The Impact of Biochar on Copoazu Growth and Production

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Abstract

Biochar is a stable, carbon rich substance that can be made from a variety of plant residues (Oliveira et. al, 2017). It is made in a process known as pyrolysis where the material is heated in a low oxygen environment (Dong et. al, 2017). Research suggests biochar may improve soil health, facilitate the presence of beneficial microorganisms (Li et. al, 2017) and promote plant growth and yield (Graber et. al, 2010, Alburquerque et. al, 2013). Biochar is being also explored for its climate change mitigation potential because it can store carbon for thousands of years (Oliveira et. al, 2017). Biochar could be especially beneficial in sustainable agroforestry models in the Amazon basin by making current plots productive for longer. This is because current land use practices involve extensive logging and inefficient use of the land's resources. The purpose of this study was to determine the impact of biochar on a locally important tree species, copoazu (*Theobroma grandiflorum*) growth as well as flower density, fruit yield, and pod number. This report details the creation of the agroforestry plot and includes a protocol for the analysis of the trees once production begins as well as a discussion of possible

outcomes including the potential for long term gain (Eyles et. al, 2015) with fewer (Bass et. al, 2016) or greater (Alburquerque et. al, 2013) short term benefits.

Introduction

Biochar is a stable, carbon rich substance (Oliveira et. al, 2017) made by heating organic materials with little to no oxygen present in a process known as pyrolysis (Dong et. al, 2017). While biochar consists mostly of carbon, it also contains some nitrogen and hydrogen as well as small amounts of potassium, calcium, and magnesium (Li et. al, 2017). However, the exact chemical composition may vary depending on what material was used to create the biochar. For example, biochar made from wood often has high levels of potassium and magnesium (Li et. al, 2017). Additionally, the components present in biochar can also change based on the pyrolysis process, such as the temperature used (Li et. al, 2017). Biochar can be made from a variety of substances including coconut shell, wood chips, rice straw, and the shells of nuts including walnuts and peanuts (Oliveira et. al, 2017).

While biochar can initially release some of the minerals mentioned previously thus providing benefits to the soil, these disappear over time as they are used by the plant or leached away (Li et. al, 2017). However, biochar can remain in the soil for thousands of years, and may continue to provide benefits long after its initial mineral release (Oliveira et. al, 2017). This is largely because biochar makes additional nutrients "bioavailable" due to the high porosity of the substance (Li et. al, 2017). Some studies have shown that soil treated with biochar is also better able to hold water due to this high porosity (Li et. al, 2017). There is also some evidence that biochar may be able to absorb and store chemical pollution from the soil, such as pesticides and insecticides (Oliveira et. al, 2017). Because of the benefits it provides to the soil, biochar can also increase the number of soil microbes in the area (Li et. al, 2017). In addition, some studies indicate biochar may increase plant growth and yield (Graber et. al, 2010, Alburquerque et. al, 2013).

By far one of biochar's most significant potential benefits that has received extensive attention recently is that its usage may serve as a climate change mitigation strategy. This is because biochar can store carbon without releasing it into the atmosphere for potentially thousands of years (Oliveira at. al, 2017). Often, excess agricultural vegetation such as plant husks are left to decompose or burn and release copious amounts of carbon dioxide into the atmosphere; biochar instead stores this carbon (Mohammadi et. al, 2016). According to Project Drawdown researchers, a group that has compiled solutions with the most potential to reduce greenhouse gas emissions, sequestration through biochar could reduce the emission of, "billions of tons of carbon dioxide every year" ("Biochar", 2019). This assumes that half the plant waste that is currently burned is instead used for biochar production, and that by 2050 about 60 million tons of biochar are being produced across about 1000 "biochar facilities" ("Biochar", 2019).

Additionally, biochar's potential positive impacts are being more closely examined because some research has suggested that biochar can be applied less heavily if it is "enriched" or charged (Mohammadi et al, 2016). This means that substances such as manure or minerals are added to the biochar that have their own benefits (Mohammadi et. al, 2016). These components could provide additional nutrients to the soil and plants, thus boosting the impact of the biochar. For example, one study that looked at the use of fertilizers and biochar in wheat production found that while on its own biochar had little effect, combined with fertilizer it became more effective at boosting the yield than fertilizer by itself (Alburquerque et. al, 2012). Thus, charging the biochar could boost its short term effects before the long term effects of biochar begin.

Researching the effects of biochar is a relatively new field of study, and there are still many unanswered questions about its usage. For instance, much is still unknown about what types are present and how exactly biochar influences microbial growth (Li et. al, 2017). Additionally, many biochar studies have only been conducted for at maximum a few years, so its long term impacts are still poorly understood (Li et. al, 2017). As mentioned previously, biochar's properties can vary based on temperature and how pyrolysis is conducted, and coupling thus with additional fertilizer use means that there is much unknown about how this process impacts soil health (Li et. al, 2017). There are also many potential substrates that can be used to create biochar, and many possible applications that have not yet been explored. This study seeks to explore one such application in an agroforestry context in the Madre de Dios region of Peru.

Biochar has the potential to be especially beneficial in sustainable agroforestry models. The Food and Agriculture Organization of the United Nations defines agroforestry as any system that combines woody species like trees and bushes with traditional agriculture (Hillbrand et. al, 2017). Agroforestry systems have been shown to have many benefits both for people and the natural environment. These include enhancing the health of the soil, providing areas of biodiversity, and creating a sustainable source of income generation (Hillbrand et. al, 2017). In the region of Peru where this study took place, unsustainable agricultural practices often involve extensive logging and inefficient use to the land's resources. This creates a cycle of additional logging and depletion of resources across the landscape. The use of biochar within an agroforestry system planted with trees that sustain the environment, such as through providing shade or nitrogen fixing, could maintain plots for longer periods of time and thus result in less land use changes to agricultural land. Agroforestry in Peru could include a range of species that are used for timber, food, soil health, and promoting biodiversity all while creating economic benefits.

Additionally, biochar could be especially well received in this area because it has been used here in the past. Although the Amazon rainforest is known for its poor soils, some evidence suggests that biochar has been used in this area before to enhance production potential (Glaser et. al, 2001). Known as Terra Preta, these parts of the Amazon with exceptionally dark soils are thought to be remnants of the work of indigenous peoples hundreds and even thousands of years ago (Glaser et. al, 2001). It is believed that through the burning of plant matter, they were able to produce their own form of biochar that even today is still enriching the soil compared to those areas not described as Terra Preta (Glaser et. al, 2001). Thus, the existence of Terra Preta suggests biochar could be quite effective in this region and although not used as much currently, would not be a completely new concept for residents of the Amazon (Glaser et. al, 2001).

This study seeks to design and create an agroforestry plot involving copoazu (*Theobroma grandiflorum*) (Figure 5), a locally important relative of cacao as well as *inga edulis* (Figure 6), a fast growing tree species known for its ability to fix nitrogen and provide shade. Copoazu is grown extensively by locals for food and drinks, and *I. edulis* fruit is also edible. Although they are important economically to the region, very little is known about the use of these two plants in a sustainable agroforestry system. This study will primarily concern the creation of the plot as well as the development of a protocol for future researchers to assess the impact of biochar on copoazu tree growth and fruit production. It is hypothesized that the trees that receive the biochar treatment will be significantly larger and produce more flowers, fruits, and pods. In the future, this plot could serve as a site for further agroforestry research to be conducted, an educational opportunity for local farmers, and a model for the direction of more agroforestry initiatives in the area.

The location of the agroforestry plot is at Finca Las Piedras, a biological research station located in the Madre de Dios region of Peru and operated by the organization Alliance for a Sustainable Amazon.

The station is 54 hectares in size and contains both terra firme lowland rainforest as well as palm swamps. It also contains a growing agroforestry area located centrally to the property in a disturbed grassland habitat near the station's buildings.

Methods

Plot design

To begin, a plot was designed that would contain 4 rows of 6 copoazu trees each. The copoazu trees were spaced 5 meters apart within the rows and 6 meters apart between the rows. Additionally, the plot design featured 5 rows of *I.edulis*, including rows between the copoazu rows and one row at either end of the copoazu rows. The *I. edulis* was spaced 1 meter apart within the rows and the rows were spaced 3 meters between the copoazu rows. At the ends of the rows, the *I. edulis* was extended an additional 2 meters beyond the last copoazu tree to provide the same benefits to edge copoazu trees as those on the interior of the rows.

After the plot characteristics were determined, an aerial photo of Finca Las Piedras was consulted to determine the site for the plot. It was decided that the plot should be located in an area of the property that is a reforestation priority because it was dominated by an invasive grass species. It was also important that the plot be close to additional future agroforestry plots. Also, the plot needed to be located somewhere that would receive ample foot traffic and therefore attention. The plot is located at 12°13'31''S, 69°6'56''W.

Planting preparations

Before planting took place, biochar was made using brazil nut shells from brazil nuts harvested in the past at Finca Las Piedras (Figure 4). This process was repeated a total of five times in an effort to produce the best resulting biochar possible. To begin, roughly crushed brazil nut shells were emptied into a pyrolizer until it was mostly full. The pyrolizer was a large metal barrel with some small holes for limited air flow and a lid containing a chimney made out of sheet metal (Figure 2). Each time biochar was to be made, a fire was built in the top of the pyrolizer on the brazil nut shells, and as soon as it became clear that the brazil nut shells were beginning to burn, the lid and chimney were added to the pyrolizer. The process was monitored generally for about 2 hours, until water splashed onto the outside of the pyrolizer revealed that burning had taken place to approximately the bottom one fourth of the barrel. Then, buckets of water were added to the biochar until the fire was out, and the biochar was left to drain.

When the biochar had drained, it was crushed with a wooden block and sifted. Unburned brazil nut shells remained extremely hard and could not be crushed and subsequently sifted, and these pieces were then added to subsequent biochar batches to attempt to get as many shells as possible to char. In total, 18 kilograms of crushed and sifted biochar were produced. To charge the biochar, in two batches of approximately 9 kilograms each, 1 liter of water mixed with 1 liter of guano was added to the biochar and well mixed. The biochar was then spread on a tarp and left to dry overnight

The area where the plot would be located was marked and workers were hired to clear the grass and small trees from the area. The grass was then raked into rows to decompose between the copoazu and *I. edulis* rows. The sites of the copoazu trees were marked out and approximately 30 cm x 30 cm x 30 cm holes were dug to plant the copoazu. *I. edulis* seeds were planted shallow in the soil and thus did not require hole digging. Copoazu trees were purchased locally as well as *I. edulis* seeds.

Planting and plot maintenance

Planting took place over a few days in late October and early to mid November when the wet season was underway to ensure that the seedlings would have a steady water supply (Figure 3). 1.5 kilograms of the previously charged biochar as well as 1 kilogram of additional guano was added to each of half the copoazu trees as soil was being filled in around the plants. The biochar and fertilizer were added slowly, taking care to not allow the fertilizer to touch and thus possibly damage the roots. The other half of the copoazu trees only received 1 kilogram of guano each as they were being planted and no biochar. The first three copoazu trees of the first row on the southern end of the plot received the biochar treatment, while the last three only received guano. In row 2, the first three copoazu trees only received guano while the last three also received biochar, and this pattern was continued for the remaining two rows. *I. edulis* seeds were planted after the copoazu making sure to clear grass and decomposing plant matter around the planting site to give the seeds more of an advantage. Additional weeding took place over the following month to assist the plants in the beginning stage. Following this, the plot will be evaluated monthly for additional weeding needs and the replacement of any *I. edulis* seedlings as needed. Chicken guano will be applied twice a year to all copoazu trees and *I. edulis* will be pruned as needed to not overshadow the copoazu, leaving trimmings in the plot. A protocol was developed to include a timeline of events for the plot as well as additional details on how the plot should be maintained, and is included at the end of this report.

Plot analysis

To evaluate all stages of copoazu growth, this study will analyze copoazu flowers, fruit, and pods as well as trunk diameter. These parameters were decided upon based on a literature review conducted of other studies evaluating agroforestry systems, especially those related to biochar. Trunk diameter evaluation will occur every 6 months beginning from the time of planting, while other evaluations will not begin until trees begin to produce, which will begin 5 years after planting. How many years data collection occurs is at the discretion of Finca Las Piedras. Many studies of biochar feature short lived species such as tomatoes and therefore include only a short collection time (Graber et. al, 2010) A study by Eyles et. al of apple orchards compared trunk diameter measurements on 3 occasions (2015), but another study of cacao yields was based on 10 years of data (Somarriba & Beer, 2010). As such, this data will most likely be evaluated on a similar timeline as some of FLP's other long term monitoring programs, such as for brazil nut trees and aguaje palms.

Trunk diameter will be measured twice a year at the same time that guano is added to the trees. This is based on the suggestion of a study by Somarriba & Beer that looked at the effects of shade trees on cacao production and monitored trunk diameter (2010) and a study by Eyles et. al for biochar use in apple orchards that also measured trunk diameter (2015). The trunk diameter should be measured at breast height, approximately 1.3 meters. A study by Somarriba & Beer for cacao trees suggests switching to measuring the trees annually after a few years time (2010). For this study, when trees begin fruiting, most likely at the 5 year point but potentially sooner, they can then be measured annually.

Other analyses of the trees will begin once copoazu begins to flower and fruit. Copoazu typically flowers from September to November of each year and fruits from November to February (J. Luna, personal communication, Nov 23, 2019). Therefore, the first analysis will be of copoazu flowers, which will occur beginning in August of most likely the 5th year, although researchers should begin checking for flowers in year 3. Although some studies focused on counting all the flowers on a tree such as in one study on cacao productivity in India (Arunkumar, 2019) others focus on counting all flowers in one area of the tree, such as in one study on almond tree yield (Tombesi et. al, 2016). For this analysis, 6 trees out of 12 of each group should be randomly selected to count all flowers on the tree. To decide which trees will be counted, a random number generator will be used. The flower analysis should occur monthly in

August, September, October, November, and December. On each of the randomly selected trees, all flowers on the tree will be individually counted, taking care to check all sides of the tree without recounting. Flower buds, dead flowers, and flowers on the ground will not be counted.

Analyzing copoazu fruit and pods will begin in November of each year and occur once per month for November, December, January, February, and March to ensure all fruits are seen. Other studies such as by Somarriba and Beer suggest more frequent harvest can occur (2010). However, that study also harvested at one month intervals at some points (Somarriba & Beer, 2010), so monthly surveys are suggested as the fruit harvest will involve all 24 trees and will therefore be the most intensive of the 4 analyses. However, if fruit is over ripening at this harvest frequency, moving to harvesting every 2 weeks can be done instead. Each month, all fruits should be harvested that have fallen from each tree as this is the most reliable method to determine that the fruits are ripe (Hernández & Hernández, 2012). The yield from each tree should be weighed individually. This survey will most likely begin in the 5th year, but checking for fruit should begin in the 3rd year.

Analyzing copoazu pods will occur in 6 of the 12 trees for each of the 2 groups. These trees should be selected randomly using a random number generator. One fruit from each tree will be randomly selected using a random number generator after total yield from the tree has been weighed. The fruit should be cut open and all pods will be counted. This analysis will occur five times, once each month, at the same time that the fruit yield analysis takes place. Like the fruit survey, this will most likely begin in the 5th year; however, checking for fruit will begin in the 3rd year, so the pod survey may also begin sooner.

Data analysis

For each of the variables being measured—trunk diameter, flower number, fruit yield, and pod number, an independent samples t test is suggested to compare the biochar and control groups. This test can be performed multiple times for the same sets of data but manipulated in different ways. For instance, all data from each year can be compiled and averaged for each of the two groups to compare differences between the groups across years. Data could also be compared from month to month for all years or for a specific month. For fruit yield, the two groups should be adjusted to have the same number of trees if some copoazu trees in the study have died and the groups are uneven. The highest equal number of trees could be randomly selected if this was the case.

Results

Much of the results of this study will not truly become apparent until there are several years of tree diameter data and the trees begins to produce fruit, which may not begin to occur for about five years. However, it is expected that the copoazu trees with the biochar treatment will have significantly larger tree diameters, a higher flower count, a greater fruit yield, and more pods per fruit. These results may not be seen for quite some time or may be seen more immediately, depending on how the biochar impacts the copoazu trees especially through changing soil characteristics. Some of the possible outcomes are discussed below.

Discussion

In order to predict and discuss the potential results of this study, it is helpful to view other research on biochar's impacts on plant growth and yield. Many studies exist that look at biochar in the short term, such as through applications on fruit and vegetable crops. These types of studies could help to illustrate what biochar's impact, if any, might be in the beginning phase of this copoazu project. One study that looked at the effect of biochar on tomato plants found that plants treated with biochar had a significantly larger stem diameter (Vaccari et. al, 2015). However, in this case, the plants produced more vegetative material rather than fruits, and yield between the control and biochar treatments were the same (Vaccari et. al, 2015). The article does suggest that biochar addition could mean that less water and fertilizer are needed to produce the same effects as the control (Vaccari et. al, 2015). This could make the sustainability of the agroforestry systems in which biochar is used much greater.

A study on pepper plants found that compared to the control, pepper plants treated with biochar grew more and produced more fruit (Graber et. al, 2010), suggesting that yield increases from biochar can occur. The number of flowers was also significantly greater (Graber et. al, 2010). Interestingly, this study also found that plants with biochar applied were less likely to succumb to diseases; further, even when these diseases were leaf-based and the biochar was applied to the soil, this pattern was observed (Graber et. al, 2010). The authors of this study believe this could be due to biochar supporting the presence of microorganisms that could assist in abating these diseases (Graber et. al, 2010). However, other short term crops showed no increases in plant growth or yield such as for an experiment with potatoes, strawberries, and barley (Jay et. al, 2015). Copoazu is not a short term crop like the ones listed above; for these reasons, biochar may be more successful in this study because its effects could be seen over the long term as suggested by Oliveira et. al (2017).

Additionally, none of the previously mentioned studies combined the biochar with other substances to charge it. One study that did do so showed that even after 2 months of growing time, wheat yields were increased when fertilizer was used in combination with biochar (Alburquerque et al, 2013). However, to show the complexities of the processes at play with biochar, another short term study using bananas and papayas did not find similar results. In this study, researchers combined compost and biochar in what is known as a COMBI (compost and biochar) system (Bass et. al, 2016). However, while certain soil characteristics including mineral levels and soil water content was improved, yield was deceased for bananas and not significantly different for papayas (Bass et. al, 2016). In the copoazu study, biochar was charged with guano; even if biochar impacts soil properties more than yield initially, this could be more useful in the copoazu project than these other studies because there will be more time to view the results. The same could be true with increasing plant foliage; although the impacts of this may not be visible with short lived species, they may be more beneficial to the copoazu trees.

The use of biochar in studies on trees could provide more information about biochar's long term effects. However, as stated in a study by Eyles et. al for biochar's use in apple orchards, "Mechanistic understanding of how biochar affects tree physiology is deficient" (2015) and more studies are needed. This study about apples is especially interesting because it looks at the effects of biochar as well as biochar mixed with compost. For tree diameter, biochar mixed with compost yielded significantly larger trunks beginning in the first year (Eyles et. al, 2015). However, biochar by itself did not yield larger trunks until the fourth year of the study (Eyles et. al, 2015) once again suggesting that biochar's singular effects may not be seen for some time. However, fruit yield was unaffected by any of the treatments (Eyles et. al, 2015). Thus, it is unclear if the biochar needed more time to become effective or was simply not effective.

Additional research on the effects of biochar on trees is extremely scarce, especially as it relates to tropical ecosystems. One recent study of a rubber tree plantation in Thailand looked at the effects of biochar over 2 years, but only in terms of soil impacts and not tree yields. Interestingly, this study found that biochar did not increase the soil water content; however, nutrients such as calcium and phosphorus did increase, while nitrogen did not (Hermann et. al, 2019). This study presents and interesting idea that biochar could be absorbing the nitrogen itself, thus not releasing it to the soil (Hermann et. al, 2019). If this is the case, planting a nitrogen fixing tree such as *I. edulis* could be especially helpful. The study also found that the bacteria and fungi present was different for soil treated with biochar compared to untreated soil (Hermann et. al, 2019). However, little is known about the interaction between these microbes and plants, so it is still unclear what effect this might have. Another study looked at the effects of biochar on loblolly pines over 2 years, but once again only impacts on the soil. This study found, unsurprisingly, increases in soil carbon, but no difference in pH or nitrogen, even though the pH of the biochar used was very high (Krapfl et. al, 2016). This suggests biochar is not sequestering nitrogen unlike in the previous study.

As can be seen by the complex findings of the studies mentioned above, there is still much that is unknown about biochar and thus many more future projects at Finca Las Piedras that can take place, or expand upon the copoazu project. For instance, looking at the use of biochar on some of the other crops at FLP including fruits and vegetables could provide valuable information on how biochar impacts short term crops in the tropical climate, as many of the studies previously sited took place in the temperate zone. Additionally, there are many more variables to be explored for biochar with agroforestry species, including amount of biochar added, how often it is added, what type of fertilizer is used to charge the biochar, and perhaps exploring other sustainable substrates for the biochar. In the copoazu plot, there are additional analyses that could be performed that were not included initially, but could be added should FLP have the interest and resources to pursue them. For instance, soil analyses on the nutrients and microbes present would provide valuable information on biochar's impacts that may not be immediately visible. Also, studies involving cacao such as Somarriba & Beer (2010) and Rajab et. al (2016) determine the dry weight in addition to weighing the fruit and pod when it was fresh, which may also provide another piece of valuable information. Ultimately, as the results of the current study become apparent over the following years and more research on other aspects of biochar is performed, there will begin to be a better understanding of how this substance could be used in sustainable agroforestry systems in the Amazon basin.

Figures & Photos

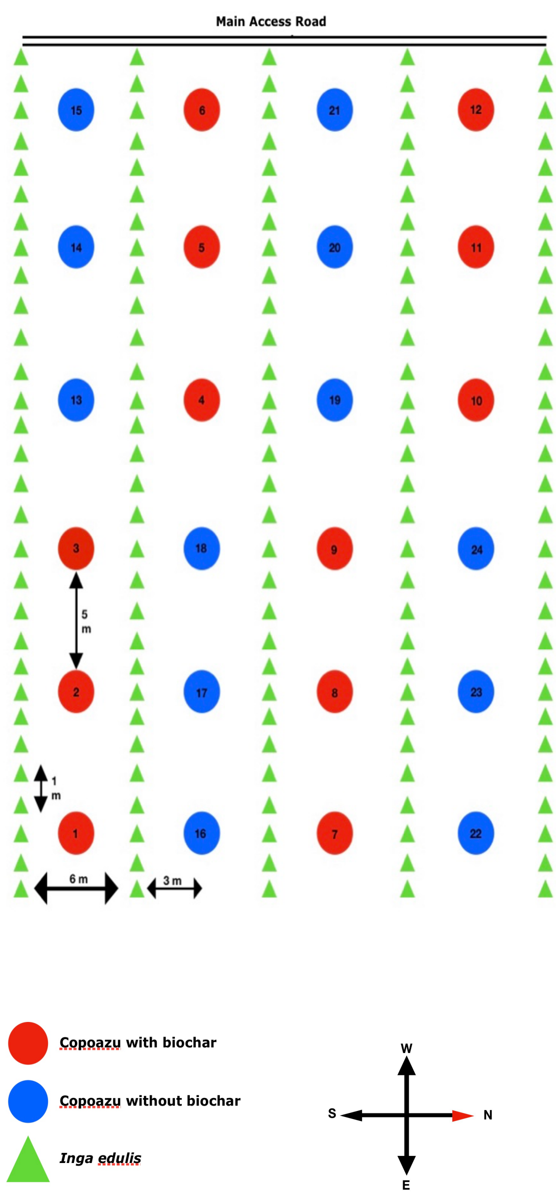

Figure 1. Diagram showing layout of copoazu plot.

Figure 2. Pyrolizer with chimney attached.

Figure 3. Visiting students helping to plant copoazu.

Figure 4. Biochar made with brazil nut shells.

Figure 5. Recently planted copoazú (*Theobroma grandiflorum*).

Figure 6. One of the recently planted *I. edulis* in this study.

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