Sustainability and Economic Feasibility of Biosolids-Based Biochar for Agricultural Applications

Authors: Riley Black, Sam Lance, Lauren Londoño, & Jessica Rodriguez

Faculty Advisor: Dr. Arturo Keller

Client: Santa Ynez Chumash Environmental Office

Bren School of Environmental Science & Management

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Riley Black, MESM

Sam Lance, MESM

Lauren Londoño, MESM

Jessica Rodriguez, MESM

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The Group Project is required of all students in the Master of Environmental Science and Management (MESM) Program. The project is a year-long activity in which small groups of students conduct focused, interdisciplinary research on the scientific, management, and policy dimensions of a specific environmental issue. This Group Project Final Report is authored by MESM students and has been reviewed and approved by:

Dr. Arturo Keller, Faculty Advisor **Date**

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Land Acknowledgement

We respectfully acknowledge that the study area for this project is, and always has been, the native homeland of the Chumash and Salinan Peoples and their ancestors. Oral histories, historical records, and archaeological evidence demonstrate that the Central Coast of California has supported Chumash and Salinan communities, cultures, and traditions for over 10,000 years.

We also recognize that the land on which we conduct our studies at the University of California, Santa Barbara, is the traditional and unceded territory of the Chumash Peoples. We extend our gratitude to the Santa Ynez Band of Chumash Indians Environmental Office nursery, as well as the plants, earth, and all beings that contributed to the growth of the plants used in this study. We further acknowledge the land and its ancestors, which sustain the UCSB Greenhouse and Bren Hall laboratories.

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Acronym Glossary

CBA	Cost-benefit analysis
GHG	Greenhouse gas
LCA	Life cycle assessment
PFAS	Per-and poly-fluoroalkyl substances
PPCP	Pharmaceuticals and personal care products
SYCEO	Santa Ynez Chumash Environmental Office
VOCs	Volatile organic compounds

Objectives

The widespread use of biosolids from wastewater treatment plants as agricultural fertilizer presents significant environmental and health challenges due to the accumulation of contaminants in soil and water sources. To address this issue, the project aimed to evaluate the feasibility and benefits of utilizing pyrolyzed biochar derived from biosolids as a safer and more sustainable alternative to applying untreated biosolids to agricultural fields. By applying biochar and biosolids as fertilizers to grow native plants and crops in collaboration with the Santa Ynez Band of Chumash Indians Environmental Office (SYCEO), the project sought to assess their effectiveness in improving overall plant yield and nutrient content. Additionally, a cost benefit analysis and literature review was conducted to understand the feasibility of using biochar as a soil amendment, paving the way for informed decision-making in agricultural practices in California.

This project aimed to evaluate whether pyrolyzed biochar from biosolids is a sustainable fertilizer alternative to using biosolids directly for selected food crops and native plants.

More specifically, the objectives were to:

1. **Design an experiment** to test biochar's effectiveness on California native plants (Basket Rush and Dogbane) and agricultural crops (lettuce and wheat) in lab and nursery settings at the Santa Ynez Chumash Environmental Office.
2. **Conduct a literature review** on biosolid-based biochar's role in agriculture, covering its business case, experimental outcomes, nutrient content, crop yield, contaminant impacts, and life cycle assessments.
3. **Perform a cost-benefit analysis**, examining when the benefit inflows from using biosolid-based biochar offset the initial cost of implementation for a farmer.

Deliverables

In addition to the required Bren School deliverables (i.e. a final report, executive summary, and final presentation), this project produced the following:

- Repository of data (including nutrient analysis, biomass, etc) from native plant and food crop experimental trials.
- Visual graphic for the SYCEO on the implications of biochar for their operations.

Significance

Any community with wastewater treatment plants, from large cities to small towns, must manage sewage sludge, which refers to the solid by-product that results from the biological treatment of water (Gopinath et al., 2021). Sewage sludge that is further treated to meet safety standards is referred to as biosolids. The U.S. EPA's 2022 Biosolids Annual Report reveals that 56% of biosolids are applied applied directly to the land for agriculture, while 43% are disposed of via landfill or incineration (EPA, 2016). Biosolids are commonly directly used as fertilizer for agricultural purposes, since they are rich in nutrients like nitrogen and phosphorus (Paz-Ferreiro et al., 2018).

Unfortunately, the routine application of biosolids to land coupled with the increasing amount of contaminants in wastewater that bypass treatment processes (e.g. microplastics, heavy metals, and pharmaceuticals), has led to an accumulation of contaminants in the soil over the years (Mohajerani and Karabatak, 2020). This presents health risks to humans since such contaminants can be transferred to crops that are harvested for consumption and runoff into sources of drinking water (Alengebawy et al., 2021). Consequently, some states have begun to regulate sludge or biosolids use as fertilizer, especially as they relate to high concentrations of PFAS (Hughes, 2023). As of now, Maine is the only state which has banned the application of biosolids for agricultural purposes (Hughes, 2023).

One potential solution is to further process the biosolids via pyrolysis to create biochar, a substance that is rich in nutrients while containing less pollutants and pathogens (Keller et al., 2024). Pyrolysis is the process of thermal decomposition of biosolids using very high temperatures in conditions without oxygen (Liu et al., 2021). This process may help eliminate pollutants such as microplastics, PFAS, chemicals in pharmaceuticals, and personal care products (Zhao et al., 2023). Besides producing biochar, pyrolysis also creates syngas and bio-oil, providing useful energy recovery (Liu et al., 2021). The recovered heat energy can ultimately be harnessed to make the biosolids drying process and pyrolysis more energy efficient in following batches.

The integration of biosolids-based biochar into agricultural practices aligns with the project's goals of promoting sustainable land management and mitigating environmental health risks. By addressing the challenges associated with conventional fertilizer applications, this project investigates a practical solution for enhancing soil health while minimizing the transfer of contaminants to crops and water systems. Additionally, the collaboration with the Santa Ynez Band of Chumash Indians Environmental Office highlights the importance of integrating traditional ecological knowledge and community priorities into innovative solutions. Ultimately, the findings from this research will contribute to a more sustainable agricultural framework in California, providing valuable insights to native plant nurseries, grain and vegetable farmers, policymakers, and environmental managers to support informed decision-making.

Background

Biochar, Biosolids, and Biosolids-based Biochar

Biochar is a highly porous material with a high surface area that is nutrient-rich and has excellent adsorption capabilities, especially for ammonia (Samuel Olugbenga et al., 2024; Carey et al., 2015). Because of these properties, biochar promotes soil microbial activity and decreases overall nitrogen volatilization (Joseph et al., 2021). As a result, biochar is a promising soil amendment to complement nitrogen fertilizers, as its sorption capabilities can improve overall nutrient composition in soil (Joseph et al., 2021). These properties can vary greatly, however, and are dependent on the type of feedstock and the particular heating process used, which could include variations in pyrolysis temperature, heating rate, and pressure (Singh et al., 2020). Potential feedstocks include many types of waste materials, including excess wood from logging, crop straw, animal manure, food waste, and sewage sludge (Joseph et al., 2021). While each feed type reduces waste products from major industries, the use of sewage-sludge and biosolids in particular introduces the possibility of addressing challenges regarding wastewater management.

Wastewater treatment is critical for handling human waste and ensuring both community and environmental health. Prior to their release back into the environment, municipal wastewaters are treated to remove pathogens, excess nutrients, pollutants, dissolved carbon and solids (Wang et al., 2008). The remaining semi-solid byproducts of these plants are anaerobically digested, and become biosolids which are often discarded into landfills (Paz-Ferreiro et al., 2018). These biosolids often are discarded in landfills, where emissions of methane and concerns of pollution are making agricultural land application of biosolids attractive (Wang et al., 2008). Because of its nutrient richness, ease of application, and low cost, biosolids have been used in agriculture for thousands of years (Lu et al., 2012). However, in the modern age contaminants such as heavy metals and emerging pollutants such as microplastics, pharmaceuticals and personal care products (PPCP), and per- and poly-fluoroalkyl substances (PFAS) are becoming a concern in biosolids due to their ubiquity and persistence in the environment (Paz-Ferreiro et al., 2018). PFAS in particular, are present in biosolids because treatment processes do not remove them (Hughes, 2023).

Pyrolysis describes the high temperature treatment of biomass, including biosolids, under anoxic conditions to reduce contaminant concentrations. Pyrolysis temperature plays an essential role in reducing contaminant concentration, with higher pyrolysis temperatures generally leading to lower concentrations of many contaminants (Wang et al., 2012). Pyrolyzing at a higher temperature requires significant energy, which should be considered when selecting a pyrolysis temperature. Another important consideration is that the concentration of nitrogen also decreases with increasing temperature, decreasing overall nutrients in the biochar (Paz-Ferreiro et al., 2018). The optimal range is often considered to be between 450°C–600°C, where most pyrolysis facilities operate (Jin et al., 2016; Thoma et al., 2022).

Several studies demonstrate pyrolysis effectively reduces contaminant concentrations to safe amounts. Thoma et al. (2022) found that when 21 distinct input biosolids with concentration of PFAS ranging from 2-85 µg/kg were pyrolyzed at 600°C, none of the resulting biochar had detectable PFAS. Microplastics have also been shown to degrade significantly with pyrolysis, with one study finding pre-pyrolysis levels to be from 550 to 969 particles/g of sludge to 1.2–2.3 particles/g of biochar when heated at a temperature of 500°C (Ni et al., 2020). Even in lower initial concentrations of 15.1 particles/g, pyrolysis reduced microplastic levels by 91–97% (Keller et al., 2024). Organic pollutants such as PPCPs have also been shown to decrease significantly when pyrolized into biochar. Alipour et al. (2022) found that when pyrolyzed at 600°C, organic pollutants were almost completely removed (99.9% removal) and even at a lower temperature of 450°C, combined concentrations of PPCPs were reduced from 45.31 ng/g to 0.94 ng/g. These reductions in concentrations suggest that biochar successfully addresses many contamination concerns of biosolid land applications.

Beyond the potential to recover nutrients from biosolids while limiting waste produced, there are other benefits to using biosolids-based biochar as a soil amendment. Biochar has been studied to be an effective agent in the remediation of organic and inorganic contaminants from groundwater by immobilizing soil contaminants (Qambrani et al., 2017). Different studies on biochar feedstocks and pyrolysis techniques have revealed that there is a range of effectiveness in contaminant remediation based on the specific biochar's porosity, surface area, ash content, alkalinity, and a few other important characteristics (Bolan et al., 2022). Depending on the chemical properties of a given potentially toxic element (PTE), volatile organic compound (VOC), or other contaminants, biochar can facilitate the chemisorption and electrostatic adsorption of the contaminant onto its surface (Bolan et al., 2022). Shen & Zhang (2019) add to this study by describing how certain activated biochars with large porous surface areas can act as a medium for the sorption of tar compounds and volatile organic compounds (VOCs) such as toluene, phenol, and other aromatic hydrocarbons.

Considerations for the Experimental Design

Plant Selection

For our study, we conducted trials using biosolids-based biochar on two crop types to test its effect on agricultural plants, and two native plant species to test its potential applicability for use at the Chumash Nursery. The crop plants selected for our greenhouse experiment are lettuce and wheat. Lettuce is an ideal candidate, as it reaches full maturity in around 2 months and generates roughly $1.25 billion in sales yearly (USDA ERS, 2023). Christou et al. (2022) conducted a study on the impacts of biosolids-based biochar on lettuce, where they found that the application of biochar led to increased soluble solids and sucrose, which improved the nutritional quality and sweetness of the produce. While wheat has a longer growing season of around 6 months, it plays a major role in the diet of Americans, with per capita wheat flour consumption at roughly 129 pounds (USDA ERS, 2024). Yonghua Liu et al. studied how biosolids-based biochar impacted the growth of Yangmai 19 wheat in a field study and found it increased overall yields and decreased the metal accumulation in crops compared to non-amended soils (Liu, 2023). Ultimately, lettuce and wheat have been chosen for our research since they are both staple foods and have shown improvement with biochar amendments.

Our experiment conducted in pot trials at the Chumash Nursery will use two plants native to California: basket rush (*Juncus textilis*) and dogbane (*Apocynum cannabinum*). To date, no studies have been conducted on the effects of biosolids-based biochar on the growth of basket rush or dogbane; however, recent literature has shown biochar's promise in restoring native species. Big bluestem, a native plant in the Central United States, was shown to increase in biomass production and height with the addition of a pine-based biochar compared to its non-native competitor, indicating biochar's possible use in prairie restoration (Adams et al., 2017). Biochar was also shown to improve soil pH, organic matter percentage, and nematode quantity, which restored the tested soils to the quality of sample remnant prairies (McCullough and Bastow, 2024). While these studies show the promise of biochar for native grasses, a study conducted on native trees and flowers in Colorado indicated biochar had little positive effect on any of the plants except for reducing their water needs (Matt et al., 2018). This study aims to contribute to the current literature by focusing on biochar applications for growing California native plants.

Biochar Amount Selection

The amount and method of biochar application varies widely. Commonly used methods for measuring application fall into two categories: measuring by weight or volume percentage of the total mixture or weight/hectare. When selecting their amounts, they range from 1–10% w/w and 10–20 tons/hectare (Christou et al., 2022; Nobile et al., 2020; Bista et al., 2019). Each noted that the addition of biochar improved the quality of the crops produced, with Fristak et. al noting that biochar amendment decreased the toxic elements in alfalfa tissues compared to soils amended directly with biosolids (Fristak et al., 2018). Paz-Ferreiro et al. similarly applied 3% biosolid-based biochar to their proso millet plants and found they increased fruit yield and overall plant biomass relative to plant-based biochar (Paz-Ferreiro et al., 2014). Another study using douglas fir based biochar tested whether the amount of biochar applied directly affects plant growth, and have found that increasing amounts of biochar application aid in plant growth until a threshold is reached at 22.4 Mg/ha (Bista, 2019). As we are uncertain how plants grown with the soil from the Native Plant Nursery will react to the application of biochar, we plan to use the range of values set by these studies, with treatments at 3%, 6%, and 9% weight/weight of biochar application to find the ideal amount. Since we will be working with native soils, we plan to add perlite to our mixtures to enhance porosity and drainage of potted trials. Due to perlite's low density, a volume approach will allow for the correct amount of perlite addition compared to weight.

Measurement Selection

Studies conducted by Rondon et al. (2007) and Kizito et al. (2019) studied green beans and yellow corn, respectively, and both showed biochar increased overall root and shoot biomass. Bin Yousaf et al. (2022) showed similar results for three different tree species (gum arabic, Indian rosewood, and eucalyptus), each amended with manure-based biochar where each tree showed increased biomass compared to the control studies. A study conducted by Hossain et al. (2010) indicated that this improvement in overall biomass and fruit yield from cherry tomatoes is increased with the application of fertilizer to the soils, indicating the presence of both has a compounding effect. Biochar has also been shown to improve the chances of survival of native plants against allelopathic invasives by improving their growth, with plants exposed to biochar exhibiting increased overall field-estimated biomass (Sujeen and Thomas, 2022).

To measure this increase in biomass, all plant samples were weighed prior to processing. For lettuce and native plant trials, aboveground and belowground biomass were measured separately. For wheat, the weight of the seeds were measured along with belowground biomass. In addition to measuring biomass, the experimenters wanted to determine whether biochar would improve the nutritional value of the crops produced, such as in Christou et. al (2022), and performed a full nutrient analysis. While many groups of nutrients were analyzed, the final results yielded nutrient profiles for sugars/sugar alcohols and organic acids.

Cost Benefit Analysis

A cost-benefit analysis, as described by the U.S. Department of Transportation (2024), is "a systematic process for identifying, quantifying, and comparing the expected benefits and costs of an investment, action, or policy." This project focuses on evaluating the economic feasibility of biosolids-based biochar for small-scale farmers rather than producers, addressing a gap in models tailored to small scale farmers' needs. By identifying the costs, benefits, and potential revenue streams for farmers, this project aims to inform adoption strategies and create the business case for biochar as a soil amendment.

The choice of feedstock significantly influences biochar properties and economic considerations. Common feedstocks include wood, crop residues, animal manure, food waste, and sewage sludge (Joseph et al., 2021). Using biosolids as a feedstock addresses challenges in wastewater management by reducing landfill waste and emissions from municipal treatment plants. McIntyre and Li (2024) emphasize the importance of evaluating large-scale pyrolysis of biosolids and developing a standardized production process. They suggest co-locating pyrolysis facilities with wastewater treatment plants to streamline the supply chain. Bioforcetech Corporation demonstrates this model by producing biochar directly at municipal wastewater treatment plants. This circular economy approach not only mitigates waste but also creates opportunities to produce biochar while addressing environmental challenges. The cost-benefit analysis model will focus on understanding the timeframe in which small-scale farmers might realize economic benefits from adopting biochar, providing insights into its long-term financial viability and potential for adoption.

Biochar can enhance profitability by improving crop yields, reducing fertilizer use, and boosting soil quality (Collison et al., 2009; Khan et al., 2022). Long-term benefits include higher agronomic value and carbon sequestration potential (Dickinson et al., 2014; Galinato et al., 2011; Latawiec et al., 2017). However, its high initial costs remain a barrier to adoption. Current biochar prices in California range depending on feedstocks used, with biosolids based biochar at an average industry price of \$500, with a range of \$300–\$900 (Bioforcetech, 2024; Saratoga Biochar, 2024). Bulk discounts of 20 to 40% could make biochar more accessible for larger farming operations. In contrast, conventional fertilizers cost \$400 to \$1000 per ton, emphasizing the need for biochar to achieve greater price competitiveness (USDA, 2024).

Case studies have examined the feasibility of biochar adoption and its role within carbon markets for farmers incorporating biochar into their operations. For instance, Dickinson et al. (2014) found biochar to be economically viable for winter wheat production when priced at \$12 per ton or when greenhouse gas offset revenue reached \$31 per ton of CO_2 equivalent. However, while biochar can theoretically be priced at this level, it may not be practical for producers due to production and supply chain constraints. This highlights the potential for carbon markets to incentivize biochar adoption. Nevertheless, the model in this project is based on data from laboratory and small-scale nursery experiments. To fully assess the broader carbon offset potential and practical applicability of biochar, further research is needed, including large-scale field trials and the development of biochar-specific carbon offset programs.

Beyond agriculture, biochar offers diverse benefits, including groundwater remediation, reduction of greenhouse gas emissions, and use as a catalyst, biofilter, or additive in construction materials (Bolan et al., 2022). It can also enhance wastewater decontamination processes based on its chemical and structural properties (Gupta et al., 2022).

Azzi et al. (2020) highlight the benefits of small-scale pyrolysis plants on Nordic farms, where biochar production is integrated with heating and energy systems. These farms reduce reliance on centralized power grids, creating circular economies for energy and waste. While this model suits biomass feedstocks, Keller et al. (2024) argue that economies of scale are essential for biosolids-based biochar. Centralized drying and pyrolysis facilities serving multiple wastewater treatment plants could optimize energy efficiency and reduce costs.

Gupta et al. (2022) highlight the economic incentives of integrating pyrolysis with wastewater treatment. WWTPs could reduce waste disposal costs and create new revenue streams by selling biosolids-based biochar to farmers and local communities. These examples demonstrate that biochar is not only an agricultural amendment but also a versatile product with potential for broader economic and environmental applications.

Outside of just agricultural use, biochar has recently been studied for its promising use as an amendment or replacement for certain construction materials such as cement. Not only is biochar a low-carbon emission alternative to clinker in concrete, but a number of studies have shown that biochar's physicochemical properties may actually improve the compression strength of mortar and improve cement hydration while also immobilizing PTES and other organic contaminants found in sediments (Bolan et al., 2022). Restuccia and Ferro contribute with their research on using biochar as a carbon nano-aggregate, which has the capability of improving flexural and compressive strength in cement, as well as increasing the fracture energy which controls the way cement cracks (Restuccia & Ferro, 2016). Gupta and Kua's (2016) analysis of biochar's improvement of cement hydration rates resulted in 28.45–50.25% compression strength at the same time as 35–38% lower water absorption (Gupta et al., 2016). Before biochar is fully integrated into standard engineering applications, however, there must be more research on the economic and production feasibility of scaling biochar-amended cement. Although not directly related to agriculture or a farmer's use for biochar, these alternative applications could be useful as pyrolysis facilities seek to diversify their revenue streams in emerging markets while hoping to gain industry and regulatory support of applying biosolids based biochar as a soil amendment.

By addressing cost barriers, exploring co-benefits, and leveraging carbon market incentives, this project aims to present the case for biosolids-based biochar in informed agricultural decisions. Thus, this group project also developed an informational graphic for the SYCEO and general farmers (in both English and Spanish) to raise awareness about biochar's costs, benefits, and potential applications (Review Appendix 1). By addressing economic, agronomic, and environmental factors, this analysis will provide actionable insights into the adoption potential of biosolids-based biochar for small-scale farming operations.

Life Cycle Assessment

Life Cycle Assessment (LCA) is a crucial environmental management tool used to evaluate the environmental and economic implications of a product throughout its life cycle (Alizadeh et al., 2024). It enables managers to compare products, identify trade-offs, and assess environmental impacts at each stage of a product's lifecycle. Conducting a LCA often involves interdisciplinary expertise and the use of specialized software tailored to specific product types, such as energy, natural resources, and agriculture (Alizadeh et al., 2024). These tools help quantify metrics such as energy consumption, water use, emissions, and pollution, with scope and assumptions depending on industry practices, market demand, and environmental conditions. For example, LCAs have been instrumental in demonstrating biosolids-based biochar as a sustainable alternative to direct land application of wastewater biosolids (Miller-Robbie et al., 2014).

LCA studies of biochar vary based on feedstock and scope but consistently address key impact categories such as water use, energy consumption, carbon footprint, human toxicity potential, and terrestrial ecotoxicity (Peters and Rowley, 2009). These factors are vital when evaluating the safety and feasibility of integrating biochar into large-scale commercial agriculture.

Water and energy use are deeply interconnected in biochar production. Processing and drying biosolids are the most energy-intensive phases, primarily due to high electricity demands, with transportation adding further energy requirements (Miller-Robbie et al., 2015). Peters and Rowley (2009) emphasize improving energy efficiency to reduce water consumption, given the significant water use in electricity production. Additionally, using biochar can offset water consumption by reducing the need for nitrogen and phosphorus fertilizers (Peters and Rowley, 2009). During application, biochar's porous, stable carbon structure enhances soil nutrient content, improves water retention, and minimizes irrigation needs (Roberts et al., 2009). These findings highlight the importance of holistically addressing water and energy use in biochar LCAs to maximize sustainability.

Miller-Robbie et al. (2015) identify incineration as the most greenhouse gas (GHG)-intensive biosolids management method, followed by waste-to-energy and landfilling. In contrast, land application reduces direct emissions and provides offsets through avoided fertilizer use and carbon sequestration. Co-producing biochar and biosolids could further lower energy consumption and GHG emissions at wastewater treatment facilities (Miller-Robbie et al., 2015). Incorporating soil carbon dynamics into LCAs, as Peters and Rowley (2009) suggest, adds complexity but is critical for assessing long-term impacts. Roberts et al. (2009) show that biochar from various feedstocks generally leads to net-negative GHG emissions, primarily due to stable carbon sequestration and reduced soil N_2O emissions. These insights highlight biochar's potential to significantly reduce agriculture's carbon footprint.

Pyrolysis effectively removes pathogens, microplastics, and toxic chemicals from biosolids while immobilizing trace heavy metals through sorption (Paz-Ferreiro et al., 2018). Biosolids can contain 800–41,000 pieces of micro- and nano-plastics per kilogram, which are challenging to filter using conventional methods (Borthakur et al., 2022). When applied to agricultural soils, microplastics and PFAS in biosolids can pose health risks to farmworkers and nearby communities via contaminated dust (Borthakur et al., 2022). Additionally, toxic chemicals and heavy metals from coal combustion and transportation fuel contribute to biosolids' ecotoxicity (Peters and Rowley, 2009). Increasing biochar production with recycled biogas at WWTPs can further reduce these contaminants while minimizing transportation emissions and fertilizer production impacts.

Building on this literature, this project aims to quantify the environmental and financial impacts of biosolids-based biochar application on small farms, emphasizing the soil application phase. By addressing these issues, the project seeks to enhance understanding of biochar’s potential to improve agricultural sustainability and reduce environmental footprints.

Approach and Methods

Lab Experiment

The purpose of conducting the experimental portion of the project was to understand the impact biosolids-based biochar can have on plant growth and nutrients. The experiment was split into two sections: agricultural plants and plants native to California. Wheat and lettuce were selected to be our agricultural plants, with one round of wheat being grown and two rounds of lettuce (referred to hereafter as Lettuce I and Lettuce II). Dogbane and basket rush were selected to be our native plants, since they are both native to California and are culturally significant to the Chumash. The following section details our process of creating the soil-biochar-perlite mixtures, planting, harvesting, and extracting the nutrients from the following plants.

Soil Mixing and Planting

Prior to mixing soil and planting, wheat was germinated and sprouted in the Keller Laboratory, being kept in water overnight before being grown for two weeks under grow lights in sphagnum moss. Lettuce I and Lettuce II were sourced from Frecker Farms, and were around 2 inches tall at the time of planting. Both dogbane and basket rush were sourced from cuttings of previous plants of various sizes at the Native Plant Nursery.

Additionally, all soil was sourced from the Native Plant Nursery, harvested in two separate rounds from the same location. This was to make all results relevant to the specific geology of the region, as biochar reacts differently to different soil compositions. Biochar was purchased from Bioforcetech in Redwood City, and is derived from wastewater byproducts. Perlite was purchased in multiple rounds from various sources and consisted solely of silicate material, without any added fertilizer. The perlite was added to every soil mixture to prevent clumping and encourage drainage, as the soil from the Native Plant Nursery had a relatively high clay content.

Soil was mixed in three major phases: Lettuce I + Wheat, Native Plants, and Lettuce II. Lettuce I + Wheat was mixed in Spring 2024 using the measurements outlined in Table 1, and involved creating small batches of soil mixtures and putting them in plastic containers on rollers to fully integrate the biochar. While effective in ideal conditions, this process would have been too time consuming for our timeline, and would have required significant modifications to the soil mixing bottles and/or roller to allow for proper mixing to occur. Native Plants and Lettuce II were mixed using the hand mixing methods, where all soil for one trial was mixed using measurements outlined in Tables 2 and 3 in a large plastic storage box by hand.

Table 1. Soil Mixtures Used in Lettuce I and Wheat Trials. Amounts of soil, biochar, and perlite added to each trial in the Lettuce I and Wheat trials in grams. This accounts for the amount of soil, biochar, and perlite used for both trials.

Experiment	Trial	Soil (g)	Biochar (g)	Perlite (g)	Mixing Method
Lettuce I and Wheat	Control	1000	-	100	Hand Rolling
	Fertilizer	1000	-	100	Hand Rolling
	3% Biochar	1000	33	100	Hand Rolling
	6% Biochar	1000	66	100	Hand Rolling
	9% Biochar	1000	99.2	100	Hand Rolling

Table 2. Soil Mixtures Used in Native Plant Trials. This accounts for the amount of soil, biochar, and perlite used for both dogbane and basket rush trials, as the soil was mixed in large batches.

Experiment	Trial	Soil (g)	Biochar (g)	Perlite (g)	Mixing Method
Native Plants	Control	16000	-	1600	Hand
	Fertilizer	16000	-	1600	Hand
	3% Biochar	16000	528	1600	Hand
	6% Biochar	16000	1056	1600	Hand
	9% Biochar	16000	1584	1600	Hand

Table 3. Soil Mixtures Used in Lettuce II Trial. This accounts for the amount of soil, biochar, and perlite used in the Lettuce II trial.

Experiment	Trial	Soil (g)	Biochar (g)	Perlite (g)	Mixing Method
Native Plants	Control	30000	-	3000	Hand
	Fertilizer	30000	-	3000	Hand
	3% Biochar	30000	990	3000	Hand
	6% Biochar	30000	1980	3000	Hand
	9% Biochar	30000	2970	3000	Hand

Lettuce I was then transplanted into containers with each soil mixture and placed in the UCSB Greenhouse which was maintained at a temperature between 70-75 degrees Fahrenheit. Wheat was similarly transplanted into soil mixtures, but was placed on risers outside the UCSB Greenhouse. This approach was taken due to multiple outbreaks of aphids—destructive sap-sucking insects—in the greenhouses, coupled with the favorable summer climate for wheat production. Dogbane and basket rush were similarly placed on risers under a sun shield at the Native Plant Nursery over the summer. Once the wheat was harvested, Lettuce II was placed on the same risers outside to prevent wilting. After all plants were transplanted, an initial dose of nitrogen fertilizer was provided to all trials since our soils and biochar were both low in nitrogen.

Over the summer, all plants were regularly monitored and all issues fixed. Every month, the monitoring group member added one tablespoon of fertilizer to the fertilizer trial. In mid July, the wheat was infested with aphids that had to be removed by hand, and all stalks soaked with a soapy solution to prevent them from returning. Weeds also sprung up regularly throughout the summer that had to be removed. Likely due to the heat and sunlight in the greenhouse, Lettuce I wilted extremely quickly over one weekend and had to be rapidly harvested and frozen to preserve remaining growth. Lettuce II multiple rounds predation from birds or vermin, which consumed the majority of the aboveground biomass.

Native plants grown at the SYCEO nursery faced significant predation and death. Several basket rush trials never sprouted and lost any evidence of propagated root stalks ever being planted. This was thought to be attributed to ground squirrels or birds foraging through unprotected pots because of other clear damages to nursery plants grown on the same shelf. Native blue-eyed grass grown directly next to the experimental basket rush was systematically decimated by pests in August, leading us to believe that destruction of several experimental plants was not a choice for pests, rather an effect of proximity to choice grass species. Future iterations of this experiment may consider growing experimental trials under wired cages to protect them from pests. The remaining maintenance only required watering each plant 2-3 times each week and applying fertilizer to the fertilizer control once each month.

Harvesting and Extraction

Each plant type was harvested either when they reached full size (or wilted in the case of Lettuce I). Lettuce I was harvested by cutting the leaves from the roots, and bagging the leaves and roots by trial (control, fertilizer, etc.). Wheat seeds were harvested by hand and were placed in Ziploc bags by trial, and weighed together. Heads of wheat per pot and total seeds were also counted and recorded. Native Plants were removed from the soil and all biomass was weighed. If no biomass was found within the soil mixtures, that was noted in our data. No further analysis was conducted on the Native Plants. Lettuce II was processed similarly to Lettuce I, but weights were taken for each individual plant prior to being bagged by trial. Once Lettuce I, Wheat, and Lettuce II were harvested, all biomass was placed into the -20 degree freezer to keep them fresh prior to grinding. Unfortunately, Lettuce I was moved to the refrigerator for roughly two weeks due to routine freezer maintenance, leading to significant rotting.

Prior to nutrient extraction, all trials needed to be ground to allow for maximum surface contact with solvents and mixing between trials. To grind the trials, biomass and liquid nitrogen was added to a mortar and pestle. This froze the material and allowed it to be ground into a fine powder. Once ground, this mixture was put into centrifuge tubes along with an extraction solution (methanol, LCMS water, formic acid). This was then vortexed, sonicated, and centrifuged until all solids collected in the bottom of the tubes and all extracted liquid remained above.

This liquid was then added to vials containing standards to extract the following nutrients: antioxidants, organic acid phenolics, amino acids, and fatty acids. After adding the organic matter solution to the antioxidant and organic acid standards, those samples were taken back to the -20 degree freezer for future analysis. The amino acid and fatty acid vials were dried until only solids remained, and reconstituted with two solutions of ACN + LCMS water and ACN + LCMS water + isopropyl alcohol respectively. These were then placed back in the -20 degree freezer for later processing. To see the full experimental process for nutrient extraction and LCMS processing created by WeiWei Li and Becca Reynolds of the Keller Laboratory, please refer to the Appendix Item 2.

Collective Nutrients of Agricultural Crops

The nutrient composition of agricultural crops was analyzed by measuring antioxidants, organic acids, nucleobases, amino acids, sugars and sugar alcohols, fatty acids, and vitamins. All samples were processed using a liquid chromatography-mass spectrometry (LC-MS) instrument. Data quality was assessed based on retention times, signal-to-noise ratios, and data completeness (i.e., the proportion of missing data). Only datasets meeting the established quality criteria were selected for further analysis.

Based on these criteria, the sugars and sugar alcohols and organic acids datasets exhibited sufficient data quality for analysis. Within these categories, specific metabolites demonstrated higher reliability than others. For sugars, the following metabolites were analyzed: ribose, xylose/arabinose, ribitol/xylitol, fructose, mannose, glucose/galactose, maltose, lactose, and raffinose. For organic acids, citric acid, malic acid, and succinic acid were identified as providing reliable data for further investigation.

Given the small sample size (n=3 per analyte per trial), all metabolites within a category (sugars or organic acids) were analyzed collectively. To ensure data integrity while preserving sample size, retention times and signal-to-noise ratios were considered but not used as exclusion criteria. However, data points in the top 5% of measured concentrations were removed to eliminate potential outliers caused by dilution errors. This filtering step reduced the total number of data points but minimized the impact of anomalous values on the final analysis.

Cost Benefit Analysis

The goal of this cost-benefit analysis was to understand the potential value of biochar from an agricultural perspective. This means that the economic feasibility of biochar production is outside the scope of the project. While other studies that look at the energy consumption and transportation of feedstock are important for establishing prices of biochar, our project looked at the few companies that already have a biochar production chain created as a reference point for prices a farmer may need to pay for bulk biochar. Based on these companies, transportation costs of biochar to the farm are included in the sale of the product and paid by the farmer within the cost of biochar.

Biochar price came from an average of two biosolid-derived biochar production companies, Bioforcetech and Saratoga Biochar, and found to be $500 per ton of biochar, with a range from $300 - $900 per ton depending on variables such as transportation and bulk pricing. This price is much higher than biochar produced from other feedstocks, where the average price for woody feedstocks were $280 per ton of biochar. This reflects the limited number of biosolid-based biochar producers currently established.

Biochar application rates in most studies range from 1-10% w/w, with 5-10 tons/hectare being common (Christou et al., 2022 ; Nobile et al., 2020; Bista et al., 2019). From these papers, a 10% biochar application rate was translated to 10 tons per hectare, which was then converted to 4.1 tons per acre. This informed the biochar application rate for the 3%, 6%, and 9% treatments in the model. For our analysis, we considered data from the project's experimental results to determine the biochar application rate for each model. The results of these treatments are explained in the experimental results sections and then were used to calculate the increase in yield resulting from biochar. These expected increases in yield were calculated using the percentage change formula for the biochar treatment chosen against the fertilizer trial, which best represents the counterfactual of a farmer continuing under business-as-usual conditions.

The costs and benefits of biochar can vary significantly across different farming operations and sizes. As such, we are focusing on the economic feasibility of implementing biochar on small-scale farming operations. According to the USDA, "a small farm is defined as an operation with a gross cash farm income under $250,000" (MacDonald, 2010). According to an article from Michigan State University, this translates to an average size of 231 acres for small family farms in the US (Dunckel, 2013). Therefore, we have chosen to focus solely on small-scale farms to ensure a more accurate analysis and have chosen to model a 231 acre farm.

For the calculation of costs, a single implementation cost was assumed, as once biochar is purchased and tilled into the soil, crops can reap benefits for decades afterwards. This cost is calculated by using a biochar application rate to determine the amount of biochar needed, the price of biochar, and average labor costs per acre. No additional maintenance costs are needed, as farmers can manage their soil the same with or without biochar.

Benefits come from the additional revenue a farmer receives from the potential increased yield from the biochar, as well as the reduction in fertilizer costs. Other biochar benefits such as soil health, nutrient availability, and water retention that may be relevant to the farmer are assumed to influence crop yield and are measured implicitly. Other benefits of biochar, such as environmental benefits including less runoff to nearby streams were not assessed as those benefits do not directly benefit the farmer.

The fertilizer savings came from a reduced fertilizer application rate under any biochar treatments. This fertilizer application rate with biochar was conservatively expected to be 25% less, as studies have shown a large range in the reductions in nitrogen leaching and subsequent fertilizer reductions associated with biochar. Nitrogen emission reductions range from 13-36%, and an average was taken for this model (Borchard et al., 2019; Dong et al., 2020; Rombel et al., 2022). Fertilizer prices were taken from the USDA database that provided a history of fertilizer prices from 2010-2014 (USDA, 2019).

Finally, the cost-benefit analysis was repeated with the assumption that there is a carbon market that will incentivize farmers to apply biochar for their carbon sequestration potential. This should not be automatically assumed, as no such marker for biochar use currently exists. Opening this market would require meeting a set of core carbon principles for a reliable carbon market (ICVCM, 2024). For biochar, this will require more research into the permanence of the carbon sequestered, and require development of cheap technology to regularly test carbon content in soils. The consideration of a carbon market, however, only impacts the initial implementation as the credits would be given upon application of the biochar to the soil. For biosolid-derived biochar, carbon content ranges from 50-80% of carbon which translates to about 3 tons of carbon dioxide sequestered for every ton of carbon (Paz-Ferreiro, 2018; Lu et al., 2013). While there are studies that suggest biochar further reduces carbon emissions from agricultural fields annually by 11-32%, more research is required to understand and quantify these mechanisms before considering these additional benefits (Paz-Ferreiro, 2018).

Despite these caveats, a sensitivity analysis was conducted to predict how carbon price can impact the years it would take for a farmer to begin earning benefits of biochar implementation. The Monte Carlo sensitivity analysis was conducted to simulate the model with a sample of 1000 carbon prices assuming a mean value of $40/ton of carbon sequestered with a standard deviation of $15. While more research is needed in this area, the purpose of this analysis is to see how much the existence of a carbon market can change the results to determine if the addition of a carbon market for farmers is worth investigating further.

Description of Wheat Data

The large variety of crops and different techniques required to grow different plants require limiting the scope further to a specific farm crop and changing the parameters to suit each crop type. While some parameters and methods remain consistent between models, some parameters beyond those informed by the experimental results were changed. Fertilizer costs is a key example of this, where ammonium-based fertilizer was chosen as this reflects what is used most in wheat farms (Nagelkirk, 2021). Additional parameters geared specifically to wheat farmers include fertilizer application rate, baseline yield, average cost per bushel of wheat, and labor costs. These parameters were informed by a literature review and when possible, these studies were limited to those about biosolid-derived biochar and those about wheat, but other studies were used as well to supplement the data. Table 4 below has an overview of different parameters used for the analysis from the point of view of a wheat farmer and the reference(s) they came from.

Table 4. Overview of Chosen Parameter Values for Wheat. Values of parameters needed for the cost-benefit analysis of a wheat farm are listed. Benefits include consideration of yield increases, fertilizer reductions, and value of carbon sequestered. Costs include biochar price and labor costs.

Parameter	Units	Value	Reference Year	Reference
farm_size	acres	231	2013	Dunckel 2013
biochar_price	$/ton	500	2024	Bioforcetech; Saratoga Biochar
biochar_application_rate	tons/acre	1.35	2019-2022	Christou et al., 2022; Nobile et al., 2020; Bista et al., 2019
baseline_yield	bushels/acre/year	47.5	2022	USDA
percent_yield_increase	%	12	2024	BIOCHARge Experiment
wheat_average_price	$/bushel	10.9	2022	USDA
baseline_fertilizer_rate	lbs/acre	40	2018	Lentz et al., 2018
fertillizer_reduction	%	25	2019	Borchard et al., 2019; Dong et al., 2020; Rombel et al., 2022
fertilizer_price	$/lb	0.278	2010-2014	USDA
discount_rate	%	7	2023	Circular A-4
labor_costs	$/acre	87.05	2018	McClure, 2019
c_sequestration	Tons C/ton biochar	3	2013-2018	Paz-Ferreiro, 2018; Lu et al., 2013

The farm size, wheat yield per acre, and average price per bushel were checked to ensure the current annual revenue of our hypothetical farm was below the $250,000 threshold that defines a small farm. The calculations resulted in a baseline revenue of $119,600, therefore the parameters and averages seem to be accurate.

Description of Lettuce Data

Like the wheat farm, specific parameters such as baseline yield, average lettuce price per hundredweight (cwt), fertilizer price, and labor costs were geared specifically for the lettuce farmer. For example, on lettuce farms, nitrate-based fertilizers are more commonly used and are less expensive than ammonium-based fertilizers used for wheat. USDA reports and literature for lettuce farmers were used to inform these parameters and Table 5 provides an overview of the values used for the analysis from the point of view of a lettuce farmer.

Table 5. Overview of Chosen Parameter Values for Lettuce. Values of parameters needed for the cost-benefit analysis of a lettuce farm are listed. Benefits include consideration of yield increases, fertilizer reductions, and value of carbon sequestered. Costs include biochar price and labor costs.

Parameter	Units	Value	Reference Year	Reference
farm_size	acres	231	2013	Dunckel, 2013
biochar_price	$/ton	500	2024	Bioforcetech; Saratoga Biochar
biochar_application_rate	tons/acre	1.35	2019-2022	Christou et al., 2022; Nobile et al., 2020; Bista et al., 2019
baseline_yield	cwt/acre/year	285	2022	USDA
percent_yield_increase	-	29	2024	BIOCHARge Experiment
lettuce_average_price	$/cwt	40	2022	USDA
baseline_fertilizer_rate	lbs/acre	60	2015	CDFA
fertillizer_reduction	-	0.25	2019	Borchard et al., 2019
fertilizer_price	$/lb	0.18	2010-2014	USDA
discount_rate	-	0.07	2023	Circular A-4
labor_costs	$/acre	304	2023	UC Davis, 2023
c_sequestration	Tons C/ton biochar	3	2013-2018	Paz-Ferreiro, 2018; Lu et al., 2013

Results

Lab Experiment

Biomass of Agricultural Crops

To evaluate the effects of biochar on the biomass of agricultural crops, we conducted a series of statistical analyses. Prior to analysis, we selected the most robust variables from our collected data. However, limitations in data collection affected our choice of biomass proxies.

Lettuce

For lettuce, the first trial was compromised due to wilting and rotting during refrigeration prior to weighing. The second trial experienced leaf predation by rats, further limiting usable biomass measurements. As a result, the most reliable metric for lettuce biomass was the diameter of the lettuce stalk. An independent analysis of the same lettuce variety grown at Frecker Farms confirmed that stalk diameter is a reliable proxy for overall lettuce biomass (Figure 1). Therefore, wheat heads per pot and lettuce stalk diameter will serve as biomass proxies throughout the remainder of this analysis.

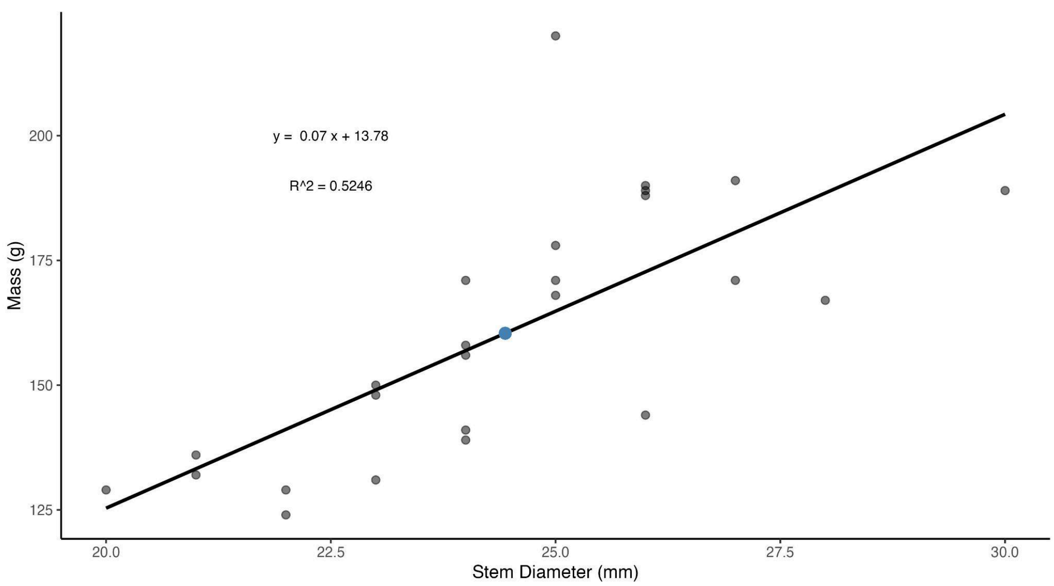

Figure 1. ***Lettuce Stalk Diameter Compared to Lettuce Biomass.*** Measurements of lettuce stalk diameter and biomass were taken in the field at Frecker Farms to assess the correlation between the two. Preliminary results indicate that stalk diameter is a viable proxy for lettuce biomass.

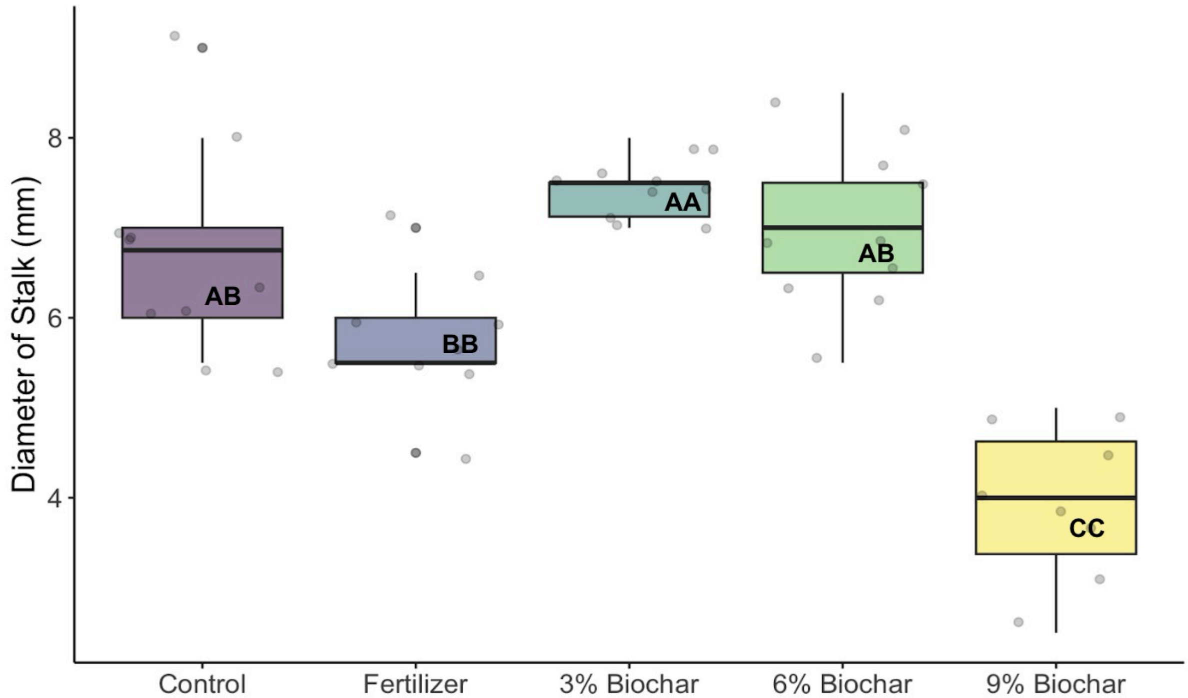

Figure 2. ***Lettuce Stalk Diameter ANOVA and Tukey's Test Results.*** The 3% and 6% Biochar trials produced significantly higher biomass than the Fertilizer trial and exhibited biomass levels comparable to the Control.

A Shapiro Test and Levene's Test were performed to test whether the lettuce data met the assumptions of an ANOVA. After passing both tests, the ANOVA revealed that at least one group was statistically different from the others (Figure 2). The 3% Biochar trials appear to have the most biomass from most of the other trials, though a Tukey's test revealed that the 3% Biochar and 6% Biochar trials were statistically similar.

A Tukey's test also revealed the following:

- The Fertilizer and Control Trials were statistically similar.
- The Control and 6% Biochar trials were statistically similar.
- The 9% Biochar trial was significantly different from other trials, with the lowest biomass.

Wheat

For wheat, an error in data collection resulted in seed weight and count being recorded by trial rather than by pot. Consequently, the most comprehensive and reliable metric available was the number of wheat heads per pot.

To analyze trends within the wheat data, we initially intended to perform an ANOVA. However, the assumption of normality was not met. Instead, we conducted a Kruskal-Wallis test, which does not require normality. This analysis indicated that one of the trials was statistically different from the others. A pairwise Wilcoxon test further revealed that the Control trial was significantly different from the Fertilizer, 3%, 6%, and 9% Biochar trials (Figure 3).

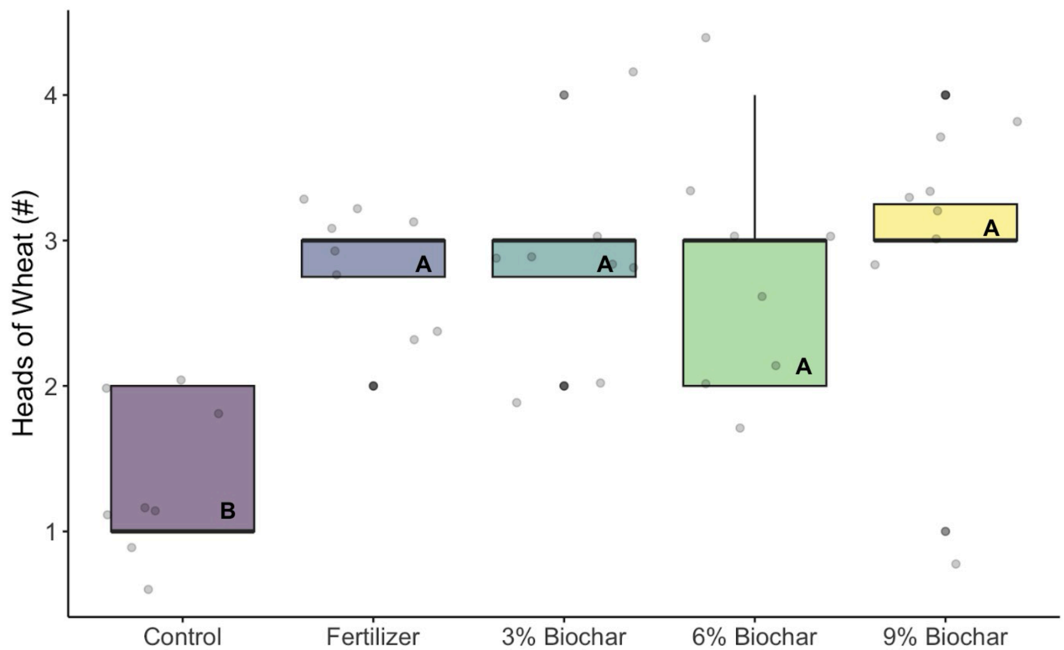

Figure 3. ***Wheat Head Kruskal- Wallis and Wilcoxon Test Results.*** The Control trial produced significantly lower heads of wheat than the other trials, which had similar means.

Collective Nutrients of Agricultural Crops

Lettuce Sugars/ Sugar Alcohols

Prior to statistical analysis, lettuce trials with sufficient data were evaluated for normality and equal variance (Figure 4). Due to a processing error, only Lettuce Round 2 contained usable data for sugar alcohols. Normality testing indicated that Lettuce Round 2 was not normally distributed, requiring the use of a Kruskal-Wallis test. The test results showed no statistically significant differences between trials, likely due to the previously mentioned data filtering process, which reduced the sample size for certain analytes.

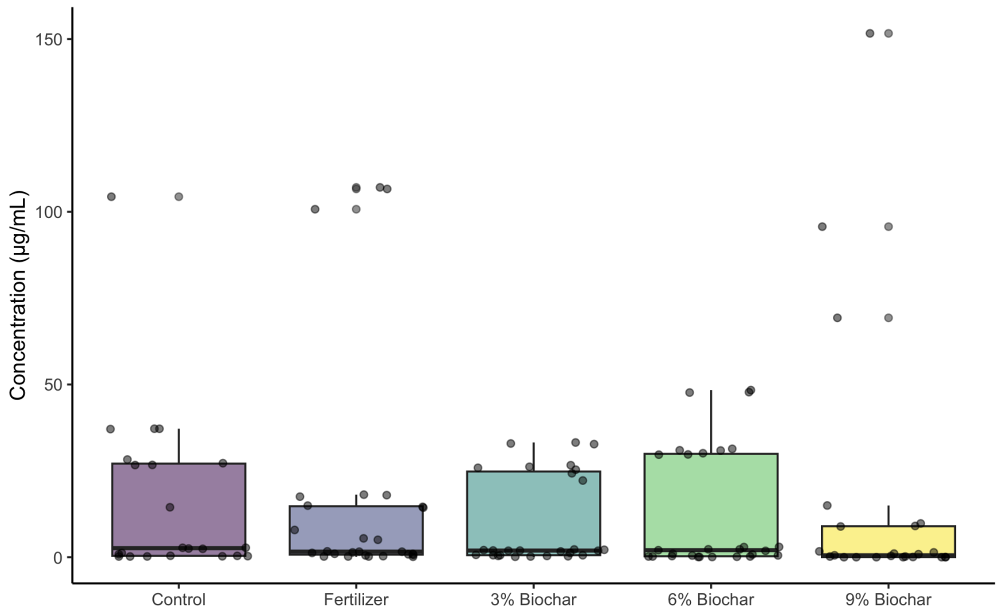

Figure 4. Visualizing Results of Sugar Alcohol Analysis in Lettuce II Trial. A Kruskall-Wallis test revealed no statistically significant difference in the amount of sugar alcohols produced between any of the trials.

Wheat Sugars and Sugar Alcohols

The wheat treatment did not meet the assumptions of normality. Consequently, a Kruskal-Wallis test was performed, revealing a statistically significant difference between trials. Pairwise comparisons using the Wilcoxon test identified significant differences between all three biochar treatments and the fertilizer treatment (p = 0.29–0.45). Visual analysis of the data suggests that biochar treatments resulted in higher concentrations of sugars and sugar alcohols compared to the fertilizer treatment, at least for some sugar analytes as will be discussed further below (Figure 5).

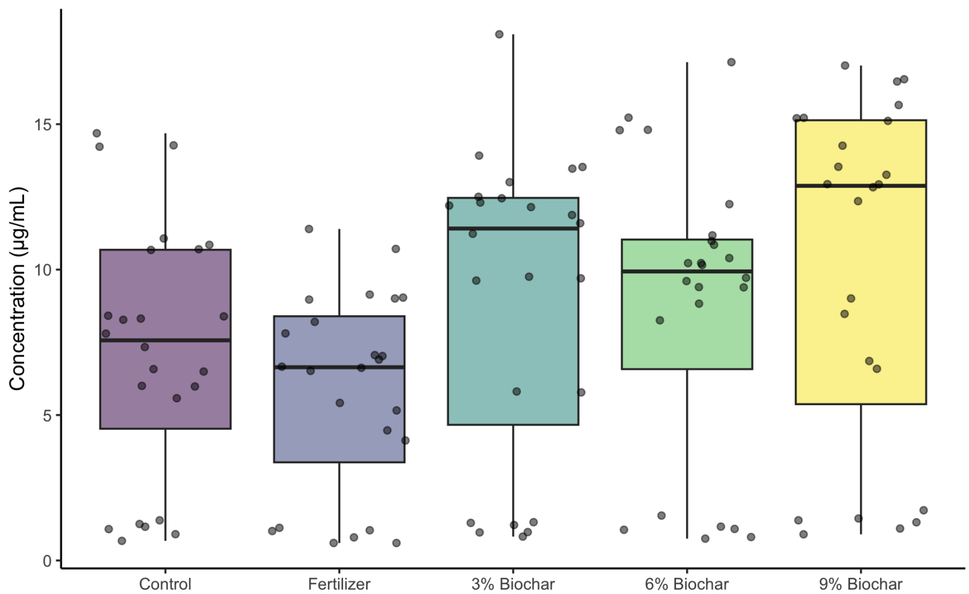

Figure 5. Visualizing Results of Sugar Alcohol Analysis in Wheat Trial. All biochar trials produced more sugar alcohols than the fertilizer trial.

Lettuce Organic Acids

The first lettuce trial met the assumptions of normality and equal variance. Consequently, an Analysis of Variance (ANOVA) was conducted to assess potential differences between trials (Figure 6). The results indicated no statistically significant differences between the groups.

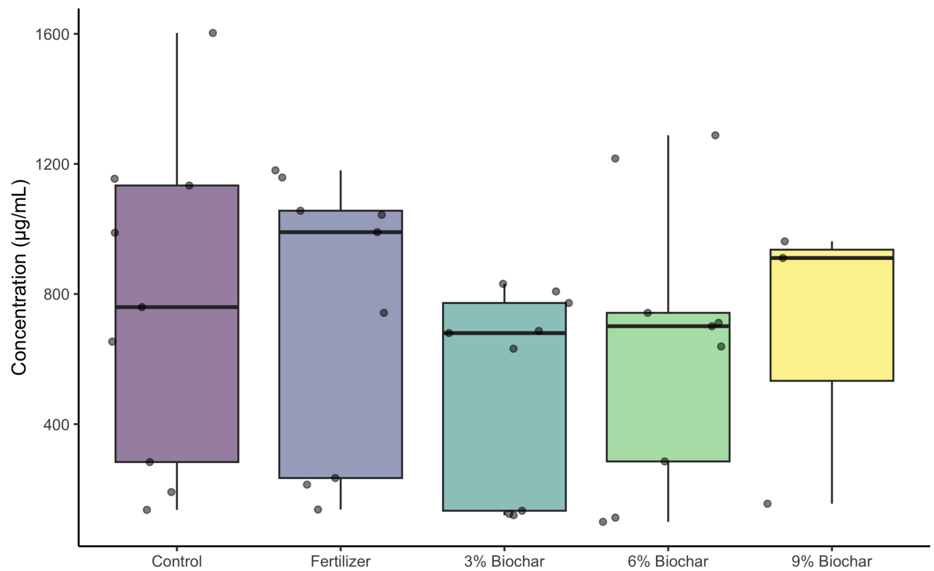

Figure 6. Visualizing Results of Organic Acid Analysis in Lettuce I Trial. An ANOVA revealed there was no statistically significant difference in the amount of sugars/sugar alcohols produced between trials.

The second lettuce trial did not meet the assumption of normality, requiring the use of a Kruskal-Wallis test. This test identified a statistically significant difference between the groups. Pairwise comparisons using the Wilcoxon test indicated that these differences occurred between the control and 9% biochar trial, as well as the fertilizer and 9% biochar trial. A visual inspection of the data distribution showed that the 9% biochar trial exhibited significantly higher levels of organic acids compared to the other trials (Figure 7).

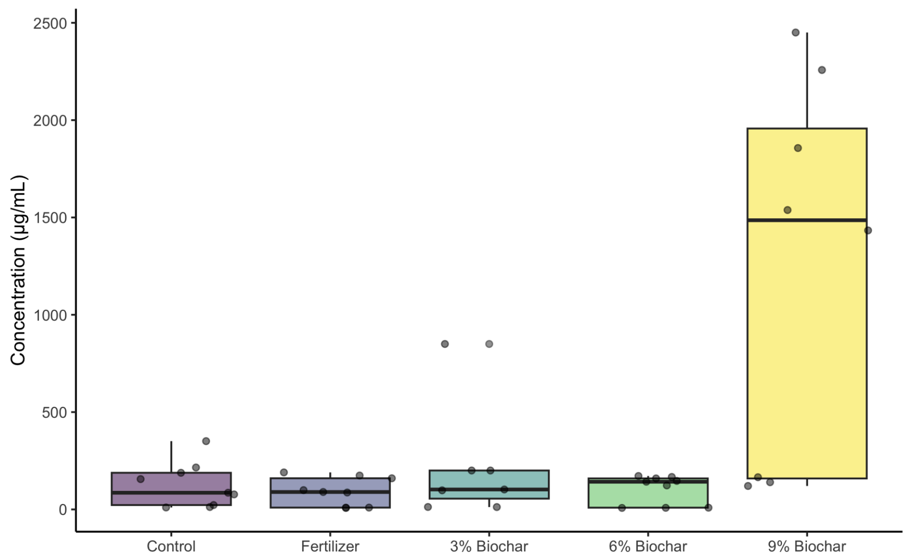

Figure 7. Visualizing Results of Organic Acid Analysis in Lettuce II Trial.
A Kruskal-Wallis test revealed statistically significant differences between the 9% trial and the control and fertilizer trials.

Wheat Organic Acids:

The wheat trial did not meet the assumption of normality and was analyzed using a Kruskal-Wallis test, which revealed no statistically significant differences between trials. Additionally, this trial experienced data loss, with all data from the 9% biochar treatment missing across analytes, potentially impacting the overall data quality (Figure 8).

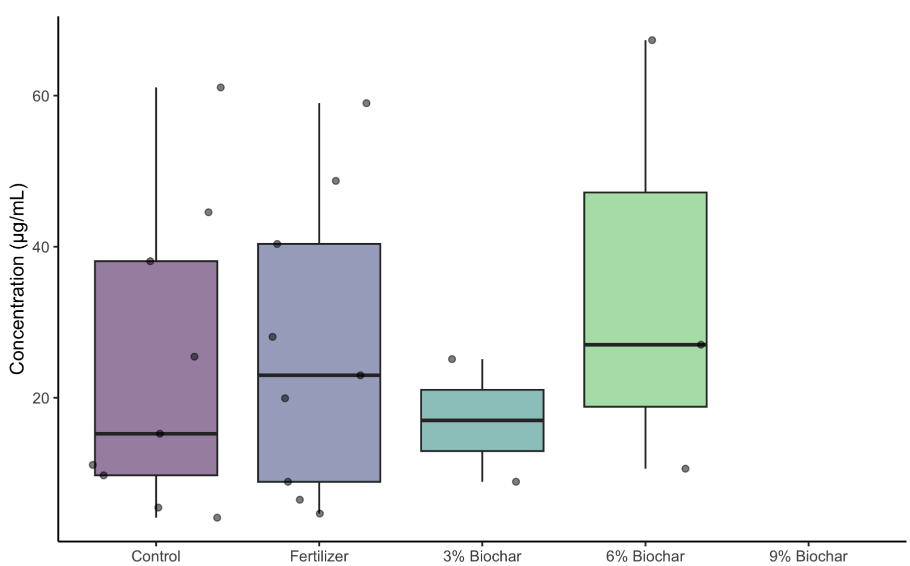

Figure 8. Visualizing Results of Organic Acid Analysis in the Wheat Trial. A Kruskal-Wallis test revealed no statistically significant difference between any of the trials.

Individual Analyte Assessment of Agricultural Crops

Data Assessment and Selection:

While analyzing nutrients by category could reveal overall trends, individual analytes were also analyzed to see if specific nutrients showed trends. While all other elements of data processing, selection, assessment remained the same, the data filtering process was altered. Due to the smaller sample size of this more granular analysis, specific samples that were viewed as outliers by the experimenters were removed by hand. This resulted in one Lettuce II control replicate and one Lettuce II 9% biochar replicate being removed for all sugar trials, as there was likely an error in sample preparation or LCMS sampling.

Lettuce Sugars/Sugar Alcohols:

For each analyte, a Shapiro Test and Levene test were performed to determine whether the data was normally distributed or had equal variances. After performing this analysis, no analytes passed both tests, so the ANOVA was not performed (Figure 9).

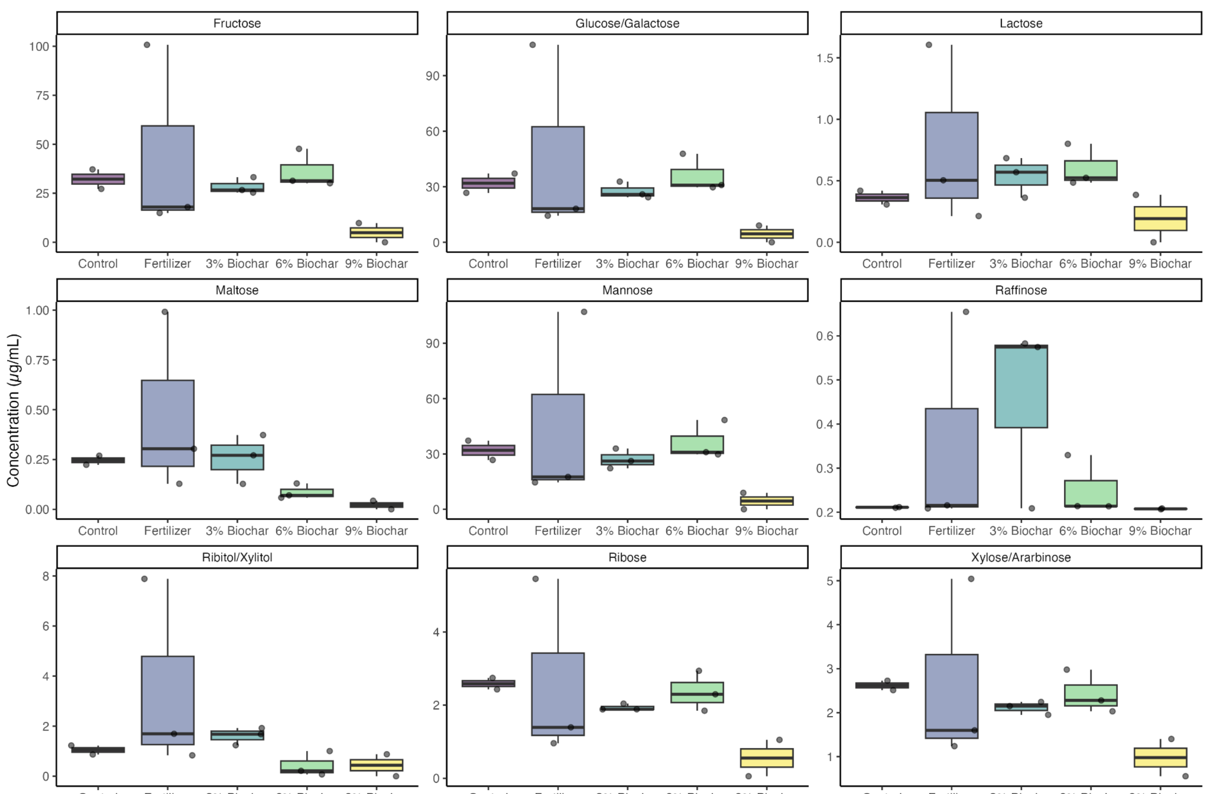

Figure 9. Visualizing Results of Individual Sugar/Sugar Alcohol Analyte Concentrations in the Lettuce II Trial. The samples were not normally distributed, therefore no ANOVA could be conducted to see if there were statistically significant differences between the means of each trial.

Wheat Sugars/Sugar Alcohols:

The same procedure was repeated for the wheat trial, which revealed Fructose, Mannose, and Glucose all passed both tests for normality and even variance (Figure 10). An ANOVA was then conducted, which revealed a p-value less than 0.05, indicating there is a significant difference in the means between trials within the dataset. A Tukey's Test revealed that for all three analytes, there is a statistically significant difference between the Fertilizer trial and the 9% Biochar trial. A visual assessment of the raw data and the distribution box plots revealed that the trials have almost identical concentrations, which contributes to them having the same results from both the ANOVA and the Tukey's test. The reason for this is unknown, but could be related to the sampling process within the LCMS machine or these nutrients appearing together often in plant tissues.

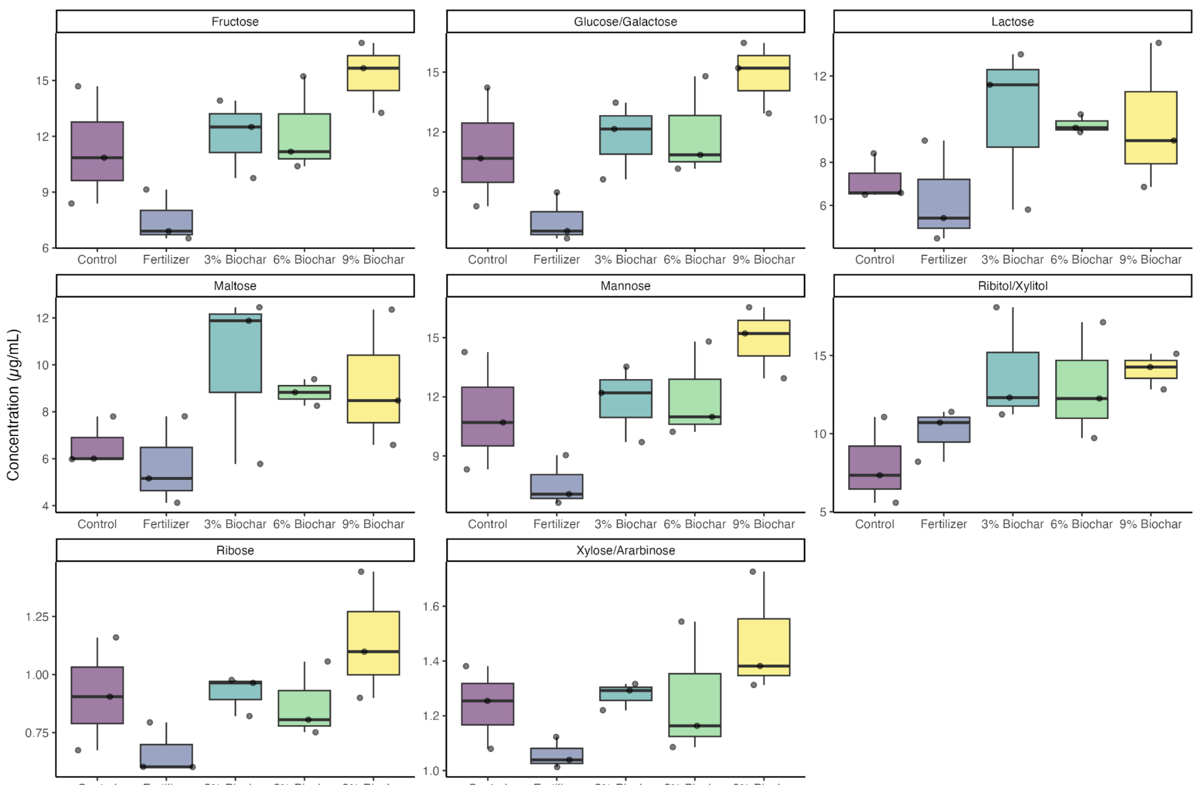

Figure 10. Visualizing Results of Individual Sugar/Sugar Alcohol Analyte Concentrations in the Wheat Trial. An ANOVA and Tukey's test revealed that for Fructose, Glucose, and Mannose there was a statistically significant difference between the Fertilizer trial and the 9% Biochar trial.

Lettuce I Organic Acids:

Both the malic acid and succinic acid analyte groups within the Lettuce I trial failed the test of normality, so further testing will not be pursued. The citric acid group passed both the tests of normality and equality of variance, so an ANOVA was conducted. Once conducted, the p-value was more than 0.05, indicating there is no significant difference between the means of the trials within this group (Figure 11).

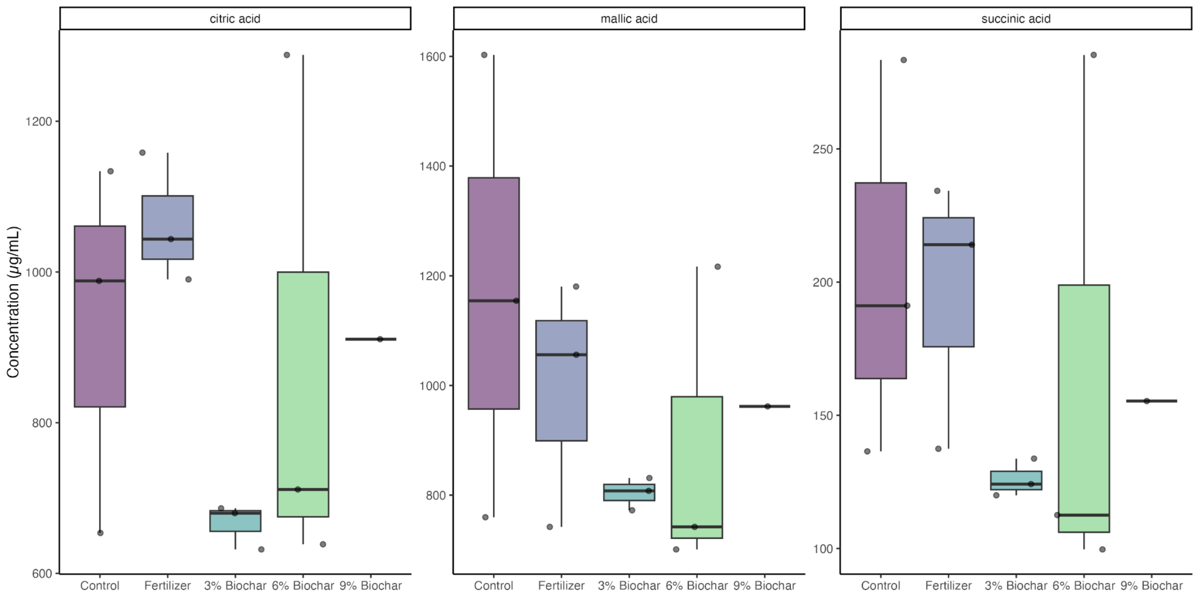

Figure 11. Visualizing Results of Individual Organic Acid Analyte Concentrations in the Lettuce I Trial. Malic Acid and Succinic Acid both failed the tests of normality so no analysis was conducted. An ANOVA revealed no statistically significant difference between any trials.

Lettuce II Organic Acids:

All three analytes failed tests of normality, indicating they are not suitable for conducting an ANOVA. No further statistical tests were performed. A visual assessment of the data reveals one unusually high sample in the 3% Biochar trial, and three unusually high values for samples in the 9% Biochar trial, indicating data from this trial might not be suitable for analysis due to errors in sample creation or processing.

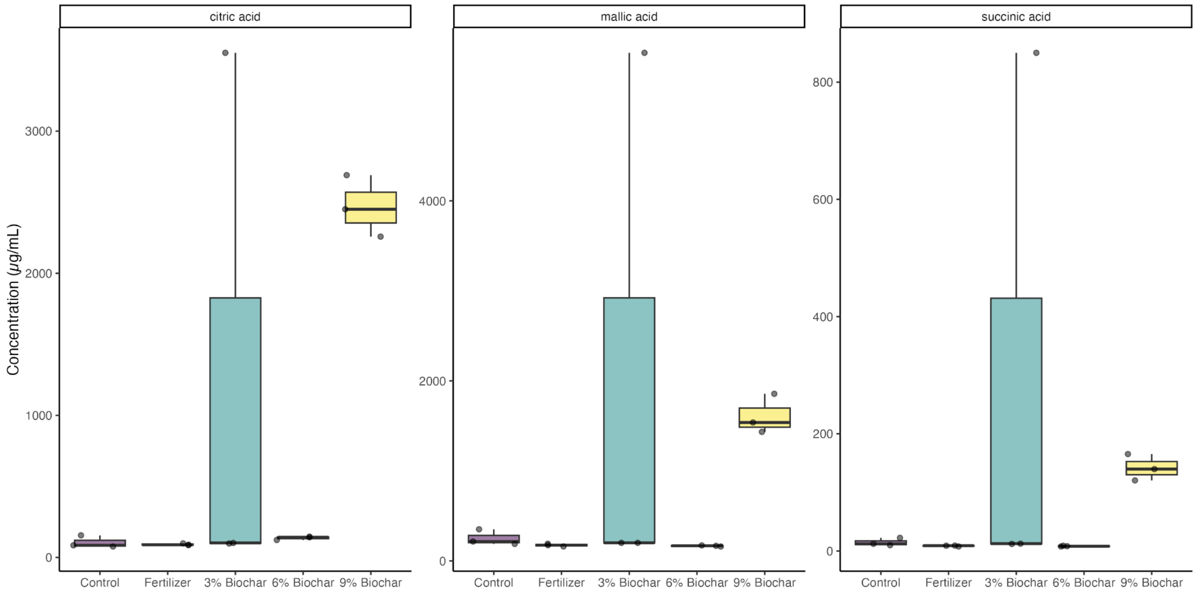

Figure 12. Visualizing Results of Individual Organic Acid Analyte Concentrations in the Lettuce II Trial. All three analytes failed tests of normality. An outlier in the 3% trial and unusually high values in the 9% trial suggest possible processing or machine errors.

Wheat Organic Acids:

Prior to conducting analysis on the Wheat Organic Acid samples, it was visually analyzed as the data itself seemed incomplete (Figure 13). This revealed that only the Control and Fertilizer samples had viable data, with all other trials being populated with NA values from the LCMS processing phase of analysis. Running statistical tests on this data would not reveal any meaningful trends, so analysis was not performed.

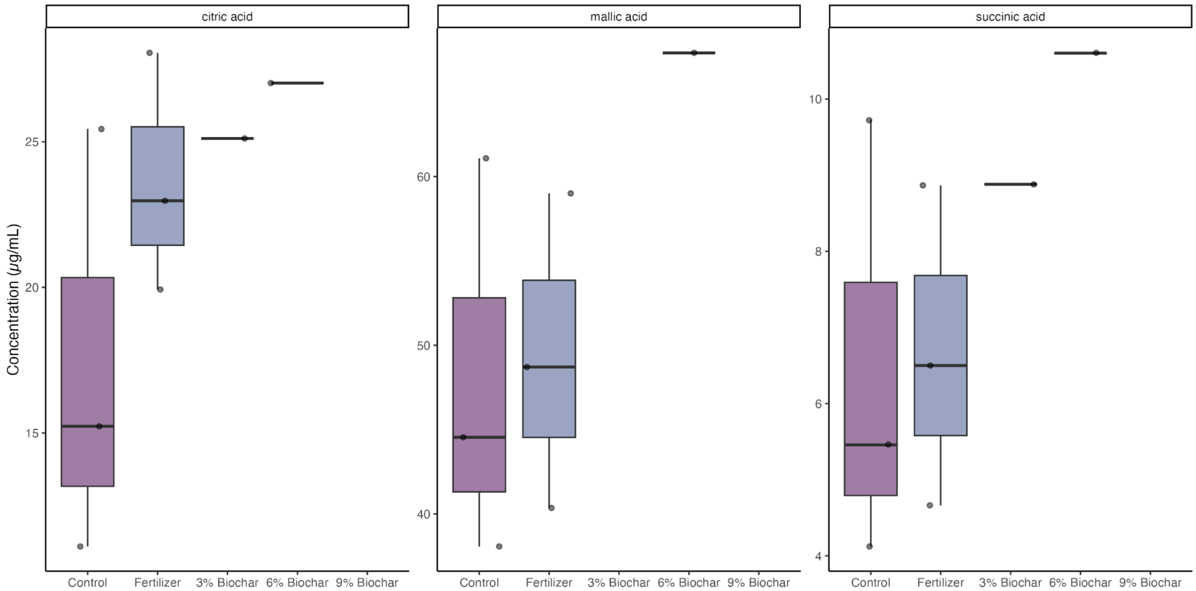

Figure 13. Visualizing Results of Individual Organic Acid Analyte Concentrations. No statistical tests were performed on this data due to significant data loss during processing.

Native Plants

Both dogbane and basket rush experienced significant predation and mortality in the Native Plant trials (Figures 14 and 15). This was likely due to the outdoor experimental conditions and the fact that the plants were propagated from cuttings rather than grown from seed. Due to the limitations in data quality and robustness, no statistical analysis was performed on this dataset.

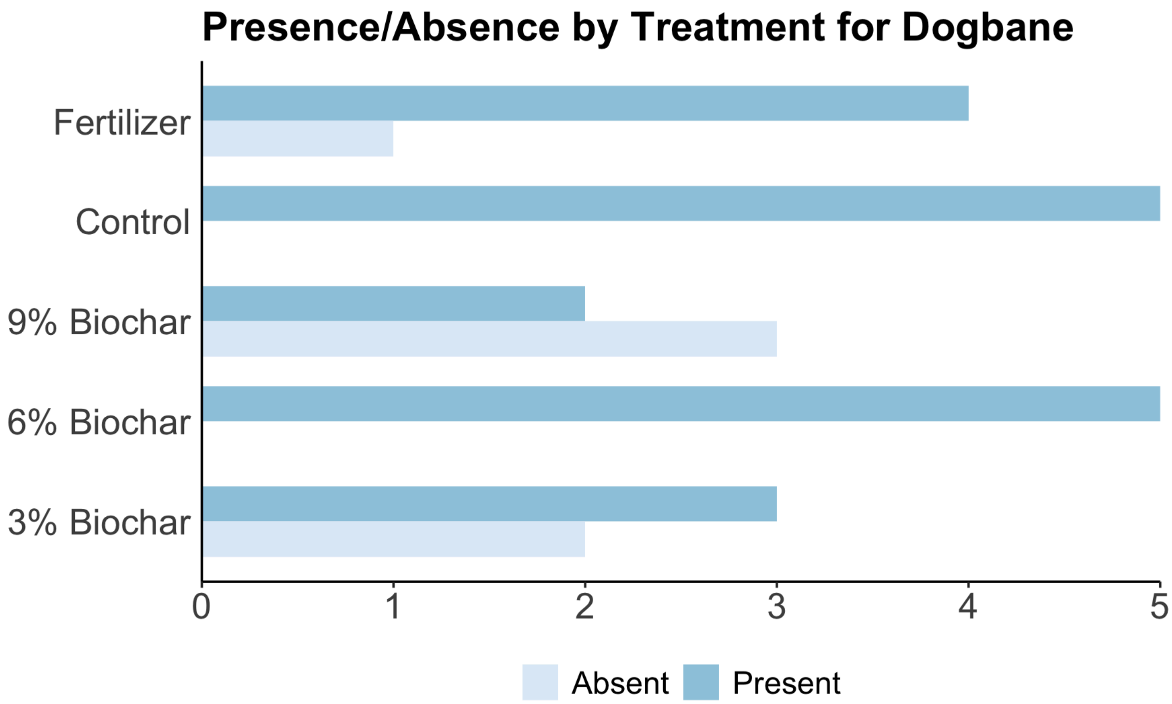

Figure 14. ***Presence/Absence for Dogbane.*** Amount of surviving dogbane treatments. The Control trial exhibited no mortality, whereas the 9% Biochar trial experienced over 50% mortality.

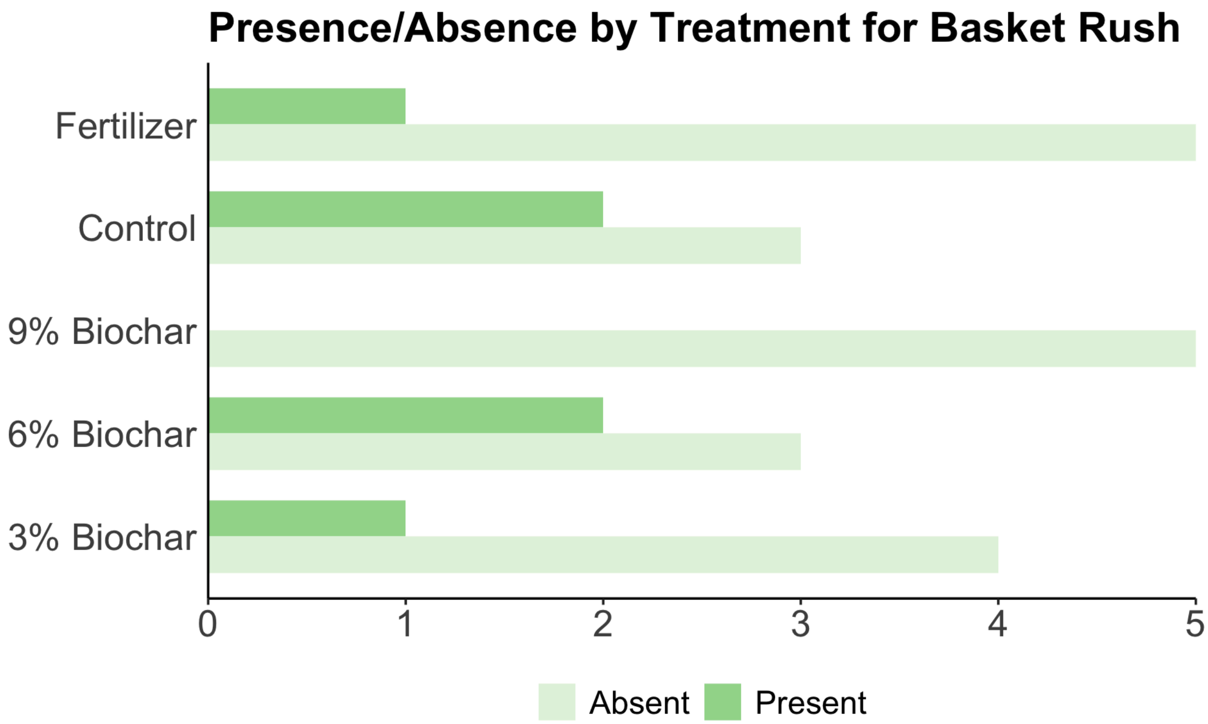

Figure 15. ***Presence/Absence for Basket Rush.*** Amount of surviving basket rush treatments. Most trials faced an over 50% mortality rate.

Cost Benefit Analysis

Lettuce Farm

The cost-benefit analysis was conducted for a lettuce farm using the results from the second lettuce trial (Lettuce II). These results indicated that the 3% and 6% biochar treatments led to statistically significant yield increases compared to the Fertilizer trial. Given these findings, a 3% biochar application rate was assumed for calculating implementation costs. The yield increase for the 3% Biochar treatment relative to the Fertilizer treatment was 28%.

Under these conditions, and in the absence of a carbon market, the lettuce farmer would achieve a positive net present value (NPV) in less than one year (Figure 16). A sensitivity analysis was not conducted, as the results remained consistent regardless of the introduction of a carbon market or variations in biochar pricing. However, it is important to note that in a field, biochar may need to be reapplied every few years rather than as a one-time application. This periodic reapplication would influence the long-term cost-effectiveness of biochar use and should be considered when evaluating the potential role of a carbon market in offsetting implementation costs.

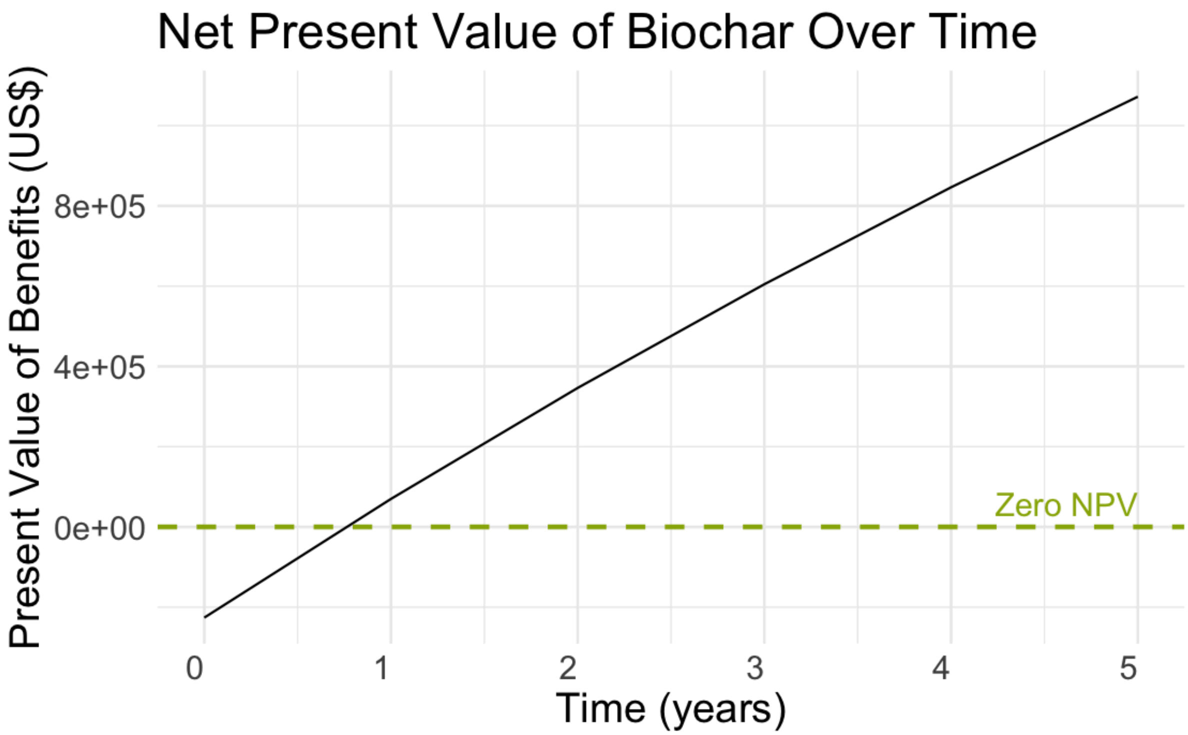

Figure 16. Net Present Value of Biochar over Time. This value was calculated assuming a single implementation cost and total benefits from increased yield as well as fertilizer savings. No carbon market was assumed for this model.

Wheat Farm

The results from the experimental portion of this study were used to inform biochar application rates and expected yield increases for wheat production. Among all trials, all of the biochar treatments produced more wheat heads per plant than the Control (Figure 3), with a statistically significant difference. When compared to the Fertilizer treatment, none of the biochar treatments resulted in a statistically significant difference in wheat heads. While there were slightly more wheat heads per plant in the 9% Biochar treatment than all others, the difference was not statistically significant. This suggests that a farmer applying biosolids-based biochar as a soil amendment could achieve comparable results at an application rate equivalent to the 3% biochar trial while minimizing implementation costs. This scenario was compared to the Fertilizer trial, which serves as the closest approximation to a business-as-usual approach in which farmers apply fertilizer at regular intervals. Given that there was no statistically significant difference in yield between the 3% Biochar and Fertilizer trials, the primary economic benefit for a wheat farmer adopting biochar would come from fertilizer cost savings.

However, the cost-benefit analysis under these conditions indicated that the benefits never outweigh the costs. Due to the discount rate, the present value of benefits from fertilizer savings remains below $1, even when projected 150 years into the future.

An evaluation of aggregate seed data across all trials showed that the 9% Biochar treatment produced a greater number of seeds compared to other trials, with a 12% increase in yield relative to the Fertilizer trial (Figure 17). Since the seeds were measured cumulatively across trials, no statistical analysis could be conducted. However, this result aligns with expectations, as a meta-analysis by Jeffery et al. (2011) found an average 10% increase in crop productivity across 14 studies involving both pot and field experiments with various biochar treatments. Given this potential yield increase, the cost-benefit model was rerun to assess whether a 9% biochar application could alter the cost-benefit outcomes for wheat farmers.

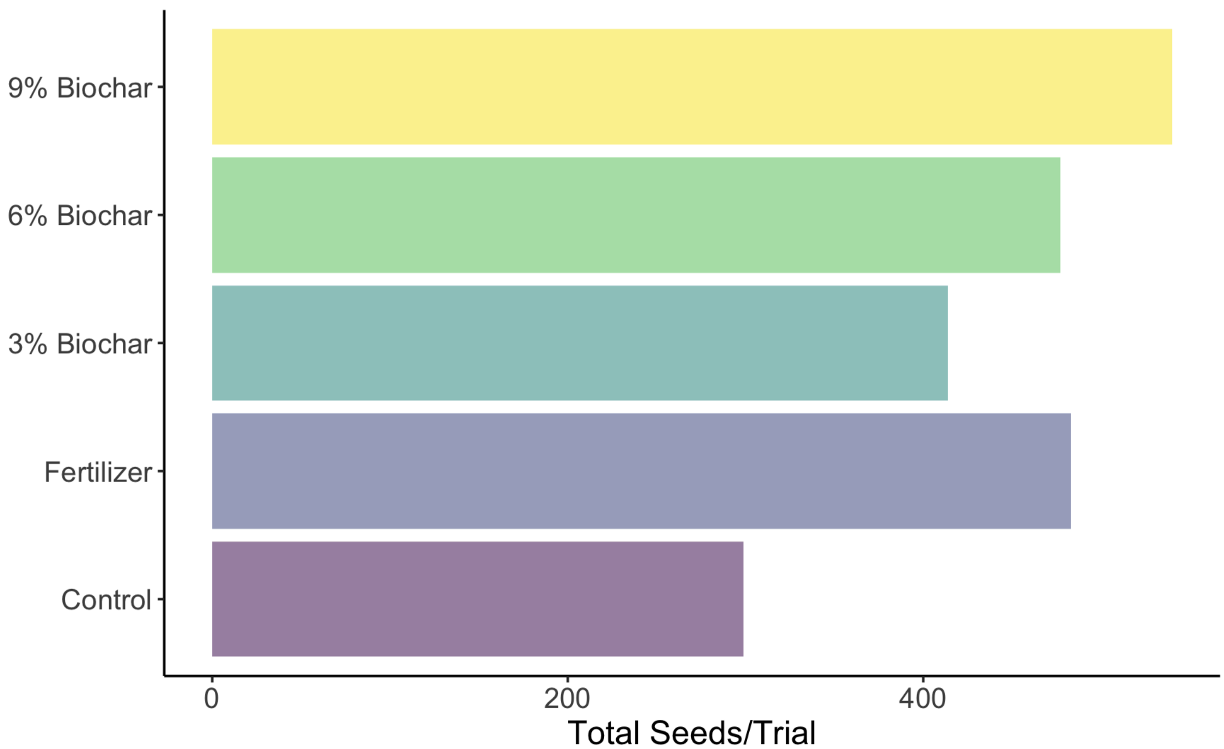

Figure 17. ***Total Seed Counts per Trial.*** The aggregated total seeds by trial. The 9% biochar trial had the most seeds than the other trials, followed by fertilizer and the 6% biochar treatment.

Based on these parameters, a farmer would require 45 years to break even from implementing biochar as a soil amendment in the absence of a carbon market (Figure 18).

The introduction of a carbon market could slightly reduce this time frame. Assuming a carbon price of $10 per ton of carbon sequestered—a conservative estimate based on EPA projections—the net present value (NPV) for the farmer becomes positive after 35 years (Figure 19). This finding suggests that at low carbon prices, the high costs of biochar application, coupled with limited yield increases, make it an economically non-viable option for most farmers.

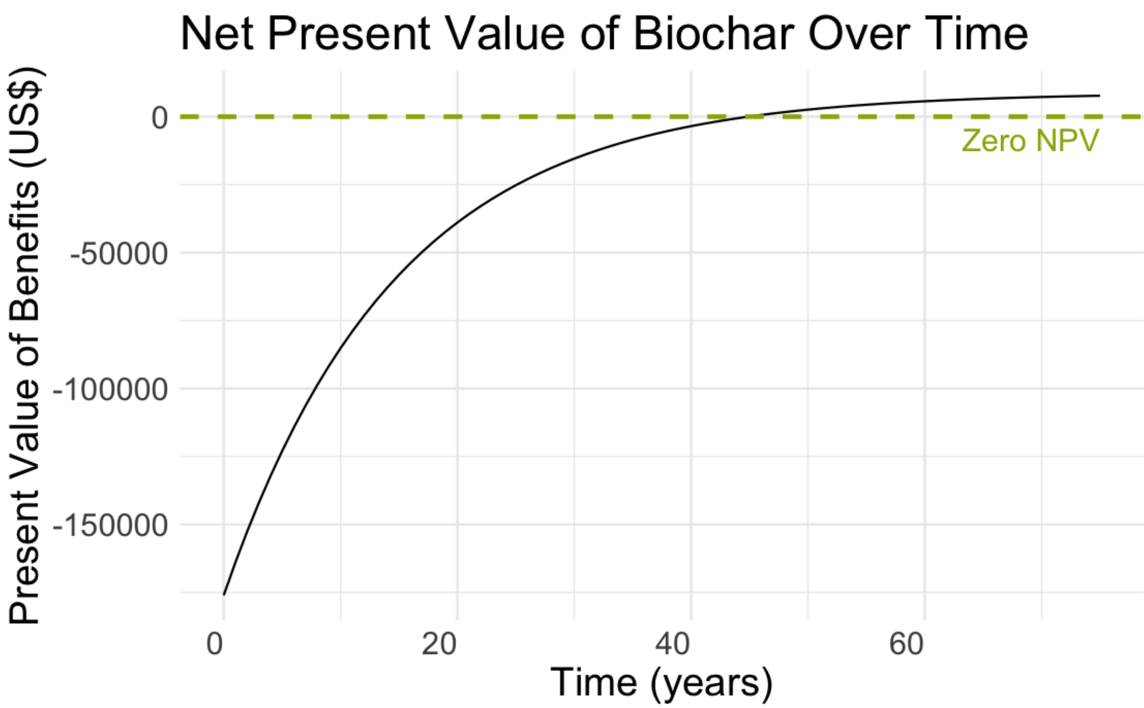

Figure 18. ***Net Present Value of Biochar over Time for Wheat without a Carbon Market.*** This value was calculated assuming a single implementation cost and total benefits from increased yield as well as fertilizer savings.

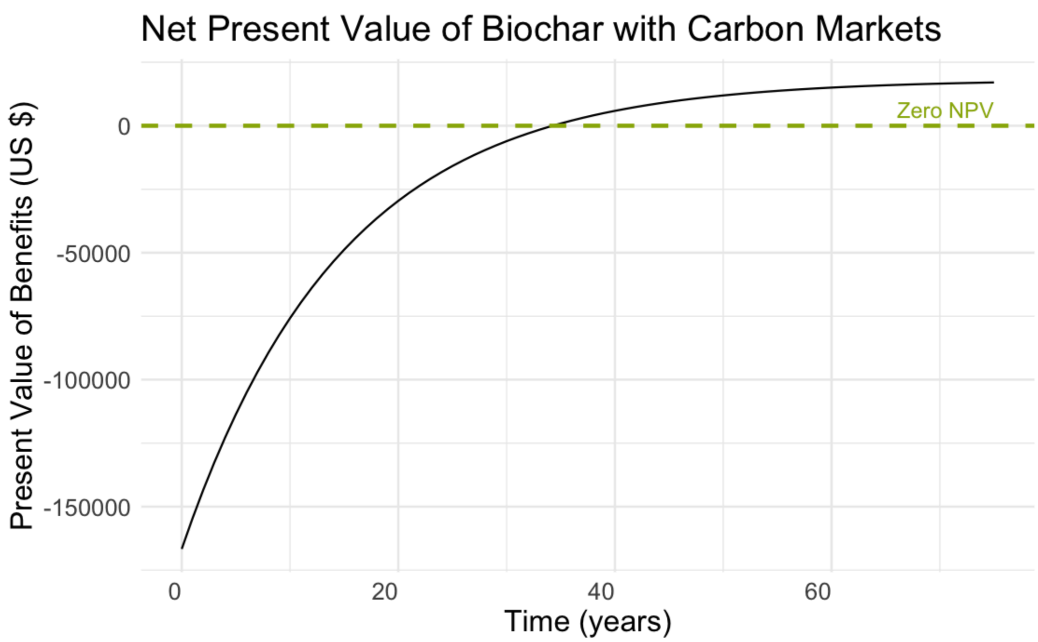

Figure 19. ***Net Present Value of Biochar for Wheat over Time with a Carbon Market.*** This value was calculated assuming a single implementation cost and total benefits from increased yield, fertilizer savings, and a value of carbon sequestration from initial implementation.

The results of the Monte Carlo sensitivity analysis testing how changing just the price of carbon in a carbon market shows that in most cases it would take 15-25 years to reach a positive NPV (Figure 20). This is the result of running the cost-benefit model with 1,000 simulated carbon markets with varied carbon prices that had a mean carbon price of $40 and a standard deviation of $15.

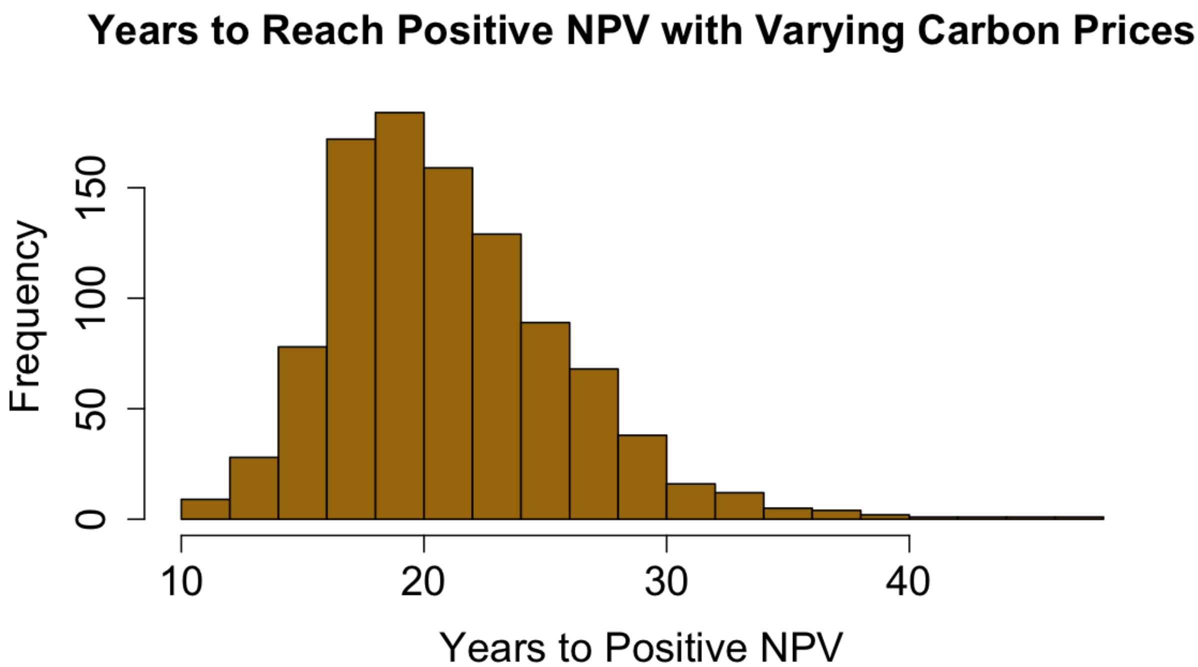

Figure 20. ***Years to Positive NPV for Wheat with Varying Carbon Prices.*** A Monte-Carlo sensitivity analysis was done on carbon prices assuming a mean of $40/ton of C sequestered with a standard deviation of $15. The X-axis shows the years to reach NPV = 0 under each simulation, and the Y-axis shows the frequency of reaching NPV=0 within each bin.

The sensitivity analysis was then repeated with a range of biochar prices with a mean of $400 and a standard deviation of $150. To understand how biochar price changes the model, this was done assuming there is no carbon market and the only change was the biochar price per ton. The results showed that the years to reach a positive net present value lies mostly within 10–30 years (Figure 21).

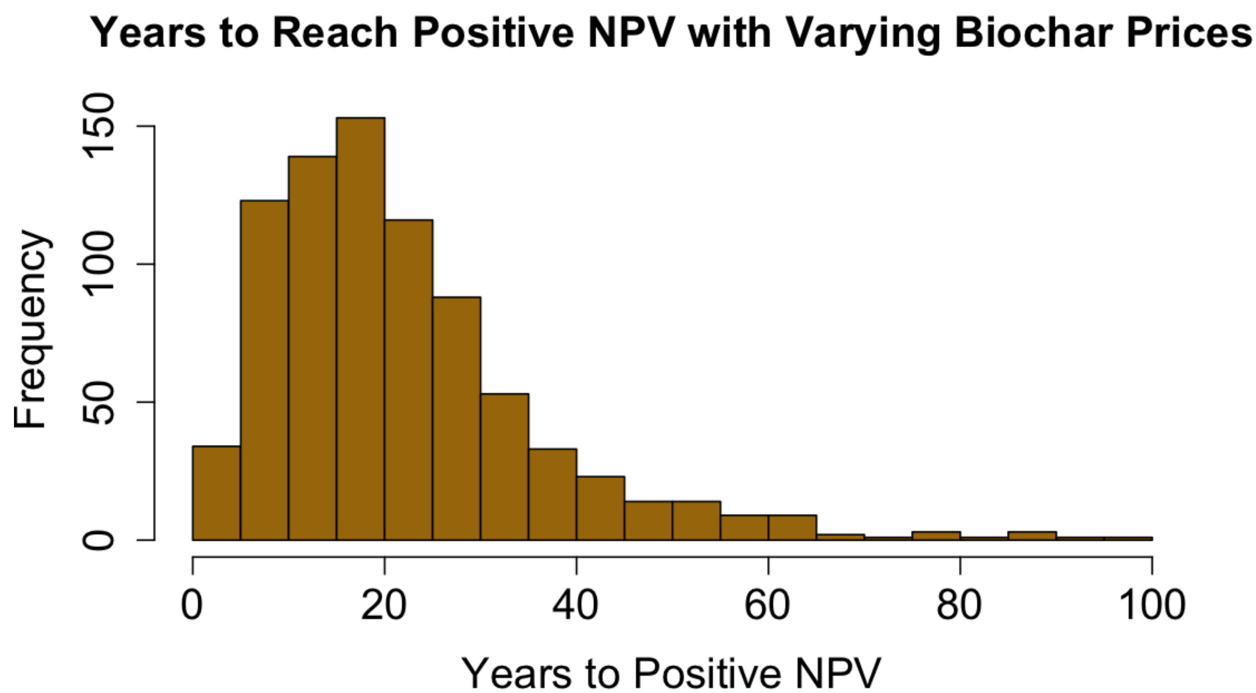

Figure 21. ***Years to Positive NPV with Varying Biochar Prices.*** A Monte-Carlo sensitivity analysis was done on biochar prices assuming a mean of $400/ton with a standard deviation of $150. The X-axis shows the years to reach NPV = 0 under each simulation, and the Y-axis shows the frequency of reaching NPV=0 within each bin.

Discussion

Lab Experiment

After completing the plant trials of lettuce and wheat, this project examined the impact of biochar amendments on the biomass production of wheat and lettuce, utilizing a controlled lab experiment to assess treatment effects. The results indicate that biochar application has the potential to enhance crop biomass, but the effects are variable depending on crop type and biochar concentration.

The Kruskal-Wallis test results demonstrate that wheat biomass, measured by the number of wheat heads per pot, was significantly affected by treatment. Notably, the Control trial yielded significantly lower wheat heads compared to all biochar and fertilizer treatments, suggesting that both biochar and fertilizer contributed to increased biomass production. However, no significant differences were observed among the Fertilizer, 3%, 6%, and 9% Biochar treatments, indicating that while biochar application improves yield relative to the Control, increasing biochar concentrations beyond 3% did not produce additional benefits. This plateau effect may suggest that biochar contributes to soil structure and nutrient retention in ways that support wheat growth, but its benefits may not increase proportionally with higher concentrations. Future research should investigate whether these effects persist across multiple growing seasons and soil conditions.

The ANOVA and Tukey's test results for lettuce biomass indicate a more complex response to biochar application. The 3% and 6% Biochar treatments produced significantly higher biomass than the Fertilizer treatment, highlighting a potential benefit of moderate biochar application. The 3% Biochar treatment, in particular, yielded the highest biomass among all treatments, suggesting that this concentration may provide optimal soil conditions for lettuce growth. Conversely, the 9% Biochar treatment resulted in significantly lower biomass than all other treatments, suggesting that excessive biochar may negatively impact lettuce growth. Potential explanations for this outcome include alterations in soil pH, nutrient availability, or water retention properties at higher biochar concentrations. Future studies should examine these mechanisms in greater detail to determine the physiological and soil-based factors influencing crop response.

Overall, these findings suggest that biochar amendments—particularly at moderate concentrations—can enhance crop biomass, with the strongest effects observed at a 3% biochar application for lettuce and positive impacts across all biochar levels for wheat. However, excessive biochar application may have adverse effects, as demonstrated by the reduced lettuce biomass at a 9% concentration. These results emphasize the need to optimize biochar application rates based on crop-specific responses to maximize benefits while avoiding potential negative impacts.

Additionally, the variability in crop yield responses to biosolid-based biochar application introduces uncertainty for farmers considering adoption. This highlights the necessity for further research to refine best practices, taking into account factors such as soil type, application frequency, and crop type. Expanding field trials, improving economic modeling, and developing policy interventions will be critical in creating a stable and financially viable pathway for biochar implementation in agriculture.

Nutrient Analysis

After completing the plant trial and cost-benefit analysis, the team analyzed lettuce and wheat data on sugars, sugar alcohols, and organic acids to assess potential nutritional differences across biochar treatment groups. This analysis aimed to determine whether biochar could impact the nutritional value of these crops, potentially offering an additional cost or benefit to farmers.

The analytes investigated were fructose, glucose/galactose, lactose, maltose, mannose, ribitol/xylitol, ribose, xylose/arabinose, citric acid, malic acid, succinic acid. Sugars and organic acids were prioritized because they are marketable nutrients for farmers. These specific analytes were then chosen based on data validation. Only three organic acids were detected at significant concentrations: citric acid, malic acid and succinic acid. This is consistent with literature that shows citric acid and malic acid are predominant in wheat and lettuce (Flores et al., 2012; Rodríguez et al., 2011). Other analytes may have resulted in no detection because of their presence in these crops in low concentrations.

The collective analysis of sugars and sugar alcohols showed no statistically significant differences between trials for Lettuce II, whereas wheat exhibited differences. All three biochar treatments resulted in higher sugar concentrations than the control and fertilizer treatments, suggesting biochar may enhance wheat sugar content. At the individual analyte level, only fructose, glucose, and mannose showed statistically significant differences between the Fertilizer and 9% Biochar trials, with the 9% Biochar treatment exhibiting higher concentrations. These sugars likely drove the observed differences in the collective analysis, presenting a potential incentive for wheat farmers to use biosolid-based biochar. However, this was not incorporated into the cost-benefit analysis due to the small sample size and resulting uncertainty.

For organic acids, the collective analysis found no statistically significant differences across treatments for Lettuce I, indicating no additional cost or benefit for farmers using biochar. Data loss and processing errors affected results for Wheat and Lettuce II, limiting conclusive findings. While individual analyte analysis revealed some trends, outliers made interpretation challenging.

Cost Benefit Analysis

The results of this analysis provide valuable insights into the economic feasibility of implementing biosolid-based biochar on small-scale wheat and lettuce farms. The findings suggest the importance of assessing the feasibility of biosolid-biochar implementation on a crop-to-crop basis as the results varied greatly for the wheat and lettuce farmer.

For instance, lettuce farmers benefit substantially from biochar application, with implementation costs fully offset within a year due to revenue increases from yield improvements. This is likely because lettuce is a high-value, high-yield crop, meaning that even modest yield increases translate into significant revenue gains based on USDA average prices. Sensitivity analyses further support this conclusion, as even a conservative 10% yield increase from the 3% biochar treatment still allows the lettuce farmer to recoup initial costs within two to three years. These results suggest that biochar may be most financially viable for crops with short growing cycles and high market values.

In contrast, the wheat results indicate that biochar application may not always be an economically sound decision. Although the 9% Biochar trial resulted in a 12% increase in wheat yield—consistent with prior research (Jeffery et al., 2011)—the long payback period presents a major barrier. Under the model assumptions, a wheat farmer would need approximately 45 years to recover the initial investment through increased crop yields and fertilizer savings. If biochar application only leads to fertilizer cost savings without a yield increase, the benefits never fully offset the initial costs. This raises concerns about the financial feasibility of biochar in lower-value, lower-yield crops like wheat, particularly in the absence of external financial incentives such as subsidies or carbon credits.

Additionally, one key assumption in the model was that biochar would be applied only once, based on literature suggesting long-term benefits (Wu et al., 2023). However, there is skepticism regarding this assumption. If biochar requires periodic reapplication, this could significantly alter cost-benefit projections, particularly for crops like wheat where the initial investment is already difficult to justify. The lettuce farmer, with a much shorter payback period, may be more willing to take on this risk, whereas for a wheat farmer, repeated applications could further reduce financial viability.

The introduction of a carbon market valuing carbon sequestration at $10 per ton reduces the payback period to 35 years, with sensitivity analyses suggesting that higher carbon prices (ranging from $50 to $100 per ton) could shorten this period to between 10 and 30 years. The sensitivity analysis conducted in this project emphasizes the need for robust and reliable carbon pricing policies, especially considering that estimates of the social cost of carbon can reach as high as $413 per ton of CO_2 (Rennert, 2022). Although the role of carbon markets was beyond the scope of this analysis, their potential as a mechanism for compensating farmers for sustainable agricultural practices warrants further consideration.

If carbon credits become a stable and predictable revenue stream, biochar adoption may increase, particularly when integrated with government subsidies or industry-supported incentive programs. However, challenges related to the verification of biochar-related carbon credits and the absence of standardized methodologies introduce significant financial uncertainty. Consequently, the model presented in this study remains theoretical and requires validation through field trials and comprehensive economic analysis.

These findings emphasize the critical role of external financial mechanisms in facilitating biochar adoption. While biochar's potential for carbon sequestration contributes to its overall value, monetizing this benefit remains complex due to uncertainties in carbon market structures and verification protocols. Further policy advancements, technological innovations, and financial incentives could enhance the economic feasibility of biochar for farmers. However, the carbon credit framework remains underdeveloped, necessitating continued research and policy refinement.

Limitations & Future Research

The food crop trials were conducted in small pots within an outdoor greenhouse on campus, limiting the direct applicability of the findings to large-scale agricultural systems. To enhance external validity, future research should incorporate field trials under real-world farming conditions to capture variations in soil composition, climate, and irrigation practices. The assumption of uniform soil and climate conditions, as well as a single wheat harvest annually, may oversimplify the complexities of diverse farming operations. Further research should explore region-specific applications, long-term soil health effects, and the scalability of biochar production to better assess its feasibility for small-scale farmers.

Data collection and interpretation were also subject to certain constraints. The wheat biomass metric was affected by errors in seed weight and count recording, necessitating the use of wheat heads per pot as a proxy. While this metric provided useful insights, future experiments should incorporate more precise biomass measurements to strengthen statistical analyses. Similarly, challenges in measuring lettuce biomass—including spoilage due to refrigeration issues in the first trial and leaf predation by rats in the second—required the use of stalk diameter as a biomass proxy. Although independent field data from Frecker Farms validated this approach, further replication is needed to confirm its robustness across different growing conditions.

As previously noted, both dogbane and basket rush experienced substantial predation and mortality in the Native Plant trials. These outcomes were likely influenced by the outdoor experimental conditions and the propagation method, as the plants were cultivated from cuttings rather than grown from seed. Given the limitations in data quality and robustness, statistical analysis was not conducted on this dataset. This project highlights the need for further research on the application of biochar for native plants, as there is currently a lack of literature demonstrating its effectiveness or feasibility.

While the findings suggest promising yield benefits of biochar, its long-term viability requires further evaluation. Discussions with experts highlighted the potential need for periodic biochar reapplication, which could alter cost-benefit projections and have implications for long-term soil health. Future research should explore the persistence of biochar benefits over multiple growing seasons and assess whether soil properties or microbial communities shift over time in response to repeated applications. Expanding research in these areas will be essential for refining best practices and ensuring the practical feasibility of biochar as a sustainable agricultural amendment.

In conducting this nutrient analysis, several factors could have contributed to the limitations in data acquisition and analysis. Measurement and processing errors with the LCMS instrument likely led to the limited data for the wheat organic acid and lettuce I sugars/sugar alcohols results that led to missing data from the 9% biochar treatment trial. Human errors is also a probable cause, as sample contamination or dilution issues could explain outliers in the data where concentration of analytes were orders of magnitude higher than other replicates. Greater experimental limitations also resulted in small sample sizes. Future research should have more replicates for each trial and plant to account for processing and measuring errors.

The project acknowledges that there is additional research that needs to be done to obtain a clearer understanding of the impacts of biochar on metabolites investigated here for wheat and lettuce crops. Additionally, the nutrient analysis was not incorporated into the CBA, meaning future research can investigate how increases or decreases due to biochar treatments can be an added benefit or cost for farmers to advertise when selling their crops.

This study provides valuable insights into the feasibility of biosolids-based biochar for agricultural applications; however, several limitations must be acknowledged, as they present opportunities for future research. One notable limitation was the observed predation of native plants at the Chumash nursery, which complicated the interpretation of biochar’s effects in that context. Future studies should investigate optimal biochar application rates for native plant species while accounting for ecological interactions.

Conclusion

The findings from this group project highlight significant differences in the impact of biosolids-based biochar on wheat and lettuce crops, emphasizing the need for crop-specific considerations in biochar application.

For lettuce, biochar treatment at 3% was sufficient to enhance crop yields, with no significant differences observed when compared to higher application rates. This result suggests that a moderate application of biochar—such as the 3% treatment—can be an effective strategy for improving yield in high-value crops like lettuce. Lettuce's high market value and relatively short growing cycle mean that even modest improvements in yield can lead to rapid financial returns, making biochar a more viable option for lettuce or high-value crop farmers.

In contrast, the effects of biochar on wheat were less pronounced, with the highest application rate (9%) yielding only a 12% increase in productivity, which aligns with prior research (Jeffery et al., 2011). However, the economic payback period for wheat farmers remained long, at around 45 years under the model assumptions. This extended payback period presents a significant barrier for wheat farmers, especially considering that wheat is a lower-value crop with a longer growing cycle. Furthermore, if biochar fails to increase wheat yield, the financial benefits may only stem from fertilizer cost savings, which are not sufficient to justify the initial investment.

These contrasting results underscore the importance of evaluating biochar application on a crop-by-crop basis. Lettuce, being a high-value, high-yield crop, presents a more favorable environment for biochar adoption, whereas wheat's lower yield and market value make biochar less economically viable unless external financial incentives (such as carbon credits or subsidies) are introduced. The difference in outcomes between the two crops can be attributed to both agronomic factors, such as the crops' respective growth cycles and yield responses, and economic considerations related to crop value and market demand.

The nutrient analysis of wheat and lettuce under different biochar treatments indicated that while biochar had no significant effect on lettuce nutrient composition, it increased sugar concentrations in wheat, particularly fructose, glucose, and mannose in the 9% Biochar treatment. These findings suggest that biosolids-based biochar may enhance wheat's nutritional value; however, further research is required to validate these effects. Limitations such as small sample sizes and data processing errors highlight the need for future studies with larger, more robust datasets and improved methodologies. A more comprehensive understanding of biochar's influence on crop nutrition could provide valuable insights into its economic implications for farmers and inform strategic crop selection.

The cost-benefit analysis underscores the interaction between biochar's agronomic and environmental benefits. Biosolid-based biochar presents a promising avenue for enhancing crop productivity, improving soil health, and contributing to climate change mitigation. Beyond agricultural benefits, its role in waste management and the repurposing of biosolids further strengthens its sustainability potential. However, widespread adoption will depend on overcoming financial barriers through mechanisms such as carbon markets, subsidies, and targeted policy support. The financial viability of biochar significantly improves when integrated into a well-functioning carbon market, while its standalone implementation remains economically risky. Given that farmers are often risk-averse and hesitant to adopt expensive new practices, policies that reduce financial uncertainty—such as tax credits, grants, or cooperative purchasing programs—could encourage broader adoption.

Furthermore, application of biosolids based biochar to offset reductions in traditional fertilizer use fits into the broader context of sustainable or regenerative agriculture. This may particularly appeal to small farmers who engage in such practices or receive organic certification from the US Department of Agriculture. Further research and policy recommendations should consider the tradeoffs of using different biochar feedstocks, including waste water treatment plant biosolids, and how they may influence funding and organic or other certifications in the future.

Despite the limitations and challenges from the native plant experiments, the data shows that there is a high mortality risk from using a high biochar treatment. For basket rush, the 9% biochar treatment resulted in the mortality of all plants. While no statistical analysis could be done on this part of the experiment, these preliminary results show that caution must be taken when considering the use of biosolid-based biochar for native plants. Too much biochar runs the risk of harming native plant growth. On the other hand, the 6% biochar treatment for basket rush had similar mortality rates to the control treatment. Similar observations are true for the dogbane plants. These results suggest there is still potential in using biochar for native plants and further research is necessary.

The SYCEO can apply this methodology to further investigate the potential of biochar as a soil amendment for native plants in the nursery. By building on the limitations and findings of this study, the SYCEO can gain a clearer understanding of whether biochar is a viable alternative and which plant species may benefit from its application. Beyond informing nursery practices, this research lays the foundation for future biochar field trials, helping to identify crops that may benefit from biochar application and evaluate its feasibility across diverse cropping and planting systems. The team looks forward to future biochar research and its role in advancing sustainable agricultural practices.

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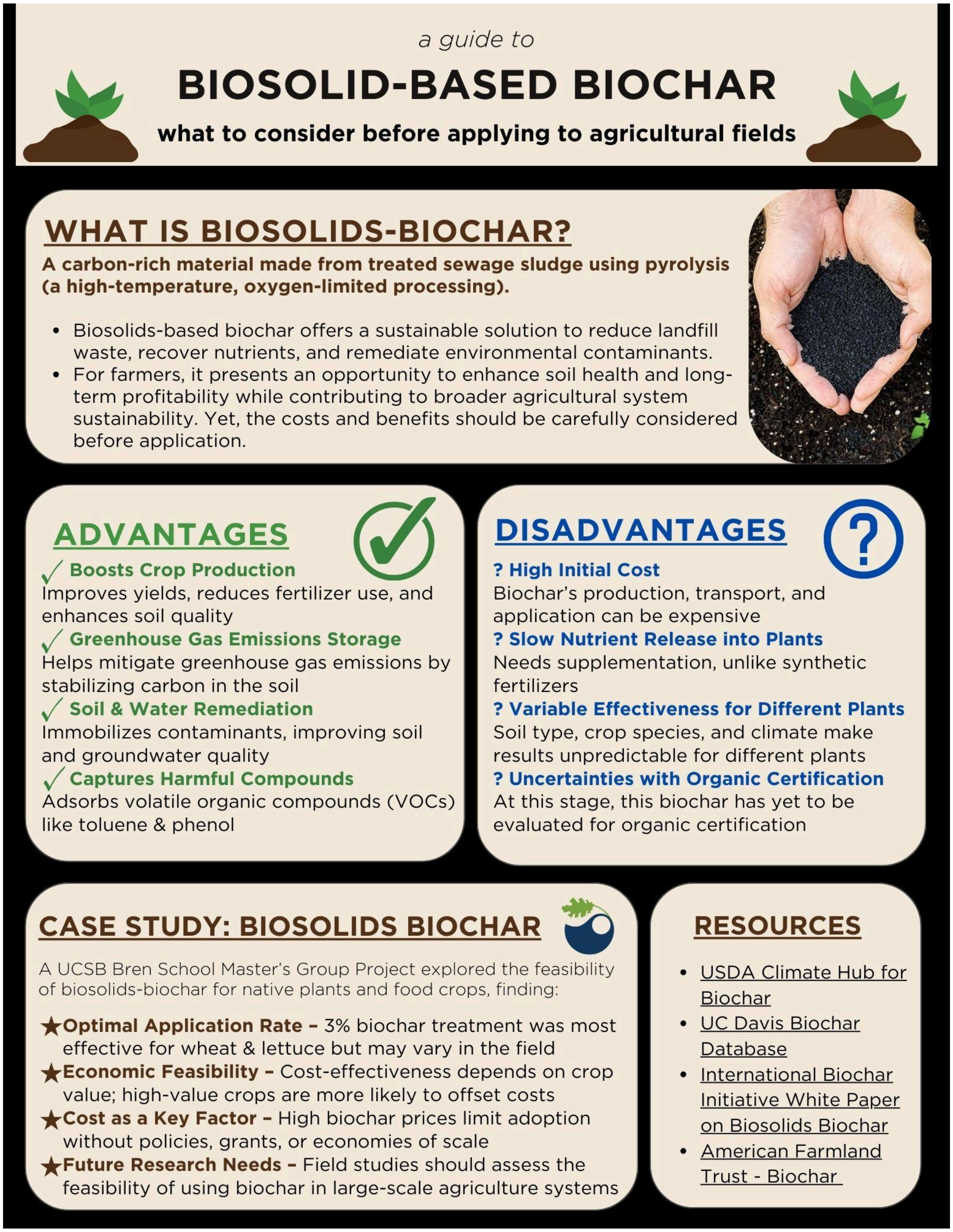
Appendix

1: Visual Infographic of Biosolids-Biochar for Educational Use



2: Experimental Protocol by WeiWei Li of the Keller Laboratory for Nutrient Extraction as Transcribed by Sam Lance

Lettuce and Wheat Processing Instructions

1. Separate leaves from roots when processing
2. Weigh leaves and roots for every plant individually
    a. Write down results on Excel sheet
3. Once weighed, separate the hard stalk from the leaves
    a. Chop up the stalk finely so will make easier to process once frozen
    b. Cover chopping board in foil to not introduce contaminants
4. Freeze all plant tissue in individual baggies in the negative 20 storage, make sure to label them well.
    a. Located across from where wheat was incubated, go in through the door, and go to the door in the back of the room, first shelf on the right
5. Retrieve liquid nitrogen from behind chem building (wooden fence with white sign with black arrow)
    a. For further instructions watch video
6. Prepare a styrofoam box with ice from room 2008 to put samples on once processed
7. Label all beakers with plant name, treatment name
8. Crunch all plant tissue in the baggies prior to mixing
9. Pour plant tissue into mortar and pestle, pour liquid nitrogen over to fully cover and let boil
10. Crush plant tissue until fine powder, put into white 50mL Falcon tubes and put on ice
    a. Note: when crushing plant tissue put all plant samples from same treatment into one storage container, don't care about individual plants just treatments as a whole
11. Once all samples are complete put back into the -20 storage as quickly as possible
12. Take three centrifuge tubes and label as follows
    a. L-T1-L-R1
    b. Code: lettuce-treatment number-leaf or root- replicate number (1-3)
13. Weigh out 0.1 grams of lettuce mixture into each tube
    a. Balance is in back right corner of the lab
    b. Be precise down to 0.100
14. Create the extraction solvent: likely will need 6 rounds of this, each day two batches so can process one round in the morning one in the afternoon
    a. Methanol: 40 mL
        i. Transfer to smaller bottle with blue top in center right lab area from large amber bottles
    b. Special LCMS Water: 10 mL
        i. Transfer to smaller bottle with blue top in center right lab area from large amber bottles
    c. Formic Acid: 1 mL

        i. Located under one of the fume hoods, take this out under the fume hood because not good
    d. Vortex afterwards to make sure fully mixed
15. Add 1mL of the solution to each of the 24 currently processing
16. Follow steps to fully mix
    a. Vortex: video for this, vortex for 20 minutes
    b. Sonicate: video for this, sonicate for 20 minutes
    c. Centrifuge: video for this, centrifuge for 20 minutes
17. Set out four foam square 24 slot tube holders and label 1, 2, 3, and 4
    a. Located in front right corner of the lab in a tall pile
    b. Vial 1 = antioxidants
    c. Vial 2 = organic acids phenolics, nucleabase/side/tides, vitamins, metabolites
    d. Vial 3 = amino acids, sugar alcohols, sugars
    e. Vial 4 = fatty acids, other metabolites
18. Put small amber vials in each of the slots for each of the four tube holders
    a. Located middle bench on the right
    b. Label each of the vials with the specific treatment, sample type, specific vial number (1,2,3,4)
        i. Example: F-L-1= fertilizer trial, lettuce, vial 1
    c. In total, you should have 60 vials, meaning each centrifuged lettuce sample will have four vials each
19. Prior to adding the lettuce solution, make the two solutions you will need for vials 3 and 4
    a. Note: some of this has already been prepared, look in back left corner cabinets
    b. Vial 3: 8 mL LCMS grade water, 32 mL ACN
    c. Vial 4: 2 mL LCMS grade water, 26 mL ACN, 12 IPA (isopropyl alcohol from brown bottles)
    d. Set aside for now
20. Take one centrifuged lettuce sample, and apply 200 uL of it to vials in each of the four foam trays
    a. Keep consistent and ensure trials are not being mixed together
    b. Change pipette tips when change samples
    c. Do not touch pipette tips to the solid matter at the bottom of the tube
21. Seal all vials in **Vial 1** foam tray and put into -20 degree freezer
22. Vial 2:
    a. Add 8uL of OA ISTD, 10 uL NAM ISTD, and 120 uL of LCMS grade water
    b. Seal all vials and put into -20 degree freezer
23. Vial 3:
    a. Add in 30 uL AA ISTD and 5 uL SA ISTD
    b. Drying:

        i. NOTE: watch video before conducting this step, this is only a brief summary
        ii. Bring into soil laboratory on first floor into the back laboratory
        iii. Place vials behind spring around the circular machine
        iv. Lower syringes just into the lip of the bottle – can lower as the process runs
        v. Turn the valve on the left wall to be horizontal
        vi. Wait 10 minutes or until all liquid is gone
        vii. Remove samples and turn off valve
    c. Reconstituting:
        i. Add 200 uL of the solution from above
        ii. Vortex all samples for at least 10 seconds
    d. Place in -20 degree freezer

24. Vial 4:
    a. Add 10 uL FA ISTD
    b. Drying:
        i. NOTE: watch video before conducting this step, this is only a brief summary
        ii. Bring into soil laboratory on first floor into the back laboratory
        iii. Place vials behind spring around the circular machine
        iv. Lower syringes just into the lip of the bottle – can lower as the process runs
        v. Turn the valve on the left wall to be horizontal
        vi. Wait 10 minutes or until all liquid is gone
        vii. Remove samples and turn off valve
    c. Reconstituting:
        i. Add 200 uL of the solution from above
        ii. Vortex all samples for at least 10 seconds
    d. Place in -20 degree freezer
25. Wrap parafilm around lids of the ISTDs, place in the -20 degree freezer
26. Let Arturo know when all samples are in freezer for laboratory tech to help run the LCMS machine