrid Poplar. It is noteworthy that all the biochar required for both trials in this experiment was produced in a single burn, thereby ensuring uniform characteristics across all samples. Figure 1 provides a visual representation of the octagonal kiln employed for biochar production in this experiment.

Figure 1

Octagonal Biochar Kiln

Crushing, Sieving, Sterilization

Following biochar production, the biochar underwent a sequence of preparatory steps. Initially, the biochar was left to dry in the open air for nearly a week. Following the drying period a 4kW hammermill was employed to crush the biochar, ensuring the generation of random particle sizes within the batch (Figure 2). Post-hammermill, the biochar was sieved using a Humboldt 120 volt sieve in order to differentiate samples by particle size (Figure 3). For Trial 1 samples were separated into sizes of less than 2mm, 2mm to 4mm, 4mm to 9.75mm, and randomized particle size distribution containing a mix of the three (Mixed mm)**.** Figures 4, 5, 6, and 7 illustrate the four different particle sizes. Each particle size was replicated twice more within the trial for a total of 12 individual samples. For Trial 2 the 4 to 9.75mm biochar particle size was substituted for inoculated soil. All samples of inoculated material in both trials were

sterilized in a lab oven for 24hrs at 65.5 degrees Celsius (150ºF) immediately prior to inoculation in order to remove any already active microbes (Figure 8).

Figure 2

Hammermill

Figure 3

Humboldt Sieve

Figure 4

Less Than 2mm Biochar (<2mm)

Figure 5

Mixed Biochar (Mixed mm)

Figure 6

2mm to 4mm Biochar (2-4mm)

Figure 7

4mm to 9.75mm Biochar (4-9.75mm)

Figure 8

Sterilization Oven

Inoculation with EM1 and Setup

After the separation of samples by particle size, the next step is to inoculate the biochar samples with EM1. Inoculation, in this context, refers to the introduction of microorganisms into the biochar samples. To minimize variability in the inoculation process, a commercially available EM1 solution was consistently employed across all samples. The EM1 solution adheres to established EM concentrate guidelines, constituting a mixture of 1 ounce of EM1 concentrate per gallon of water. The EM1 solution, “expanded EM”, was made in a singular batch prior to the incorporation of the biochar and should be made at least 5 days prior to the incorporation of biochar to allow proper time for the EM concentrate to expand. In this case, the use of EM1 concentrate ensures that all biochar samples receive the same microorganisms, leaving particle size as the sole difference between samples. Though, the inoculation process varied slightly

between trials 1 and 2 due to the incorporation of soil in Trial 2. Figure 9 illustrates the EM1 concentrate that was used for the experiment.

Figure 9

EM1 Concentrate

Trial 1

In Trial 1, biochar samples, each with a dry weight of 20 ounces, were individually enclosed within paint strainer bags and placed within separate 7.56 liter (2-gallon) buckets. Each bucket received 3.78 liters (1 gallon) of EM1 solution in order to submerge the samples in the inoculant equally for a period of 2 days. Figure 10 illustrates the biochar inoculation for Trial 1. After the 2-day inoculation period, the samples were removed from the EM1 solution and allowed to strain for 12 hours. Subsequently, the contents of the strainer bags were delicately emptied into their respective buckets and their weights were recorded before the commencement of testing. Notably, there were slight variations in sample weights following the inoculation (Table 1) with the smaller particle sizes absorbing slightly more inoculant than the larger ones.

Figure 10

Biochar Inoculation Trial 1

Trial 2

For Trial 2, 10oz of each of the <2mm, 2-4mm, Mixed mm, and dry potting soil samples were dosed with 10 oz of EM1 solution and stirred to inoculate at a 1:1 rate (Figure 11). Every sample had a standardized weight of 20oz after inoculation. Inoculated samples were left to sit for two days before being incorporated into wet soil with roughly 30% moisture content. The inoculated biochar was added a rate of 10% by weight consistently. Figure 12 depicts the composition of the Carolina organics gardening soil. Each bucket contained 30 ounces of moist potting soil and received 3 ounces of the inoculated material. Each sample was stirred for 30 seconds after the mixing of the potting soil and inoculated material. Following this samples were left to settle for one day before testing began.

Figure 11

Biochar Inoculation Trial 2

Figure 12

Soil Composition Trial 2

Microbial Activity Testing

To evaluate the level of microbial activity in the distinct samples, CO_2 flux data was collected using a LI-COR 8200-01s soil gas flux smart chamber in conjunction with the LI-870 gas analyzer. This LI-COR instrumentation enabled the real-time monitoring of respired CO_2 levels within the individual biochar samples. By measuring the amount of CO_2 flux at a given time, microbial activity can be evaluated as CO_2 respiration levels are representative of microbial activity and population. These measurements were consistently recorded using the LI-COR equipment, which quantifies respiration in terms of CO_2 flux, measured in units of μmol m-2 s-1. For both Trial 1 and Trial 2, data collection was carried out daily, within the timeframe of 4 to 6 pm, for a duration of four weeks. This approach allowed for more precise data and reduced any respiration variation based on time. To analyze the respired levels of CO_2 in the biochar samples, the LI-COR system uses Infrared gas analyzer technology**.** LI -COR biosciences based out of Lincoln, Nebraska provides the following Spec sheets LI-COR 8200-01s (Figure 13) and LI -870 systems (Figure 14). Figure 15 illustrates the LI-COR 8200 in tandem with the LI-870 gas analyzer.

Figure 13

LI-COR 8200 and LI-870

Figure 14

LI-870 Specifications

General

Case dimensions: 28.4 cm L × 27.9 cm W × 12.4 cm H (11.2 in × 11 in × 4.9 in)
Weight: 2.31 kg (5.1 lbs.)
Measurement rate: 1 per second (1 Hz)
Operating temperature range: -20 to 45 °C, without solar loading
Relative humidity range: 0 to 95% RH, non-condensing
Measurement principle: Non-dispersive infrared (NDIR)
Operating pressure range: 50 to 110 kPa
Flow rate (nominal): 0.75 liters min^{-1}
Power Requirements:

- **Input voltage:** 10-17 VDC, 2 A max
- **Power source**: 8200-01S Smart Chamber

CO_2 Measurements

Measurement range: 0 to 20,000 ppm
Accuracy: Within 1.5% of reading

H_2O Measurements

Measurement range: 0 to 60 mmol mol^{-1}
Accuracy: Within 1.5% of reading

Figure 15

LI-COR 8200s Specifications

Bowl Diameter: 20 cm
Chamber Volume: 4244.1 cm^3
Soil Area: 317.8 cm^2 (49.3 $inches^2$)
Operating Temperature Range: -20 to 50 °C
Air Temperature Thermistor
Operating Temperature Range: -20 to 70 °C
Accuracy: ±0.5 °C over 0 to 70 °C
Barometric Pressure Sensor
Operating Pressure Range: 50 - 110 kPa
Accuracy: +/- 0.4 kPa
Resolution: 1.5 Pa (typical)
Power Out: 10-17 VDC ⎓, Center-Positive ⊖—(—⊕ Battery-Unregulated, Self-Resetting Fuse Rating of 2 Amp.
Battery: 4S Lithium-ion, 98Wh, Smart Battery with Protection
Battery Life:
Without LI-870 CO_2/H_2O Analyzer: 34 hours (2 batteries; 17 hours per battery with 2 min per collar active time and 20 collars total per hour.
With LI-870 CO_2/H_2O Analyzer: 20 hours (2 batteries; 10 hours per battery with 2 min per collar active time and 20 collars total per hour).
Thermocouple Port: For measuring soil temperature using Omega Soil Temperature Thermocouple or other Type-E thermocouple.
Weight: 4.3 kg (9.6 lbs) including battery
Size: 34 × 32 × 29 cm (W × H × D) with handle lowered and HydraProbe dock installed

Data Collection

In this study CO_2 flux data was collected once daily between 4 and 6pm from each of the different samples over a four-week period. Each individual inoculated sample was placed in a 2-gallon bucket with the lid resting on top. A custom bucket lid was constructed using a soil collar sealed to the surface in order to pair the LI-COR smart chamber with the samples. This innovation was necessary to accommodate the LI-COR smart chamber, originally designed to rest on a soil collar. A visual representation of this data collection mechanism can be observed in Figure 16.

Two measurements were obtained for each individual sample daily, with the second measurement taken immediately after the first. To account for the slight variations often observed between the initial and subsequent measurements, the two values were meticulously averaged to derive the daily respiration value, ensuring the precision of the results. Flux rates were collected on a daily basis, rather than at weekly intervals, in order to offer a more nuanced understanding of the dynamic changes occurring within the biochar samples concerning microbial activity. The LI-COR system has a measurement window of 120 seconds per sample and uses a 20cm chamber. Additionally, it has a built-in wifi local network to relay the necessary information to a personal device in real time. As data was received, it was downloaded to and organized in the SoilFluxPro software tool that accompanies the LI-COR Smart Chamber. Subsequently, the necessary data was downloaded to Microsoft Excel for interpretation.

Figure 16

Data Collection Mechanism

Data Analysis

The analysis of the CO_2 flux data, accrued throughout the four-week testing period from the diverse biochar samples, was executed utilizing the in-house software provided by LI-COR, known as SoilFluxPro, in conjunction with Microsoft Excel. SoilFluxPro allowed for the data provided by the LI-COR equipment to be seamlessly downloaded to a platform where it can be organized and transferred to a CSV file. Microsoft Excel enabled the development of comparative graphs and tables in order to analyze and display the data in a way that is easily comprehensible.

Given that each of the four variables in both trials featured two replicants (resulting in 12 individual samples, with three for each variable), data sets were methodically averaged, barring any unusual outliers. Final charts and graphs were derived from the average values of the sample and its replicants rather than individual ones. Formulation of tables and graphs enabled the visual

determination of which biochar sample, distinguished by particle size, yielded the greatest levels of CO_2 flux, consequently representing microbial activity. The biochar sample that achieved the highest CO_2 respiration levels over the testing period can be identified as the most effective microbial carrier. Recognizing that microbial activity is directly linked to soil health and quality, it is plausible to hypothesize that the particle size yielding the highest microbial activity is the optimal choice for biochar inoculated with EM1 in commercial agricultural applications.

Results

Trial 1

Results

The dataset presented in Figure 17 is derived from the average CO_2 flux values of the three samples within each of the four distinct particle sizes of inoculated biochar over a 4-week measurement period. The lines on the chart correlate to the colors on the legend which indicates the sample they represent. Along the y-axis, are the CO_2 flux values measured in units of F CO_2 (umol m^{-2} s^{-1}), the x-axis represents days during the trial. Complementing Figure 17, Table 1 provides insight into the sample weights both before and after inoculation.

Figure 17

Trail 1 Average CO_2 Flux vs. Time

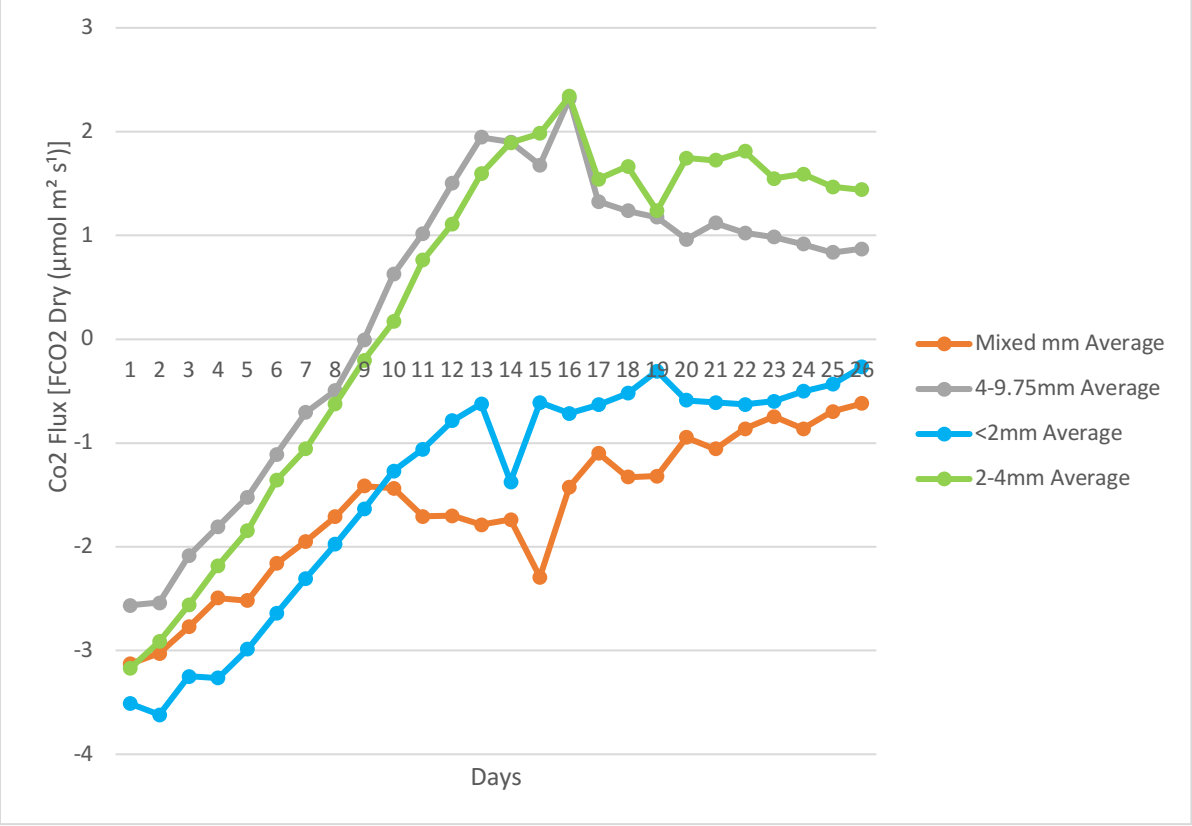

Table 1

Trial 1 Sample Weights Pre and Post Inoculation

Sample	Weight Oz Pre Inoculation	Weight Oz Post Inoculation	Average Particle Size Weight Oz
Bucket 1 <2mm	20	38.3	
Bucket 2 <2mm	20	37.8	38.3
Bucket 3 <2mm	20	38.8	
Bucket 1 2-4mm	20	36.3	
Bucket 2 2-4mm	20	36.9	36.23333
Bucket 3 2-4mm	20	35.5	
Bucket 1 Mix mm	20	37.4	
Bucket 2 Mix mm	20	37.6	37.68
Bucket 3 Mix mm	20	38.1	
Bucket 1 4-9.75mm	20	35.6	
Bucket 2 4-9.75mm	20	36.1	36
Bucket 3 4-9.75mm	20	36.3	

Based on the evaluation of Figure 17, CO_2 flux values were not consistent across the different particle sizes of biochar. Although, the similar shape of the trend lines indicates that the behavior over time was fairly consistent across all the samples. Notably, all 12 individual samples displayed initial measurements with negative values, which persisted for approximately 10 days, following which flux values exhibited a gradual increase. The mixed mm samples and the <2mm samples remained negative for the entire duration of the experiment. Conversely, the samples of the 4-9.75mm and 2-4mm particle sizes flipped positive around day 9-10 and continued to increase until all samples began to level off near day 20. Figure 17 distinctly reveals that the samples characterized by larger particle sizes (2-4mm & 4-9.75mm) of inoculated biochar outperformed their counterparts in terms of CO_2 flux values in the absence of soil. In contrast, the variations in weights post-inoculation, as illustrated in Table 1, suggest that the

samples housing smaller particle sizes (mixed mm and <2mm) exhibited a superior capacity to absorb and retain the inoculant in comparison to their larger particle size counterparts.

Statistical Analysis

A one-way Anova test was performed to determine if the differences between the 4 sample group averages was significant or not (Table 2). The null hypothesis states that there are no statistically significant differences between the mean CO_2 flux values in the averages of the 4 different sample groups. In this case the P value is far below the Alpha of .05, and the null hypothesis is rejected. A Scheffe Post Hoc test was used to make all possible contrasts between group means. The Scheffe Post Hoc determines the source of the statistical significance between sample groups (Table 3).

Table 2

Anova Single Factor Between Groups Trial 1

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Mixed mm avg	26	-42.775	-1.645	0.526		
<2mm avg	26	-36.691	-1.411	1.273		
2-4mm avg	26	9.718	0.374	3.021		
4-9.75mm avg	26	8.608	0.331	2.098		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	92.691	3.000	30.897	17.864	0.001	2.696
Within Groups	172.956	100.000	1.730			
Total	265.647	103.000				

Table 3

Scheffe's Test Between Groups Trial 1

Scheffe Test CV		8.0866	Trial 1
Pairs		CVs	Difference (Y or N)
Mixed mm	<2mm	0.4116	N
Mixed mm	2-4mm	30.6385	Y
Mixed mm	4-9.75mm	29.3565	Y
<2mm	2-4mm	23.9475	Y
<2mm	4-9.75mm	22.8157	Y
2-4mm	4-9.75mm	0.0137	N

In Trial 1 there is a significant difference between all sample groups except the mixed mm and the <2mm, and the 2-4mm and 4-9.75mm. This result correlates with the similar trendlines of these two pairs illustrated in Figure 17. The mean CO_2 production was lower in the samples containing the smallest particle size (<2mm and Mixed mm). The lack of significant difference between these two pairs can be determined to be a product of the consistent factor between them. In the case of the <2mm and the mixed mm, the <2mm is the consistent factor. With the 2-4mm and the 4-9.75mm, a particle size of 4mm is the consistent factor. Ultimately, the results show that there was a significant difference between the performances of the smaller (mixed mm & <2mm) and larger (2-4mm & 4-9.75mm) particle sizes.

An additional set of one-way Anova tests was performed individually within each of the four sample groups. The results showed that there were no significant differences between the three individual replicants in each sample group. The P value for each group (.643, .875, .439, .453) was far above the alpha of .05 for those tests. In other words, all three of the individual replicants in each of the four sample groups performed similarly to one another in Trial 1. Illustrating a degree of consistency in the performance of the individual samples in each sample group.

Lastly, a set of t-Tests performed on the back to back daily measurement for each individual sample showed that there was no statistically significant difference between the first and second repetition of consecutive measurements in Trial 1. In all cases we fail to reject the null hypothesis, there is not have sufficient evidence to say that the two-population means are different. This concludes that using the average of the two consecutive daily measurements was warranted, or that only one measurement per sample per day was necessary when testing biochar alone because there wasn't any significant variation between the first and second consecutive measurements.

Trial 2

Results

Like Trial 1, the dataset illustrated in Figure 18 emerges from the average CO_2 flux values of the three replicant samples within each of the four distinct sample groups featuring inoculated material. The trial extended over a four-week measurement period. The lines on the chart correlate to the colors on the legend which indicates the sample they represent. The y-axis on the chart signifies the respiration values, measured in units of F CO_2 (umol m^{-2} s^{-1}), while the x-axis tracks the progression of days during the trial. This trial introduced an added dimension by measuring an equally weighted sample solely composed of soil and devoid of any inoculated material, which is also depicted on the chart. Complementing Figure 18, Table 4 provides insight into the sample weights both before and after inoculation.

Figure 18

Trial 2 Average CO_2 Flux vs. Time

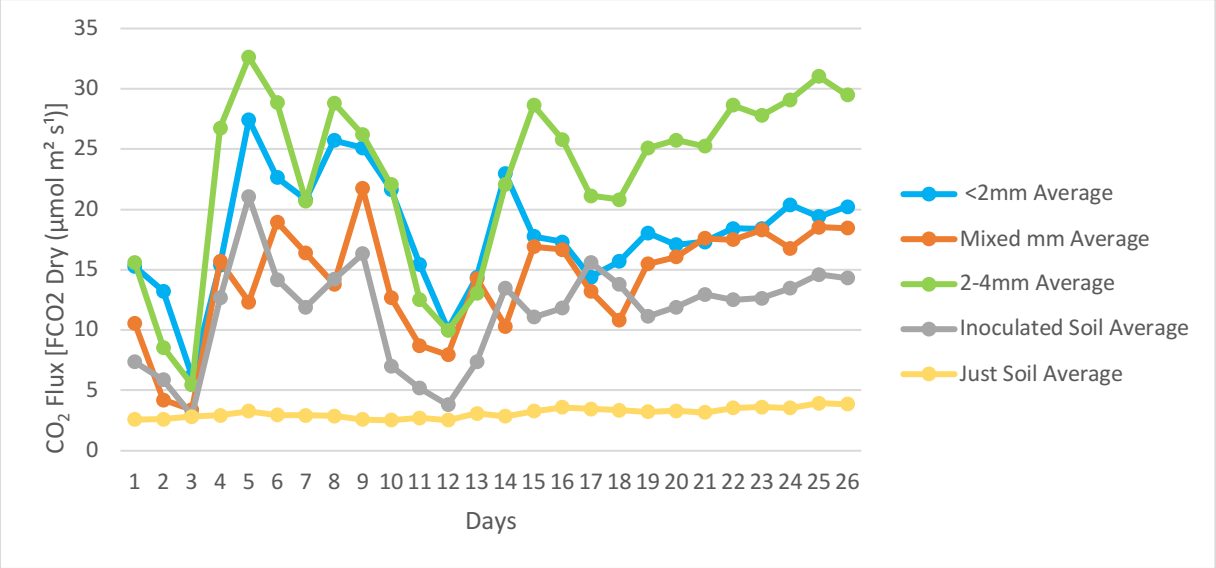

Table 4

Trial 2 Sample Weights Pre and Post Inoculation

Sample	Weight Oz Pre Inoculation	Weight Oz Post Inoculation	Average Particle Size Weight Oz
Bucket 1 <2mm	30	33	
Bucket 2 <2mm	30	33	33
Bucket 3 <2mm	30	33	
Bucket 1 2-4mm	30	33	
Bucket 2 2-4mm	30	33	33
Bucket 3 2-4mm	30	33	
Bucket 1 Mix mm	30	33	
Bucket 2 Mix mm	30	33	33
Bucket 3 Mix mm	30	33	
Bucket 1 Inoc soil	30	33	
Bucket 2 Inoc soil	30	33	33
Bucket 3 Inoc soil	30	33	
Bucket 1 wet soil (no EM1)	30	33	
Bucket 2 wet soil (no EM1)	30	33	33

Based on the evaluation of Figure 18, CO_2 flux values were again not consistent across the four different sample groups. Although, the similar shape of the trend lines once again indicates a degree of consistency in the behavior across all the samples over time. A common pattern emerges as all sample groups achieve their peak flux values around day 5, followed by a marked decline leading into days 11-12, eventually culminating in a leveling-off phase. Notably, no negative values were measured throughout the entire duration of Trial 2. All sample groups containing inoculated material demonstrated significantly higher flux values in comparison to the batch that received no inoculated material. Among these, the three sample groups featuring biochar outperformed the single sample group containing inoculated soil. The <2mm and mixed mm sample groups leveled off with very similar flux values, though the <2mm samples experienced higher peaks. The superior performance, in terms of the highest CO_2 flux values, was consistently observed in the sample group characterized by the largest particle size (2-4mm). Complimenting Figure 18, Table 4 shows that all samples had uniform weights prior to testing due to the change in inoculation process in Trial 2.

Statistical Analysis

A one-way Anova test was performed to determine if the differences between the 4 sample group averages was significant or not (Table 5). The null hypothesis states that there are no statistically significant differences between the mean CO_2 flux values in the different samples. In this case the P value is far below the Alpha of .05, and the null hypothesis is rejected. Meaning, there is a statistically significant difference between the averages of the four sample groups. A Scheffe post hoc test was used to make all possible contrasts between group means (Table 6). The Scheffe post hoc test determines the source of the statistical significance within groups. In Trial 2 there is a significant difference between all pairs except the mixed mm and the

inoculated soil, and following suit with Trial 1, the mixed mm and the <2mm. The lack of significant difference between the mixed mm group and the inoculated soil needs to be further explored.

Table 5

Anova Single Factor Between Groups Trial 2

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Mixed mm Avg	26	367.336	14.128	20.812		
2mm Avg	26	470.730	18.105	22.343		
2-4mm Avg	26	591.757	22.760	56.105		
Inoculated Soil Avg	26	299.220	11.508	17.670		
Just Soil Avg	26	81.330	3.128	0.172		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5667.536	4.000	1416.884	60.498	2.46E-28	2.444
Within Groups	2927.544	125.000	23.420			
Total	8595.080	129.000				

Table 6

Scheffe's Test Between Groups Trial 2

Scheffe Test CV		9.7766	Trial 2
Pairs		CVs	Difference (Y or N)
Mixed mm	<2mm	8.7778	N
Mixed mm	2-4mm	41.3550	Y
Mixed mm	Inoc Soil	3.8098	N
<2mm	2-4mm	12.0273	Y
<2mm	Inoc Soil	24.1534	Y
2-4mm	Inoc Soil	134.7830	Y
Just Soil	Inoc Soil	38.9834	Y
Just Soil	<2mm	124.5073	Y
Just Soil	2-4mm	213.9295	Y
Just Soil	Mixed mm	67.1669	Y

An additional set of one-way Anova tests was performed within each of the sample groups. Contrary to Trial 1, the results of these tests gave P values far below the alpha of .05 and showed that there was in fact a significant difference between the individual samples within each of the sample groups, except the group that received no inoculant (Just Soil). In other words, all three of the replicants in each of the four sample groups did not consistently perform similarly to one another in Trial 2. Illustrating a degree of inconsistency in the performance of the individual samples in each of the four sample groups. A set of Scheffe post hoc tests was conducted to determine the source of this inconsistency for each sample group.

Table 7

Mixed mm Anova Single Factor

ANOVA			Mixed mm			
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6216.659	8.000	777.082	19.395	4.41E-22	1.980
Within Groups	8974.760	224.000	40.066			
Total	15191.419	232.000				

Table 8

Mixed mm Scheffe Test

Scheffe Test CV		15.8391	Mixed mm
Pairs		CVs	Difference (Y or N)
B1 (rep 1)	B1 (rep 2)	5.0637	N
B1 (rep 1)	B1 (avg)	1.2659	N
B1 (rep 1)	B2 (rep 1)	11.1957	N
B1 (rep 1)	B3 (rep 1)	63.6088	Y
B1 (rep 2)	B1 (avg)	1.2659	N
B1 (rep 2)	B2 (rep 2)	8.6455	N
B1 (rep 2)	B3 (rep 2)	36.5064	Y
B1 (avg)	B2 (avg)	9.8794	N
B1 (avg)	B3 (avg)	48.9388	Y
B2 (rep 1)	B2 (rep 2)	3.4025	N
B2 (rep 1)	B2 (avg)	0.8506	N
B2 (rep 1)	B3 (rep 1)	21.4323	Y
B2 (rep 2)	B2 (avg)	0.8506	N
B2 (rep 2)	B3 (rep 2)	9.6207	N
B2 (avg)	B3 (avg)	14.8414	N
B3 (rep 1)	B3 (rep 2)	0.1003	N
B3 (rep 1)	B3 (avg)	0.0210	N
B3 (rep 2)	B3 (avg)	0.0294	N

Table 9

<2mm Anova Single Factor

ANOVA			<2mm			
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14180.926	8.000	1772.616	34.736	2.78E-35	1.980
Within Groups	11380.043	223.000	51.032			
Total	25560.969	231.000				

Table 10

<2mm Scheffe Test

Scheffe Test CV		15.8406	<2mm
Pairs		CVs	Difference (Y or N)
B1 (rep 1)	B1 (rep 2)	3.3773	N
B1 (rep 1)	B1 (avg)	0.8443	N
B1 (rep 1)	B2 (rep 1)	47.8440	Y
B1 (rep 1)	B3 (rep 1)	100.0162	Y
B1 (rep 2)	B1 (avg)	0.8443	N
B1 (rep 2)	B2 (rep 2)	32.1331	Y
B1 (rep 2)	B3 (rep 2)	79.6242	Y
B1 (avg)	B2 (avg)	39.5990	Y
B1 (avg)	B3 (avg)	87.8594	Y
B2 (rep 1)	B2 (rep 2)	0.3474	N
B2 (rep 1)	B2 (avg)	0.0868	N
B2 (rep 1)	B3 (rep 1)	9.5102	N
B2 (rep 2)	B2 (avg)	0.0868	N
B2 (rep 2)	B3 (rep 2)	10.5926	N
B2 (avg)	B3 (avg)	9.4898	N
B3 (rep 1)	B3 (rep 2)	0.5779	N
B3 (rep 1)	B3 (avg)	0.0849	N
B3 (rep 2)	B3 (avg)	0.2197	N

Table 11

2-4mm Anova Single Factor

ANOVA			2-4mm			
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	37784.966	8.000	4723.121	37.653	2.19E-37	1.980
Within Groups	27847.383	222.000	125.439			
Total	65632.349	230.000				

Table 12

2-4mm Scheffe Test

Scheffe Test CV		15.8422	2-4mm
Pairs		CVs	Difference (Y or N)
B1 (rep 1)	B1 (rep 2)	4.5017	N
B1 (rep 1)	B1 (avg)	1.1254	N
B1 (rep 1)	B2 (rep 1)	55.0089	Y
B1 (rep 1)	B3 (rep 1)	110.1795	Y
B1 (rep 2)	B1 (avg)	1.1254	N
B1 (rep 2)	B2 (rep 2)	42.4747	Y
B1 (rep 2)	B3 (rep 2)	79.3599	Y
B1 (avg)	B2 (avg)	48.5395	Y
B1 (avg)	B3 (avg)	93.1806	Y
B2 (rep 1)	B2 (rep 2)	1.4937	N
B2 (rep 1)	B2 (avg)	0.3734	N
B2 (rep 1)	B3 (rep 1)	9.4854	N
B2 (rep 2)	B2 (avg)	0.3734	N
B2 (rep 2)	B3 (rep 2)	5.7174	N
B2 (avg)	B3 (avg)	7.2144	N
B3 (rep 1)	B3 (rep 2)	0.2846	N
B3 (rep 1)	B3 (avg)	0.0471	N
B3 (rep 2)	B3 (avg)	0.1000	N

Table 13

Inoculated Soil Anova Single Factor

ANOVA			Inoculated Soil			
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7487.223	8.000	935.903	21.044	1.17E-23	1.980
Within Groups	9917.656	223.000	44.474			
Total	17404.879	231.000				

Table 14

Inoculated Soil Scheffe Test

Scheffe Test CV		15.8406	Inoc. Soil
Pairs		CVs	Difference (Y or N)
B1 (rep 1)	B1 (rep 2)	6.4085	N
B1 (rep 1)	B1 (avg)	1.6021	N
B1 (rep 1)	B2 (rep 1)	41.4578	Y
B1 (rep 1)	B3 (rep 1)	64.7109	Y
B1 (rep 2)	B1 (avg)	1.6021	N
B1 (rep 2)	B2 (rep 2)	20.5421	Y
B1 (rep 2)	B3 (rep 2)	35.6024	Y
B1 (avg)	B2 (avg)	30.0913	Y
B1 (avg)	B3 (avg)	48.8925	Y
B2 (rep 1)	B2 (rep 2)	0.3907	N
B2 (rep 1)	B2 (avg)	0.0976	N
B2 (rep 1)	B3 (rep 1)	2.5777	N
B2 (rep 2)	B2 (avg)	0.0976	N
B2 (rep 2)	B3 (rep 2)	2.0575	N
B2 (avg)	B3 (avg)	2.2703	N
B3 (rep 1)	B3 (rep 2)	0.2060	N
B3 (rep 1)	B3 (avg)	0.0456	N
B3 (rep 2)	B3 (avg)	0.0577	N

Lastly, a set of t-Tests was performed on the back to back daily measurement for each individual sample. Contrary to Trial 1, the t-Tests performed on repetitions one and two of the

daily measurements for each individual sample showed that there was in fact a statistically significant difference between the back to back measurements. This result was consistent for each individual sample in Trial 2. Furthermore, this result creates the need for discussion regarding the consecutive daily measurements in Trial 2 and whether the second measurement was necessary.

Discussion

The results of Trial 1 and Trial 2 provide insights into the impact of biochar particle size on microbial activity and CO_2 flux, both in the absence of soil (Trial 1), and when incorporated into potting soil (Trial 2). The results provide additional insight into the mechanisms and methodology with which CO_2 flux and microbial activity should be examined in biochar. Prior to data collection this study hypothesized that the smaller particle sizes (<2mm) would act as better inoculum carriers and provide better habitat for microbial colonization, In-turn providing higher CO_2 flux values. The results from the study (Figures 17 & 18) did not follow suit with this hypothesis at first glance, but further discussion is necessary to make this determination. Trial 1 was originally conducted as a standalone experiment, Trial 2 was conducted as a follow up study in an attempt to validate the findings from Trial 1. This discussion will analyze the findings from each trial separately and then draw overarching conclusions based on the combined results.

Trial 1

In Trial 1, the focus of the study was on inoculated biochar alone, with 4 different particle sizes tested. The data revealed an inconsistency in CO_2 flux values across the different particle sizes of biochar, suggesting that some performed better than others. The statistical analysis performed on the data set confirms this result. Although, the data depicted similar trend lines across all the different particle sizes indicating a consistency in the behavior of all the samples. All 4 particle sizes saw a trend that had CO_2 flux gradually increasing over time before eventually leveling off. The shape of the trend lines is likely due to microbial life cycle and succession. The consistent behavior but differing CO_2 flux values indicate that particle size directly factored into the results. The two larger particle sizes (2-4mm & 4-9.75mm) performed similarly and had higher CO_2 flux values than the two other particle sizes. The 2-4mm particle

size had values slightly higher than the 4-9.75mm particle size, potentially indicating it is the best for microbial habitation.

The less than 2mm and mixed mm particle sizes also performed similarly to each other which is interesting because these two sample groups were quite different from each other physically. Statistical analysis confirmed a significant similarity in the performance of these two sample groups. The less than 2mm samples were purely a very fine particle size of biochar, and the mixed mm particle sizes contained a mix of all the of the other particle sizes, including the larger ones. Through this examination it can be determined that the less than 2mm particle size was responsible for this similarity in behavior and CO_2 flux as it is the factor that was consistent across the two sample groups. Additional insight can be provided to this determination when examining the negative CO_2 flux values that were measured in all the samples.

All 12 individual samples started with negative CO_2 flux values that were within a range of 1-2 micro moles of each other, these negative values persisted for approximately 10 days in the two larger particle sizes and through the duration of the experiment for the other two. Upon reaching out to LI-CORs support team, they confirmed that negative flux values will be reported for the cases of net carbon uptake. This initial period of negative values suggests that the biochar was pulling in more CO_2 than it was emitting in the atmosphere that contained the samples. A process known as adsorption, or CO_2 adhering to the surface of the biochar (Guo et al., 2022). This phenomenon has been observed in instances where biochar has been added to concrete (Li & Shi, 2023). Li and Shi (2023) observed biochar absorbing atmospheric CO_2 by up to 23% by weight when incorporated into concrete. Subsequently, the flux values gradually increased over time, which could be indicative of microbial activity and respiration overtaking the biochar's ability to sequester carbon. Biochar's inherit carbon negative properties and ability to store

carbon for a number of years have been widely studied (Hunt et al., 2010), indicating it is unlikely that the biochar itself was responsible for the gradual decrease in carbon influx over the short period of time. Rather, the microbial activity in the samples was responsible for a gradual increase in carbon efflux over time, making less negative, and in the case of the two larger particle sizes, eventually positive CO_2 flux values.

While all particle sizes initially exhibited negative values, the <2mm and mixed mm samples remained negative throughout the experiment. In contrast, the samples of the 4-9.75mm and 2-4mm particle sizes flipped to positive values around day 9-10. This change could indicate that certain particle sizes, in this case the larger ones, supported more microbial activity over time in this specific study. However, it may also indicate the larger particle sizes resulted in less CO_2 adsorption during the study. A longer trial could have shown an equalizing effect. Therefore, this result cannot be blanketed over all biochar studies and does not necessary determine that larger particle sizes are better for microbial activity than smaller ones. This is because the conditions within the samples likely directly affected the microbial activity and CO_2 respiration occurring.

The differing weights post inoculation displayed in Table 1 show that the samples containing the less than 2mm particle size (mixed mm & <2mm) had a slightly better ability to absorb and hold the inoculant. This indicates that smaller particle sizes may provide better inoculant or liquid retention. Relating to general knowledge of soils, this is consistent with the idea that a finer or sandy soil is going to retain more moisture than a gravel one for example (De Jesus Duarte et al., 2019). However, this ability to hold more liquid potentially had adverse effects on the CO_2 respiration values in this Trial. The inoculant in this case, the liquid EM1 solution, was mixed at the recommended ratio of 100:1 water to EM1 concentrate. The additional

inoculant in the less than 2mm and mixed mm particle sizes, combined with the smaller biochar's greater ability to hold liquid, created "waterlogged" conditions within the samples which likely directly affected the microbial activity and CO_2 respiration.

Figures 19 and 20 depict the mixed mm particle size and the less than 2mm particle size near the end of the trial. Figure 21 depicts the 2-4mm particle size. What is clearly visible from the examination of these figures is that the samples depicted in Figures 19 and 20 look much wetter and more clumped together than the sample in Figure 21. Waterlogged conditions likely created an anaerobic environment within the samples. The sample in Figure 21 shows depicts an environment that looks visibly more aerated and aerobic than the samples in Figures 19 and 20.

Figure 19

<2mm Biochar

Figure 20

Mixed mm Biochar

Figure 21

2-4mm Biochar

While the microbes contained in EM1 are capable of both anerobic and aerobic respiration, aerobic is far more efficient and yields greater respiration (Abd El-Mageed et al., 2020). Abd El-Mageed et al. (2020) explains that the microbes in EM1 are facultative anaerobic, meaning if conditions are ideal, they will be aerobic, and anaerobic if not. This condition is potentially what was responsible for the differences in CO_2 flux values across the samples. The gradual increases in CO_2 flux over time likely indicates that as the amount of water in the samples decreased, the microbial activity increased. Due to the smaller particle size ability to hold more liquid and the absence of any soil to provide any additional aeration, the less than 2mm and mixed mm particle sizes were not able to dry out enough for the levels of microbial activity and CO_2 respiration to outweigh the biochar's inherit ability to sequester carbon during this shorter duration study. Figure 17 depicts that as all the samples began to level off around day 20, the less than 2mm and mixed mm particle sizes were still gradually increasing. A longer duration of examination could have possibly yielded these particle sizes flux values eventually flipping positive and overtaking the larger particle sizes. Therefore, the results from this trial cannot effectively characterize that in every case a larger particle size of biochar, or more specifically a 2-4mm particle size, is the most effective inoculum carrier. That result is specific to this study and the conditions within it.

Trial 2

Trial 2 was implemented as a continuation of Trial 1. In this trial the focus shifted to inoculated biochar incorporated into potting soil in an attempt to counteract the anaerobic conditions that were created in Trial 1 by testing biochar alone. The different particle sizes of inoculated biochar were added at 10% by weight to the Carolina Organics gardening soil and

mixed in their respective 7.56 liter (2-gallon buckets). Literature on biochar suggest that a 10% incorporation rate is recommended (Garbowski et al., 2023; Traunfeld, 2020).

Trial 2 substituted the 4-9.75mm particle size from Trial 1 for inoculated soil rather than biochar. The 4-9.75mm was a fairly large particle size and was deemed to be uncommon in biochar application. The inoculated soil was the same Carolina Organics gardening soil and was inoculated and mixed into the samples under the same exact procedures as the biochar. The purpose for substituting the 4mm-9.75mm particle size for the inoculated soil was to get an idea of what the CO_2 flux values would look like without the addition of biochar. An additional sample that received 10% uninoculated soil was tested to further this examination and get an idea of the effect of the inoculant on the samples versus the natural CO_2 flux from the wet soil. Trial 2 used a slightly different inoculation process than Trial 1 in order to combat the differing sample weights that were seen post inoculation in Trial 1. Rather than submerging the samples in the EM1 solution, all samples in Trial 2 were inoculated with the EM1 solution at a 1:1 rate by weight, mixed, and then left to sit for a day. This change in the process ensured all the inoculated samples weighed the same prior to their incorporation into the soil, and that there was no additional liquid inoculant in any of the samples.

Like Trial 1, the CO_2 flux values in Trial 2 were not consistent across the four different samples. However, the trend lines showed a similar shape, suggesting that the behavior of CO_2 flux or microbial activity over time was relatively consistent across all samples. The samples peaked in CO_2 flux values around day 5 and then experienced a sharp decline into day 11-12 before rising back up and beginning a steady climb with small fluctuations. The consistency in this pattern indicates that microbial activity reached a maximum level around day 5, saw rapid decline, and then stabilized. This pattern is again likely due to microbial life cycle and

succession. Rapid microbial succession would account for the initial spike and sharp decline in the trendlines before culminating into a leveling off phase. However, an alternative explanation could be that the initial spike in CO_2 flux values seen in all the samples potentially could have been due to an increased carbon efflux from the soil due to the recent agitation from the mixing. This phenomenon has been observed in soil following agitation like tilling (Pimentel, 2006). Although, that explanation would not account for why some samples spiked higher than others. That said, it is likely that the biochar influenced these values, and the particle sizes played a role in the differences between them.

Unlike Trial 1, no negative values were measured throughout the entire duration of Trial 2. Likely due to the natural carbon release from the soil, but this suggests that when biochar was incorporated into potting soil, it consistently promoted CO_2 efflux, indicating microbial activity. Comparing the three sample groups containing biochar to the one sample group containing inoculated soil, all the biochar groups performed significantly better as seen in the statistical analysis. This suggests that biochar, regardless of particle size, was more effective in supporting microbial activity compared to the inoculated soil alone. Previous literature on biochar suggests this same thing (Zhao et al., 2020; Bamdad et al., 2021). Additionally, all the inoculated samples had higher CO_2 flux values than the sample that received no inoculant, affirming that the microbial activity, life cycle, and succession, occurring in the inoculated samples was responsible for the increased CO_2 flux values.

The less than 2mm and mixed mm sample groups in Trial 2 had leveled off at similar flux values, with the less than 2mm samples experiencing higher peaks. This was consistent with the results from Trial 1 and again confirmed by the statistical analysis between sample groups. Unlike Trial 1, The statistical analysis performed on the replicants within the sample groups

showed a difference between the replicants. The volatility of the carbon release from the soil likely played a role in these differences because the experimental procedures were the same across all the individual samples. This result potentially knocks the credibility of the results in Trial 2 as the addition of the soil might not have had a uniform effect, and played a role in the differences between the replicants in the four sample groups. Additionally, examination of the t-Tests performed on the data illustrates a difference between the back-to-back daily measurements in Trial 2. Indicating that potentially only one measurement should have been taken, rather than using the average of two. The second measurement often displayed lower values than first in Trial 2, indicating that providing just the first measurement might be a more accurate representation of the data. Overall, the samples containing the largest particle size (2-4mm) performed the best in terms of having the highest CO_2 flux values, reaffirming the impact of particle size on microbial activity in this study.

Combined Analysis

In this study particle size of biochar had an impact on microbial activity and the CO_2 flux values measured. The 2-4mm particle size consistently performed better in terms of CO_2 flux indicating greater microbial activity. The 2-4mm biochar's physical properties likely allowed for a balance between inoculant retention and aeration within the environment, leading the microbes within the samples being exposed to increased oxygen and hence respiring more. However, the smaller particle size (<2mm) demonstrated greater inoculant retention. Similar studies to this one have suggested that smaller particle sizes of biochar encourage greater microbial activity due to the high specific surface area of the smaller particles (Chen et al., 2017; Sarfraz et al., 2020). At first glance the results of this study did not follow suit with that result, nor did they confirm the original hypothesis that hypothesized just that.

Although, this study was potentially limited by the length of the study, and it is possible that over time the smaller particle sizes would have encouraged greater microbial activity due to the increased inoculant retention observed. This is because the bigger particle sizes saw higher microbial activity more rapidly in both trials, but the smaller particle size increased microbial activity from a more stable perspective. Microbial life cycle and rapid microbial succession accounts for the flux in CO_2, that flux is less common in the smaller size because they are more stable and encourage a steady growth rather than rapid growth and end exhibited in the larger particle sizes.

Regardless of particle size, the trend lines showed consistent behavior over time. This suggests that once microbial activity started, it followed a similar pattern of peaking and then stabilizing regardless of particle size. Biochar, even with varying particle sizes, outperformed inoculated soil in promoting microbial activity. This result follows suit with the literature and highlights the potential of biochar as a carrier for beneficial microorganisms (Zhao et al., 2020; Zhou et al., 2017). These findings have practical implications for agricultural applications, suggesting that biochar can play a significant role in enhancing soil health and crop productivity when used in combination with microbial inoculants. Further research can delve into the specific mechanisms behind these observations and explore the long-term effects on soil quality and plant growth.

Future Directions

Recent studies have highlighted the impact of biochar on soil health over centuries and in various global regions (Hunt et al., 2010). Despite this evidence, there remains a significant gap in the understanding of biochar's long-term effects on soil health, nutrient cycling, and plant growth. Therefore, there is a clear need for more extensive, long-term research on the subject.

Additionally, future research should explore various feedstocks and production methods to tailor biochar for specific soil needs as it is essential to recognize that not all biochar is the same. Biochar properties such as feedstock, production temperature, inoculation, and particle size can be modified to address specific soil deficiencies, from drainage issues to low nutrient values (Tomczyk et al., 2020).

The current study was confined to a lab experiment that sought to characterize how biochar particle size effects microbial activity and respiration. While providing valuable insights, this study was limited to a relatively short four-week duration. In order to gain a more comprehensive understanding of this subject, further lab experiments with longer durations and additional testing mechanisms are required. A longer duration study is recommended because it would potentially illustrate the smaller particle sizes over take the larger ones in terms of CO_2 flux. Additionally, field experiments must be conducted in order to observe the effect on soil health and plant growth. Further experiments, with different feedstocks, pyrolysis temperatures, and inoculants will enable a more precise understanding of how factors like particle size influence microbial respiration and plant-available nutrients over time.

Specific to the LI-COR CO_2 flux testing mechanism, future research can be conducted to determine the effects increases or decreases in flux values has on soil and plant health. Further microbial analysis should be conducted to gain better understanding of the relationship between microbial respiration versus the natural CO_2 efflux of soil. Future studies examining microbial respiration in biochar across different metrics will help characterize biochar's potential as a soil amendment. Microbial life is largely responsible for the uptake of plant available nutrients and is one of the most important factors in healthy soils (Bamdad et al., 2021). Ultimately, the goal of biochar is to render healthier soils.

If the opportunity to conduct this study again was presented a more comprehensive and longer duration study of 6-8 weeks would be recommended before anything. Rather than testing biochar alone, an additional variable, like soil, is likely necessary to accurately test the biochar due to its carbon adsorption properties. Conducting a simultaneous plant growth experiment would greatly add to the significance of the results. Additionally, a field experiment examining the effects of particle size on plant growth would produce the most beneficial results. Ultimately, examining microbial activity in this aspect is important because it facilitates reactions that are vital to the rhizosphere and plant growth.

Conclusion

This research set out to explore the relationship between biochar particle size and microbial activity. The aim was to characterize the effects biochar particle size has on microbial activity and determine the optimal particle size for biochar that promotes the highest levels of microbial activity after inoculation with effective microorganisms (EM1). The study consisted of two trials, each featuring four sample groups of different particle sizes. Trial 1 focused on biochar alone, while Trial 2 introduced a mixture of biochar and soil. Microbial activity was monitored using CO_2 flux measurements collected over a four-week period.

The results of these trials offer insights into the impact of biochar particle size on microbial activity and CO_2 flux, both in the absence of soil (Trial 1) and when combined with soil (Trial 2). In Trial 1, the data showed that CO_2 flux values were not uniform across different particle sizes, but the behavior over time displayed similar trend lines indicating microbial activity played a significant role in the values. The short duration study revealed that larger particle sizes (2-4mm and 4-9.75mm) outperformed smaller sizes, contradicting the initial hypothesis that smaller particle sizes would be more effective. Although, this result was likely due to both the conditions presented in Trial 1 and rapid microbial succession in the larger particle sizes. The smaller particle sizes were more effective at retaining inoculant, although they exhibited lower CO_2 flux values. This is possibly due to lower amounts of oxygen moving through the samples leading to less microbial respiration, but more likely due to the facilitation of a more stable microbial community, confirming the original hypothesis.

It was observed that all samples initially exhibited negative CO_2 flux values, indicating that biochar was absorbing more CO_2 than it was releasing, likely due to its carbon-sequestering properties. Over time, microbial activity appeared to outpace carbon sequestration in the larger

particle sizes more rapidly, resulting in positive flux values quicker than the smaller particle sizes. Trial 2 introduced a different dimension by incorporating biochar into soil. The results showed that, regardless of particle size, biochar consistently outperformed inoculated soil in promoting microbial activity and CO_2 flux. The 2-4mm particle size continued to demonstrate superior performance. Notably, no negative values were observed during Trial 2 likely due to the increased carbon release from the soil. Although, it suggests that the combination of biochar with soil provided a more favorable environment for testing microbial activity in biochar due to the physical properties of the soil.

The combined analysis of both trials highlights the influence of biochar particle size on microbial activity and CO_2 flux. The 2-4mm particle size consistently performed better, likely due to its balanced properties that facilitated inoculant retention and aeration. However, the smaller particle size (<2mm) exhibited greater inoculant retention but had a limited impact on microbial activity in the shorter study duration. It is highly likely that a longer study would have seen the smaller particle size start to perform better as they held more inoculant over time and facilitated a more stable microbial community with less rapid succession.

This study reaffirmed the potential of biochar, irrespective of particle size, as an effective carrier for beneficial microorganisms, aligning with existing literature. These findings have practical implications for agricultural applications, suggesting that biochar can enhance soil health and crop productivity when combined with microbial inoculants. Although, specific to the question this study set out to answer, these findings can be deemed inconclusive. Suggesting that further research is needed to delve into the effects of biochar particle size on microbial respiration and the specific mechanisms behind these observations. Further research is needed to explore the long-term effects of biochar on not only microbial respiration but on soil quality and

plant growth as well, providing valuable insights for sustainable agriculture and environmental stewardship.

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Appendix A
Raw Data Sets

Co2 Flux [FCO2 Dry (µmol m^2 s^1)]	mix mm										<2mm												2-4mm												4mm-9.75mm											
days	B1 Mix mm	rep2	B1 avg	B2 Mix mm	rep2	B2 avg	B3 Mix mm	rep 2	B3 avg	mix mm avg	B1 <2mm	rep2	B1 avg	B2 <2mm	rep2	B2 Avg	B3<2mm	rep 2	B3 Avg	<2mm avg	B1 2-4mm	rep 2	B1 Avg	B2 2-4mm	rep 2	B2 Avg	B3 2-4mm	rep 2	B3 avg	2-4mm avg	B1 >4mm	rep 2	B1 avg	B2 >4mm	rep 2	B2 avg	B3 >4mm	rep2	B3 avg	4mm avg
1	-2.96	-3.10	-3.03	-2.29	-2.75	-2.52	-4.14	-3.52	-3.83	-3.13	-3.22	-2.85	-3.04	-4.17	-3.98	-4.08	-3.46	-3.38	-3.42	-3.51	-2.98	-3.31	-3.15	-2.24	-2.71	-2.48	-4.12	-3.67	-3.90	-3.17	-2.11	-2.45	-2.28	-2.74	-2.51	-2.63	-2.91	-2.66	-2.79	-2.56
2	-2.89	-2.67	-2.78	-2.67	-2.58	-2.63	-3.86	-3.49	-3.68	-3.03	-3.09	-3.16	-3.13	-4.01	-4.31	-4.16	-3.39	-3.74	-3.57	-3.62	-3.44	-2.77	-3.11	-2.16	-2.33	-2.25	-3.59	-3.18	-3.39	-2.91	-2.09	-2.17	-2.13	-2.81	-2.69	-2.75	-2.59	-2.88	-2.74	-2.54
3	-2.78	-2.49	-2.64	-2.33	-2.61	-2.47	-3.13	-3.28	-3.21	-2.77	-2.86	-2.97	-2.92	-3.56	-3.81	-3.69	-3.22	-3.07	-3.15	-3.25	-2.56	-2.98	-2.77	-1.91	-1.77	-1.84	-3.02	-3.13	-3.08	-2.56	-2.24	-2.02	-2.13	-2.02	-1.89	-1.96	-2.02	-2.32	-2.17	-2.09
4	-2.51	-2.44	-2.48	-2.08	-2.21	-2.15	-2.74	-2.97	-2.86	-2.49	-2.71	-2.89	-2.80	-3.69	-3.58	-3.64	-3.44	-3.26	-3.35	-3.26	-2.41	-2.59	-2.50	-1.66	-1.52	-1.59	-2.56	-2.34	-2.45	-2.18	-1.71	-1.66	-1.69	-1.54	-1.97	-1.76	-1.89	-2.06	-1.98	-1.81
5	-2.18	-2.26	-2.22	-2.31	-1.96	-2.14	-2.84	-3.56	-3.20	-2.52	-3.01	-2.66	-2.84	-3.01	-3.39	-3.20	-2.99	-2.86	-2.93	-2.99	-2.09	-2.21	-2.15	-1.08	-1.21	-1.15	-2.11	-2.37	-2.24	-1.85	-1.83	-1.49	-1.66	-1.41	-1.49	-1.45	-1.56	-1.34	-1.45	-1.52
6	-1.76	-1.92	-1.84	-1.81	-2.02	-1.92	-2.63	-2.82	-2.73	-2.16	-2.73	-2.59	-2.66	-2.56	-2.81	-2.69	-2.71	-2.44	-2.58	-2.64	-1.67	-1.81	-1.74	-0.99	-0.65	-0.82	-1.45	-1.57	-1.51	-1.36	-1.15	-1.32	-1.24	-1.01	-0.74	-0.88	-1.19	-1.24	-1.22	-1.11
7	-1.69	-1.54	-1.62	-1.69	-1.77	-1.73	-2.59	-2.41	-2.50	-1.95	-2.22	-2.41	-2.32	-2.27	-2.09	-2.18	-2.28	-2.55	-2.42	-2.30	-1.31	-1.09	-1.20	-0.78	-0.83	-0.81	-1.02	-1.30	-1.16	-1.06	-0.67	-0.91	-0.79	-0.46	-0.31	-0.39	-0.99	-0.89	-0.94	-0.71
8	-1.19	-1.24	-1.22	-1.71	-1.59	-1.65	-2.18	-2.35	-2.27	-1.71	-1.89	-1.94	-1.92	-2.11	-1.84	-1.98	-1.99	-2.07	-2.03	-1.97	-0.72	-0.89	-0.81	-0.45	-0.21	-0.33	-0.67	-0.81	-0.74	-0.63	-0.99	-1.13	-1.06	0.19	0.02	0.11	-0.45	-0.61	-0.53	-0.50
9	-1.09	-0.93	-1.01	-1.33	-1.51	-1.42	-1.87	-1.75	-1.81	-1.41	-1.59	-1.77	-1.68	-1.66	-1.90	-1.78	-1.51	-1.38	-1.45	-1.64	-0.54	-0.39	-0.47	0.01	0.22	0.12	-0.37	-0.15	-0.26	-0.20	-0.64	-0.49	-0.57	0.76	0.59	0.68	-0.09	-0.16	-0.13	0.00
10	-1.12	-1.26	-1.19	-1.66	-1.49	-1.58	-1.61	-1.48	-1.55	-1.44	-1.34	-1.29	-1.32	-1.34	-1.58	-1.46	-0.85	-1.21	-1.03	-1.27	0.16	0.02	0.09	0.56	0.31	0.44	-0.12	0.09	-0.02	0.17	0.14	0.22	0.18	1.09	0.99	1.04	0.54	0.78	0.66	0.63
11	-1.67	-1.44	-1.56	-1.72	-1.70	-1.71	-1.77	-1.91	-1.84	-1.70	-1.08	-1.21	-1.15	-0.95	-1.29	-1.12	-0.98	-0.84	-0.91	-1.06	1.21	1.03	1.12	0.79	0.61	0.70	0.34	0.59	0.47	0.76	0.74	0.69	0.72	1.49	1.24	1.37	0.89	1.05	0.97	1.02
12	-1.33	-1.71	-1.52	-1.38	-1.52	-1.45	-2.08	-2.19	-2.14	-1.70	-0.93	-0.81	-0.87	-0.88	-0.72	-0.80	-0.66	-0.71	-0.69	-0.79	1.41	1.50	1.46	1.25	1.09	1.17	0.84	0.58	0.71	1.11	1.08	1.24	1.16	1.67	1.81	1.74	1.54	1.68	1.61	1.50
13	-1.64	-1.51	-1.58	-1.49	-1.22	-1.36	-2.39	-2.47	-2.43	-1.79	-0.68	-0.84	-0.76	-0.79	-0.64	-0.72	-0.27	-0.51	-0.39	-0.62	2.38	1.65	2.02	1.87	1.72	1.80	0.93	1.01	0.97	1.59	1.71	1.46	1.59	2.32	1.99	2.16	2.03	2.16	2.10	1.95
14	-1.57	-1.34	-1.46	-1.31	-1.24	-1.28	-2.56	-2.41	-2.49	-1.74	-0.71	-0.57	-0.64	-3.01	-2.58	-2.80	-0.75	-0.62	-0.69	-1.37	2.98	3.28	3.13	1.68	1.74	1.71	0.89	0.79	0.84	1.89	1.65	1.23	1.44	2.01	1.85	1.93	2.48	2.18	2.33	1.90
15	-1.60	-1.31	-1.46	-1.52	-1.45	-1.49	-4.42	-3.45	-3.94	-2.29	-0.44	-0.37	-0.41	-0.98	-0.88	-0.93	-0.46	-0.53	-0.50	-0.61	3.94	3.45	3.70	1.08	1.24	1.16	1.20	0.98	1.09	1.98	1.16	1.32	1.24	1.58	1.79	1.69	1.97	2.24	2.11	1.68
16	-1.17	-0.85	-1.01	-1.16	-1.38	-1.27	-2.08	-1.89	-1.99	-1.42	-0.58	-0.29	-0.44	-0.74	-0.71	-0.73	-1.23	-0.74	-0.99	-0.72	4.84	3.70	4.27	1.80	1.72	1.76	0.94	1.04	0.99	2.34	1.75	1.38	1.57	1.94	1.32	1.63	4.29	3.21	3.75	2.32
17	-0.66	-0.62	-0.64	-1.01	-0.84	-0.93	-1.89	-1.56	-1.73	-1.10	-0.54	-0.21	-0.38	-0.79	-0.64	-0.72	-0.99	-0.62	-0.81	-0.63	1.85	2.73	2.29	0.96	1.57	1.27	1.13	1.02	1.08	1.54	0.73	0.98	0.86	0.82	0.43	0.63	2.67	2.33	2.50	1.33
18	-0.95	-1.21	-1.08	-1.37	-1.19	-1.28	-1.53	-1.71	-1.62	-1.33	-0.47	-0.31	-0.39	-0.73	-0.52	-0.63	-0.45	-0.64	-0.55	-0.52	2.45	2.39	2.42	1.97	1.58	1.78	0.87	0.74	0.81	1.67	0.60	0.87	0.74	0.49	0.86	0.68	2.40	2.21	2.31	1.24
19	-1.02	-0.83	-0.93	-1.06	-0.95	-1.01	-2.29	-1.75	-2.02	-1.32	-0.25	-0.19	-0.22	-0.48	-0.32	-0.40	-0.29	-0.31	-0.30	-0.31	1.89	1.76	1.83	1.59	0.83	1.21	0.57	0.78	0.68	1.24	0.57	0.69	0.63	1.00	0.68	0.84	2.36	1.75	2.06	1.18
20	-0.75	-0.64	-0.70	-0.79	-0.66	-0.73	-1.24	-1.59	-1.42	-0.95	-0.44	-0.51	-0.48	-0.74	-0.95	-0.85	-0.47	-0.42	-0.45	-0.59	2.49	2.25	2.37	1.61	1.71	1.66	1.26	1.16	1.21	1.75	0.46	0.59	0.53	0.79	0.62	0.71	1.49	1.82	1.66	0.96
21	-0.87	-0.79	-0.83	-0.82	-0.69	-0.76	-1.51	-1.65	-1.58	-1.06	-0.64	-0.59	-0.62	-0.69	-0.56	-0.63	-0.51	-0.67	-0.59	-0.61	2.01	2.17	2.09	1.81	1.56	1.69	1.51	1.29	1.40	1.73	0.78	0.62	0.70	1.06	0.91	0.99	1.77	1.58	1.68	1.12
22	-0.60	-0.59	-0.60	-0.61	-0.72	-0.67	-1.37	-1.29	-1.33	-0.86	-0.52	-0.44	-0.48	-0.81	-0.69	-0.75	-0.71	-0.59	-0.65	-0.63	2.56	2.09	2.33	1.79	1.63	1.71	1.47	1.33	1.40	1.81	0.54	0.71	0.63	0.97	0.72	0.85	1.51	1.69	1.60	1.02
23	-0.43	-0.61	-0.52	-0.59	-0.64	-0.62	-1.03	-1.17	-1.10	-0.75	-0.71	-0.55	-0.63	-0.63	-0.71	-0.67	-0.43	-0.55	-0.49	-0.60	1.71	1.95	1.83	1.41	1.59	1.50	1.38	1.24	1.31	1.55	0.39	0.47	0.43	0.89	0.75	0.82	1.76	1.64	1.70	0.98
24	-0.72	-0.57	-0.65	-0.88	-0.70	-0.79	-1.32	-0.99	-1.16	-0.86	-0.39	-0.48	-0.44	-0.57	-0.53	-0.55	-0.34	-0.69	-0.52	-0.50	1.82	2.04	1.93	1.50	1.62	1.56	1.19	1.37	1.28	1.59	0.31	0.36	0.34	0.61	0.82	0.72	1.61	1.79	1.70	0.92
25	-0.41	-0.49	-0.45	-0.54	-0.69	-0.62	-0.89	-1.16	-1.03	-0.70	-0.36	-0.51	-0.44	-0.66	-0.39	-0.53	-0.27	-0.42	-0.35	-0.44	1.69	1.81	1.75	1.32	1.48	1.40	1.21	1.30	1.26	1.47	0.67	0.51	0.59	0.41	0.57	0.49	1.49	1.36	1.43	0.84
26	-0.34	-0.51	-0.43	-0.49	-0.63	-0.56	-0.71	-1.04	-0.88	-0.62	-0.24	-0.17	-0.21	-0.38	-0.29	-0.34	-0.34	-0.19	-0.27	-0.27	1.57	1.75	1.66	1.39	1.54	1.47	0.99	1.41	1.20	1.44	0.48	0.57	0.53	0.69	0.53	0.61	1.44	1.51	1.48	0.87

Figure A1. This figure is a screen shot of the raw data set table from Trial 1.

Co2 Flux [	Mixed mm										<2mm										2-4mm										innoculated soil										Just soil		
days	B1 Mix mm	rep2	B1 avg	B2 Mix mm	rep2	B2 avg	B3 Mix mm	rep 2	B3 avg	Mix mm a	B1 <2mm	rep2	B1 avg	B2 <2mm	rep2	B2 avg	B3 <2mm	rep 2	B3 avg	<2mm avg	B1 2-4mm	rep 2	B1 avg	B2 2-4mm	rep 2	B2 avg	B3 2-4mm	rep 2	B3 avg	2-4mm av	B1 soil	rep 2	B1 avg	B2 soil	rep 2	B2 avg	B3 soil	rep2	B3 avg	Inoc. Soil	soil1	soil2	Just Soil avg
1	11.24	7.86	9.55	10.69	8.13	9.41	14.95	10.52	12.74	10.57	15.12	19.70	17.41	16.20	13.80	15.00	14.06	12.81	13.44	15.28	27.30	18.90	23.10	11.55	8.74	10.15	14.85	12.28	13.57	15.60	11.50	9.78	10.64	4.96	4.12	4.54	6.66	7.10	6.88	7.35	3.06	2.13	2.60
2	2.90	2.20	2.55	6.74	5.39	6.07	4.22	3.77	4.00	4.20	19.50	20.01	19.76	18.20	13.04	15.62	4.49	4.19	4.34	13.24	19.81	15.47	17.64	4.14	3.66	3.90	4.54	3.74	4.14	8.56	11.94	7.60	9.77	5.05	4.14	4.60	2.89	3.61	3.25	5.87	2.89	2.36	2.63
3	1.24	1.17	1.21	3.22	2.77	3.00	6.16	5.78	5.97	3.39	6.63	5.14	5.89	5.96	4.75	5.36	8.16	6.57	7.37	6.20	8.68	6.57	7.63	1.86	1.93	1.90	6.89	6.89	6.89	5.47	1.38	1.81	1.60	2.63	1.62	2.13	5.43	4.78	5.11	2.94	2.96	2.71	2.84
4	21.88	18.28	20.08	19.69	17.28	18.49	9.47	7.64	8.56	15.71	22.46	16.94	19.70	16.87	15.59	16.23	12.23	8.40	10.32	15.42	37.84	28.14	32.99	41.63	35.21	38.42	10.13	7.53	8.83	26.75	12.11	10.33	11.22	18.39	15.53	16.96	11.95	7.65	9.80	12.66	3.29	2.60	2.95
5	28.16	17.34	22.75	10.35	8.69	9.52	4.84	4.53	4.69	12.32	42.21	25.73	33.97	47.53	38.62	43.08	5.73	4.85	5.29	27.45	54.23	64.35	59.29	44.74	25.29	35.02	3.63	3.51	3.57	32.63	41.80	26.56	34.18	24.52	24.11	24.32	4.84	4.53	4.69	21.06	3.27	3.27	3.27
6	31.15	20.39	25.77	32.26	18.64	25.45	6.19	5.04	5.62	18.95	44.11	32.87	38.49	15.43	13.76	14.60	17.99	11.65	14.82	22.64	35.94	23.88	29.91	59.23	29.77	44.50	14.70	9.62	12.16	28.86	47.10	20.67	33.89	4.53	3.83	4.18	4.65	4.26	4.46	14.17	2.65	3.26	2.96
7	13.98	13.92	13.95	33.64	19.34	26.49	8.68	8.68	8.68	16.37	38.11	31.73	34.92	15.68	13.38	14.53	12.98	12.98	12.98	20.81	35.69	21.83	28.76	22.55	17.84	20.20	13.05	13.06	13.06	20.67	26.25	16.27	21.26	10.87	6.86	8.87	5.59	5.59	5.59	11.91	2.80	3.08	2.94
8	17.65	15.83	16.74	16.08	22.69	19.39	4.98	5.57	5.28	13.80	57.60	41.07	49.34	19.29	20.56	19.93	8.87	6.95	7.91	25.72	66.93	41.59	54.26	27.73	16.43	22.08	12.08	8.14	10.11	28.82	45.48	21.47	33.48	2.12	2.10	2.11	8.28	5.83	7.06	14.21	2.61	3.13	2.87
9	39.99	26.11	33.05	22.16	16.74	19.45	11.70	13.67	12.69	21.73	48.73	34.33	41.53	21.17	18.28	19.73	14.06	14.06	14.06	25.11	54.94	37.93	46.44	21.06	13.13	17.10	15.05	15.05	15.05	26.19	37.11	17.05	27.08	19.50	9.71	14.61	7.33	7.33	7.33	16.34	2.16	3.02	2.59
10	17.95	11.01	14.48	20.89	12.21	16.55	8.07	6.05	7.06	12.70	46.87	37.25	42.06	19.64	17.23	18.44	5.24	3.72	4.48	21.66	58.59	40.46	49.53	12.80	10.44	11.62	6.04	4.05	5.05	22.06	7.51	6.00	6.76	9.32	6.53	7.93	7.60	5.10	6.35	7.01	2.68	2.41	2.55
11	12.21	6.96	9.59	11.89	7.08	9.49	7.22	6.95	7.09	8.72	33.20	19.88	26.54	15.40	10.01	12.71	7.77	6.54	7.16	15.47	26.99	18.18	22.59	8.99	7.19	8.09	7.61	6.09	6.85	12.51	6.78	4.44	5.61	5.98	4.56	5.27	5.23	4.09	4.66	5.18	2.79	2.68	2.74
12	10.74	7.44	9.09	8.07	5.48	6.78	9.42	6.48	7.95	7.94	17.07	19.54	18.31	9.11	7.47	8.29	4.15	2.87	3.51	10.04	14.96	15.70	15.33	7.67	8.47	8.07	5.98	7.06	6.52	9.97	5.40	3.84	4.62	3.67	3.37	3.52	3.85	2.81	3.33	3.82	2.63	2.42	2.53
13	22.91	16.40	19.66	19.47	13.60	16.54	7.47	5.85	6.66	14.28	30.00	22.90	26.45	11.95	10.72	11.34	5.99	4.58	5.29	14.36	29.50	20.37	24.94	7.60	8.40	8.00	7.41	5.13	6.27	13.07	9.40	11.29	10.35	5.55	6.13	5.84	7.16	4.63	5.90	7.36	3.21	2.98	3.10
14	19.40	13.43	16.42	12.62	9.84	11.23	3.38	3.23	3.31	10.32	42.04	38.70	40.37	12.07	15.55	13.81	17.30	12.03	14.67	22.95	60.09	35.78	47.94	15.02	11.38	13.20	5.84	4.44	5.14	22.09	44.15	20.54	32.35	4.81	4.15	4.48	4.06	3.19	3.63	13.48	3.19	2.52	2.86
15	39.86	20.23	30.05	14.75	11.71	13.23	8.28	6.67	7.48	16.92	36.13	31.50	33.82	11.25	9.70	10.48	10.36	7.57	8.97	17.75	82.11	42.23	62.17	12.64	10.28	11.46	15.42	9.11	12.27	28.63	15.22	22.41	18.82	10.36	6.22	8.29	7.21	5.01	6.11	11.07	3.88	2.69	3.29
16	32.11	27.48	29.80	11.88	12.65	12.27	8.11	7.79	7.95	16.67	29.81	35.40	32.61	10.81	10.44	10.63	8.34	9.09	8.72	17.32	50.80	59.09	54.95	11.24	12.90	12.07	12.19	8.65	10.42	25.81	14.89	16.09	15.49	12.18	10.94	11.56	8.78	8.05	8.42	11.82	3.33	3.89	3.61
17	25.34	22.98	24.16	9.13	10.10	9.62	4.84	6.99	5.92	13.23	20.88	26.19	23.54	11.98	13.40	12.69	6.77	7.09	6.93	14.39	35.78	39.11	37.45	15.43	17.80	16.62	11.04	7.69	9.37	21.14	27.71	23.09	25.40	15.48	13.75	14.62	6.67	7.01	6.84	15.62	3.14	3.78	3.46
18	16.68	14.88	15.78	9.55	11.93	10.74	5.17	6.64	5.91	10.81	19.77	21.33	20.55	17.70	14.93	16.32	9.54	10.90	10.22	15.70	27.22	34.40	30.81	22.99	19.84	21.42	9.89	10.57	10.23	20.82	24.18	26.52	25.35	12.67	10.88	11.78	4.34	4.15	4.25	13.79	3.16	3.55	3.36
19	32.10	18.99	25.55	14.90	10.66	12.78	8.33	7.98	8.16	15.49	26.81	24.97	25.89	15.30	16.99	16.15	12.28	11.92	12.10	18.05	41.69	45.01	43.35	17.84	18.22	18.03	14.98	12.71	13.85	25.08	16.56	19.02	17.79	9.08	11.42	10.25	5.76	4.99	5.38	11.14	2.99	3.49	3.24
20	26.76	22.19	24.48	18.11	13.33	15.72	7.77	8.20	7.99	16.06	27.40	22.05	24.73	16.06	18.44	17.25	10.11	8.39	9.25	17.08	52.85	40.99	46.92	19.45	21.04	20.25	9.45	10.75	10.10	25.76	22.89	17.17	20.03	7.23	10.78	9.01	6.83	6.44	6.64	11.89	3.09	3.53	3.31
21	25.83	27.77	26.80	18.49	15.93	17.21	9.64	7.91	8.78	17.60	24.95	23.40	24.18	17.34	16.92	17.13	11.87	9.37	10.62	17.31	44.76	41.55	43.16	22.36	21.05	21.71	11.54	10.25	10.90	25.25	19.13	21.60	20.37	10.66	11.69	11.18	7.10	7.56	7.33	12.96	2.86	3.47	3.17
22	23.85	27.82	25.84	19.34	15.99	17.67	9.86	8.13	9.00	17.50	25.09	27.85	26.47	15.35	17.83	16.59	11.43	12.93	12.18	18.41	51.29	49.40	50.35	21.78	20.29	21.04	15.43	13.65	14.54	28.64	20.77	22.85	21.81	10.22	8.90	9.56	6.33	5.99	6.16	12.51	3.67	3.44	3.56
23	24.73	29.12	26.93	19.34	16.40	17.87	10.23	9.99	10.11	18.30	26.12	25.99	26.06	17.83	16.44	17.14	10.74	13.34	12.04	18.41	48.54	45.09	46.82	24.55	23.77	24.16	11.49	13.23	12.36	27.78	21.46	20.88	21.17	9.88	9.44	9.66	7.21	7.07	7.14	12.66	3.52	3.71	3.62
24	22.64	23.88	23.26	18.90	17.33	18.12	8.34	9.56	8.95	16.78	33.13	26.98	30.06	16.97	18.34	17.66	12.94	13.97	13.46	20.39	52.87	51.11	51.99	22.11	20.09	21.10	13.45	14.97	14.21	29.10	21.21	23.90	22.56	10.27	9.79	10.03	8.12	7.55	7.84	13.47	3.41	3.68	3.55
25	26.93	28.34	27.64	18.96	15.99	17.47	9.94	11.10	10.52	18.54	27.34	29.34	28.34	18.32	15.34	16.83	13.95	12.12	13.04	19.40	54.23	59.50	56.87	23.94	22.78	23.36	12.54	13.19	12.87	31.03	25.87	23.79	24.83	10.11	9.75	9.93	9.31	8.76	9.04	14.60	4.09	3.81	3.95
26	24.72	28.22	26.47	19.11	16.13	17.62	11.65	10.93	11.29	18.46	25.99	31.61	28.80	17.34	18.86	18.10	14.54	12.98	13.76	20.22	51.24	56.88	54.06	24.96	21.21	23.09	11.89	10.67	11.28	29.48	22.31	23.40	22.86	10.43	10.09	10.26	10.08	9.62	9.85	14.32	3.93	3.79	3.86

Figure A2. This figure is a screen shot of the raw data set table from Trial 2.

Appendix B

Materials List	
Materials	Quantity
Hardwood Biochar	76 liters (20 gal)
Organic Gardening Soil	13.6 kg (30 lbs)
Dr. Higa's EM1 Concentrate	3.78 liters (1 gal)
7.56 liter (2 gal) Buckets	13
LI-COR 8200 Smart Chamber	1
LI-COR LI-870 Gas Analyzer	1
4kW Hammermill	1
120V Humboldt Sieve	1
Sterilization Oven	1
Paint Strainer Bags	12
Measuring Cup	1
Large Scale	1

Appendix B. Materials list for the study.

Vita

Clayton James Pope was born in San Ramon, California to James and Stacey Pope. He attended Appalachian State University in the fall of 2017, and in 2021 he earned a Bachelor's degree in Sustainable Development. In the spring of 2022, he began to pursue a Master of Technology degree at Appalachian State University which is expected to be conferred in December 2023.

Clayton's interests have long been vested in sustainability and environmental sciences, but through his time at Appalachian State he developed a deep appreciation for technology as well. More specifically, sustainable technologies. Following the completion of his degree, he plans to contribute to the agricultural technologies industry and reside in Raleigh, North Carolina with his beloved dog Magic.