Evaluating the Long-term Effects of Biochar on Copoazu Growth and Microbial Activity Levels in a Tropical Agroforestry System

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Abstract

Biochar, a charcoal-like substance used as a soil amendment, has the potential to increase agricultural productivity through the modification of soil properties, while simultaneously acting as a carbon sequestration strategy. Research indicates that biochar can build up soil organic matter, increase soil pH, improve hydro-structural properties of the soil, and increase cation exchange capacity and soil nutrient retention. These soil improvements are important for the highly weathered tropical soils of the Amazon, where they have the potential to increase the longevity of existing agricultural land and consequently help fight the cycle of deforestation driven by agricultural expansion. Despite the growing body of research on biochar, its effects – particularly in agroforestry systems – remain poorly understood, with mixed results observed in plant growth and microbial activity. The objective of this study was to evaluate the effects of biochar on copoazu (*Theobroma grandiflorum*) growth and microbial activity levels in a tropical agroforestry system in Madre de Dios, Peru, five years after an initial biochar application. Copoazu growth was assessed by measuring the diameter at breast height (DBH) and trunk diameter at a height of 2 inches. Microbial activity was monitored by measuring the mass loss of tea bags buried in the soil. Results indicated that the average DBH and trunk diameter at 2 inches of copoazu plants treated with biochar was greater than those in the control sites; however, these results were not statistically significant ($p > 0.05$). Additionally, no significant differences in decomposition rates were observed between biochar-treated and control sites. While these findings did not reach statistical significance, they contribute to the growing body of knowledge regarding the complexities of biochar's effects on soil properties and ecosystem services.

Introduction

The planet's tropical forests have a large potential to store carbon (and therefore mitigate climate change) due to their high biomass (Rahman et al., 2017). However, tropical deforestation is occurring at an alarming rate, releasing this stored carbon from biomass and soils back into the atmosphere (Asner et al., 2009). In the Amazon basin, the largest driver of deforestation is expanding agricultural activities (Riquetti et al., 2023). Farmers oftentimes will clear and burn forested lands, cultivate them for one or two growing seasons until the nutrients are depleted from the soil, and then move on to clear more rainforest for new agricultural land (Weil & Brady, 2017). One proposed strategy to combat this agriculture-driven deforestation in the tropics is increasing the productivity of land that is already being used for agriculture (Bass et al., 2016; Colman de Azevedo Junior et al., 2022).

Biochar is a carbon-rich, charcoal-like substance that is the byproduct of pyrolysis (thermal treatment) of organic material or biomass in an oxygen-limited environment (Basak et al., 2022). The discovery of the "Terra Preta" soils, which are ancient tropical soils in Amazonia recognized by their dark color, provide evidence that ancient peoples purposely introduced biochar to tropical soils as a soil amendment (Allohverdi et al., 2021). A considerable amount of research in recent years has demonstrated that biochar can build up soil organic matter, increase soil pH, improve hydro-structural properties of the soil, and increase cation exchange capacity and soil nutrient retention (Li et al., 2018; Basak et al., 2022). These properties give biochar the potential to enhance plant growth in tropical agricultural systems.

Additionally, the lack of oxygen during the pyrolysis process in biochar production causes incomplete combustion of the original biomass – so instead of leaving plant biomass from agricultural waste to decompose or be burned through the complete combustion process (which releases carbon dioxide back into the atmosphere), pyrolysis stores this as solid carbon in the resulting biochar. Therefore, biochar's carbon storage abilities give it the potential as a carbon sequestration strategy.

There is a significant amount of research supporting biochar's abilities to improve crop growth and yields (Biederman & Harpole, 2013). However, most research on biochar is limited to small-scale studies in laboratory or greenhouse settings (Nair et al., 2017), so little is known about biochar's effects in the context of agroforestry systems. Additionally, little is confirmed about its effects on soil microbes, which play large roles in nutrient cycling and organic matter composition (Deshoux et al., 2023) as well as maintenance of soil structure and suppression of pests and diseases (Alkharabsheh et al., 2021). Many studies have been conducted measuring responses in soil microbial activity in response to biochar applications, but results are varied, due to different biochar characteristics, soil properties, and experiment conditions, making it difficult to identify its general effects (Zhang et al., 2018; Singh et al., 2022; Deshoux et al., 2023; Vannini et al., 2023).

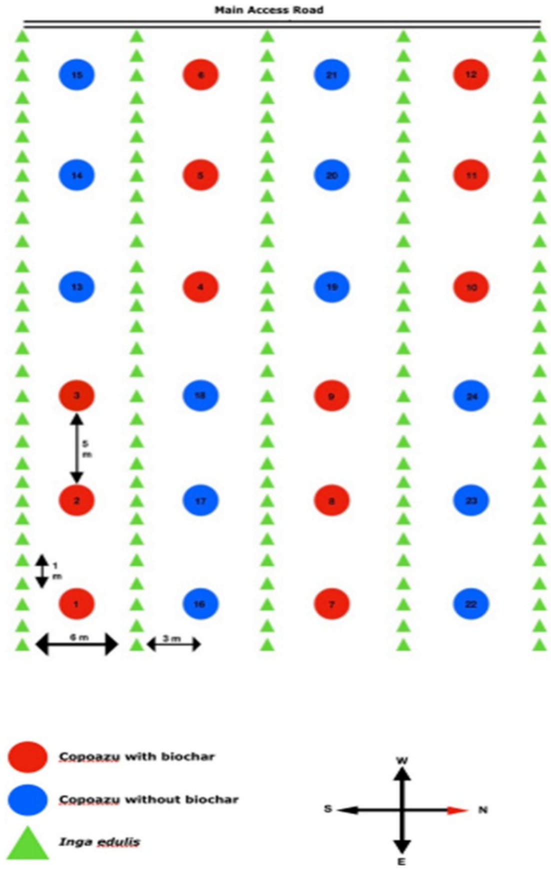
The aim of this study is to measure the long-term effects of biochar on copoazu growth and microbial activity levels in a tropical agroforestry system five years after an initial biochar application. A measurement of copoazu trunk diameters at two different heights was taken to measure plant growth, and a simplified litter bag experiment was conducted which involved monitoring mass loss of tea bags (representative of dead plant material) buried in the soil over a period of time to measure microbial activity levels. I hypothesize that copoazu plants treated with biochar will have larger trunk diameters, while tea bags in sites treated with biochar will have a higher rate of decomposition (and therefore higher microbial activity levels) as compared to control sites.

Methods

Study site

The study site is located at Finca Las Piedras biological research station in Madre de Dios, Peru (Latitude -12.22789; Longitude: -69.11119). The field site is an agroforestry plot that was planted in October and November of 2019 by a previous intern of the Alliance for a Sustainable Amazon, Allison Leight. The plot contains 24 copoazu trees (*Theobroma grandiflorum*) planted in four rows of six plants each, interspersed with rows of *Inga edulis* trees. Copoazu is a local relative of cacao, while *I. edulis* is a fast-growing tree that is known for its nitrogen-fixing capabilities. Biochar was applied at the time of planting to the plot in a block design (Figure 1). The biomass used to produce the biochar was Brazil nut shells, an agricultural waste product from previous harvests at Finca Las Piedras. The initial biochar application was charged with chicken guano, and there were no additional applications of biochar (Leight, 2019). One row of copoazu plants, containing two plants treated with biochar and two plants treated without biochar, was chosen randomly to test decomposition rates (Figure 1).

Figure 1: Map of the agroforestry plot with biochar applied in a block design (Leight, 2019). Decomposition rates were measured at sites containing copoazu plants 4, 10, 13, and 19.



Copoazu growth

Following the plot protocol that Leight created (Leight, 2019), the trunk diameter of each copoazu plant was measured at breast height (approximately 1.3 meters) using a soft measuring tape wrapped around the tree at this height. For copoazu plants that had multiple stems (n = 10), the DBH was determined by using the quadratic sum method (below), as recommended by the New York State Department of Environmental Conservation:

$$= \sqrt{(DBH\ Stem\ 1)^2 + (DBH\ Stem\ 2)^2 + (DBH\ Stem\ 3)^2}$$

Additionally, the trunk diameter was measured at a height of 2 inches, which was selected because it represented the minimum height common to all copoazu plants before they split into multiple stems. This measurement was taken to supplement the DBH data.

These measurements were entered into a data sheet, and bar graphs with standard error bars showing the means of the DBH and trunk diameter at 2-inch height in biochar-treated versus control sites were created using an R script. A one-way ANOVA test was run in R to determine significant differences between these means. At the time of this study, the copoazu plants had not started to flower or produce fruit, so yield measurements (flower densities, fruit yield, and pod numbers) were not able to be recorded.

Decomposition rates

The methodology chosen to evaluate microbial activity levels in soils amended with biochar versus control soils is based on the Tea Bag Index (TBI). The original Tea Bag Index was proposed by Keuskamp et al. (2013). This study proposed a simplified litter bag experiment which involved monitoring mass loss by decomposition of tea bags (representative of dead plant material) buried in the soil over a ninety-day period to monitor soil biological activity levels. While the TBI has traditionally been used to compare soil microbial activity across different ecosystems, it is less frequently used to determine differences in soil quality due to agricultural management practices (Gmach et al., 2024). However, the accessible and low-cost approach of the TBI makes it an attractive potential tool for farmers, who may not have the time, resources, or means to conduct expensive soil tests, to determine the effects that different management practices may have on their soils.

The original TBI uses two specific kinds of rooibos and green tea manufactured by Lipton, intending to create an easily reproducible experiment from which a worldwide database of decomposition data can be created using the same, standardized brand of tea (Keuskamp et al., 2013). In this approach, tea bags of each variety are weighed and buried underneath the soil. After 90 days, the tea bags are taken out, dried, and weighed. The decomposition rate (k) is calculated using the remaining weights of the tea bags and fitting this to a decomposition curve represented by:

$$W(t) = ae^{-k*t} + (1-a)$$

Where *W(t)* is the relative remaining mass of the substrate as a function of how many days (t) it has been buried in the soil, k is the decomposition rate of the sample, a represents the labile fraction of the substance (the fraction of tea that is easily broken down before decomposition plateaus), and (1-a) represents the recalcitrant fraction of the substance (Middelanis et al., 2023).

Recently, the specific green and rooibos Lipton tea bags originally used by the Tea Bag Index were discontinued by the manufacturer, making its goal of worldwide standardization no longer an option. Middelanis et al. (2023) tested four different flavors and brands of alternative teas and found that black tea had particularly low standard errors in comparing the decomposition curves of different brands. Consequently, the tea bags chosen for this experiment were a Peruvian black tea manufactured by La Fidelia, flavored “Eternal Desire.” The La Fidelia tea brand was chosen because its bags are made of woven (as opposed to nonwoven) mesh material, which is consistent with both Middelanis et al. (2023) and the original TBI (Keuskamp et al., 2013).

Thirty-six tea bags were weighed initially and given a unique identifier code on their tags. At each of the four sites, nine tea bags were buried 8cm deep in the soil and marked with a wooden piece in a circle surrounding the copoazu plant, making sure to place the bags 20 cm apart from each other, per recommendation from Middelanis et al. (2023). After 20 days in the soil, the first three tea bags from each site were unburied, dried in the sun for two days, and weighed to the nearest hundredth of a gram. This process was repeated with an additional three tea bags from each site after 40 days in the soil, and then repeated for the final three tea bags from each site after 90 days in the soil. Therefore, the measurement of the remaining masses of the tea bags was done three times throughout the decomposition period, rather than just once as in the original TBI procedure, to assess the decomposition rate (k) more precisely (Middelanis et al., 2023). These masses were recorded into a data sheet.

A decomposition curve was modeled for each site using the remaining masses of the tea substrates after 20, 40, and 90 days, using an R script. A one-way ANOVA test was run in R to determine significant differences between *k* values for sites treated with biochar versus control sites.

Results

Copoazu growth

Figure 2 shows the mean DBH (in) of copoazu plants in biochar-treated versus control sites. While the mean DBH of plants in the biochar-treated sites was larger (1.36 in) as compared to control sites (1.24 in), the results were not found to be statistically significant ($p > 0.05$). Similarly, the mean trunk diameter at 2-inch height in biochar-treated versus control sites was found to be larger in biochar-treated sites (2.37 in) as compared to control sites (2.33 in), but the means were also not found to be statistically significant ($p > 0.05$) (Figure 3).

Decomposition rates

Figure 4 shows decomposition curves of the relative remaining mass of tea bags between biochar-treated sites (Site B4, Site B10) and control sites (Site R13, Site R19). The *k* values for the biochar sites were 0.055 and 0.044, while the control sites had *k* values of 0.049 and 0.071, respectively. While the average of the *k* values for our biochar sites (0.0495) was technically found to be lower than for our control sites (0.06), it is important to note that each average is derived from only two observations. The ANOVA test results indicated there was no significant difference between *k* values between the biochar-treated sites and control sites ($p > 0.05$).

Discussion

This study aimed to determine the effects of biochar on copoazu growth and microbial activity levels in a tropical agroforestry system five years after an initial biochar application, measured by trunk diameter measurements and the decomposition rates of bags of black tea buried in the soil. We found that although biochar-treated copoazu plants had higher average DBH and trunk diameter at a 2-inch height compared to control sites, these differences, along with decomposition rates

Figure 2: Mean DBH (in) of copoazu plants treated with biochar (red, n = 12) versus control treatments (blue, n = 12) (p > 0.05).

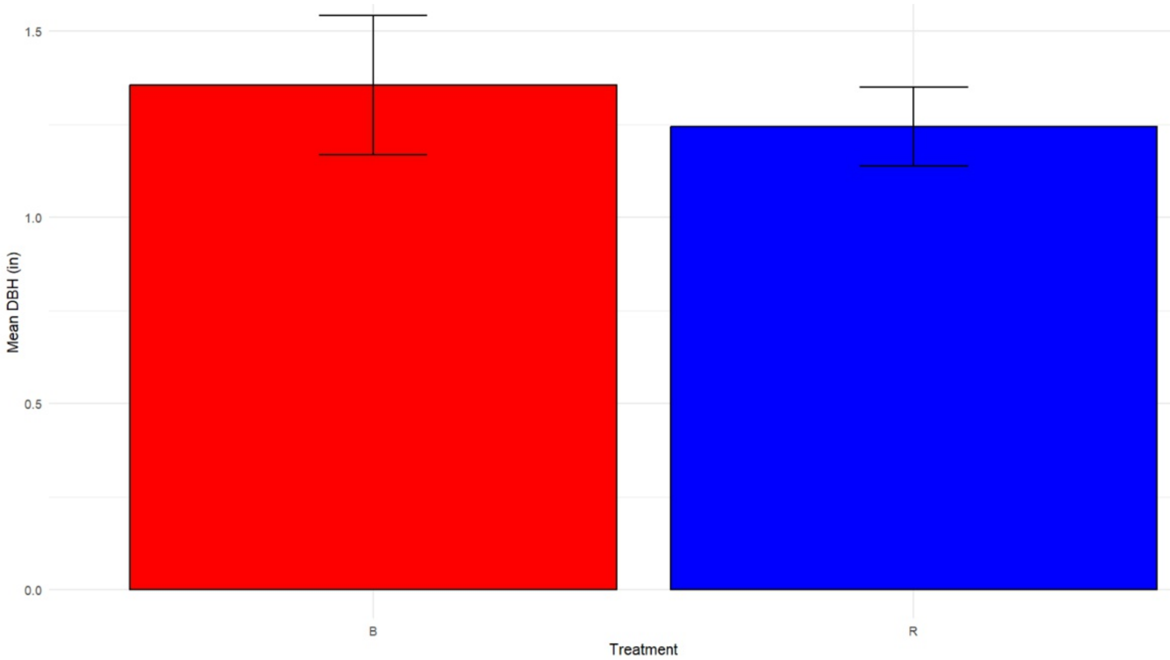


Figure 3: Mean diameter at 2 in height (in) of copoazu plants treated with biochar (red, n = 12) versus control treatments (blue, n = 12) ($p > 0.05$).

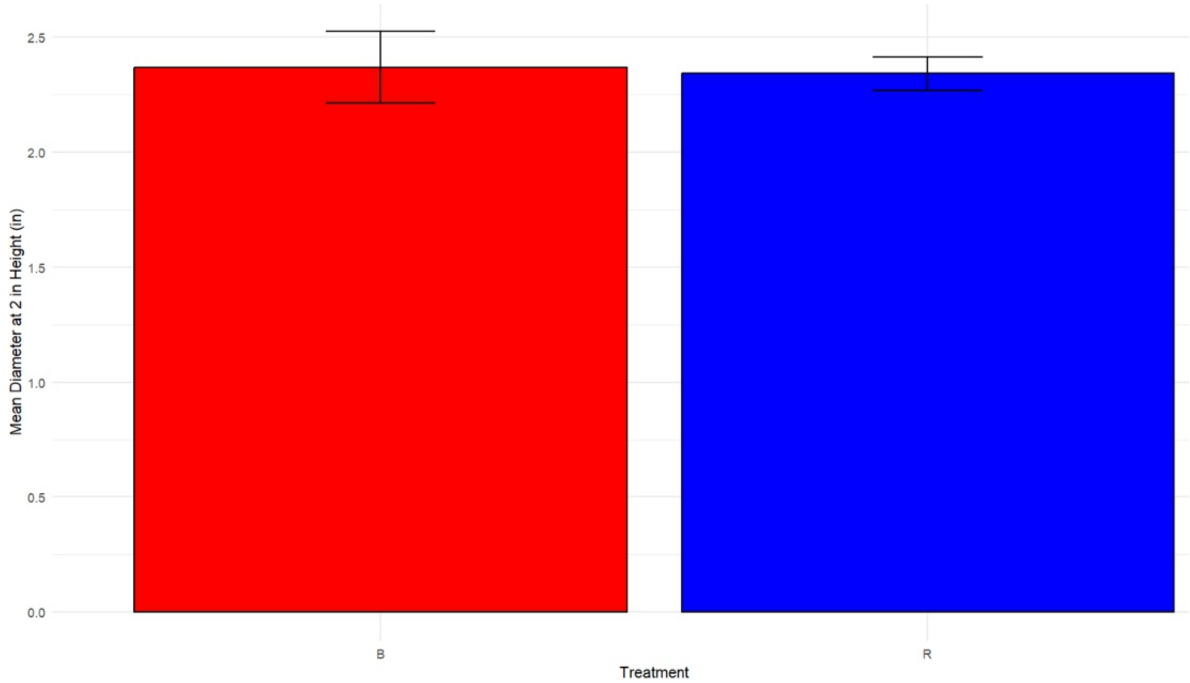
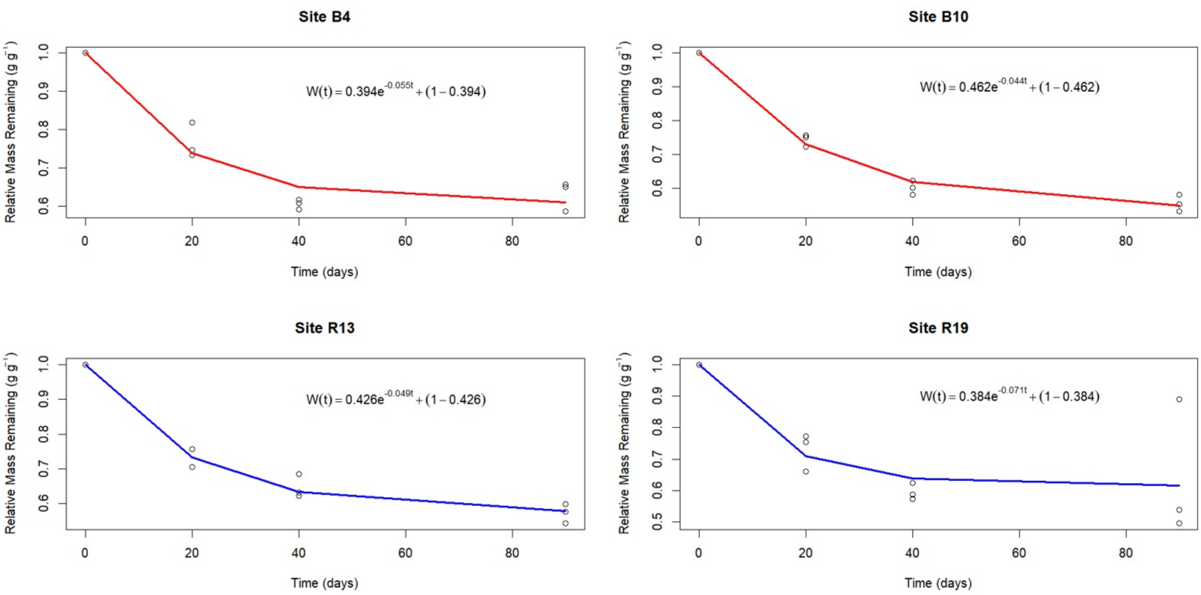


Figure 4: Relative remaining mass of teabags after 20, 40, and 90 days in the soil. Dots show measured values and lines are fitted using an R equation. The colors of the lines represent the treatment (red = biochar, blue = control).



between sites, were not statistically significant.

A few overarching limitations may have influenced the results of this study. First, the relatively small sample size, as we were limited by the materials we had available, likely reduced the statistical power to detect significant differences. Additionally, there is a significant amount of research that suggests biochar's effects on microbial activity are affected by feedstock type and pyrolysis temperature of the biochar (Zhou et al., 2017; Li et al., 2018; Li et al., 2020; Deshoux et al., 2023). In this study, the feedstock type was Brazil nut shells, but the absence of recorded pyrolysis temperature when the biochar was first produced five years ago introduces uncertainty about its properties, potentially affecting plant growth and soil decomposition dynamics.

Copoazu growth

The observed increases in DBH and trunk diameter align with existing research suggesting that biochar can enhance plant growth. Studies on biochar's influence in agricultural settings frequently show increases in crop yields, but in the limited amount of studies performed in agroforestry systems, results are mixed, with the treatment of biochar resulting in increased plant growth (Thomas et al., 2019; Victoria et al., 2023) inconsistent effects (Krapfl et al., 2016), or no detectable effects (Deng et al., 2017). Our findings add to this nuanced picture and suggest that biochar's effects in agroforestry systems may be context-dependent, due to differences in biochar properties, soil types, or other environmental conditions.

Future studies should aim to assess additional growth parameters, such as plant height, and productivity parameters, such as flower densities, fruit yield, and pod numbers (when the plants start to fruit), to provide a more comprehensive understanding of biochar's effects on growth. Additionally, we recommend that plants should be monitored periodically from the time they are first planted to give insights into any changes or patterns in growth dynamics over time.

Decomposition rates

Only one study was found (Vannini et al., 2023) that tested the effects of biochar application on the decomposition of litter using the Tea Bag Index method, conducted in European beech forest soils. They found that biochar did not affect the decomposition of green tea but significantly slowed the decomposition of rooibos tea. When measuring the decomposition dynamics of different tea varieties, Middelanis et al. (2023) found that black tea decomposed faster than rooibos tea but slower than green tea. Since our study used black tea, the faster decomposition rate may have made biochar's effects harder to detect as compared to rooibos tea. Additionally, differences in environmental conditions between European beech forests and tropical soils could have influenced the results.

There are also other considerations that may have influenced the results. During the weighing of tea bags on day 40 and day 90, many of the bags exhibited holes and root growth. The roots and soil particles were removed as best as possible, but some roots had penetrated the bag and could not be removed without risking damage, potentially affecting the recorded weights. Additionally, tea material may have been lost through the holes in the bag. In future tea bag decomposition studies, it may be beneficial to open each tea bag, weigh its content, then combust that content at 550°C and subtract what is left from the content weight, to ensure that the final weight represents the tea substrate only (not additional soil and roots) (Keuskamp et al., 2013). Additionally, a more precise scale would improve the accuracy of weight measurements (our scale only measured up to a hundredth of a gram). Increasing the sample size could provide more robust data.

While each parameter presented distinct challenges, addressing general issues such as biochar characterization and increasing sample sizes will be essential in future studies to more accurately assess biochar's role in agroforestry systems. Despite the limitations, this study contributes to the growing body of knowledge about biochar's effects on plant growth and decomposition rates. These findings advance our understanding of the complex ways that biochar interacts with our environment and offers a foundation for future investigations that can give insight into biochar's potential in agroforestry systems.

Conclusion

This study found that, although biochar-treated copoazu plants had higher average DBH and trunk diameter at a 2-inch height compared to control sites, these differences, along with decomposition rates, were not statistically significant. These findings may result from factors such as limited sample size and variations in biochar properties related to feedstock and pyrolysis temperature. Future research should explore biochar's effects in agroforestry systems across different feedstock and pyrolysis conditions, increase sample sizes, and include additional measurement parameters to clarify its impact on plant growth and soil microbial activity. Understanding these effects is crucial for leveraging biochar's potential enhance plant growth and soil health while sequestering carbon in agroforestry systems.

Acknowledgements

Thank you to the staff at Finca Las Piedras, especially my supervisor, Joana Duran, for guidance throughout this project and helping me obtain all the supplies I needed. Thank you to Dr. Selfa, my honors thesis advisor from SUNY-ESF, for helping me to write this manuscript. Thank you to Betsy and Jesse Fink from the Career Fellowships Program at SUNY-ESF for funding my research internship in Peru.

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